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D13808F · ISSN 0947-0867

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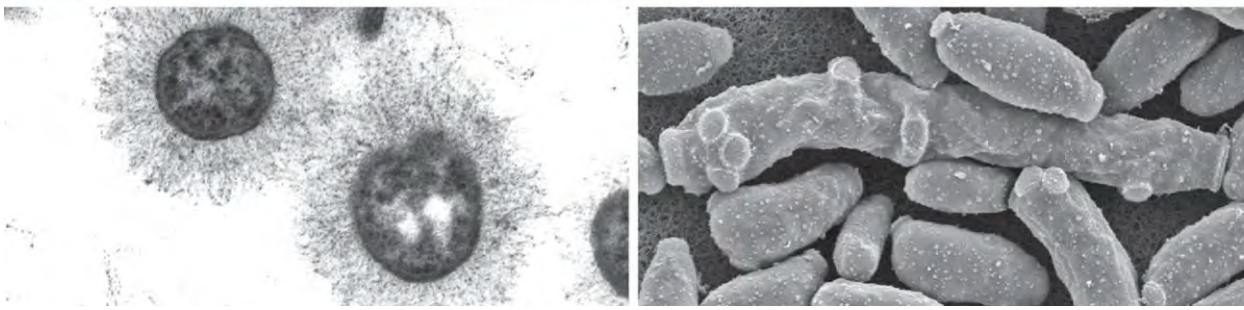

2014
Sonderausgabe

Tagungsband zur 4. Gemeinsamen Konferenz von DGHM und VAAM

VAAM-Jahrestagung 2014
66. Jahrestagung der DGHM

Dresden, 05.–08. Oktober 2014

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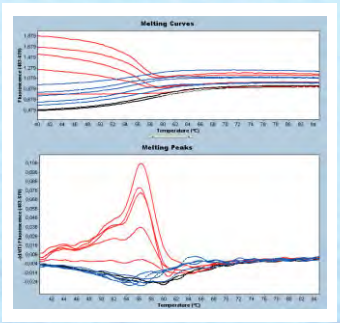

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Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)
Deutsche Gesellschaft für Hygiene und Mikrobiologie e.V. (DGHM)

Tagungsband zur 4. Gemeinsamen Konferenz von DGHM und VAAM

05.–08. Oktober in Dresden

Venue and Date: International Congress Center Dresden (ICD), Ostra-Ufer 2/Devrienstraße 10–12, 01067 Dresden
5–8 October 2014

Conference Homepage: www.dghm-vaam-2014.de

Conference Chairs: DGHM – Prof. Dr. med. Volkhard A. J. Kempf, Goethe University Frankfurt, Institute for Med. Microbiology and Infection Control, Paul-Ehrlich-Str. 40, 60596 Frankfurt a. M.
VAAM – Prof. Dr. Gerold Barth, Technical University Dresden, Institute of Microbiology, Zellescher Weg 20b, 01217 Dresden

Scientific Committee: M. Ansorge-Schumacher (Dresden), M. Göttfert (Dresden), S. Göttig (Frankfurt a. M.), E. Jacobs (Dresden), P. Kraiczy (Frankfurt a. M.), M. Rother (Dresden), C. Brandt (Frankfurt), M. Schlömann (Freiberg), K.-P. Stahmann (Senftenberg), K.-H. van Pée (Dresden), T. Wichelhaus (Frankfurt a. M.)

Hauptprogramm

Abstracts

Liebe Freunde und Kollegen, liebe Gäste,

im Namen der Vorstände der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) und der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM) heißen wir Sie herzlich zu unserer 4. gemeinsamen Jahreskonferenz „Microbiology and Infection“ willkommen.

Wir setzen mit unserer gemeinsamen Konferenz eine mit der Jahrtausendwende begonnene Tradition fort: die DGHM und die VAAM, die beiden größten deutschen Fachgesellschaften auf dem Gebiet der Mikrobiologie, halten in regelmäßigen Abständen ihre Jahrestagung als eine Gemeinschaftstagung ab. Nach den gemeinsamen Kongressen in München (2000), Göttingen (2005) und Hannover (2010) findet die Konferenz in diesem Jahr in Dresden, der Landeshauptstadt Sachsens, statt.

Renommierte internationale und nationale Gastsprecher werden neueste Forschungsergebnisse aus allen Gebieten der allgemeinen, angewandten und medizinischen Mikrobiologie präsentieren. Wir haben die Themengebiete „Metabolismus und Transport“, „Mikrobielle Biotechnologie“, „Mikrobielle Pathogenität“, „Lebensmittelmikrobiologie und -hygiene und Krankenhaushygiene“, „Mikrobielle Stressantwort“, „Mikrobiom in Medizin und Natur“ und „Hypoxie und Anaerobiosis“ als Kernthemen des Kongresses ausgewählt. Diese Kernthemen werden im Zentrum intensiver Diskussionen in Vortrags-Sessions, Symposien, Fortbildungs-Workshops, Postersitzungen und den Fachgruppentreffen stehen. Eine große Industrieausstellung gibt den Teilnehmern Gelegenheit, sich über neue Technologien, Geräte, Dienstleistungen sowie Wirksubstanzen in der Mikrobiologie und Infektionsbiologie sowie der Diagnose und Behandlung von Infektionskrankheiten zu informieren. Ein besonderes Anliegen war es, Themen aus dem Bereich „Infektionsprävention und Hygiene“ einzubinden, und so stellt das Themengebiet „Hygiene“ ein wesentliches Element dieser Veranstaltung dar.

Der diesjährige Veranstaltungsort, das hochmoderne Congress Center des Maritim Hotels, bietet durch seine außergewöhnliche Architektur und das professionelle Equipment, verbunden mit der herausragenden Technik, optimale Bedingungen, um den Ansprüchen von Teilnehmern und Ausstellern gerecht zu werden. Wir danken schon an dieser Stelle sehr herzlich unseren eingeladenen Sprechern und allen Autoren für ihre Beiträge zu dieser Konferenz. Wir sind außerordentlich dankbar für die großzügige Unterstützung durch unsere Hauptsponsoren und Industriepartner, sowie durch die Deutsche Forschungsgemeinschaft (DFG), die European Society of Clinical Microbiology and Infectious Diseases (ESCMID) und die Federation of European Microbiological Societies (FEMS).

Wir erwarten mehr als 1.800 Teilnehmer in Dresden. Besonders wichtig ist uns die Teilnahme von Doktorandinnen und Doktoranden sowie Nachwuchswissenschaftlerinnen und Nachwuchswissenschaftlern. Wie berechtigt dieses Anliegen ist, belegt der Eingang von über 1.000 Beiträgen aus allen Bereichen der Mikrobiologie eindrucksvoll.

Wir freuen uns auf eine interdisziplinäre, interaktive und hoffentlich trendsetzende Tagung in Dresden und hoffen, dass Sie die Gelegenheit wahrnehmen werden, neue wissenschaftliche Impulse zu erhalten, den Austausch zwischen unseren beiden Gesellschaften voranzutreiben, alte Freunde zu treffen sowie neue Kontakte zu knüpfen. Für Ihre Freizeit hat Dresden ein Angebot in Kunst und Kultur, wie kaum eine andere Stadt. Besonders die Altstadt ist mit der Frauenkirche, dem Zwinger und der Semperoper ein Touristenanziehungspunkt.

Willkommen in Dresden.

Volkhard A. J. Kempf (DGHM)

Gerold Barth (VAAM)

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Dear friends and colleagues, Dear guests,

On behalf of the executive boards of the German Society for Hygiene and Microbiology (DGHM) and the Association for General and Applied Microbiology (VAAM) we welcome you warmly to our 4th Joint Conference "Microbiology and Infection".

We continue a tradition begun with the turn of the millennium with our joint conference: the DGHM and the VAAM, the biggest German societies in the area of microbiology, hold their annual conference as a joint conference in regular intervals. After the joint conferences in Munich (2000), Goettingen (2005) and Hanover (2010) this year's conference takes place in Dresden, the capital of Saxony.

We are proud to welcome prestigious international and national guest speakers who will present the newest results of research from all areas of the general, applied and medical microbiology. We have selected the subject areas "Metabolism and Transport", "Microbial Biotechnology", "Microbial Pathogenicity", "Food and Hospital Hygiene-Quo Vadis?", "Microbial Stress Responses", "Microbiome in Medicine and Nature" and "Hypoxia and Anaerobes" as core subjects of the conference. These subjects will be central to intensive discussions in lecture sessions, symposia, advanced training workshops, poster sessions and the special group meetings. Further a large industrial exhibition gives opportunity to the participants to find out about new technologies, devices, services as well as active substances in microbiology and infection biology as well as diagnostic and treatment of infectious diseases. A special concern was to integrate subjects from the area "Infection Prevention and Hygiene". Thus, the subject "Hygiene" represents an essential part of this event.

This year's venue, the very modern Congress Centre of the Maritim Hotel, offers by its unusual architecture and the professional equipment linked with the outstanding technology, best conditions to meet the participants and exhibitors requirements.

We would like to take this opportunity to thank our invited speakers and all authors very warmly for their contributions to this conference. We are exceptionally grateful for the generous support by our main sponsors and industrial partners, as well as by the German Research Foundation (DFG), the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Federation of European Microbiological Societies (FEMS).

We expect more than 1,800 participants in Dresden. The participation of doctoral candidates as well as young researchers is especially important to us. The receipt of more than 1,000 contributions from all areas of microbiology proved impressively the importance of this matter.

We look forward on an interdisciplinary, interactive and hopefully trend-setting conference in Dresden and hope that you will agree to the opportunity to receive new scientific impulses, to promote the exchange between both our societies, to meet old friends as well as to socialise with new contacts.

For your spare time Dresden offers art and culture like almost no other town in Germany. Particularly the historic city is a tourist centre of attraction with the Dresden Frauenkirche, the Zwinger Place and the Semperoper.

Welcome to Dresden.

Volkhard A. J. Kempf (DGHM)

Gerold Barth (VAAM)

Breakthrough solutions to improve health and advance science.

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■ Wir freuen uns, dass sich DGHM und VAAM bereits 4 Jahre nach der letzten gemeinsamen Tagung in Hannover nun in Dresden wieder zu einer gemeinsamen Tagung zusammenfinden und möchten Sie in dieser faszinierenden Stadt sehr herzlich willkommen heißen.

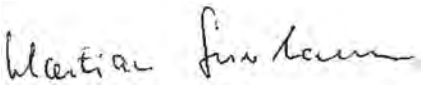
Diese 4. gemeinsame Jahrestagung findet erstmals unter dem Kongressnamen „Microbiology and Infection“ statt, der in Zukunft als Dachmarke für die gemeinsamen Kongresse von DGHM und VAAM verwendet wird. Wir wollen damit der immer enger werdenden Verzahnung der beiden Gesellschaften sowie den immer häufiger werdenden Kooperationen innerhalb der Mikrobiologie gerecht werden. Die Mikrobiologie hat sich heute in ein weitläufiges interdisziplinäres Fach entwickelt, das in seiner Breite auf dem Kongress dargestellt werden soll.

Die enge thematische Verbundenheit der DGHM und VAAM zeigt sich auch in dem formalen Zusammenschluss der beiden Fachgruppen „Mikrobielle Pathogenität“ und „Lebensmittelmikrobiologie“, die nach langjähriger Kooperation in Dresden ein gemeinsames Sprecherteam wählen werden. Wir hoffen, dass dieser Schritt dann in naher Zukunft auch von weiteren thematisch ähnlichen Fachgruppen beschlossen wird und die beiden Gesellschaften immer weiter zusammenwachsen.

Ergänzt wird diese Vernetzung durch zahlreiche persönliche Kontakte unter den Mitglieder sowie einige gemeinsam organisierte Schwerpunktprogramme der Deutschen Forschungsgemeinschaft. Von den mehr als 1.000 wissenschaftlichen Abstracts wurde eine erfreulich große Anzahl von jungen Doktorandinnen und Doktoranden eingereicht. Mit dieser gemeinsamen Tagung bieten wir dem naturwissenschaftlichen und medizinischen Nachwuchs eine gemeinschaftliche Plattform auf der sie ihre Arbeiten einem breiten Publikum präsentieren und diskutieren können.

Unser herzlicher Dank gilt auch unseren Hauptsponsoren, Industriepartnern und der Deutschen Forschungsgemeinschaft für ihre großzügige Unterstützung sowie den Tagungspräsidenten und dem Organisationskomitee für die hervorragende Zusammenstellung des Tagungsprogramms.

Wir wünschen allen Teilnehmerinnen und Teilnehmern eine erfolgreiche Tagung mit konstruktiven Diskussionen, Anregungen und neuen Kooperationen. ■



Prof. Dr. Sebastian Suerbaum
Präsident der DGHM

Prof. Dr. Dieter Jahn
Präsident der VAAM

■ We are glad that only 4 years after the last joint conference in Hanover, DGHM and VAAM meet again.. We would like to warmly welcome you in the mesmerising city of Dresden.

For the first time, this 4th joint annual conference takes place under the title “Microbiology and Infection”, which in the future will be used as family brand for the joint conferences of the DGHM and VAAM. We want to comply to the increasingly close relationship of both societies, as well as to the growing number of cooperations within microbiology. Today microbiology has developed into an extensive and interdisciplinary field which in its broadness will be represented at the conference.

The close thematic relatedness of the DGHM and VAAM is also mirrored in the formal union of both scientific groups “Microbial Pathogenesis“ and “Food Microbiology”. According to the intensive cooperation in Dresden for many years, each of these unions will choose a joint speaker’s team. We hope that this example will be followed by other thematically similar scientific groups in the near future, so that as a result, the increasing similarities of both societies will also find their expression through these mergers in Dresden.

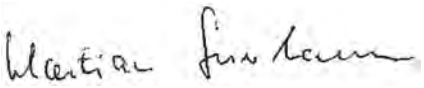
This interconnection is complemented by numerous personal contacts among the members as well as some jointly organised areas of research for the German Research Foundation.

From more than 1,000 scientific abstracts a pleasantly high number was submitted by doctoral candidates. With this joint conference we offer to the young scientists a collaborative platform where contributions can be presented and discussed in front of a large audience.

We would also like to express our thanks to our main sponsors, industrial partners and the German Research Foundation for their generous support as well as the conference presidents and the organising committee for the excellent arrangement of the conference programme.

We wish all participants a successful conference with inspiring discussions as well as some new cooperations.

And last but not least: Don’t forget to enjoy the marvellous city of Dresden! ■



Prof. Dr. Sebastian Suerbaum
Präsident der DGHM

Prof. Dr. Dieter Jahn
Präsident der VAAM

Mikrobiologie – das bewährte Laborhandbuch

Eckhard Bast
Mikrobiologische Methoden
3., überarb. u. erg. Aufl. 2014, XVIII, 472 S. 31 Abb. Brosch.
ISBN 978-3-8274-1813-5
€ (D) 39,95 | € (A) 41,11 | *sFr 50,00

Neuauflage

Dieses bewährte Laborhandbuch richtet sich an Studierende und Dozenten der Biologie, Biotechnologie und Medizin, an Biologielehrer, an technische Assistenten und an Wissenschaftler in Forschung, Industrie und Untersuchungslabors. Es bietet auch in der Neuauflage präzise und reproduzierbare „Man-nehme"-Vorschriften der wichtigsten mikrobiologischen Methoden sowie theoretische Grundlagen und Hinweise zur Auswertung, zur Leistungsfähigkeit und zu den Grenzen der behandelten Arbeitstechniken. Das Buch hat sich als unentbehrlicher Begleiter für alle erwiesen, die erste Erfahrungen im Umgang mit Mikroorganismen, insbesondere mit Bakterien, sammeln und sich über die gängigen Standardmethoden informieren wollen.

Für die **3. Auflage** wurde der Text überarbeitet und an zahlreichen Stellen ergänzt. Unter anderem wurden die Regeln der Biostoffverordnung, Schnelltests zur Gramfärbung und die Epifluoreszenzmikroskopie mit einer Reihe von Färbeverfahren neu aufgenommen.

Registration

Please register online at www.dghm-vaam-2014.de.

Conference Tickets

	Regular Registration
Member DGHM/VAAM/GBM/DECHEMA[1,2]	230 EUR
Non-Member[1]	295 EUR
Student (Member DGHM/VAAM/GBM/DECHEMA)[1,2]	85 EUR
Student (Non-Member), MTLA, VMTA[1,2]	110 EUR

Day Tickets

Member DGHM/VAAM/GBM/DECHEMA[1,2]	110 EUR
Non-Member	135 EUR
Student (Member DGHM/VAAM/GBM/DECHEMA)[1,2]	50 EUR
Student (Non-Member), MTLA, VMTA[1,2]	60 EUR

[1] Welcome Reception and Mixer are included.

[2] Proof of status required. Please send via e-mail at registrierung@conventus.de, via Fax +49 3641 31 16-244 or postal at Conventus GmbH · Keyword: DGHM/VAAM 2014 · Carl-Pulfrich-Straße 1 · 07745 Jena

Social Evening

Welcome Reception, 05 October 2014	18 EUR
Mixer, 07 October 2014	18 EUR

Workshop – MTAs/Doctors Training course

„Molekulare Diagnostik & Externe Qualitätssicherung von molekularen und serologischen Diagnostikverfahren“
07 October 2014

	with congress attendance	without congress attendance
Member DGHM/VAAM/GBM/DECHEMA[2]	15 EUR	30 EUR
Non-Member	100 EUR	130 EUR

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Payment/Confirmation of Payment

Please process the payment after receipt of the invoice, making reference to the invoice number. Payment via credit card is also possible (Master-/Eurocard, AmericanExpress, Visa Card). Should you transfer your invoice amount within 10 days of the start of the event, please present your transfer remittance slip at the Check-In desk as proof of payment.

Registration fees include:

- Participation in the scientific programme as well as access to the industrial exhibition
- Opening event
- Conference documents (programme, abstract book, etc.)
- Beverages within the scope of the breaks given in the programme

The conference fee also includes the provision of snacks and drinks at the get together as well as buffet and beverages at the social evening.

General Terms and Conditions

You can find the general terms and conditions on our conference website www.dghm-vaam-2014.de.

Check-In

The Check-In is located at the entrance of the International Congress Center. Please find a detailed plan on page 18.

No longer waiting lines at the counter!

With our Quick Check-In you can check in fast and comfortably by - yourself. After your invoice is paid you receive a QR code (approximately 2 weeks prior to the congress itself). Please hold it under the scanner at the Quick Check-In counter on site. Your name-badge will be printed out directly in seconds and your registration is completed.

For your information: At the end of the congress you are able to print your certificate of attendance the same way.

Name Tag

Admittance to the congress and industrial exhibition is only allowed for those with a name tag. Name tags should be worn at all time. Name tags for exhibitors will be given to the exhibit personnel.

Evaluation

Please turn in your completed and legible evaluation form to the Check-In on the last day. Thank you for your active participation and constructive criticism.

Industrial Exhibition

An extensive industrial exhibition accompanies the 4th Joint Conference of the DGHM and VAAM. The exhibitors would be glad about your visit and present a comprehensive spectrum of innovative products to you. The booth plan can be found on page 20.

Opening Hours

	Sunday	Monday	Tuesday	Wednesday
Industrial Exhibition	15^{00}–21^{00} h	08^{30}–18^{30} h	08^{30}–22^{30} h	08^{30}–13^{30} h
Poster Exhibition		08^{30}–18^{30} h	08^{30}–18^{30} h	08^{30}–10^{30} h
Check-In	12^{00}–19^{30} h	08^{00}–19^{00} h	08^{00}–19^{00} h	08^{00}–15^{00} h
Media Check-In	12^{00}–19^{30} h	08^{00}–18^{00} h	08^{00}–18^{00} h	08^{00}–13^{00} h
Child Care	as required	08^{00}–18^{00} h	08^{00}–18^{00} h	08^{00}–14^{30} h

Cloakroom

There is the possibility to leave your clothes and light luggage in the cloakroom at the entrance.

Internet

We can offer you the following opportunities for internet usage:

1. Hotspots

Wi-Fi is sponsored by T-Mobile. The costs for 24h are 4.95 EUR. Please log in by yourself.

2. Voucher

Every participant has the opportunity to buy a voucher at the Check-In. The voucher is valid for 24 h and costs 5.50 EUR per device.

3. Internet Lounge

There will be offered an internet lounge free of charge.

Bring your copy of the BIOspektrum Sonderausgabe 2014; at the meeting it will cost 20 Euro

Conference Language

English is the conference language, selected sessions are held in German.

DGHM & VAAM Poster Awards

Poster prizes will be awarded to the best posters. Ten posters will be awarded with 150 EUR.

The poster prizes will be awarded after the last Joint Plenary Session of DGHM & VAAM in the Plenary Hall "Großer Saal" on Wednesday, 8 October 2014.

Education Credits and Certification of the Scientific Programme

The 4th Joint Conference of the German Society for Hygiene and Microbiology (DGHM) and the Association for General and Applied Microbiology (VAAM) is applied for by the Medical Association of Saxony as follows:

Medical Association of Saxony

Scientific programme (CME Points 32)

Sunday, 5 October 2014	16^{00}–20^{00} h	Category B	CME Points 5
Monday, 6 October 2014	08^{30}–12^{00} h	Category B	CME Points 4
	13^{30}–18^{00} h	Category B	CME Points 4
Tuesday, 7 October 2014	08^{30}–12^{00} h	Category B	CME Points 4
	13^{30}–18^{30} h	Category B	CME Points 4
Wednesday, 8 October 2014	08^{30}–12^{30} h	Category B	CME Points 4
	12^{30}–15^{30} h	Category B	CME Points 3

Lunch Symposia

Monday, 6 October 2014	12^{15}–13^{15} h	Category A	CME Points 2
Tuesday, 7 October 2014	12^{15}–13^{15} h	Category A	CME Points 2

MTAs/Doctors Training course

Tuesday, 8 October 2014	08^{30}–18^{00} h	Category A	CME Points 4

The certification regulations of the Medical Association Saxony require a presence control via bar coding.

Please scan twice a day (in the morning and in the afternoon) your advanced training numbers (EFN) at the terminal to credit the certification points on your account.

The presence control of the lunch symposia and training courses takes place in the corresponding room.

The participation certificates will be handed out when leaving the conference at the Check-In desk or directly after the lunch symposia or training courses by the instructor/organiser.

German Institute for Improvement of Technical Assistants in Medicine e. V. (DIW-MTA)

Scientific Programme (CME points 18)

Sunday, 5 October 2014	16^{00}–20^{00} h	Category B	CME Points 3
Monday, 6 October 2014	08^{30}–18^{30} h	Category B	CME Points 6
Tuesday, 7 October 2014	08^{30}–18^{00} h	Category B	CME Points 6
Wednesday, 8 October 2014	08^{30}–14^{30} h	Category B	CME Points 3

MTAs/Doctors Training course

Tuesday, 7 October 2014	08^{30}–18^{00} h	Category A	CME Points 4

The list of participants is located in the lecture room. The participation certificates will be handed out when leaving the conference at the Check-In desk

Academy for Veterinary Improvement

Scientific programme 5– 8 October 2014	16 Training Hours
Day Ticket Monday	6 Training Hours
Day Ticket Tuesday	6 Training Hours
Day Ticket Wednesday	4 Training Hours

Travel and City Map

Travel by Car

Navigational address:
Maritim Hotel & Internationales Congress Center Dresden
Ostra-Ufer 2/Devrientstraße 10–12 · 01067 Dresden

Highway A4 · Exit Dresden Neustadt

- Washingtonstraße (straight ahead at the commercial area "ELBEPARK" for approx. 3 km
- cross the "Flügelwegbrücke"
- get into the left lane at the stop light and turn onto Hamburger Straße (B 6)
- turn left onto Bremer Straße after approx. 150 m (B 6)
- follow the main street direction "ZENTRUM" (down town)
- turn left at the next traffic light onto Devrienstraße
- the Maritim Hotel is located on the left side

Highway A4 · Exit Dresden Altstadt

- turn right into "Meißner Landstraße" at the traffic light
- take the left straight ahead lane after approx. 3 km at the forking, straight ahead on "Hamburger Straße" (B 6)
- turn left at the forking onto "Bremer Straße" after approx. 150 meters
- follow the main street direction "ZENTRUM" (down town)
- turn left at the next traffic light onto "Devrientstraße"
- the Maritim Hotel is located on the left side

Travel by Public Transport

From main station

Departure:	Main Station North, Tram 11 direction "Dresden Waldschlösschen" (distance: 11 minutes)
Stops:	"Walpurgisstraße" – "Prager Straße" – "Postplatz" – "Am Zwingerteich"
Destination:	Dresden Kongresszentrum/Haus der Presse

From airport

Departure:	Airport, city bus 77 direction "Klotzsche Infineon" (distance: 7 minutes)
Destination:	Infineon Nord
Departure:	Infineon Nord, Tram 7 direction "Pennrich Gleisschleife" (distance: 16 minutes)
Destination:	"Albertplatz"
Departure:	"Albertplatz", Tram 6 direction "Wölfnitz" (distance: 6 minutes)
Destination:	Kongresszentrum/Haus der Presse

DER HYGIENEINSPEKTOR

Die Fachzeitschrift des Bundesverbandes der Hygieneinspektoren e.V.

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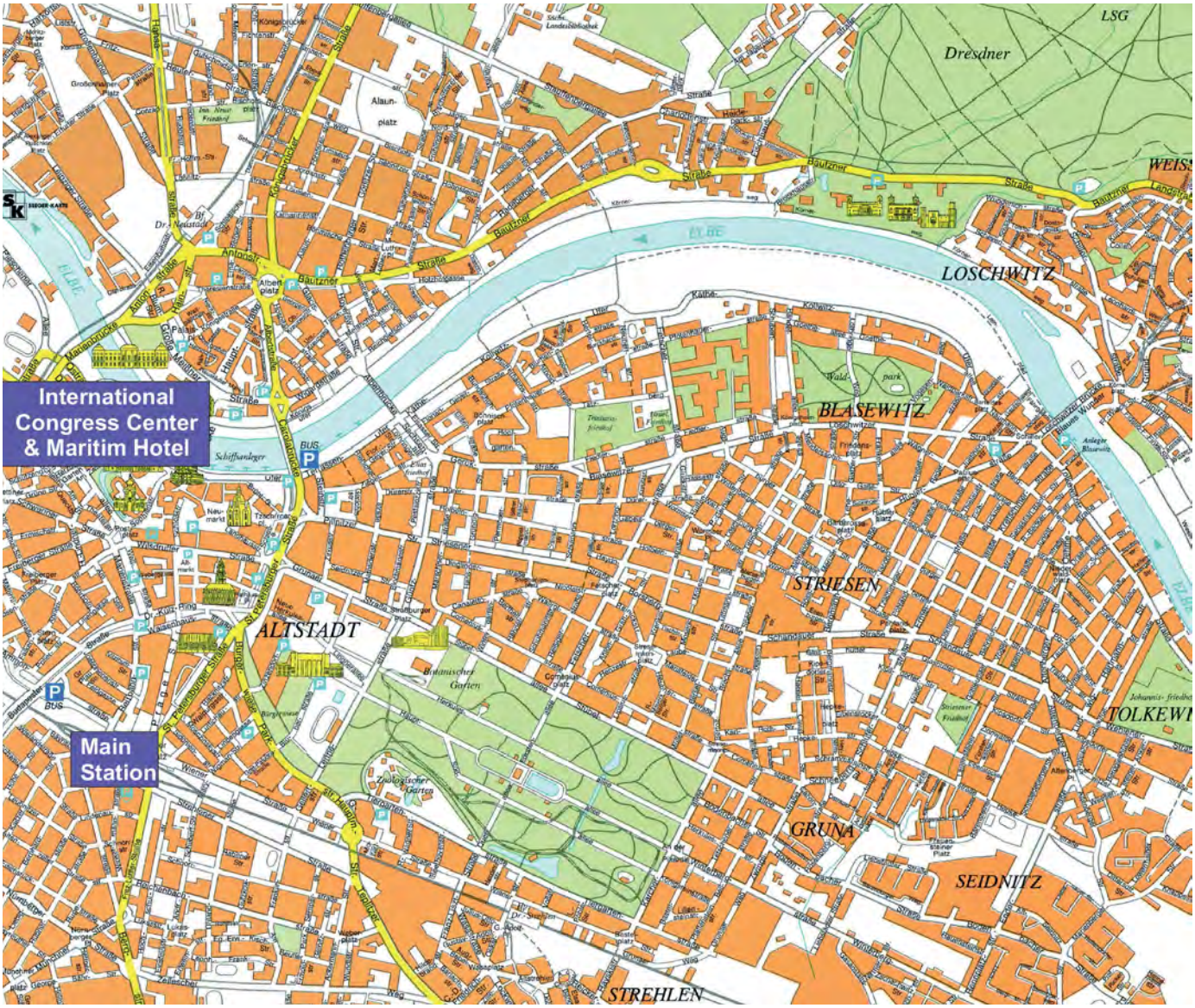

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Social Programme

Sunday, 5 October 2014 · Welcome Reception
The organisers welcome all participants of the conference at the industrial exhibition area. Meet your colleagues and other participants while having fresh drinks and snacks.
Time 19^{00} h
Place Industrial Exhibition

Tuesday, 7 October 2014 · MIXER
We like to invite you to the MIXER for speakers, participants and exhibitors. The wonderful music group band "ROSA" will play for your entertainment and a buffet will be served.
Time 19^{30} h
Place Industrial Exhibition, Posterexhibition on ground floor, Grosser Saal

Media Cooperations

Berufsverband der Ärzte für Mikrobiologie, Virologie und Infektionsepidemiologie e. V. (Stuttgart)
"Der Mikrobiologe"

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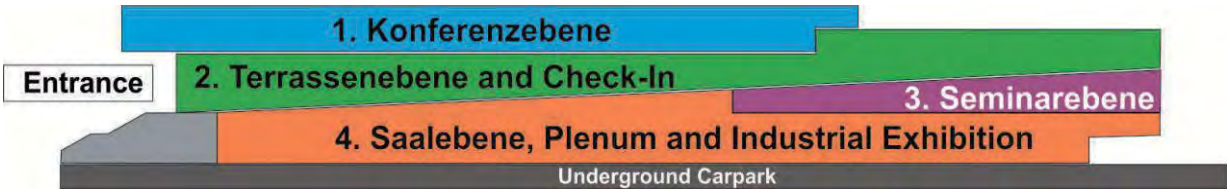
Overview



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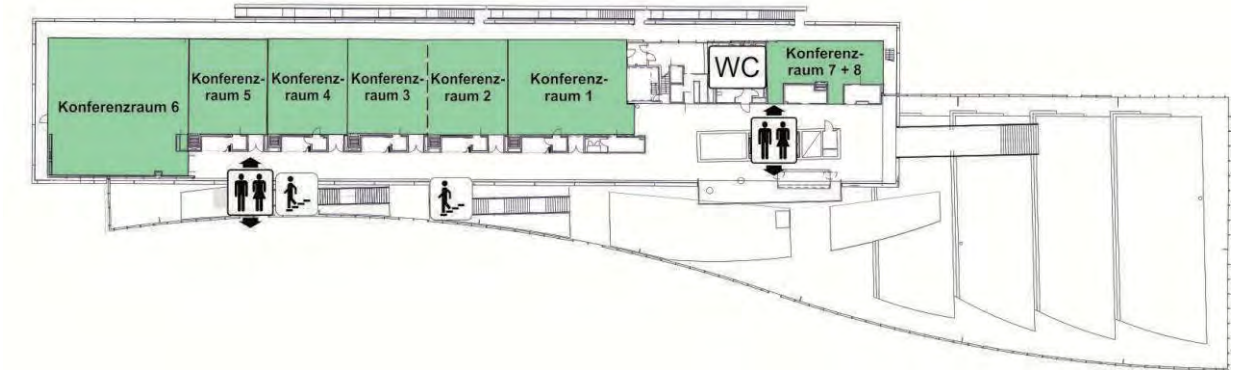

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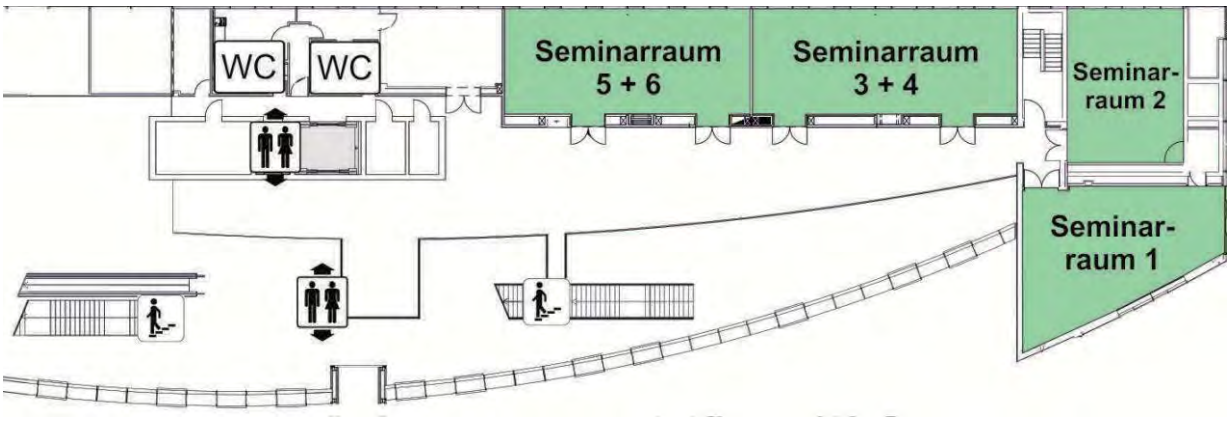
3. Seminarebene



4. Saalebene, Plenum and Industrial Exhibition

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Ärzte ohne Grenzen	2.10
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Hain Lifescience GmbH	2.12
Expedeon Ltd.	2.13
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Curetis AG	2.17
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Anagostics Bioanalysis GmbH	5.10
Nationale Forschungsplattformen	5.11
IBA Lifescience GmbH	5.12

Einladung zur Mitgliederversammlung der DGHM

Liebe Mitglieder,

im Namen des Präsidenten der DGHM, Herrn Prof. Dr. Sebastian Suerbaum, lade ich Sie ganz herzlich zur Mitgliederversammlung der DGHM anlässlich der 4. Gemeinsamen Tagung von DGHM und VAAM am Montag, den 6. Oktober 2014, 18^{30} Uhr ein.

Tagesordnung

TOP 1: Annahme der Tagesordnung
TOP 2: Bericht des Präsidenten
TOP 3: Bericht des Schriftführers
TOP 4: Bericht des Schatzmeisters
TOP 5: Wissenschaftlicher Beirat
– Bericht des Sprechers
TOP 6: Bericht der Kassenprüfer
TOP 7: Wahl von 2 neuen Kassenprüfern
TOP 8: Wahl des Präsidenten
TOP 9: Wahl des Schatzmeisters
TOP 10: Wahl der beiden Vizepräsidenten
TOP 11: Verschiedenes

Über Ihre Teilnahme würde ich mich sehr freuen!

Mit freundlichen Grüßen

Nicole von Maltzahn

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am Dienstag, den 7. Oktober, um 18^{00} Uhr im Saal 1 des Kongresszentrums Dresden stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 12. März 2013 in Bremen (siehe BIO*spektrum* 3/13, Seiten 302 und 303)
2. Bericht aus dem Präsidium und Vorstand, u.a. Haushalt 2013 und Haushaltsplan 2014, Fachgruppen, BIO*spektrum, VBIO, DGHM, Mikrobe des Jahres, Ort* und Zeit der nächsten Jahrestagung
3. Bericht der Kassenprüfer
4. Entlastung des Vorstandes
5. Änderung der Geschäftsordnung (Gemeinsame Fachgruppen)
6. Verschiedenes

Im Anschluss: Verleihung der Ehrenmitgliedschaft an Volkmar Braun, Tübingen

Reisekostenzuschüsse für studentische Mitglieder können bei fristgerecht eingegangenen Anträgen und bei Vorliegen der sonstigen Voraussetzungen nur persönlich Dienstag, den 7. Oktober, von 13^{00} bis 16^{00} Uhr und Mittwoch, den 8. Oktober 2014, von 10^{00} bis 13^{00} Uhr im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Änderung der Geschäftsordnung

Alt:

V. Einrichtung von Fachgruppen

Die Mitglieder der Fachgruppen wählen auf einer Mitgliederversammlung aus ihrer Mitte – jeweils auf 2 Jahre – einen Sprecher der Gruppe und seinen Stellvertreter, was durch das Präsidium der VAAM zu bestätigen ist. Wiederwahl ist zulässig.

Neu:

Die Mitglieder der Fachgruppen wählen auf einer Mitgliederversammlung **oder online** aus ihrer Mitte – jeweils auf 2 Jahre – eine/n **Sprecher/in** der Gruppe und seine/n **Stellvertreter/in**, was durch das Präsidium der VAAM zu bestätigen ist. Wiederwahl ist zulässig.

...

Auf Beschluss der Mitgliederversammlung einer Fachgruppe und auf begründeten Antrag von mindestens 25 ordentlichen Mitgliedern einer bestehenden Fachgruppe kann diese in eine Gemeinsame Fachgruppe mit einer thematisch ähnlich ausgerichteten Fachgruppe einer anderen Fachgesellschaft übergeleitet werden. Der Vorstand der VAAM entscheidet mit einfacher Mehrheit über diesen Antrag. Die Mitglieder der Gemeinsamen Fachgruppe wählen aus ihrer Mitte auf einer Mitgliederversammlung oder online – jeweils auf 2 Jahre – eine/n Sprecher/in der Gruppe und seine/n Stellvertreter/in, wobei beide Fachgesellschaften repräsentiert sein sollten. Sprecher/in und Stellvertreter/in sind durch das Präsidium der VAAM zu bestätigen. Bei Bedarf können weitere Mitglieder der Fachgruppe in die Leitung gewählt werden. Gemeinsame Fachgruppen haben ansonsten die gleichen Rechte und Pflichten wie die übrigen Fachgruppen der VAAM.

5.–8. OKTOBER 2014
DRESDEN

Microbiology and Infection 2014

66. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM)

Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

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Fusion der Fachgruppen Mikrobielle Pathogenität der DGHM und VAAM

Die Fachgruppen Mikrobielle Pathogenität der DGHM und VAAM sind seit 2014 in einer gemeinsamen Fachgruppe Mikrobielle Pathogenität vereinigt. In Sitzungen auf der letztjährigen DGHM in Rostock und Treffen der VAAM haben die Mitglieder der Fachgruppen Mikrobielle Pathogenität die Zusammenführung der Fachgruppen mit ihren jeweiligen Mitgliedern diskutiert und insgesamt ein positives Votum für die Vereinigung erfahren. Die beiden Fachgruppen haben aufgrund der gemeinsamen Thematiken und Forschungsthemen über die vergangenen Jahre bereits sehr intensiv und erfolgreich zusammengearbeitet und möchten die Vereinigung für eine noch effizientere gemeinsame Zukunft in der Erforschung von Pathogenitätsmechanismen und Erreger-Wirt-Interaktionen nutzen. Die gemeinsamen Symposien, wie z.B. das alle zwei Jahre stattfindende *Symposium Mikrobielle Pathogenität* mit ca. 60 Teilnehmern, und die gemeinsamen Sessions auf den Jahrestagungen der DGHM und VAAM waren sehr erfolgreich und werden in Zukunft in gemeinsamer Arbeit fortgeführt sowie intensiviert.

In der Übergangsphase hat ein „Doppelvorstand" aus den derzeitigen Sprechern der jeweiligen Fachgruppen zusammengearbeitet, der zeitnah durch Neuwahlen wieder auf 3 Vorstandsmitglieder reduziert wird. Eine wichtige Aufgabe des neuen Vorstands ist die Zusammenarbeit mit der DGHM und VAAM. Daher ist beabsichtigt, dass mindestens ein Mitglied des Vorstands Naturwissenschaftler bzw. Mediziner ist und eine Mitgliedschaft in der DGHM sowie VAAM vorhanden ist.

Die Fachgruppe Mikrobielle Pathogenität ist auch auf ResearchGate unter folgendem Link (www.researchgate.net/project/FG_Mikrobielle_Pathogenitaet) vertreten. Dort können aktuelle Informationen oder Programme zu Veranstaltungen der Fachgruppe gepostet werden. Diese Seite kann weiterhin zum Austausch von Informationen dienen.

Fachgruppe Lebensmittelmikrobiologie und -hygiene

Die Fachgruppen Lebensmittelmikrobiologie und -hygiene der DGHM und Lebensmittelmikrobiologie der VAAM möchten Ihre Zusammenarbeit verstärken und sich zu einer gemeinsamen Fachgruppe beider mikrobiologischen Gesellschaften umbilden. Die Hauptgründe hierfür sind die gemeinsame Thematik und die Möglichkeit die Forschung auf diesem Gebiet in Deutschland besser und effektiver voranzubringen. Um dies besser auf kurzen Wegen steuern zu können, ist ein gemeinsamer Vorstand geplant.

Die Mitglieder beider Fachgruppen sind bereits hierüber informiert worden und während des 15. Fachsymposium Lebensmittelmikrobiologie vom 15. bis 17. April 2015 im Bildungszentrum Kardinal-Döpfner-Haus in Freising wird das Vorgehen endgültig mit den Mitgliedern abgestimmt. Bereits auf der Mitgliederversammlung der DGHM auf der gemeinsamen DGHM/VAAM Haupttagung wird hierüber gesprochen und alle Mitglieder sind eingeladen aktiv diesen Vorgang mitzugestalten.

Legende/Key

Fachgruppe(-nsession) (FG), Ständige Arbeitsgemeinschaft (StAG), Kommission (KOM), Sektion (SEK), Short Lecture (SL)	Poster-/Vortrags-ID
Anaerobic Metabolism (SL AM)	AM
Antimicrobial Resistence and Drugs, Infection Prevention (FG PR)	PR
Archaea (SL AR)	AR
Biology of Conventional and Non-Conventional Yeasts (FG YE)	YE
Biology of Filamentous Fungi (FG FU)	FU
Bioenergetics (SL BE)	BE
Biotechnology (incl. Microbial Biocatalysis) (SL BT)	BT
Clinical Microbiology and Infectious Diseases (StAG KM)	KM
Diagnostic Microbiology (FG DV)	DV
Eukaryotic Pathogens (FG EK)	EK
Food Microbiology and Food Hygiene (FG LM)	LM
Free Topics	FT
Gastrointestinal Pathogens (FG GI)	GI
General and Hospital Hygiene (StAG HY)	HY
Genomics and Metagenomics (SL GM)	GM
Host-Microbe Interactions (SL HM)	HM
Infection Epidemiology and Population Genetics (FG MS)	MS
Infection Immunology (FG II)	II
Intracellular Transport and Secretion (SL TS)	TS
Metabolism and Metabolic Networks/Metabolomics (SL MM)	MM
Microbial Cell Biology (SL CB)	CB
Microbial Diversity and Ecology (incl. Soil and Water Microbiology) (SL DE)	DE
Microbial Pathogenesis (FG MP)	MP
Microbiota, Probiotics and Host (FG PW)	PW
National Reference Laboratories and Consiliary Laboratories (StAG RK)	RK
Quality Management in Diagnostic Microbiology (StAG QS)	QS
Regulation and Signaling (incl. Stress Responses) (SL RS)	RS
Secondary Metabolism (SL SM)	SM
Zoonoses (FG ZO)	ZO

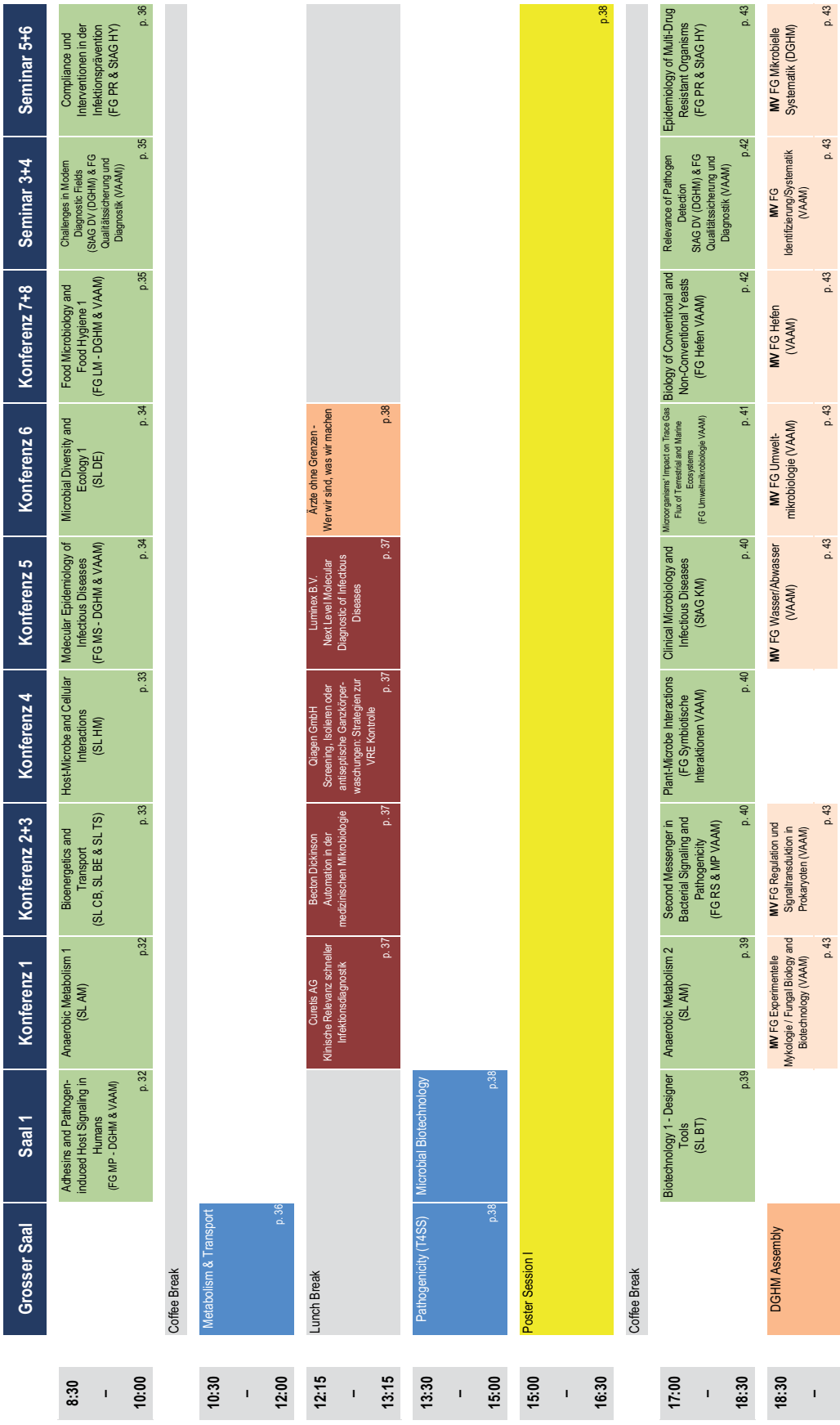
Conference Programme · Overview

Colour Key	
	Plenary Session
	Oral Session / Working Group Session
	Lunch Symposia
	Poster Session
	Workshop for Technical Assistants in Medicine
	Opening, DGHM/VAAM Lecture, Award Sessions, General Assembly of the Societies, Special Group Meetings
	Social Event
	Meetings DGHM (FG, StAG, Komm.) and VAAM (FG)
	Particularly interesting Sessions for Hospital Hygienist

Programme Overview · Sunday · October 05, 2014

	Grosser Saal
11:00 – 14:30	
15:00 – 16:30	Opening of Conference DGHM Lecture Hans-Günter-Schlegel-Lecture (VAAM) p. 31
	Coffee Break
17:00 – 19:00	Award Presentation DGHM and VAAM p. 31
19:00 – 22:00	Welcome Reception (Industrial Exhibition) p. 31

Programme Overview · Monday · October 06, 2014



Programme Overview · Tuesday · October 07, 2014

Programme Overview · Wednesday · October 08, 2014

	Grosser Saal	Saal 1	Konferenz 1	Konferenz 2+3	Konferenz 4	Konferenz 5	Konferenz 6	Konferenz 7+8	Seminar 3+4	Seminar 5+6
8:30 – 10:00		Pathomechanisms and Regulatory Strategies of Bacteria (FG MP - DGHM & VAAM) p. 56	Primary and Secondary Metabolism (SL MM & SM) p. 56	Regulation and Signalling 1 (SL RS) p. 57	Host-Microbe Interactions: Pathogen Induced Host Responses (SL HM) p. 57	Genomics and Metagenomics 1 (SL GM) p. 58	Biotechnology 3 - Pathways to Added-Value (SL BT) p. 58	Archaea (SL AR) p. 59	Microbial Diversity and Ecology 2 (SL DE) p. 59	ABS - Antibiotic Stewardship and Development of Resistance (FG PR & SAG HY) p. 60
	Coffee Break									
10:30 – 12:00	Hypoxia and Anaerobiosis p. 60									
	Poster Prize Awards p. 60									
12:30 – 13:30	Lunch Break									Late Breaker (FG PR & SAG HY) p. 60
13:30 – 15:00		Infection Immunology 2 (FG II) p. 61	Metabolism and Metabolomics (SL MM & SM) p. 61	Regulation and Signalling 2 (SL RS) p. 62	Host-Microbe Interactions: Virulence Factors an Infection Models (SL HM) p. 62	Genomics and Metagenomics 2 (SL GM) p. 63	Enzyme Catalysis (SL BT) p. 63	Microbial Cell Biology 2 (SL CB, SL BE & SL TS) p. 64	Microbial Diversity and Ecology 3 (SL DE) p. 64	Antibiotic Stewardship und Krankenhaushygiene gehören zusammen! (FG PR & SAG HY) p. 65
	End of Meeting									

SCIENTIFIC PROGRAMME, SUNDAY, 5 OCTOBER 2014

VAAM-Jahrestagung 2014 / 66. Jahrestagung der DGHM

13^{30}–15^{00} **Registration & Industrial Exhibition**

Room Industrial Exhibition

15^{00}–16^{30} **Opening**

Room Grosser Saal

15^{00} Grußwort des Prorektors für Wissenschaft der TU Dresden
G. Rödel (Dresden)

15^{10} Grußwort der Tagungspräsidenten
V. A. J. Kempf (Frankfurt a. M.), G. Barth (Dresden)

15^{20} Grußwort der Präsidenten der Gesellschaften
S. Suerbaum (Hannover), D. Jahn (Braunschweig)

15^{30} DGHM Lecture – Assembly of the Envelope of Gram-Positive Bacterial Pathogens
O. Schneewind (Chicago, IL/US)

16^{00} Hans-Günter-Schlegel-Lecture – Outer Membrane Biogenesis in Gram-Negative Bacteria
T. Silhavy (Princeton, NJ/US)

16^{30}–17^{00} Coffee Break/Industrial Exhibition

17^{00}–19^{15} **Award Session**

Room Grosser Saal

17^{00} **Awards DGHM Stiftung**

Förderpreis DGHM
C. Rüter (Münster)

Förderpreis DGHM
C. Weidenmaier (Tübingen)

bioMérieux Diagnostikpreis
F. Schaumburg (Münster)

Becton Dickinson Forschungspreis
M. Lappann (Würzburg)

Awards DGHM

Promotionspreis
M. Saleh (Greifswald)

Promotionspreis
K. Dichtl (München)

DZIF Promotionspreis
I. Finsel (München)

17^{40} **VAAM-Forschungspreis**
INV01 Antibacterial strategies and bacterial defenses
J. E. Bandow (Bochum)

18^{20} **DGHM-Hauptpreis**
INV02 Pathogenicity mechanisms of *Candida albicans* and *Aspergillus fumigatus*
B. Hube, A. Brakhage (Jena)

19^{00}–21^{00} **Welcome Reception**
Room Industrial Exhibition

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08^{30}–10^{00} Oral Session 1
Adhesins and Pathogen-Induced Host Cell Signaling in Humans (FG MP – DGHM & VAAM)

Room: Saal 1
Chair: C. Josenhans (Hannover), P. Dersch (Braunschweig)

08^{30} MPV01
Autopenetrating bacterial effector proteins as biological therapeutics
A.-S. Stolle, M. A. Schmidt, C. Rüter (Münster)

08^{45} MPV02
Deletion of the galU gene abolishes the impaired immune response to uropathogenic *E. coli*
C. Meyer, M. Messerer, C. Hoffmann, R. Haas, Sö. Schubert (München)

09^{00} MPV03
SPI-4 mediated adhesion to polarized epithelial cells: is signaling by chemotaxis sensors involved?
T. Wille, K. Blank, S. Hoffmann (Wernigerode), M. Hensel (Osnabrück), **R. G. Gerlach** (Wernigerode)

09^{15} MPV04
The regulation of c-Abl activity in *Helicobacter pylori* infected gastric epithelial cells
G. Posselt, M. Österbauer, S. Wessler (Salzburg/AT)

09^{30} MPV05
Evaluation of *Acinetobacter baumannii* trimeric autotransporter adhesin as an adhesion and virulence factor
C. Makobe, D. Frank, S. Christ, G. Enders (Frankfurt a. M.), G. Wilharm (Wernigerode), D. Linke (Tübingen)
T. A. Wichelhaus, **S. Göttig**, V. A. J. Kempf (Frankfurt a. M.)

09^{45} MPV06
Physical determinants on surface protein deformation and staphylococcal attachment – a cautionary tale
H. Peisker, P. Jung (Homburg), N. Thewes (Saarbrücken), P. Loskill (Berkeley, CA/US), M. Bischoff (Homburg)
K. Jacobs (Saarbrücken), M. Herrmann (Homburg)

08^{30}–10^{00} Oral Session 2
Anaerobic Metabolism 1 (SL AM)

Room: Konferenz 1
Chair: I. Berg (Freiburg), T. Schubert (Jena)

08^{30} AMV01
A Regulatory Link Between Nitrate and Oxygen Respiration in *Streptomyces coelicolor*
D. Falke, M. Fischer, G. Sawers (Halle)

08^{45} AMV02
Comparing benzene degradation under sulphate- and nitrate-reducing conditions
A. H. Keller, C. Roy, S. Kleinsteuber, C. Vogt (Leipzig)

09^{00} AMV03
Identification of naphthalene carboxylase subunits of the sulfate-reducing culture N47
J. Kölschbach, H. Mouttaki, J. Merl, R. U. Meckenstock (Neuherberg)

09^{15} AMV04
Iron-sulfur/flavoproteins involved in the anaerobic degradation of naphthalene
S. Estelmann, I. Blank, A. Feldmann, C. Eberlein, M. Boll (Freiburg)

09^{30} AMV05
Simultaneous involvement of a phenylacetaldehyde dehydrogenase and an aldehyde – ferredoxin oxidoreductase in anaerobic phenylalanine metabolism
G. Schmitt, C. Debnar-Daumler, J. Heider (Marburg)

09^{45} AMV06
Anaerobic activation of p-cymene in denitrifying betaproteobacteria – methyl group hydroxylation vs. addition to fumarate
A. Strijkstra, **K. Trautwein** (Oldenburg, Bremen), R. Jarling (Potsdam), L. Wöhlbrand, M. Dörries (Oldenburg)
R. Reinhardt (Köln), B. Golding (Newcastle upon Tyne/GB), H. Wilkes (Potsdam)
R. Rabus (Oldenburg, Bremen)

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08^{30}–10^{00} Oral Session 3
Bioenergetics and Transport (SL CB, SL BE & SL TS)

Room	Konferenz 2+3
Chair	A. Diepold (Oxford/GB), G. M. Seibold (Köln)
08^{30} TSV01	Functional dissection of the tetracysteine motif of the competence ATPase PilF in *Thermus thermophilus* HB27 **R. Salzer**, H. Knoblich, B. Averhoff (Frankfurt a. M.)
08^{45} TSV02	In vivo dynamics and regulation of the type III secretion injectisome **A. Diepold**, J. Armitage (Oxford/GB)
09^{00} TSV03	Structure and Function of a G+ Bacterial Type IV Secretion System (T4SS) **W. Keller**, C. Fercher, N. Goessweiner-Mohr (Graz/AT), I. Probst (Freiburg), K. Arends (Berlin) E. Grohmann (Freiburg)
09^{15} TSV04	A Type VI Secretion System Adaptor Protein Facilitates Translocation of Diverse Effectors **D. Unterweger**, B. Kostiuk, N. Atanasova, S. Pukatzki (Edmonton/CA)
09^{30} BEV01	Characterization of the full set of cation/proton antiporters in the soil bacterium *Corynebacterium glutamicum* **A. M. Bartsch**, G. M. Seibold, R. Krämer (Köln)
09^{45} BEV02	Flavin based electron bifurcation – A mechanistic approach **N. Pal Chowdhury** (Marburg), U. Ermler (Frankfurt a. M.), W. Buckel (Marburg)

08^{30}–10^{00} Oral Session 4
Host-Microbe and Cellular Interactions (SL HM)

Room	Konferenz 4
Chair	S. P. Glaeser (Gießen), S. Zehner (Dresden)
08^{30} HMV01	Analysis of the localization of glycolytic enzymes in *Mycoplasma pneumoniae* **A. Gründel**, E. Jacobs, R. Dumke (Dresden)
08^{45} HMV02	Characterization of the metal ion-inducible autocleavage (MIIA) domain conserved in a small set of pathogenic and symbiotic bacteria **S. Zehner**, J. Schirrmeister, M. Hoppe, M. Göttfert (Dresden)
09^{00} HMV03	The gut microbiome of phytopathogenic root fly larvae: insights into the detoxification of plant secondary 3metabolites by insect-associated microbes **C. Welte**, R. de Graaf, N. van Dam, H. Op den Camp, M. Jetten (Nijmegen/NL)
09^{15} HMV04	Colonization pattern of the beneficial endomycotic bacterium *Rhizobium radiobacter* in plant roots **S. P. Glaeser**, J. Imani, I. Alabid, H. Guo, N. Kumar (Gießen), M. Rothballer (Gießen, Neuherberg) A. Hartmann (Neuherberg), P. Kämpfer, K.-H. Kogel (Gießen)
09^{30} CBV01	The septal cross walls in *filamentous cyanobacteria* is perforated by a nanopore array enabling cell-cell communication and cell differentiation **I. Maldener**, J. Lehner, J. Bornikoel, K. Faulhaber, K. Forchhammer (Tübingen)
09^{45} CBV02	Insights into the molecular basis of cell-cell interactions in phototrophic consortia **P. Henke** (Braunschweig), G. Wanner (München), M. Rohde, S. Huang, J. Overmann (Braunschweig)

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08^{30}–10^{00} Oral Session 5
Molecular Epidemiology of Infectious Diseases (FG MS – DGHM & VAAM)

Room: Konferenz 5
Chair: S. Niemann (Borstel), U. Nübel (Braunschweig)

08^{30} MSV01
Whole genome sequencing approach to determine the acquisition of vanB gene clusters in epidemic strains of *Enterococcus faecium* ST192 in Germany
J. Bender, A. Kalmbach, M. Steglich (Wernigerode), U. Nübel (Braunschweig), I. Klare
G. Werner (Wernigerode)

08^{45} MSV02
Whole-genome sequence comparision of salmonella entertidis strains isolated from samples associated with almond outbreaks suggests a reduction in genome complexity over time
C. Parker (Albany, CA/US), K. Cooper, A. Oliver (Northridge, CA/US), S. Huynh (Albany, CA/US)

09^{00} MSV03
Population dynamics of *Staphylococcus aureus* recovered from the airways of cystic fibrosis patients during a longitudinal prospective observational multicenter study
N. Braun, T. Janssen, C. Vogel, Ka. Becker, G. Peters, **B. Kahl** (Münster)

09^{15} MSV04
Establishing a molecular test assay based on genomic analysis of multidrug-resistant *Mycobacterium tuberculosis* outbreak strains from Gabon
P. Beckert (Borstel), E. Bruske (Tübingen), A. N. Traoré (Lambaréné/GA), D. Kombila
A. Alabi (Lambaréné/GA), H. Lay, J. Frick (Tübingen), S. Janssen (Amsterdam/NL), B. Lell, M. Grobusch
P. Kremsner (Tübingen), T. A. Kohl, S. Rüsch-Gerdes (Borstel), M. Frank (Tübingen), S. Niemann (Borstel)

09^{30} MSV05
Association of meningococcal type with disease outcome
J. Elias (Würzburg), W. Hellenbrand (Berlin), H. Claus, M. Frosch, U. Vogel (Würzburg)

09^{45} MSV06
Bayesian phylogeography analysis of *Clostridium difficile* in Germany – a critical evaluation
M. Steglich (Wernigerode), A. Aleksandar Radonić, A. Nitsche (Berlin), T. A. Kohl, S. Niemann (Borstel)
L. von Müller, M. Herrmann (Homburg), U. Nübel (Wernigerode, Braunschweig)

08^{30}–10^{00} Oral Session 6
Microbial Diversity and Ecology 1 (SL DE)

Room: Konferenz 6
Chair: S. Kolb (Bayreuth), M. Pester (Konstanz)

08^{30} DEV01
High activity of acteogenic bacteria in anaerobic biogas fementation plants using complex lignin degradation derivatives
J. Frerichs, V. Müller (Frankfurt a. M.)

08^{45} DEV02
Investigation of the archaeal diversity during anaerobic digestion of maize silage and municipal waste water
J. Theiss (Leipzig), M. Rother (Dresden), K. Röske (Leipzig)

09^{00} DEV03
Investigation of H_2-dependent methanogenesis in biogas plants
T. Kern, M. Linge, A. Begenau, T. Enders, M. Rother (Dresden)

09^{15} DEV04
Population dynamics in thin stillage based biogas reactors
I. Röske, W. Sabra, K. Sahm (Hamburg), H. Nacke, R. Daniel (Göttingen), A.-P. Zeng
G. Antranikian (Hamburg)

09^{30} DEV05
Potential Interactions of Anaerobes in Fen Rhizospheres
S. Hunger, O. Schmidt, M. Hilgarth, A. S. Gößner, H. L. Drake (Bayreuth)

09^{45} DEV06
Isolation and environmental distribution of type Ib methane oxidizing bacteria
C. Lüke (Nijmegen/NL), P. Frenzel (Marburg), S. Dedysh (Moskau/RU), P. Bodelier (Wageningen/NL)
H. Op den Camp, M. Jetten (Nijmegen/NL)

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08^{30}–10^{00} Oral Session 7
Food Microbiology and Food Hygiene 1 (FG LM – DGHM & VAAM)

Room: Konferenz 7+8
Chair: H. Schmidt (Stuttgart), S. Müller-Herbst (Freising)

08^{30} LMV01
Live and let die – how the interaction of *Listeria monocytogenes* and *Acanthamoeba spp.* affects growth and distribution of the foodborne pathogen
M. Schuppler (Zürich/CH), D. Doyscher (Oberschleissheim), L. Fieseler (Wädenswil/CH) M. J. Loessner (Zürich/CH)

08^{45} LMV02
Comparison of the bacterial community structure of *Listeria monocytogenes* positive and negative food samples from the retail market
M. Noll (Coburg, Berlin), S. Kleta (Berlin), J. Rau (Fellbach), S. Al Dahouk (Berlin)

09^{00} LMV03
Detection and Identification of *Pseudomonas* Species on Oak Leaf Lettuce
S. Nübling, H. Schmidt, A. Weiss (Stuttgart)

09^{15} LMV04
Highly sensitive loop-mediated isothermal amplification for rapid phage detection in dairy samples
E. Brinks, N. Wagner (Kiel), M. Samtlebe, Z. Atamer, J. Hinrichs (Stuttgart), H. Neve, K. Heller (Kiel)

09^{30} LMV05
Rapid quantification of viable *Legionella*
A. Pöcking, K. Müller-Zahm, S. Henze, I.-G. Richter, P. Miethe (Bad Langensalza)

09^{45} LMV06
Fast and effective killing of *Bacillus atrophaeus* endospores using a new generation of light-activated vitamin B2 derivatives
A. Eichner, A. Gollmer, A. Späth, W. Bäumler, J. Regensburger, T. Maisch (Regensburg)

08^{30}–10^{00} Oral Session 8
Challenges in Modern Diagnostic Fields StAG DV, FG QS (DGHM) & FG Qualitätssicherung und Diagnostik (VAAM)

Room: Seminar 3+4
Chair: K. Becker (Münster), S. Prowe (Berlin)

08^{30} DVV01
Endotoxin Masking in Common Formulations of Bio-Pharmaceuticals
J. Reich, H. Grallert (Bernried)

09^{00} QSV01
Microbiological testing and assessment of cell based medicinal products – An area of conflict between pharmaceutical and clinical microbiology?
U. Schurig, J.-O. Karo, U. Sicker, E. Spindler-Raffel, I. Spreitzer (Langen)

09^{15} DVV02
Microbial Diagnostics in Pharmaceutical Quality management – Is Molecular Biology improving or replacing Microbial Diagnostics in quality management?
C. Scheuermann, C. Farrance (Lyon/FR)

09^{30} DVV03
Lower MALDI Biotyper Threshold Values are Appropriate for Nontuberculous Mycobacteria
A. B. Pranada (Dortmund), M. Timke (Bremen), E. Witt (Dortmund), M. Kostrzewa (Bremen)

09^{45} DVV04
Microbead-Chip Read for Next Generation Quantitative Multiplex Real-Time PCR
S. Rödiger, C. Schmidt, C. Deutschmann (Senftenberg), H. Hanschmann, W. Lehmann (Lipten/Bronkow) P. Schierack (Senftenberg)

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08^{30}–10^{00} **Oral Session 9**
Compliance und Interventionen in der Krankenhaushygiene (FG PR & StAG HY)

Room Seminar 5+6
Chair F. Mattner (Köln), H.-M. Just (Nürnberg)

08^{30} HYV01 Problems in the pre-analytic stages of blood culture diagnostics in German hospitals – a qualitative study using focus groups
A. Karch, A. Duddeck, H. Raupach-Rosin, M. Gehrlich, R. Mikolajczyk (Braunschweig)

08^{45} HYV02 The positive association between self-reported cooperation on one's ward and hand hygiene compliance among physicians on intensive care units – Is it based on superiors, colleagues, or patients' relatives?
T. von Lengerke, B. Lutze, K. Graf, C. Krauth, B. Kröning, K. Lange, L. Schwadtke, J. Stahmeyer I. F. Chaberny (Hannover)

09^{00} HYV03 Hand hygiene compliance rates in 27 ICUs – Does hospital size, profession, working shifts or indication specific opportunities has an effect?
C. Alefelder (Wuppertal), H. Niggemann (Jena), G. Horstmann, H. Rüden (Berlin)

09^{15} HYV04 Implementing Link Nurses for Infection Control in German Hospitals – Difficulties and Shortcomings during the process – interim report from the HYGPFLEG-Project
D. Peter (Köln), M. Meng (Witten), G. Braun, R. Galante (Köln), C. Krüger, C. Kugler (Witten), F. Mattner (Köln)

09^{30} HYV05 Chlorhexidine-containing dressings for external ventricular drainages in order to reduce meningoventriculitis rates – a before after trial
S. Scheithauer (Göttingen), H. Schulze-Steinen, H. Häfner, A. Höllig, G. Marx, S. Lemmen (Aachen)

09^{45} HYV06 Reduction of Nosocomial Blood Stream Infections (BSI) and Nosocomial Vancomycin-Resistant *Enterococcus faecium* (VRE) Colonisation on an Intensive Care Unit (ICU) after the Introduction of Antiseptic (Octenidine-based) Bathing – An Interrupted Time Series Analysis
F. Mattner (Köln), I. Klare (Wernigerode), F. Wappler (Köln), G. Werner (Wernigerode), U. Ligges (Dortmund) S. Sakka, S. Messler (Köln)

10^{00}–10^{30} Coffee Break/Industrial Exhibition

10^{30}–12^{00} **Plenary Session 1**
Metabolism and Transport

Room Grosser Saal
Chair M. Hensel (Osnabrück), V. Müller (Frankfurt a. M.)

10^{30} INV03 Keeping membrane lipids in balance – deciphering the metabolic flux of fatty acids in yeast
S. Kohlwein (Graz/AT)

11^{00} INV04 Diversity and regulation of amino acid transporters in *Saccharomyces cerevisiae*
B. Andre (Gosselies/BE)

11^{30} INV05 Structural studies of autotransport and immune evasion in gram-negative bacteria
A. Goldman (Leeds/GB)

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12^{15}–13^{15} **Lunchsymposium – Curetis AG**
Klinische Relevanz schneller Infektionsdiagnostik

curetis

Room Konferenz 1
Chair H. Fickenscher (Kiel)

12^{15} Bessere Diagnostik von Lungeninfekten durch Multiplex-PCR?
R. Frei (Basel/CH)

12^{45} Klinische Relevanz von schnellen molekularen Tests
M. Deja (Berlin)

12^{15}–13^{15} **Lunchsymposium – Becton Dickinson GmbH**
Automation in der medizinischen Mikrobiologie

BD

Room Konferenz 2+3
Chair D. Mack (Ingelheim)

12^{15} Moving to full lab automation – What to take into consideration?
J. van Eldere (Leuven/BE)

12^{35} Automatisierung des mikrobiologischen Labors – Klinischer Impact?
N. Mutters (Heidelberg)

12^{55} BD Kiestra™ Work Cell Automation – erste Erfahrungen aus einem Universitätsklinikum
J. Rödel (Jena)

12^{15}–12^{35} **Screening, Isolieren oder antiseptische Ganzkörperwaschungen – Strategien zur VRE Kontrolle**

Room Konferenz 4
Chair F. Mattner (Köln), C. Brandt (Frankfurt a. M.)

12^{15} VRE – Diagnostik und Epidemiologie
G. Werner (Wernigerode)

12^{35}–13^{15} **Lunchsymposium – Qiagen GmbH**
Screening, Isolieren oder antiseptische Ganzkörperwaschungen – Strategien zur VRE Kontrolle

QIAGEN®

Room Konferenz 4
Chair F. Mattner (Köln), C. Brandt (Frankfurt a. M.)

12^{35} Ob, wann, wie lange und wen screenen – Wo ist der Break-point?
M. Dettenkofer (Freiburg)

12^{55} Isolierung versus antiseptische Ganzkörperwaschung
S. Scheithauer (Göttingen)

12^{15}–13^{15} **Lunchsymposium – Luminex B.V.**
Taking Molecular Diagnostics to the Next Level

Luminex

Room Konferenz 5
Chair J. Willuhn (s-Hertogenbosch/NL)

Innovative molecular diagnostics to help prevent HCAI's associated with Infectious Intestinal Disease
J. Anson (Liverpool/UK)

Multiplex NAT in stool samples and next generation Luminex chemistry
H. Hirsch (Basel/CH)

The future of Molecular Diagnostics is now: NxTAG™ and Aries™
S. Dunbar (Austin, TX/US)

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12^{15}–13^{15} Symposium „Ärzte ohne Grenzen“

Room: Konferenz 6

Ärzte ohne Grenzen – Wer wir sind, was wir machen
Dany Balke (Berlin)

13^{30}–15^{00} Plenary Session 2 Microbial Pathogenicity (Type IV Secretion Systems)

Room: Grosser Saal
Chair: S. Suerbaum (Hannover), A. Lührmann (Erlangen)

13^{30} INV06 Modulation of host cell functions by *Bartonella* type IV secretion effectors
C. Dehio (Basel/CH)

14^{00} INV07 Type IV secretion systems in *Helicobacter pylori*
R. Haas (München)

14^{30} INV08 Biological diversity of bacterial type IV secretion systems
P. J. Christie (Houston, TX/US)

13^{30}–15^{00} Plenary Session 3 Microbial Biotechnology

Room: Saal 1
Chair: M. Ansorge-Schumacher (Dresden), M. Hofrichter (Zittau)

13^{30} INV09 Metabolic engineering of yeasts to optimize the production of biofuels and other bio-based chemicals
E. Boles (Frankfurt a. M.)

13^{52} INV10 Engineering by design – systems biology based development of microbial production strains
D. Mattanovich (Wien/AT)

14^{14} INV11 Pathway engineering in *Corynebacterium glutamicum* for the production of amino acids and other value-added products
B. J. Eikmanns (Ulm)

14^{36} INV12 White biotechnology for black gold – BASF-Wintershall initiatives for enhanced oil recovery
A. Herold (Ludwigshafen)

15^{00}–16^{30} Postersession 1

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	Infection Epidemiology and Population Genetics (MS) (MSP01-MSP15)	page 316
	Infection Immunology (II) (IIP01-IIP17)	page 273
	Microbial Diversity and Ecology (incl. Soil and Water Microbiology) (DE) (DEP01-DEP58)	page 195
	Microbiota, Probiotics and Host (PW) (PWP01-PWP17)	page 342
15^{00}–17^{00}	**Coffee Break/Industrial Exhibition**	

17^{00}–18^{30} Oral Session 10
Biotechnology 1 – Designer Tools (SL BT)

Room: Saal 1
Chair: M. Steinhagen (Dresden), T. Drepper (Juelich)

17^{00} BTV01
A new reporter protein for Extremophiles
K. Rabe (Eggenstein-Leopoldshafen)

17^{15} BTV02
Cage me if you can! Optogenetic bacterial gene regulation using photocaged inducers
D. Binder, A. Grünberger, A. Loeschcke, C. Probst, C. Bier, J. Pietruszka, W. Wiechert, D. Kohlheyer
K.-E. Jaeger, T. Drepper (Jülich)

17^{30} BTV03
A multigene assembly strategy for improved heterologous expression of lanthipeptides in *Escherichia coli* allows for efficient in vivo peptide engineering
A. Kuthning, E. Mösker, R. D. Süssmuth (Berlin)

17^{45} BTV04
Exploiting unconventional secretion in *Ustilago maydis*
P. Sarkari, J. Stock, M. Terfrüchte, M. Reindl, M. Feldbrügge, **K. Schipper** (Düsseldorf)

18^{00} BTV05
Gluconobacter factory – development of a platform strain for oxidative biotransformations
I. Kiefler, S. Bringer, M. Bott (Jülich)

18^{15} BTV06
Heterologous production of an electron transport chain and electrode-interaction in microbial electrochemical cells enables *Escherichia coli* to perform unbalanced fermentations
K. Sturm-Richter, **F. Golitsch**, G. Sturm (Karlsruhe), E. Kipf (Freiburg), A. Dittrich (Karlsruhe)
S. Kerzenmacher (Freiburg), J. Gescher (Karlsruhe)

17^{00}–18^{30} Oral Session 11
Anaerobic Metabolism 2 (SL AM)

Room: Konferenz 1
Chair: M. Perner (Hamburg), T. Goris (Jena)

17^{00} AMV07
The crystal structure of the cobamide-containing tetrachloroethene reductive dehalogenase of *Sulfurospirillum multivorans*
C. Kunze (Jena), M. Bommer, J. Fesseler (Berlin), T. Schubert (Jena), H. Dobbek (Berlin), G. Diekert (Jena)

17^{15} AMV08
Anaerobic transformation of brominated phenolic aromatics by *Dehalococcoides mccartyi* strain CBDB1
C. Yang, A. Kublik, L. Adrian (Leipzig)

17^{30} AMV09
Elucidation of anaerobic benzene and alkylated aromatic compound degradation pathways and associated proteins by proteomic and metabolomic analyses
R. Starke (Hohenheim, Leipzig), N. Jehmlich, C. Vogt, S. Kleinsteuber, M. von Bergen (Leipzig)
J. Seifert (Hohenheim)

17^{45} AMV10
2,3-butanediol production using acetogenic bacteria grown on syngas
C. Erz, P. Dürre (Ulm)

18^{00} AMV11
Heme d1 – Biosynthesis and Insertion into Cytochrome cd1 Nitrite Reductase NirS in *Pseudomonas aeruginosa*
J. Adamczack, M. Hoffmann, U. Papke, T. Nicke, G. Layer (Braunschweig)

18^{15} AMV12
Novel insights into FHL, the *Escherichia coli* formate hydrogenlyase complex
C. Pinske, F. Sargent (Dundee/GB)

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17^{00}–18^{30} Working Group Session 1 (VAAM)
Second Messenger in Bacterial Signaling and Pathogenicity (FG RS & MP)

Room: Konferenz 2+3
Chair: A. Peschel (Tübingen), T. Mascher (München)

17^{00} MPV07
c-di-AMP signalling in *Staphylococcus aureus*
A. Gründling (London/GB)

17^{30} MPV08
Structural analysis of c-di-AMP synthesis by the DAC domain and downstream c-di-AMP recognition
G. Witte, Mart. Müller, T. Deimling (München)

17^{50} RSV01
The stringent response and its impact on rRNA regulation in *S. aureus*
B. Kästle, F. Gratani, T. Geiger, C. Wolz (Tübingen)

18^{10} RSV02
Oxygen control of alginate production in *Pseudomonas aeruginosa* by a signaling module comprising an oxidoreductase, a hydratase and a cyclic di-GMP synthase
A. Schmidt, A. S. Hammerbacher (Tübingen), M. Merighi (Boston, MA/US), K. Lapouge (Lausanne/CH)
Mi. Bastian, F. Aktürk (Tübingen), A. Garbe (Hannover), M. Ulrich (Tübingen), G. B. Pier (Boston, MA/US)
V. Kaever (Hannover), S. Lory (Boston, MA/US), D. Haas (Lausanne/CH), S. Schwarz, G. Döring (Tübingen)

17^{00}–18^{30} Working Group Session 2 (VAAM)
Plant-Microbe Interactions (FG Symbiotische Interaktionen)

Room: Konferenz 4
Chair: S. Schnell, M. Cardinale (Gießen)

17^{00} HMV05
Deciphering the plant microbial network
G. Berg (Graz/AT)

17^{30} HMV06
Isolation and characterization of endophytic bacteria associated with root-nodules *Medicago sativa* in Al-Ahasa region
A. Khalifa (Al-Ahsaa, Beni-Suef/SA), M. AlMalki (Al-Ahsaa/SA)

17^{45} HMV07
Effects of bacterial inoculation on plant fitness under salt stress
M. Cardinale, S. Ratering, C. Suarez Franco, A. M. Zapata, S. Schnell (Gießen)

18^{00} HMV08
Biochemical and in planta characterization of the compartment-specific enzymatic activities of the extracelluar exzymes, LscB and LscC, of the plant pathogen *Pseudomonas syringae*
A. Mehmood, M. Ullrich (Bremen)

18^{15} HMV09
Conserved and Divergent Transcriptional Responses of Two Plant Species on PGPB *Kosakonia radicincitans* Colonisation
K. Witzel, B. Berger, S. Ruppel (Großbeeren)

17^{00}–18^{30} Oral Session 12
Clinical Microbiology and Infectious Diseases (StAG KM)

Room: Konferenz 5
Chair: S. Zimmermann (Heidelberg), C. von Eiff (Münster)

17^{00} KMV01
Abandonment of prophylactic antibiotic therapy after kidney transplantation leads to a reduction in urinary tract infection with ESBL producing bacteria
S. Scheithauer (Göttingen), A. Mühlfeld, F. Wenzel (Aachen), J. Flöge (Aachen, Göttingen)

17^{15} KMV02
Is the implementation of a microbiological surveillance screening beneficial in neonatal intensive care units?
I. Schmeh, A. Welk, t. Schwanz, E. Siegel, A. Diefenbach, R. Metz, B. Jansen, E. Mildenberger (Mainz)

17^{30} KMV03
(1→3)-β-D-Glucan kinetics in patients with candida blood stream infection
J. Held (Erlangen, Freiburg), A. Busse Grawitz, T. Epting, S. Dräger, R. Friedrich, E. Rappold (Freiburg)

17^{45} KMV04
The microbial diversity of the female urogenital tract in the context of STIs and female infertility
S. Graspeuntner, K. Gillmann (Lübeck), R. Speer (Berlin), M. K. Bohlmann, I. R. König (Lübeck)
J. F. Baines (Plön, Kiel), J. Rupp (Lübeck)

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18^{00} KMV05	Characterization of bacterial persistence and phenotypic drift in an acute-chronic murine sepsis model **N. Jbeily** (Jena), R. Oertel (Dresden), A. Kather (Jena), A. Moter (Berlin), R. A. Claus, M. Pletz (Jena)
18^{15} KMV06	Molecular genotyping of 1200 community acquired commensal and clinical *Staphylococcus aureus* isolates of the African-German StaphNet multicenter study **U. Ruffing** (Homburg), A. Abraham (Lambaréné/GA), S. Abdulla (Bagamoyo/TZ), R. Akulenko (Saarbrücken) H. von Briesen, A. Germann (Sulzbach), M. Grobusch (Amsterdam/NL), V. Helms (Saarbrücken) T. Kazimoto (Bagamoyo/TZ), W. Kern (Freiburg), I. Mandomando (Maputo/MZ), A. Mellmann (Münster) L. von Müller (Homburg), G. Peters, F. Schaumburg (Münster), Sa. Schubert (Homburg) D. Vubil (Maputo/MZ), L. Wende, M. Herrmann (Homburg)

17^{00}–18^{30} **Working Group Session 3 (VAAM)**
Microorganisms' impact on trace gas flux of terrestrial and marine ecosystems (FG Umweltmikrobiologie)

Room	Konferenz 6
Chair	S. Kolb (Bayreuth/Jena), M. A. Horn (Bayreuth)
17^{00} DEV07	Bacterial metabolism of isoprene – a much neglected atmospheric trace gas J. C. Murrell (Norwich/GB)
17^{45} DEV08	Targeting methanotrophs in environmental samples using stable isotope probing combined with metatranscriptomics M. Dumont (Marburg)
18^{00} DEV09	Unveiling the microbial sink of methanol in terrestrial ecosystems **S. Kolb** (Bayreuth/Jena), M. Morawe (Bayreuth), A. Stacheter (Bayreuth/Coburg)
18^{15} DEV10	A metaproteogenomic approach for functional investigation of carbon cycling by marine methylotrophs **M. Taubert**, C. Grob, A. Howat, O. Burns (Norwich/GB), Y. Chen (Warwick/GB), N. Jehmlich M. von Bergen (Leipzig), J. C. Murrell (Norwich/GB)
18^{30} DEV11	Novel syntrophic species link fermentation and methanogenesis in peat **O. Schmidt**, L. Hink, M. A. Horn, H. L. Drake (Bayreuth)
18^{45} DEV12	Nitrous oxide consumption by a remarkably diverse fen denitrifier community K. Palmer, **M. A. Horn** (Bayreuth)

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17^{00}–18^{30} Oral Session 13 / Working Group Session 4 (VAAM) Biology of Conventional and Non-Conventional Yeasts (FG Hefe)

Room: Konferenz 7+8
Chair: K.-D. Entian (Frankfurt a. M.), C. Otto (Dresden)

17^{00} YEV01
Short-chain carboxylic acid transporters in *Yarrowia lipolytica*
C. Otto, G. Barth (Dresden)

17^{15} YEV02
Engineering of glucose transporters into specific xylose transporters for simultaneous fermentation of glucose-xylose mixtures
E. Boles (Frankfurt a. M.)

17^{30} YEV03
A novel yeast killer toxin attacking ribosomal RNA
A. Kast, F. Meinhardt (Münster)

17^{45} YEV04
Ribosome Biogenesis in Yeast: Base Modifications in 18S rRNA
K.-D. Entian, B. Meyer, S. Sharma, P. Kötter, P. Wurm, Y. Yang, J. Wöhnert (Frankfurt a. M.)

18^{00} YEV05
Identification of novel base methyltransferases of the 25S rRNA in *Saccharomyces cerevisiae*
S. Sharma, J. Yang, P. Watzinger, P. Koetter, K.-D. Entian (Frankfurt a. M.)

18^{15} YEV06
Biotechnological production of ω-hydroxy fatty acids with the help of metabolically engineered *Yarrowia lipolytica* strains
M. Gatter, G. Barth (Dresden)

18^{30} YEV07
Lipids from lignocellulose-grown oleaginous yeasts for food and biodiesel production
V. Passoth, J. Blomqvist, J. Brandenburg (Uppsala/SE), N. Bonturi (Campinas/BR), U. Rova (Luleå/SE) J. Pickova, M. Sandgren (Uppsala/SE)

18^{45} YEV08
The fraction of cells that resume growth after acetic acid addition is a strain dependent parameter of acetic acid tolerance in *Saccharomyces cerevisiae*
S. Swinnen, M. Fernández Niño (Bremen), D. González-Ramos (Delft/NL), A. J. A. van Maris (Delft)
E. Nevoigt (Bremen)

17^{00}–18^{30} Oral Session 14 Relevance of Pathogen Detection StAG DV, FG QS (DGHM) & FG Qualitätssicherung und Diagnostik (VAAM)

Room: Seminar 3+4
Chair: U. Reischl (Regensburg), A. Seiffert-Störiko (Frankfurt a. M.)

17^{00} DVV05
The German NAK, the local branch of the EUCAST
S. Gatermann, M. Kresken (Bochum)

17^{30} DVV06
Development of a protein microarray based test for the rapid and multiplex detection of *Burkholderia pseudomallei* –specific antibodies
C. Kohler (Greifswald), E. Müller, R. Ehricht (Jena), I. Steinmetz (Greifswald)

17^{45} DVV07
Chip-based Isolation of Pathogens for Subsequent Raman Spectroscopic Identification
K. Weber, S. Pahlow, S. Kloss, S. Stoeckel, P. Rösch, D. Cialla-May, J. Popp (Jena)

18^{00} DVV08
FISH in medical biofilms – closing the gap between bench and bedside
J. Kikhney, B. Gocht, A. Petrich, P. Rojas, J. Schulze, S. Sutrave, A. Wießner, R. Hetzer, **A. Moter** (Berlin)

18^{15} QSV02
You will never talk alone – Bacteria on smartphone touchscreens in a German university setting and evaluation of two popular cleaning methods
K. Späth, K. Weik, H. Kunzelmann (Villingen-Schwenningen), C. Horn (Singen)
M. Kohl (Villingen-Schwenningen), F. Blessing (Villingen-Schwenningen, Singen)
M. Egert (Villingen-Schwenningen)

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17^{00}–18^{30} **Oral Session 15**
Epidemiology of Multi-Drug Resistant Organisms
(FG PR & StAG HY)

Room Seminar 5+6
Chair T. Eckmanns, L. Wieler (Berlin)

17^{00} Distribution of Methicillin-resistant *Staphylococcus aureus* clonal lineages in bacteraemia isolates from
PRV01 North Rhine-Westphalia, 2011-2013
R. Koeck, D. Harmsen, I. Daniels-Haardt, A. Jurke (Münster), W. Witte, C. Cuny (Wernigerode)

17^{15} Mupirocin susceptibility in clinical staphylococcal isolates from Germany
PRV02 **B. Strommenger**, F. Layer, F. Erdmann, I. Klare, G. Werner (Wernigerode)

17^{30} Occurrence of carbapenemase producing *Enterobacteriaceae* (CPE) isolated from pig-fattening farms throughout Germany
PRV03 **N. Roschanski**, C. von Salviati (Berlin), J. Hering (Hannover), A. Friese, B. Guerra, A. Käsbohrer (Berlin) L. Kreienbrock (Hannover), U. Rösler (Berlin)

17^{45} Emerging carbapenemase-producing multi-drug-resistant bacteria in University Hospital Bonn
PRV04 **M. Parcina**, E. Hoffmann (Bonn), M. Kaase (Bochum), A. Hörauf, I. Bekeredjian-Ding (Bonn)

18^{00} Staphylococci living in aquatic environments with inducible resistance to macrolides, lincosamides and streptogramin B antibiotics and the capacity of the metabolite anhydroerythromycin to induce cross-resistance
PRV05 against these antibiotic classes
S. Heß, C. Gallert (Karlsruhe)

18^{15} Quantitative microbial risk assessment in the „safe Ruhr“ Project
HYV07 **L. Jurzik**, I. A. Hamza, M. Leifels (Bochum), C. Timm, T. Kistemann (Bonn), M. Wilhelm (Bochum)

18^{30}–19^{30} **DGHM Mitgliederversammlung**
Room Grosser Saal
Tagesordnungspunkte (siehe Seite 23)

18^{30}–19^{00} **Treffen Fachgruppen VAAM/DGHM**

Konferenz 1
FG Experimentelle Mykologie/Fungal Biology and Biotechnology (VAAM)

Konferenz 2+3
FG Regulation und Signaltransduktion in Prokaryoten (VAAM)

Konferenz 5
FG Wasser und Abwasser (VAAM)

Konferenz 6
FG Umweltmikrobiologie (VAAM)

Konferenz 7+8
FG Hefen (VAAM)

Seminar 3+4
Identifizierung und Systematik (VAAM)

Seminar 5+6
FG Mikrobielle Systematik (DGHM)

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08^{30}–10^{00} Oral Session 16
Host-pathogen Interactions of Gram-Positive and Gram-Negative Bacteria (FG MP – DGHM & VAAM)

Room: Saal 1
Chair: S. Hammerschmidt (Greifswald), V. A. J. Kempf (Frankfurt a. M.)

08^{30} MPV09
A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization
S. Baur, M. Rautenberg (Tübingen), M. Faulstich (Würzburg), T. Grau, Y. Severin (Tübingen)
T. Rudel (Würzburg), I. Autenrieth, **C. Weidenmaier** (Tübingen)

08^{45} MPV10
Clostridium perfringens Enterotoxin Targeted Pancreatic Cancer Therapy using *Clostridium sporogenes*
M. Mahmoud, S. König, D. Meisohle, P. Dürre (Ulm)

09^{00} MPV11
Crystal structure of pneumococcal carboxypeptidase DacB and the impact of carboxypeptidases on pathogenesis
M. Abdullah (Greifswald), J. Gutiérrez-Fernández (Madrid/ES), T. Pribyl (Greifswald), N. Gisch (Borstel)
M. Saleh (Greifswald), M. Rohde (Braunschweig), L. Petruschka, G. Burchhardt (Greifswald)
D. Schwudke (Borstel), J. A. Hermoso (Madrid/ES), S. Hammerschmidt (Greifswald)

09^{15} MPV12
Characterization of the protein translocation channel of bacterial type III secretion systems
S. Wagner (Tübingen)

09^{30} MPV13
Identification and functional characterization of plasminogen-binding proteins of *Acinetobacter baumannii*
A. Koenigs, S. Goettig, T. A. Wichelhaus, B. Averhoff, P. Kraiczy (Frankfurt a. M.)

09^{45} MPV14
Reprogramming of Myeloid Angiogenic Cells by *Bartonella henselae* leads to microenvironmental regulation of pathological angiogenesis
F. O'Rourke (Frankfurt a. M.), T. Mändle (Tübingen), C. Urbich, S. Dimmeler, U. R. Michaelis, R. P. Brandes
C. Döring, M.-L. Hansmann (Frankfurt a. M.), M. Flötenmeyer (Tübingen), K. Lauber (Grosshadern)
W. Ballhorn, V. A. J. Kempf (Frankfurt a. M.)

08^{30}–10^{00} Working Group Session 4 (DGHM & VAAM)
Evolutionary genomics (FG MS & FG Identifizierung und Systematik)

Room: Konferenz 1
Chair: U. Nübel (Braunschweig), H.-J. Busse (Wien/AT)

08^{30} MSV07
Sexual transmission of *meningococci* may account to an outbreak of meningococcal disease among men who have sex with men
M. K. Taha (Paris/FR), M. Lappann (Würzburg), F. Veyrier (Paris/FR), H. Claus (Würzburg), D. Harmsen
K. Prior (Münster), A.-E. Deghmane (Paris/FR), A. Otto (Greifswald), I. Parent du Châtelet (Saint Maurice/FR)
W. Hellenbrand (Berlin), D. Becher (Greifswald), **U. Vogel** (Würzburg)

09^{00} MSV08
Sequencing bacterial genomes from clinical samples without cultivation
H. Seth-Smith, S. Harris, N. Thomson, R. Schlapbach, L. Vaughan (Zürich/CH)

09^{30} MSV09
Assessment of the microbial diversity in groundwater used for drinking water abstraction by 16S-tag pyrosequencing and physiological analysis of enrichment cultures
M. Mühling, C. Steinbrenner, M. Liebig (Freiberg), H. Fischer, S. Rösner (Halsbrücke), A. Thürmer
R. Daniel (Göttingen), M. Schlömann (Freiberg)

09^{45} MSV10
A next generation sequencing approach to understand the evolution and global success of *Mycobacterium tuberculosis* complex strains of the Beijing lineage
M. Merker, T. A. Kohl (Borstel), C. Blin, S. Mona (Paris/FR), N. Duforet-Frebourg (Grenoble/FR), S. Lecher
E. Willery (Lille/FR), M. Blum (Grenoble/FR), S. Rüsch-Gerdes (Borstel), I. Mokrousov (St. Petersburg/RU)
P. Supply (Lille/FR), S. Niemann (Borstel), T. Wirth (Paris/FR)

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Oral Session 17
Eukaryotic Pathogens (FG EK & Deutsche Gesellschaft für Parasitologie (DGP))

08^{30}–10^{00}

Room: Konferenz 2
Chair: C. Lüder (Göttingen), F. Ebel (München)

08^{30} EKV01
In vivo imaging of disseminated murine *Candida albicans* infection reveals unexpected host sites of fungal persistence during antifungal therapy
I. Jacobsen, A. Lüttich, O. Kurzai, B. Hube, M. Brock (Jena)

08^{45} EKV02
The transcriptional regulators Nrg1 and Tup1 have a different impact on the morphology of the human fungal pathogen *Candida albicans*
R. Martin, A. Haeder, K. Meinhardt, D. Hellwig, M. Böhringer, S. Brunke, I. Jacobsen, K. Hünniger B. Hube (Jena), J. Morschhäuser (Würzburg), O. Kurzai (Jena)

09^{00} EKV03
Fitness costs of drug resistance in *Candida albicans*
C. Popp, C. Sasse, J. Morschhäuser (Würzburg)

09^{15} EKV04
Critical impact of the negative host cell cycle regulator Tspyl2 on stage differentiation of the protozoan parasite Toxoplasma gondii
I. Swierzy, F. Verworrn, **C. Lüder** (Göttingen)

09^{30} EKV05
Mechanisms of complement evasion by malaria parasites
C. J. Ngwa, **T. F. A. Rosa** (Aachen), V. Agarwal, A. M. Blom (Malmö/SE), P. F. Zipfel, C. Skerka (Jena) G. Pradel (Aachen)

09^{45} EKV06
Manganese Superoxide Dismutase Mimics Inhibit the Proliferation of Leishmania Parasites
S. Beez, M. Filipović, I. Ivanović-Burmazović, U. Schleicher, C. Bogdan (Erlangen)

Oral Session 18
Food Microbiology and Food Hygiene 2 (FG LM – DGHM & VAAM)

08^{30}–10^{00}

Room: Konferenz 3
Chair: M. Wenning (München), E. Hauser (Stuttgart)

08^{30} LMV07
Inhibitory effect of nitrite on growth and survival of pathogens – molecular analysis of a preservation method
S. Müller-Herbst, A. Mühlig, D. Kaspar (Freising), S. Wüstner (München), J. Kabisch (Kiel) R. Pichner (Kulmbach), S. Scherer (Freising, Kulmbach)

08^{45} LMV08
Characterization of *staphylococcus carnosus* strains for the application in raw ham production
A. Mueller, G. Fogarassy, A. Bajac, R. Reichhardt, H. Schmidt, A. Weiss (Stuttgart)

09^{00} LMV09
Who lives out there? – Molecular monitoring of the smear microbiota composition and diversity of surface-ripened red-smear cheese by high-throughput sequencing
J. Ritschard (Zürich/CH)

09^{15} LMV10
Growth behavior of different lactic acid bacteria in lupin flour and lupin protein isolate
C. Fritsch, R. F. Vogel, S. Toelstede (Freising)

09^{30} LMV11
Non-thermal atmospheric plasmas for food decontamination
U. Schnabel, R. Niquet (Greifswald), O. Schlüter (Potsdam), J. Ehlbeck (Greifswald)

09^{45} LMV12
Impact of different measures on virus inactivation in meat products
T. Albert, A. Lange-Starke, C. Jarke, A. Petereit, J. Manteufel, J. Straube, J. Heinze, P. Braun, U. Truyen K. Fehlhaber (Leipzig)

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Oral Session 19
Microbial Cell Biology 1 (SL CB, SL BE & SL TS)

08^{30}–10^{00}

Room: Konferenz 4
Chair: J. Pané-Farré (Greifswald), A. Möll (Boston/USA)

08^{30} CBV03
Regulation of amidase-mediated peptidoglycan hydrolysis in *Vibrio cholerae*
A. Möll, T. Dörr (Boston, MA/US), L. Alvarez-Munoz (Umeå/SE), M. C. ChaoB. M. Davis (Boston, MA/US) F. Cava (Umeå/SE), M. K. Waldor (Boston, MA/US)

08^{45} CBV04
Another brick in the wall – Evidence for peptidoglycan in the cell walls of planctomycetes
O. Jeske (Braunschweig), M. Schüler (Martinsried), P. Schumann, C. Boedeker (Braunschweig) A. Schneider (Tübingen), M. Rohde, M. Jogler, S. Spring (Braunschweig), H. Engelhardt (Martinsried) C. Mayer (Tübingen), C. Jogler (Braunschweig)

09^{00} CBV05
The response of *Bacillus pumilus* to hydrogen peroxide provoked oxidative stress
S. Handtke, R. Schroeter, K. Methling, B. Jürgen, D. Albrecht, M. Lalk, T. Schweder, M. Hecker
B. Voigt (Greifswald)

09^{15} CBV06
A dynamin-like protein that reacts to membrane stress in *Bacillus subtilis*
P. Sawant, M. Bramkamp (Martinsried)

09^{30} CBV07
Analysis of the alkaline shock protein 23 (Asp23) in *Staphylococcus aureus*
Marr. Müller, S. Reiß, R. Schlüter, U. Mäder, A. Beyer, W. Reiß (Greifswald), J. Marles-Wright (Edinburgh/GB) R. J. Lewis (Newcastle/GB), H. Pförtner (Greifswald), R. J. Lewis (Newcastle/GB), U. Völker, K. Riedel M. Hecker (Greifswald), S. Engelmann (Braunschweig), J. Pané-Farré (Greifswald)

09^{45} CBV08
The zinc-buffering system of *Cupriavidus metallidurans* retards efficient zinc allocation in a ΔzupT mutant
M. Herzberg, D. Dobritzsch, S. Baginski, D. H. Nies (Halle a. S.)

Oral Session 20
Cases in Clinical Microbiology (StAG KM)

08^{30}–10^{00}

Room: Konferenz 5
Chair: S. Zimmermann (Heidelberg), S. Scheithauer (Göttingen)

08^{30} KMV07
TED Fall 1
H. Frickmann (Hamburg, Rostock), R. Hinz, R. M. Hagen, D. Wiemer (Hamburg)

08^{52} KMV08
TED Fall 2
A. Zajac, **S. Buder**, P. Müller, P. K. Kohl (Berlin)

09^{14} KMV09
TED Fall 3
C. Pilz, R. Wolf, H. Hanso, I. Dzialowski, M. Wolz, **B. Rolinski** (Meißen)

09^{36} KMV10
TED Fall 4
V. Forsbach-Birk (Ravensburg)

Oral Session 21
Biotechnology 2 – Environment & Sustainability (SL BT)

08^{30}–10^{00}

Room: Konferenz 6
Chair: A. Wobus (Dresden), D. Tischler (Freiberg)

08^{30} BTV07
Biogas production from coumarin-rich plants – Impact of coumarin on process parameters and microbial community
D. Popp, H. Sträuber, H. Harms (Leipzig)

08^{45} BTV08
Isolation of *Cellulomonas uda* from biogas reactor sludge
C. Gabris, F. Bengelsdorf, P. Dürre (Ulm)

09^{00} BTV09
Continuous Benzene and Ammonium Removal from Contaminated Groundwater using a Microbial Fuel Cell
M. Wei, F. Harnisch, P. Bombach, J. Ahlheim, C. Vogt, H. H. Richnow (Leipzig)

09^{15} BTV10
Evaluation of process parameters for the development of a biological treatment concept for sulfate and heavy metal containing mine drainage and waste waters
R. Klein (Freiberg), E. Janneck, T. Aubel (Halsbrücke), M. Schlömann, M. Mühling (Freiberg)

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09^{30} BTV11	Microbial leaching of rare earth elements from fluorescent phosphor **S. Mey**, S. Kutschke, K. Pollmann (Dresden)
09^{45} BTV12	Analysis of different leaching parameters as preliminaries for in-situ leaching **N. Gelhaar**, M. Schlömann, S. Schopf (Freiberg)

08^{30}–10^{30} Praxisorientierter Workshop – organisiert von der StAG Diagnostische Verfahren und INSTAND e.V. Molekulare Diagnostik & Externe Qualitätssicherung von molekularen und serologischen Diagnostikverfahren – Teil 1

Room: Konferenz 7+8
Chair: Ka. Becker (Münster), T. Meyer (Hamburg)

MALDI-TOF MS „ausreizen" – Ultraschnelle MALDI aus Mikrokolonien
E. Idelevich (Münster)

Molekularer Nachweis von Blutparasiten
E. Tannich (Hamburg)

Diagnostik und Epidemiologie des van Alphabets in Enterokokken
G. Werner (Wernigerode)

Prävalenz der *Chlamydia trachomatis* Infektion seit Einführung des Chlamydien Screenings in Deutschland: Daten des *C. trachomatis*-Laborsentinels
V. Bremer, S. Dudareva-Vizule (Berlin)

Bedeutung der genetischen Variabilität von *C. trachomatis* für die molekulare Diagnostik
T. Meyer (Hamburg)

08^{30}–10^{00} Oral Session 22 / Working Group Session 5 (DGHM & VAAM) Fungal friends and foes (FG EK & FG Fungal Biology and Biotechnology, VAAM)

Room: Seminar 1
Chair: V. Meyer (Berlin), S. Krappmann (Erlangen)

08^{30} FUV01	The role of the pore-forming toxin Ece1 of *Candida albicans* during translocation through the intestinal epithelial barrier **T. Förster** (Jena), D. Wilson (Jena; Aberdeen/GB), F. Mayer (Jena; Vancouver/CA), S. Mogavero, L. Kasper S. Höfs, B. Hube (Jena)
08^{45} FUV02	Scrutinizing the *Aspergillus fumigatus* Mating-Type Idiomorphs Y. Yu, C. Will (Erlangen), E. Szewczyk, J. Amich (Würzburg), **S. Krappmann** (Erlangen)
09^{00} FUV03	The transcription factor TaSte12 mediates the regulatory role of the Tmk1 MAP kinase in mycoparasitism and vegetative hyphal fusion in *Trichoderma atroviride* S. Zeilinger, **S. Gruber** (Wien/AT)
09^{15} FUV04	Velvet components in the industrial penicillin producer *Penicillium chrysogenum* – Regulation of secondary metabolism and morphology **S. Bloemendal**, Ko. Becker, K. Kopke, B. Hoff, U. Kück (Bochum)
09^{30} FUV05	*Aspergillus niger* as Expression Platform for Secondary Metabolite Production **S. Boecker**, L. Richter, F. Wanka, D. Storm, R. D. Süssmuth, V. Meyer (Berlin)
09^{45} FUV06	Marine Fungi – Understanding the biology of marine fungi for the production of secondary metabolites to establish a natural product library J. Timm, B. Jacobsen, A. Kramer, **A. Labes** (Kiel)

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08^{30}–10^{00} Oral Session 23
Gastrointestinal Infections and Microbiota 1 (FG GI & FG PW)

Room: Seminar 3+4
Chair: R. Haas (München), J. Frick (Tübingen)

08^{30} GIV01
Utilization of host-derived sulphur components enables *Campylobacter jejuni* to overcome its restricted cysteine metabolism
H. Vorwerk, J. Mohr, O. Wensel (Hannover), K. Schmidt-Hohagen (Braunschweig), C. Huber (München) E. Gripp, C. Josenhans (Hannover), D. Schomburg (Braunschweig), W. Eisenreich (München) D. Hofreuter (Hannover)

08^{45} GIV02
High throughput mapping of transcription start sites and non-coding RNAs in the pAA plasmid of enterohemorrhagic *Escherichia coli* O104:H4
P. Zhelyazkova (Münster), K. U. Förstner, C. M. Sharma, J. Vogel (Würzburg), H. Karch A. Mellmann (Münster)

09^{00} GIV03
Bidirectional gene exchange between *Helicobacter pylori* strains from a family in Coventry, United Kingdom
J. Krebes (Hannover), X. Didelot (London/GB), L. Kennemann, S. Suerbaum (Hannover)

09^{15} PWV01
Complete genome sequence of the probiotic *Escherichia coli* strain Nissle 1917
C. Liang, T. Dandekar (Würzburg), U. Sonnenborn (Herdecke), S. Rund, **T. Oelschlaeger** (Würzburg)

09^{30} PWV02
The oral microbiome as a source of bacterial genes for insights into human population structure
S. Latz, K. Henne, M. Kiesow (Aachen), M. Stoneking, J. Li (Leipzig), G. Conrads, **H.-P. Horz** (Aachen)

09^{45} PWV03
Comparative study of the microbiome of the abomasum in cattle with or without abomasal ulcers
M. Dzieciol, A. Hund, M. Wagner, T. Wittek, S. Schmitz-Esser (Wien/AT)

08^{30}–10^{00} Working Group Session 6 (DGHM)
Forschung zur Prävention nosokomialer Infektionen und multiresistenter Erreger (FG PR & StAG HY)

Room: Seminar 5+6
Chair: P. Gastmeier (Berlin), M. Herrmann (Homburg/Saar)

08^{30}
Inventory der Forschung zur Prävention nosokomialer Infektionen und MRE in Deutschland
P. Gastmeier (Berlin)

08^{52}
Inventory of research in prevention of nosocomial infections and multiresistant organisms in the Netherlands
J. Kluytmans (Breda/NL)

09^{14}
Criteria for good studies for the control of nosocomial infections and multiresistant organisms – What are the needs for a good research?
E. Tacconelli (Tübingen)

09^{36}
Das Ethik-Dilemma in der Infektionspräventionsforschung zwischen Infektionsschutz, Datenschutz, Qualitätssicherung, und Publikationspflicht
F. Mattner (Köln)

10^{00}–10^{30} Coffee Break/Industrial Exhibition

10^{30}–12^{00} Plenary Session 4
Microbiome in Medicine and Nature

Room: Grosser Saal
Chair: I. Autenrieth (Tübingen), M. Göttfert (Dresden)

10^{30} INV13
Colonization resistance – A ménage à trois with the host?
A. Bäumler (Davis, CA/US)

11^{00} INV14
Ecosystems biology – from integrated omics to control strategies for mixed microbial communities
P. Wilmes (Belval/LU)

11^{30} INV15
Response of *Candida albicans* to the infection environment
A. P. Mitchell (Pittsburgh, PA/US)

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12^{00}–13^{30} **Industrielle Mikrobiologie (VAAM)**

Room Konferenz 6
Chair G. Schmid (München), Ch. Lang (Berlin)

12^{00} Cell factories – engineering yeast for terpene production
IMV01 Ch. Lang (Berlin)

12^{22} Pseudomonas exotoxin A fusion proteins for tumor therapy
IMV02 U. Brinkmann (Penzberg)

12^{44} "ESETEC" – teaching an old workhorse new tricks – bioprocess innovations with *E.coli*
IMV03 G. Seidel (München)

13^{06} Autodisplay – a new dimension in surface display for whole cell biocatalysis and bioanalytics
IMV04 R. Maas (Düsseldorf)

12^{15}–13^{15} **Lunchsymposium – Cepheid GmbH**
News & Views on Spread of Carbapenemase-Producing Bacteria

Cepheid. A better way.

Room Konferenz 1
Chair M. Kaase (Bochum)

Carbapenemase-producing Enterobacteriaceae in Germany: the situation so far
M. Kaase (Bochum)

Carbapenemase-producers Enterobacteriaceae: experiences from an endemic scenario
E. Carretto (Reggio Emilia/IT)

12^{15}–13^{15} **Lunchsymposium – Qiagen GmbH**
Molecular Diagnostics for Healthcare-Associated Infections (HAI)

QIAGEN®

Room Konferenz 3
Chair A. Plasche-Schlütter (Hilden)

12^{15} Healthcare Associated Infections and related issues of Antimicrobial Resistance in Europe: current situation
B. Cookson (London/GB)

12^{35} Implementation of PCR as Routine Method for Detection of HAI related Infections
G. Plum (Köln)

12^{55} Implementation of Routine Molecular Diagnostic Work Flows for Stool Samples:
Platform Consolidation & Lean Aspects
M. Wasner (Dessau)

12^{15}–13^{15} **Lunchsymposium – Bruker GmbH**
MALDI-Biotyper – Changing Microbiology

BRUKER

Room Konferenz 4
Chair M. Kostrzewa (Bremen)

12^{15} Validierung und QS der MALDI MS gestützten Identifizierung im veterinärmedizinisch-klinischen Bereich der Routine-Diagnostik
I. Stamm (Ludwigsburg)

12^{35} Entwicklung und Analysen MALDI MS gestützter Bakterieller Resistenz- Untersuchungen
J. Jung (München)

12^{55} Streptokokken: Pneumokokken und Strep. mitis : Ähnlichkeiten, Unterschiede und/oder Pathogenität
M. P. G.van der Linden (Aachen)

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13^{30}–15^{00} **Plenary Session 5**
Microbial Stress Response

Room	Grosser Saal
Chair	E. Jacobs (Dresden), T. Mascher (München)
13^{30} INV16	Cyclic-di-GMP signaling and bacterial biofilm architecture R. Hengge (Berlin)
14^{00} INV17	Physiological genomics of yeast response and resistance to stress I. Sa-Correia (Lissabon/PT)
14^{30} INV18	Molecular mechanisms of fungal pathogen – host interactions & stress response K. Kuchler (Wien/AT)

13^{30}–15^{00} **Plenary Session 6**
Food and Hospital Hygiene – Quo Vadis?

Room	Saal 1
Chair	H. Schmidt (Stuttgart), I. F. Chaberny (Hannover)
13^{30} INV19	Do we have to face superbugs in food and food-producing animals? B. Guerra Román (Berlin)
14^{00} INV20	Viruses in water and food, new techniques and emerging pathogens R. Girones (Barcelona/ES)
14^{30} INV21	Antimicrobial resistance – the role of antibiotic use in animals J. Kluytmans (Breda/NL)

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15^{00}–16^{30} **Postersession 2**

15^{00}–17^{00} **Coffee Break/Industrial Exhibition**

16^{30}–18^{30} **Praxisorientierter Workshop – organisiert von der StAG Diagnostische Verfahren und INSTAND e. V.**
Molekulare Diagnostik & Externe Qualitätssicherung von molekularen und serologischen Diagnostikverfahren – Teil 2

Room: Konferenz 7+8
Chair: U. Reischl (Regensburg), K.-P. Hunfeld (Frankfurt a. M.)

PCR Diagnostik von Pneumocystis- und Mucorales-Infektionen
R. Bialek (Geesthacht)

Differenzierter Nachweis von Carbapenemase Genen
M. Kaase (Bochum)

Aktueller Stand der Legionellendiagnostik
C. Lück (Dresden)

Neues zum bakteriologischen Ringversuch – EUCAST und Gramfärbung
S. Ziesing (Hannover)

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17^{00}–18^{30} Oral Session 24
National Reference Laboratories (FG RK)

Room: Konferenz 1
Chair: U. Vogel (Würzburg), V. A. J. Kempf (Frankfurt a. M.)

17^{00} RKV01
Comparative genome analysis of 9 toxigenic *Corynebacterium ulcerans* strains by NGS reveals zoonotic transmission and rapid acquisition of pathogenic islands
D. Meinel, G. Margos, R. Konrad, C. König, A. Berger (Oberschleißheim), S. Krebs, H. Blum (München) A. Sing (Oberschleißheim)

17^{11} RKV02
Outcome of phenotypic and genotypic resistance guided *Helicobacter pylori* eradication therapy in German individuals
S. Dräger (Freiburg), N. Wueppenhorst (Hamburg), M. Kist, E. Glocker (Freiburg)

17^{22} RKV03
Reference diagnostics and research at the new national reference center for invasive fungal infection NRZMyk
K. Voigt, M. von Lilienfeld, K. Kaerger, G. Walther, R. Martin, **O. Kurzai** (Jena)

17^{33} RKV04
Asymptomatic bacterial carriage in the elderly – a multicentre prevalence study of the network Invasive Bacterial Infections (IBI)
T.-T. Lam, K. Hubert, M. Drayß, K. Thiel, H. Claus, U. Vogel (Würzburg)

17^{44} RKV05
Serotype distribution and burden of pneumococcal disease in adults in Germany – Reaching the limit of herd protection?
M. Imöhl, **M. van der Linden** (Aachen)

17^{55} RKV06
Diagnostic and epidemiologic aspects on *Clostridium difficile* infections in Germany – experiences from a multi-centre bi-annual point prevalence study in European countries (EUCLID)
L. von Müller, D. Zevallos, A. Nimmesgern, M. Herrmann (Homburg/Saar)

18^{06} RKV07
Bartonella henselae serology – not as easy as commonly believed as
J. F. A. Bofinger (Frankfurt a. M.), M. Lipkowski (Lübeck), T. Schmidgen, H. Podlich, W. Ballhorn V. A. J. Kempf (Frankfurt a. M.)

18^{17} RKV08
Microbiological Investigation during an Outbreak of *Legionellosis* in Warstein, Germany, August 2013
C. Lück, M. Petzold, K. Lück (Dresden), A. Brockmann (Lippstadt), S. Pleischl, M. Exner (Bonn)

17^{00}–18^{30} Oral Session 25
Microbial Pathogenicity of Pro- and Eukaryotes (FG MP & FG EK – DGHM & VAAM)

Room: Konferenz 2
Chair: I. Jacobsen (Jena), S. Wagner (Tübingen)

17^{00} EKV07
Pneumocystis jirovecii can be productively cultured in differentiated CuFi-8 airway cells
V. Schildgen, S. Mai, S. Khalfaoui, R.-L. Tillmann, M. Brockmann, O. Schildgen (Köln)

17^{15} EKV08
Ece1 – a *Candida albicans* pore-forming toxin
S. Hoefs (Jena), D. Wilson (Aberdeen/GB), S. Mogavero (Jena), D. Moyes (London/GB) T. Gutsmann (Borstel), O. Bader (Göttingen), J. Naglik (London/GB), B. Hube (Jena)

17^{30} EKV09
Survival and proliferation of human pathogenic *Candida* species within macrophages
L. Kasper, K. Seider, S. Allert, F. Gerwien, C. Zubiria-Barrera, S. Hoefs (Jena) D. Wilson (Aberdeen/GB), T. Schwarzmüller (Wien/AT), L. Ames (Exeter/GB), M. K. Mansour J. M. Vyas (Boston, MA/US), A. Haas (Bonn), K. Haynes (Exeter/GB), K. Kuchler (Wien/AT), B. Hube (Jena)

17^{45} MPV15
Influence of the virulence factor Mip on the secretome profile of *Legionella pneumophila*
J. Rasch, N. Heinsohn, A. Klages, S. Tran, J. Hoppe, C. Ünal (Braunschweig), S. Fuchs, D. Zühlke, F. Bonn K. Riedel (Greifswald), M. Steinert (Braunschweig)

18^{00} MPV16
Improved plaque assay identifies a novel anti-Chlamydia ceramide derivative with altered intracellular localization
S. Banhart, E. M. Saied, S. Koch, L. Aeberhard, C. Arenz, D. Heuer (Berlin)

18^{15} MPV17
Correlative super resolution/atomic force microscopy unravels the localization of two protein secretion systems during invasion of polarized epithelial cells by *Salmonella enterica*
B. Barlag, O. Beutel, D. Janning, N. Hansmeier, C. Richter, J. Piehler, M. Hensel (Osnabrück)

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Oral Session 26
Infection Immunology 1 (FG II)

17^{00}–18^{30}

Room: Konferenz 3
Chair: R. Lang (Erlangen), B. Opitz (Berlin)

17^{00} IIV01
Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) treatment ameliorates *Toxoplasma gondii*-induced encephalitis in mice
A. Parlog, L. Möhle, A. Biswas (Magdeburg), D. Reglodi (Pecs/HU), I. R. Dunay (Magdeburg)
M. M. Heimesaat (Berlin)

17^{15} IIV02
Analysis of gene defects in severe congenital neutropenia
S. Kirschnek, S. Gautam, M. Dold, G. Häcker (Freiburg)

17^{30} IIV03
Novel Role for Pro-Coagulant Microvesicles in the Early Host Defense against *Streptococcus pyogenes*
S. Oehmcke-Hecht (Rostock)

17^{45} IIV04
A new function of CD11b/CD18 – Complement Receptor 3 (CR3) is critical in late defense against intracellular *Chlamydia psittaci* in mouse lung infection
P. Dutow, B. Fehlhaber, M. Bothe, R. Laudeley, C. Rheinheimer, S. Glage, **A. Klos** (Hannover)

18^{00} IIV05
The quorum sensing molecule farnesol as a modulator of dendritic cell function
I. Leonhardt, S. Spielberg, M. Weber, D. Albrecht-Eckhardt, K. Hünniger, D. Barz, O. Kurzai (Jena)

18^{15} IIV06
Epithelial cells directly contribute to attraction and activation of neutrophils during infection with *Chlamydia trachomatis* L2
S. Lehr, S. Kirschnek, G. Häcker (Freiburg)

Oral Session 27
Zoonoses (FG ZO)

17^{00}–18^{30}

Room: Konferenz 4
Chair: R. Bauerfeind (Gießen), L. Wieler (Berlin)

17^{00} ZOV01
Effect of Prophage Genes on the Growth of enterohemorrhagic *Escherichia coli* O157:H7 with 5-N-Acetyl-9-O-Acetylneuraminic Acid as a Carbon Source
N. Schairer, H. Schmidt (Stuttgart)

17^{15} ZOV02
Multilocus Sequence Typing (MLST) for the identification of transmission routes of *Streptococcus gallolyticus* subsp. Gallolyticus
J. Dumke, D. Hinse (Bad Oeynhausen), J. Schulz (Hannover), C. Knabbe, J. Dreier (Bad Oeynhausen)

17^{30} ZOV03
Humoral immune response against different surface and virulence-associated *Chlamydia abortus* antigens in ovine and human abortion
J. B. Hagemann, U. Simnacher (Ulm), D. Longbottom (Penicuik/GB), K. Sachse (Jena), J. Maile
G. Göttner (Neuried), A. Essig (Ulm)

17^{45} ZOV04
Antigenic mycobacterial lipopeptides – investigations in the guinea pig model
E. Kaufmann, C. Spohr (Langen), M. Gilleron (Toulouse/FR), R. Bauerfeind (Gießen), E. Balks, K. Cussler
Ma. Bastian (Langen)

18^{00} ZOV05
Interaction of the *Streptococcus canis* M-like Protein SCM with host proteins
S. Bergmann, M. Rohde, G. S. Chhatwal (Braunschweig), M. Fulde (Hannover)

18^{15} ZOV06
Shiga toxin glycosphingolipid receptors of dog kidney epithelial MDCK II cells and their association with lipid rafts
N. Legros, A. Bauwens, G. Pohlentz, H. Karch, J. Müthing (Münster)

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Oral Session 28
New Antimicrobial Agents (FG PR & StAG HY)

Room: Seminar 1
Chair: U. Vogel (Würzburg), H. Rohde (Hamburg)

17^{00} PRV06
The antibiotic roseoflavin from *Streptomyces davawensis*: Mechanism of action and resistance
M. Mack, D. Pedrolli, F. Jankowitsch, S. Langer, J. Schwarz (Mannheim)

17^{15} PRV08
Antimicrobial peptides delocalize peripheral membrane proteins and elicit a hypoosmotic shock response in *Bacillus subtilis*
M. Wenzel (Amsterdam/NL), A. I. Chiriac (Bonn), A. Otto (Greifswald), D. Zweytick (Graz/AT)
C. May (Bochum), C. Schumacher (Düsseldorf), P. Prochnow (Bochum), R. Gust (Innsbruck/AT)
H. B. Albada (Jerusalem/IL), M. Penkova, U. Krämer, R. Erdmann, N. Metzler-Nolte (Bochum)
S. K. Straus (Vancouver/CA), E. Bremer (Marburg), D. Becher (Greifswald), H. Brötz-Oesterhelt (Düsseldorf)
H.-G. Sahl (Bonn), J. E. Bandow (Bochum)

17^{30} PRV09
Artilysins – Combination of a novel antibacterial mode of action, high effectivity against multidrug-resistant strains and persisters of *Pseudomonas aeruginosa* with a low risk of resistance formation
Y. Briers, R. Lavigne (Leuven/BE), **S. Miller** (Regensburg)

17^{45} PRV11
The undiscovered power of vitamins – fast and effective killing of multiresistant bacteria by light activation of Riboflavin derivatives
T. Maisch, A. Eichner, A. Späth, A. Gollmer, B. König, J. Regensburger, W. Bäumler (Regensburg)

18^{00} PRV12
Using PhiSigns for rapid identification of bacteriophage isolates with lytic activity against multi-drug resistant pathogens
S. Latz, N. Liedke, W. Pier, K. Ritter, **H.-P. Horz** (Aachen)

18^{15} PRV13
The inhibitory effect of a novel NA inhibitor on bacterial growth and biofilm formation of *Streptococcus pneumoniae*
M. Richter, E. Walther (Jena), S. Savina, V. A. Makarov (Moskau/RU), S. Nietzsche, A. Sauerbrei
M. Schmidtke (Jena)

Oral Session 29
Gastrointestinal Infections and Microbiota 2 (FG GI & FG PW)

Room: Seminar 3+4
Chair: B. Stecher (München), H. Rüssmann (Berlin)

17^{00} PWV04
Proteome analysis of human sebaceous follicles reveals health- and disease-associated proteins of human and microbial origin
H. Lomholt, M. Bek-Thomsen, C. Scavenius, J. Enghild, **H. Brüggemann** (Aarhus/DK)

17^{15} PWV05
Colicin M biology – Does ColM have a lethal effect on its producing bacteria?
S. Spriewald, M. Diehl, Bä. Stecher (München)

17^{30} PWV06
Quantitative Determination of Neurochemicals and their Derivatives in Dairy Products by HPLC with Amperometric and Fluorometric Detectors
A. Oleskin, O. Zhilenkova, V. Kudrin, P. Klodt, B. Shenderov (Moscow/RU)

17^{45} GIV04
A small RNA which is regulated by the acid-responsive ArsRS two-component system in *Helicobacter pylori*
P. H. S. Tan, D. Beier, C. M. Sharma (Würzburg)

18^{00} GIV05
"Drugs from Bugs" – the bacterial effector protein YopM is a 'self-delivering' anti-inflammatory agent
C. Rüter, J. Bertrand, K. Loser (Münster)

18^{15} GIV06
The role of serine protease HtrA in acute ulcerative enterocolitis and extra-intestinal immune responses during *Campylobacter jejuni* infection of gnotobiotic IL-10 deficient mice
M. M. Heimesaat, M. Alutis, U. Grundmann, A. Fischer (Berlin), N. Tegtmeyer, M. Böhm (Erlangen), A. A. Kühl
U. B. Göbel (Berlin), S. Backert (Erlangen), S. Bereswill (Berlin)

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17^{00}–18^{30} Oral Session 30
Ausbrüche in der Hämatologie und Neonatologie (FG PR & StAG HY)

Room: Seminar 5+6
Chair: I. F. Chaberny (Hannover), C. Brandt (Frankfurt a. M.)

17^{00} PRV07
Outbreak control of multi-resistant *Pseudomonas aeruginosa* in a hematology and bone marrow transplant unit
U. Vogel, H. Claus, J. Elias, G.-U. Grigoleit (Würzburg), D. Harmsen (Münster), U. Heinrich, W. Heinz
A. Menzer, S. Mielke (Würzburg), S. Niemann (Borstel), C. Reiners, A. Ullmann, H. Einsele (Würzburg)

17^{15} HYV12
Nosocomial transmission of MDR-*Pseudomonas aeruginosa* among patients isolated in a stem cell transplantation unit is linked to bathroom reservoirs
M. Wiegner, S. Göttig, M. Hogardt, T. A. Wichelhaus, V. A. J. Kempf, C. Brandt (Frankfurt a. M.)

17^{30} PRV10
Molecular typing of Toxic shock syndrome toxin-1- and Enterotoxin A-producing Methicillin-sensitive *Staphylococcus aureus* isolates from a neonatal intensive care unit
F. Layer (Wernigerode), A. Sanchini (Berlin; Stockholm/SE), B. Strommenger (Wernigerode), A.-C. Breier
H. Proquitté, C. Bührer, K. Schenkel, J. Bätzing-Feigenbaum, B. Greutelaers (Berlin), U. Nübel (Wernigerode)
P. Gastmeier, T. Eckmanns (Berlin), G. Werner (Wernigerode)

17^{45} HYV13
Occurence and genetic relationship of *Klebsiella pneumoniae* isolates from two neonatal intensive-care units ofa university medical center
J. Liese, M. Marschal, C. Gille (Tübingen)

18^{00} HYV14
Outbreak of multiresistant *Escherichia coli* in a neonatal intensive care unit (NICU)
U. Vogel, J. Wirbelauer, H. Claus, A. Menzer, C. Speer (Würzburg)

18^{15} PRV14
Estimation of the burden of common sequelae due to healthcare-associated neonatal sepsis
S. Haller (Berlin), A. Cassini (Stockholm/SE), M. Abu Sin, T. Eckmanns, T. Harder (Berlin)

17^{00}–17^{45} VAAM Promotionspreise PhD Awards

Room: Saal 1
Sponsored by BASF SE, Sanofi Aventis Deutschland GmbH, Bayer Schering Pharma,
New England Biolabs GmbH, Evonik Degussa GmbH

18^{00}–19^{30} VAAM Mitgliederversammlung

Room: Saal 1
Tagesordnungspunkte (siehe Seite 24)

18^{30}–19^{30} Treffen Fachgruppen DGHM/VAAM

Konferenz 2: FG Mikrobielle Pathogenität (DGHM & VAAM)

Konferenz 3: FG Infektionsimmunologie

Konferenz 4: FG Zoonosen (DGHM)

Konferenz 5: FG Eukaryotische Krankheitserreger (DGHM)

Konferenz 6: FG Microbiota, Probiota und Wirt (DGHM)

Konferenz 7+8: AG Nationale Referenzzentren und Konsiliarlaboratorien (DGHM)

Seminar 3+4: AG Diagnostische Verfahren in der Mikrobiologie (DGHM)

Seminar 5+6: AG Allgemeine und Krankenhaushygiene (DGHM)

19^{30} Mixer

Room: Industrial Exhibition

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Oral Session 31
Pathomechanisms and Regulatory Strategies of Bacteria (FG MP – DGHM & VAAM)

08^{30}–10^{00}

Room: Saal 1
Chair: C. Wolz (Tübingen), W. Ziebuhr (Würzburg)

08^{30} MPP17
The mutltifunctional adhesins PavB and PspC are major human thrombospondin-1-binding proteins of *Streptococcus pneumoniae*
U. Binsker, T. Kohler, S. Kohler, S. Hammerschmidt (Greifswald),

08^{45} MPV18
Functional characterization of the small non-coding RNA 26 in *Streptococcus pyogenes*
R. Pappesch, B. Hammerbacher, A. Wisniewska-Kucper (Rostock), T. Hain (Gießen), B. Kreikemeyer
N. Patenge (Rostock)

09^{00} MPV19
A differentially expressed small regulatory RNA influences metabolism and biofilm matrix production in a hypervariable *Staphylococcus epidermidis* strain
W. Ziebuhr, Cl. Lange, M. Lerch, S. Schoenfelder, D. Kozlova, J. Wax (Würzburg)

09^{15} MPV20
Proteomic profiling of longitudinal *Pseudomonas aeruginosa* isolates from cystic fibrosis patients
D. Zühlke (Greifswald), M. Lindegaard (Hørsholm/DK), C. Lassek (Greifswald), H. K. Johansen
S. Molin (Hørsholm/DK), K. Riedel (Greifswald)

09^{30} MPV21
A novel membrane-bound phospholipase B of *Pseudomonas aeruginosa*
F. Bleffert, **F. Kovacic**, J. Granzin (Jülich), L. Rahme (Boston, MA/US), R. Batra-Safferling (Jülich)
K.-E. Jaeger (Jülich)

09^{45} MPV22
Itaconate degradation promotes pathogenesis of *Salmonella Typhimurium*
J. Sasikaran (Freiburg), L. Maier, B. Periaswamy, M. Barthel, W. Hardt (Zürich/CH)
I. Berg (Freiburg)

Oral Session 32
Primary and Secondary Metabolism (SL MM & SM)

08^{30}–10^{00}

Room: Konferenz 1
Chair: K.-H. van Pée (Dresden)

08^{30} SMV01
Proteomic analysis of *Scopulariopsis brevicaulis* LF580 and its mutant strain M26 for the production of the cyclodepsipeptides scopularide A and B
A. Kramer (Kiel), H. C. Beck (Odense/DK), A. Kumar, A. Labes, J. F. Imhoff (Kiel)

08^{45} SMV02
Engineering of *Aspergillus niger* for the production of secondary metabolites
V. Meyer, F. Wanka, S. Boecker, K. Tutku, Ö. Vural, L. Richter, R. D. Süssmuth (Berlin)

09^{00} SMV03
Elucidation of genes involved in the biosynthesis of the secondary metabolite sodorifen in *S. plymuthica* 4Rx13
D. Domik, T. Weise (Rostock), A. Thürmer (Göttingen), S. von Reuß (Jena), W. Francke (Hamburg)
G. Gottschalk (Göttingen), B. Piechulla (Rostock)

09^{15} SMV04
Analysis of the biosynthesis of chlorinated pyrrole moieties from *Aster tataricus*, *Penicillium islandicum* and *Streptomyces albogriseolus*
L. Flor, K.-H. van Pée (Dresden), T. Weber (Tübingen), L. Jahn (Dresden), T. Schafhauser (Tübingen)
J. Kalinowski (Bielefeld)

09^{30} MMV01
Characterization of the *Chlamydia pneumoniae* transcriptome under hypoxic conditions
I. Kaufhold (Lübeck), T. Weinmaier (Wien/AT), L. Rodrigo, A. Conesa (Valencia/ES), T. Rattei (Wien/AT)
J. Rupp (Lübeck)

09^{45} MMV02
Characterization of mercaptosuccinate dioxygenase from *Variovorax paradoxus* strain B4, a novel cysteine dioxygenase homologue
U. Brandt, M. Schürmann, A. Steinbüchel (Münster)

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Oral Session 33
Regulation and Signaling 1 (SL RS)

08^{30}–10^{00}

Room: Konferenz 2+3
Chair: M. Burghartz (Braunschweig), J. Frunzke (Juelich)

08^{30} RSV03
RNase E and the small RNA SinZ affect the 5' UTR of the acyl-homoserine lactone synthase gene in *Sinorhizobium meliloti*
K. Baumgardt (Gießen), K. Novotná (Prague/CZ), P. Charoenpanich, M. McIntosh (Marburg)
G. Klug (Gießen), A. Becker (Marburg), E. Evguenieva-Hackenberg (Gießen)

08^{45} RSV04
Phosphatase activity of the histidine kinases ensures pathway specificity of heme-responsive two-component systems in *Corynebacterium glutamicum*
E. Hentschel, C. Gätgens, M. Brocker, J. Frunzke (Jülich)

09^{00} RSV05
Signaling across the membrane in *Escherichia coli* DcuS
C. Monzel, G. Unden (Mainz)

09^{15} RSV06
Understanding structural interactions and signal transduction within two component systems
S. Hunke, P. Hörnschemeyer (Osnabrück)

09^{30} RSV07
Dialkylresorcinols as novel bacterial cell-cell communication molecules in the human pathogen *Photorhabdus asymbiotica*
S. Brameyer, R. Heermann (München), H. B. Bode, D. Kresovic (Frankfurt a. M.)

09^{45} RSV08
New insights in DNA binding modalities and gene regulation of the transcriptional regulator ComA in *Bacillus subtilis*
D. Wolf, V. Rippa (Heidelberg), J.-C. Mobarec (Marburg), P. Sauer, L. Adlung (Heidelberg), P. Kolb (Marburg)
I. Bischofs (Heidelberg)

Oral Session 34
Host-Microbe Interactions – Pathogen Inducued Host Responses (SL HM)

08^{30}–10^{00}

Room: Konferenz 4
Chair: A. Peschel (Tübingen), R. G. Gerlach (Berlin)

08^{30} HMV10
Face-to-face RNA-Seq analysis of the *Candida*-neutrophil interaction
M. J. Niemiec (Umeå/SE), C. Grumaz, K. Sohn (Stuttgart), C. Urban (Umeå/SE)

08^{45} HMV11
Activation of Ran GTPase by a Legionella effector promotes microtubule stabilization, pathogen vacuole motility and infection
E. Rothmeier, G. Pfaffinger, C. Hoffmann, C. F. Harrison, H. Hilbi (München)

09^{00} HMV12
Targeting of host cell autophagy by *Yersinia enterocolitica*
M. J. Valencia Lopez, B. Holstermann, R. Reimer, M. Aepfelbacher, K. Ruckdeschel (Hamburg)

09^{15} HMV13
Neisseria meningitidis infection induces a cyclin independent S-phase arrest in human brain microvascular endothelial cells
W. Oosthuysen, A. Schubert-Unkmeir (Würzburg)

09^{30} HMV14
Restriction of *Salmonella* replication in the intestinal mucosa by NAIP/NLRC4 inflammasome-driven expulsion of infected enterocytes
M. Sellin, B. Felmy, T. Dolowschiak, M. Diard (Zürich/CH), A. Tardivel, K. Maslowski (Lausanne/CH)
W. Hardt (Zürich/CH)

09^{45} HMV15
Inhibition of host immune responses by an effector protease of enteropathogenic *E. coli*
S. Mühlen (Melbourne/AU), A. Bankovacki (Parkville/AU), J. S. Pearson (Melbourne/AU)
U. Nachbur (Parkville/AU), Y. Zhang (Melbourne/AU), G. N. Schroeder (London/GB), J. Silke (Parkville/AU)
E. L. Hartland (Melbourne/AU)

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Oral Session 35
Genomics and Metagenomics 1 (SL GM)

08^{30}–10^{00}

Room: Konferenz 5
Chair: J. A. Müller (Leipzig), D. Tischler (Freiberg)

08^{30} GMV01
Towards unravelling the origin of the unique natural compound sodorifen of *Serratia plymuthica* by a metagenomic approach
S. Piepenborn, D. Domik, T. Weise, B. Piechulla (Rostock)

08^{45} GMV02
Identification and characterization of genes coding for enzymes with cellulolytic activities in metagenomes from agricultural soil
M. de Vries, A. Schöler, J. Ertl (München), Z. Xu (Kopenhagen/DK), M. Schloter (München)

09^{00} GMV03
Methanotrophic bacteria of the termite gut
J. Reuß, S. Dröge, H. König (Mainz)

09^{15} GMV04
Genome analysis of acidophilic sulfate reducing bacteria
P. Petzsch (Freiberg), A. Poehlein (Göttingen), D. B. Johnson (Bangor/GB), R. Daniel (Göttingen), B. Joffroy M. Schlömann, M. Mühling (Freiberg)

09^{30} GMV05
The genome of Beggiatoa alba B18LDT, a large sulfur-oxidizing Gammaproteobacterium
P. M. Nguyen (Leipzig), L. Goodwin (California/US), K. P. Samuel (Baltimore, MD/US)
A. Teske (Chapel Hill, NC/US), J. A. Müller (Leipzig)

09^{45} GMV06
The genome of *Variovorax paradoxus* strain TBEA6 provides new insights for the 3,3′-hiodipropionic acid catabolism and hence the biotechnical production of polythioesters
J. H. Wübbeler, S. Hiessl (Münster), J. Schuldes, R. Daniel (Göttingen), A. Steinbüchel (Münster)

Oral Session 36
Biotechnology 3 – Pathways to Added-Value (SL BT)

08^{30}–10^{00}

Room: Konferenz 6
Chair: N. Wierckx (Aachen), R.-J. Fischer (Rostock)

08^{30} BTV13
Process development of glutathione enriched yeast production
E. Lorenz, M. Senz, U. Stahl (Berlin)

08^{45} BTV14
Alternative pathway for itaconic acid biosynthesis in the basidiomycetous fungus *Ustilago maydis*
S. K. Przybilla (Marburg), E. Geiser (Aachen), A. Friedrich, W. Buckel (Marburg), N. Wierckx
L. Blank (Aachen), M. Bölker (Marburg)

09^{00} BTV15
Biotechnological application of the ethylmalonyl-CoA pathway – de novo synthesis of mesaconic and methylsuccinic acid from methanol using *Methylobacterium extorquens* AM1
F. Sonntag (Frankfurt a. M.), P. Kiefer, J. Müller (Zürich/CH), **M. Buchhaupt** (Frankfurt a. M.)
J. Vorholt (Zürich/CH), J. Schrader (Frankfurt a. M.; Zürich/CH)

09^{15} BTV16
Synthesis of Poly (3HB-co-3HV) from unrelated carbon sources in engineered *Rhodospirillum rubrum*
D. Heinrich, M. Raberg, A. Steinbüchel (Münster)

09^{30} BTV17
Genetic manipulation of *Clostridium ljungdahlii* – Reduction of Biomass Intermediates to Tailor-Made Fuels
B. Molitor, A. W. Henrich, T. M. Kirchner, M. Agler-Rosenbaum (Aachen)

09^{45} BTV18
Development of a Highly Efficient Gene Delivery System for Syngas Fermenting Clostridia
G. Philipps, S. de Vries, B. Engels, C. Janke, N. Schnaß, S. Jennewein (Aachen)

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Oral Session 37
Archaea (SL AR)

08^{30}–10^{00}

Room: Konferenz 7+8
Chair: R. Rachel (Regensburg), L. Randau (Marburg)

08^{30} ARV01
In-meso crystal structure of a novel membrane-bound nitrite reductase from the *crenarchaeum Ignicoccus hospitalis*
K. Parey (Frankfurt a. M.), A. J. Fieldings (Manchester/GB), J. Flechsler, R. Rachel, H. Huber
C. Rajendran (Regensburg), C. Ziegler (Frankfurt a. M., Regensburg)

08^{45} ARV02
Unique metabolic and morphological features of the ammonia-oxidizing archaeon *Nitrososphaera viennensis sp. nov.*
M. Stieglmeier, M. Mooshammer (Wien/AT), A. Klingl (Marburg), R. Alves, N. Leisch, W. Wanek, A. Richter
C. Schleper (Wien/AT)

09^{00} ARV03
Ammonia-oxidizing archaea use an energy efficient variant of the hydroxypropionate/hydroxybutyrate cycle for CO_2 fixation
M. Könneke (Bremen), D. Schubert, P. Brown (Freiburg), M. Hügler (Karlsruhe), T. Schwander
L. Schada von Borzyskowski, T. Erb (Zürich/CH), I. Berg (Freiburg)

09^{15} ARV04
Adaptation of *Natrialba* species towards ecological habitats of distinct features
R. L. Hahnke, H.-P. Klenk (Braunschweig)

09^{30} ARV05
Protein phosphorylation in *Sulfolobus acidocaldarius* – What is the role of the atypical protein kinases?
D. Esser (Essen), S.-V. Albers (Marburg), B. Siebers (Essen)

09^{45} ARV06
The intestinal archaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* activate human immune responses
C. Bang, K. Weidenbach (Kiel), T. Gutsmann, H. Heine (Borstel), R. A. Schmitz (Kiel)

Oral Session 38
Microbial Diversity and Ecology 2 (SL DE)

08^{30}–10^{00}

Room: Seminar 3+4
Chair: J. Gescher (Karlsruhe), S. Schopf (Freiberg)

08^{30} DEV13
Transcriptomics reveals the regulatory response to light mediated stationary phase transition in the ubiquitous freshwater bacterium *Polynucleobacter necessarius*
S. P. Glaeser (Gießen), K. U. Förstner (Würzburg), H.-P. Grossart (Neuglobsow), **J. Glaeser** (Gießen)

08^{45} DEV14
Influence of photosynthesis an its adaption strategy in the aerobic anoxygenic phototrophic bacteria *Dinoroseobacter shibae*
S. Heyber, J. Jacobs, I. Wagner-Döbler, R. Münch, D. Jahn (Braunschweig)

09^{00}
Development of a labeling system for microorganisms based on antimicrobial peptides

DEV15
R. Barthen, K. Mickein, S. Kutschke, K. Pollmann (Dresden), J. Kulenkampff, M. Gründig
J. Lippmann-Pipke (Leipzig)

09^{15}
From monitoring to steering microbiomes using single cell analysis

DEV16
C. Koch, S. Müller, F. Harnisch (Leipzig)

09^{30} DEV17
Hunting for active degraders: Novel screening approach combining substrate-specific radiolabelling of cells and their separation in microcompartments
H. Beck, R. Sigrist, S. Mosler, J. A. Müller, M. Kästner (Leipzig)

09^{45} DEV18
Plasmid curing and the loss of grip – RepA-I type replicons of the *Roseobacter* group are essential for biofilm formation, motility and the colonization of marine algae
J. Petersen (Braunschweig)

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08^{30}–10^{00} **Oral Session 39**
ABS – Antibiotic Stewardship and Development of Resistance (FG PR & StAG HY)

Room: Seminar 5+6
Chair: T. Eckmanns (Berlin), Ka. Becker (Münster)

08^{30} PRV15
Evaluation of the adjustment of antimicrobial therapy to microbiological results at an intensive care unit in a regional hospital
C. Schulke, W. Beil (Hannover), F. Schmitz (Hildesheim), S. Ziesing, R.-P. Vonberg (Hannover)

08^{45} PRV16
Variability of linezolid concentrations after standard dosing in critically ill patients – a prospective observational study
M. Zoller, B. Maier, C. Hornuss, C. Neugebauer, G. Döbbeler, D. Nagel, L.-M. Holdt, M. Bruegel, T. Weig B. Grabein, L. Frey, D. Teupser, M. Vogeser, **J. Zander** (München)

09^{00} PRV17
S. aureus develops increased resistance against antibiotics by forming dynamic small colony variants during chronic osteomyelitis
C. Kreis, L. Tuchscherr, V. Hörr, L. Flint, M. Hachmeister (Münster), M. Kiehntopf (Jena)
E. Medina (Braunschweig), T. Fuchs, M. Raschke, G. Peters, **B. Löffler** (Münster)

09^{15} PRV18
Emergence of daptomycin non-susceptible, vancomycin resistant *Enterococcus faecium* isolates in colonizing populations during empiric daptomycin therapy in patients after bone marrow transplantation
G. Franke, H. Lellek, C. Ruckert, L. Berneking, M. Christner, M. Alawi, N. Kröger, H. Rohde (Hamburg)

09^{30} HYV15
Carbapenemase producing and not producing enterobacteriaceae (4-MR-GNE) – Differences in clinical outcomes?
C. Wessels, D. Peter (Köln), M. Kaase (Bochum), **S. Messler**, F. Mattner (Köln)

09^{45} PRV19
Impact of sub-inhibitory antibiotic concentrations on *Pseudomonas aeruginosa* physiology
A. Khaledi, E. Surges, W.-R. Abraham (Braunschweig), S. Häußler (Braunschweig, Hannover)

10^{00}–10^{30} Coffee Break/Industrial Exhibition

10^{30}–12^{00} **Plenary Session 7**
Hypoxia and Anaerobiosis

Room: Grosser Saal
Chair: V. A. J. Kempf (Frankfurt a. M.), M. Rother (Dresden)

10^{30} INV22
Hypoxia and response to infection
R. S. Johnson (Cambridge/GB)

10^{52} INV23
Hypoxia induction during *shigella* infection
B. Marteyn (Paris/FR)

11^{14} INV24
Insights into an ancient anaerobe – *Methanococcus maripaludis*
W. Whitman (Athens, GA/US)

11^{36} INV25
The gut microbiota of termites – evolutionary origin and functional adaptions
A. Brune (Marburg)

12^{00}–12^{15} **Poster Prize Awards**
(Awardees will be informed in time with a short notice on their Poster)

12^{30}–13^{30} **Lunch Break/Industrial Exhibition**

12^{30}–13^{30} **Late Breaker Session**

Abstracts containing new data and information that could not have been submitted in due time, can now be submitted and will be considered as „Late Breaker Abstracts".

Late Breaker Abstracts are very competitive; only abstracts describing unknown, new and/or acute onsets (e.g. hospital hygiene) can be considered. Retrospective studies will not be considered. Due to the nature of Late Breaker Abstracts only a few submissions will be eligible for presentation.

This session will only take place if there were submitted acute cases which have to be dealed with.

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Oral Session 40
Infection Immunology 2 (FG II)

13^{30}–15^{00}

Room — Saal 1
Chair — G. von Zandbergen (Langen), C. Hölscher (Zürich/CH)

13^{30} IIV07 — Development and Evaluation of an mRNA-based Vaccine Against *Staphylococcus aureus*
J. Hantzschmann, C. Stein, I. Bekeredjian-Ding (Bonn)

13^{45} IIV08 — Comprehensive transcriptome and quantitative proteome analyses of a macrophage-intrinsic type I and II IFN-dependent defense pathway that restricts an intracellular bacterium in the lung
J. Naujoks, C. Tabeling (Berlin), B. Dill (Dundee/GB), C. Hoffmann (München), A. Brown (Melbourne/AU) M. Kunze, A. Peter, H.-J. Mollenkopf, A. Dorhoi, O. Kershaw, A. Gruber, L. E. Sander (Berlin) S. Herold (Gießen), M. Witzenrath (Berlin), E. L. Hartland (Melbourne/AU), N. Suttorp (Berlin) S. Bedoui (Melbourne/AU), H. Hilbi (München), M. Trost (Dundee/GB), B. Opitz (Berlin)

14^{00} IIV09 — Live cell imaging of autophagy in *Leishmania* infected human primary macrophages
G. van Zandbergen, M. Thomas (Langen)

14^{15} IIV10 — Induction of collagen IV autoimmunity by streptococcal M protein is PARF-specific
G. Gulotta, R. Bergmann (Braunschweig), V. Sagar (Chandigarh/IN), **D. P. Nitsche-Schmitz** (Braunschweig)

14^{30} IIV11 — CD1b restricted T cell antigens of *Mycobacterium tuberculosis* – a particular focus on mannosylated Lipoarabinomannan
C. Spohr, S. Battenfeld, E. Kaufmann (Langen), M. Gilleron (Toulouse/FR), S. Stenger (Ulm)
Ma. Bastian (Langen)

14^{45} IIV12 — Effects of bacterial infection on primary human macrophage subpopulations
A. Sedlag, C. Riedel (Ulm)

Oral Session 41
Metabolism and Metabolomics (SL MM & SM)

13^{30}–15^{00}

Room — Konferenz 1
Chair — J. Frerichs (Frankfurt a. M.), C. Jogler (Braunschweig)

13^{30} MMV03 — The lipidome of *Myxococcus xanthus*
T. Ahrendt, W. Lorenzen, H. B. Bode (Frankfurt a. M.)

13^{45} MMV04 — The first 2D GC/MS non-targeted metabolomic analysis of bacteria and archaea by use of the new version of the automated software MetaboliteDetector
C. Schauer, C. Nieke, C. Nienhagen, J. Wolf, D. Schomburg (Braunschweig)

14^{00} MMV05 — New insights into methylotrophy of the thermophilic methylotroph *Bacillus methanolicus*
B. Litsanov, J. Müller, A. Ochsner, Fa. Meyer, P. Schürch, M. Bortfeld-Miller, P. Kiefer, J. Vorholt (Zürich/CH)

14^{15} MMV06 — The essential putative cysteine desulfurase MPN487 from *M. pneumoniae* is a H2S producing enzyme and involved in virulence
S. Großhennig, J. Busse, J. Stülke (Göttingen)

14^{30} MMV07 — Sulfoglycolysis in *Escherichia coli* K-12 closes a gap in the biogeochemical sulfur cycle
D. Schleheck, M. Weiss, A.-K. Felux, K. Denger, A. Cook (Konstanz)

14^{45} MMV08 — Disinfectants as environmental pollutants: Novel mechanisms of microbial biotransformation and detoxification
R. Schlüter, A. Mikolasch, F. Schauer (Greifswald)

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Oral Session 42
Regulation and Signaling 2 (SL RS)

13^{30}–15^{00}

Room: Konferenz 2+3
Chair: C. Welte (Nijmegen/NL), R. Heermann (München)

13^{30} RSV09
Multitasking by PII Proteins – energy state sensing differentially affects NtcA activation and arginine synthesis in *Cyanobacteria*
K. Forchhammer (Tübingen)

13^{45} RSV10
Regulation of the *Bacillus subtilis* ECF-sigma factor σX
J. Heinrich, K. Schäfer, M. Mildner, T. Wiegert (Zittau)

14^{00} RSV11
Interdependence between different layers of the cell envelope stress response in *Bacillus subtilis*
J. Radeck, P. Orchard, M. Kirchner, C. Höfler, S. Gebhard, T. Mascher (Planegg-Martinsried)
G. Fritz (Planegg-Martinsried, München)

14^{15} RSV12
Pathogens talking to each other in dental biofilms – Crossfeeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*
I. Wagner-Döbler, H. Sztajer, S. Szafranski, J. Tomasch, M. Reck, M. Nimtz, M. Rohde (Braunschweig)

14^{30} RSV13
Elucidating the function of the mazEF Toxin-Antitoxin System from *Staphylococcus aureus*
C. F. Schuster (Tübingen), K. U. Förstner, C. M. Sharma (Würzburg), R. Bertram (Tübingen)

14^{45} RSV14
Synechocystis sp. PCC 6803, a representative expressing multiple clock protein homologs
A. Wiegard (Düsseldorf), A. K. Dörrich (Gießen), C. Beck, S. Hertel (Berlin), A. Wilde (Freiburg)
I. M. Axmann (Düsseldorf, Berlin)

Oral Session 43
Host-Microbes Interactions – Virulence Factors and Infection Models (SL HM)

13^{30}–15^{00}

Room: Konferenz 4
Chair: D. Hofreuter (Hannover), K. Ohlsen (Würzburg)

13^{30} HMV16
Tissue specific colonization pattern as consequence of metabolic diversity between *C.jejuni* and *C.coli*
J. Mohr, H. Vorwerk, P. Grüning (Hannover), K. Methling (Greifswald), A. von Altrock, O. Wensel (Hannover)
S. Bhuju, K. Schmidt-Hohagen, D. Schomburg (Braunschweig), M. Lalk (Greifswald), P. Valentin-Weigand
D. Hofreuter (Hannover)

13^{45} HMV17
The immune system of the carpenter ant *Camponotus floridanus* is involved in the surveillance of its bacterial endosymbiont
M. Kupper, J. Vanselow, S. K. Gupta, T. Dandekar, A. Schlosser, R. Gross (Würzburg)

14^{00} HMV18
Interplay of *Streptococcus suis* und swine influenza virus during co-infection in a porcine ex vivo precision-cut lung slice model
M. Seitz (Hannover), F. Meng, N.-H. Wu, G. Herrler, P. Valentin-Weigand (Hannover)

14^{15} HMV19
Functional high-throughput screening identifies the miR-15 microRNA family as cellular restriction factors for *Salmonella* infection
C. Maudet, U. Sunkavalli, M. Sharan, K. U. Förstner (Würzburg), M. Mano (Trieste/IT), **A. Eulalio** (Würzburg)

14^{30} HMV20
Novel immunostimulatory flagellin-like protein FlaC in unsheathed *Helicobacter* and *Campylobacter* species
E. Leno, E. Gripp (Hannover), S. Maurischat (Berlin), B. Kaspers (München), K. Tedin (Berlin), S. Klose
C. Josenhans (Hannover)

14^{45} HMV21
Identification by TnSeq of a novel *Staphylococcus aureus* virulence regulator orchestrating leukocyte cytotoxicity
S. Das, B. Österreich, K. U. Förstner, A.-C. Winkler (Würzburg), R. Reinhardt (Köln), K. Ohlsen, J. Vogel
T. Rudel, **M. Fraunholz** (Würzburg)

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Oral Session 44
Genomics and Metagenomics 2 (SL GM)

13^{30}–15^{00}	
Room	Konferenz 5
Chair	S. Niemann (Borstel), U. Nübel (Braunschweig)
13^{30} GMV07	Characterization of the *Campylobacter* coli Methylome **W. Masanta**, A. M. Goldschmidt, M. Dilcher, N. L.-A. Mund, R. Lugert, U. Groß, A. E. Zautner (Göttingen)
13^{45} GMV08	Whole-genome sequencing of *Coxiella burnetii* without prior culture or amplification **M. Walter** (Neuherberg, Freising), M. Runge (Braunschweig, Hannover), M. Ganter (Hannover) D. Frangoulidis (München)
14^{00} GMV09	Diversity of resistance plasmids in bacteria from the Warsaw wastewater treatment plant **M. Adamczuk**, L. Dziewit, D. Bartosik (Warsaw/PL)
14^{15} GMV10	Genome analyses of human pathogenic *Lichtheimia* species **V. U. Schwartze**, S. Winter, E. Shelest, F. Horn, V. Valiante, J. Linde (Jena), M. Sammeth (Barcelona/ES) K. Voigt (Jena)
14^{30} GMV11	Comparative genome sequencing reveals within-host evolution of *Neisseria meningitidis* during invasive disease J. Klughammer (Wien/AT), M. Dittrich (Würzburg), J. Blom, A. Goesmann (Gießen), U. Vogel M. Frosch (Würzburg), C. Bock (Wien/AT), T. Müller (Würzburg), **C. Schoen** (Würzburg)
14^{45} GMV12	Recent changes in the MG-RAST metagenome analysis portal Fo. Meyer (Argonne, IL/US)

Oral Session 45
Biotechnology 4 – Enzyme Catalysis (SL BT)

13^{30}–15^{00}	
Room	Konferenz 6
Chair	S. Kara (Dresden), J. Rudat (Karlsruhe)
13^{30} BTV19	Development of a two-enzyme system comprising of an aryl-alcohol oxidase and a dye-decolorizing peroxidase **I. Galperin**, C. Lauber, A. Javeed, H. Zorn, M. Rühl (Gießen)
13^{45} BTV20	Synthesis of chiral β-amino acids via Lipase/Transaminase reaction cascade **S. Dold**, C. Syldatk, J. Rudat (Karlsruhe)
14^{00} BTV21	Investigation of structural determinants for substrate specificity in zinc dependent alcohol dehydrogenases **C. Loderer**, M. Ansorge-Schumacher (Dresden)
14^{15} BTV22	Full surfactant resistance landscape of *Bacillus subtilis* lipase A **A. Fulton** (Jülich), J. Frauenkron-Machedjou (Aachen), P. Skoczinski (Jülich), U. Schwaneberg (Aachen) K.-E. Jaeger (Jülich)
14^{30} BTV23	Synthetic biology as means for enzyme optimization: improving *Thermoanaerobacter tengcongensis esterase* by incorporation of non-canonical amino acids **A. Krüger** (Hamburg), M. G. Hoesl (Berlin), A. Peters (Hamburg), N. Budisa (Berlin), G. Antranikian (Hamburg)
14^{45} BTV24	Dried fungal Enzymes, a Lipase, a Threonine Aldolase, and a Laccase were entrapped in Epoxy Resin to coat a solid Support **S. Barig** (Senftenberg), S. Merting, K. Schnitzlein (Cottbus), K.-P. Stahmann (Senftenberg)

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Oral Session 46
Microbial Cell Biology 2 (SL CB, SL BE & SL TS)

13^{30}–15^{00}	
Room	Konferenz 7+8
Chair	A. Treuner-Lange, F. Dempwolff (Marburg)
13^{30} CBV09	PPK1 and PPK2-independent formation of polyphosphate in *Ralstonia eutropha* **T. Tumlirsch**, A. Kaufmann, D. Jendrossek (Stuttgart)
13^{45} CBV10	Bacterial membrane domains in super resolution **F. Dempwolff**, A. Stroh (Marburg), N. Takeshita, R. Fischer (Karlsruhe), C. Stürmer (Konstanz) P. L. Graumann (Marburg)
14^{00} CBV11	The structure of the halobacterial gas vesicle by cryo-electron tomography **D. Bollschweiler** (Martinsried), F. Pfeifer, K. Faist (Darmstadt), F. Beck, R. Danev, H. Engelhardt (Martinsried)
14^{15} CBV12	Analyses of chromosome and replisome dynamics in *Myxococcus xanthus* reveal a novel chromosome arrangement **A. Treuner-Lange**, A. Harms, D. Schumacher, L. Søgaard-Andersen (Marburg)
14^{30} CBV13	The role of the rhomboid protease in virulence factor secretion by *Listeria monocytogenes* **E. Sysolyatina**, Y. Chalenko, S. Ermolaeva (Moskau/RU), A. Surin (Puschino/RU)
14^{45} CBV14	Swarming Bacteria as Freight Haulage Systems – how *Paenibacillus vortex* physically transports antibiotic resistant cargo bacteria to the mutual advantage of both species C. Ingham (Utrecht/NL)

Oral Session 47
Microbial Diversity and Ecology 3 (SL DE)

13^{30}–15^{00}	
Room	Seminar 3+4
Chair	L. Adrian (Leipzig), M. Mühling (Freiberg)
13^{30} DEV19	Water-uptake by desiccated terrestrial Nostoc commune colonies from a saturated NaCl solution: consequences for the recultivation of associated heterotrophic bacteria **N. Feyh** (Berlin), J. Jänchen (Wildau), U. Szewzyk (Berlin)
13^{45} DEV20	Solutions to the public goods dilemma in bacterial biofilms **K. Drescher**, C. Nadell, H. Stone, N. Wingreen, B. Bassler (Princeton, NJ/US)
14^{00} DEV21	Assessing the ecological niches of not-yet-cultured *Acidobacteria* in situ **J. Sikorski**, V. Baumgartner, J. Overmann (Braunschweig)
14^{15} DEP09	In-situ Protein-SIP in a constructed wetland model system reveals Ralstonia as the key genus for aerobic toluene degradation V. Lünsmann, U. Kappelmeyer, R. Benndorf, H. J. Heipieper, J. A. Müller, M. von Bergen (Leipzig), M. Kästner, **N. Jehmlich** (Leipzig)
14^{30} DEV23	How rare biosphere members contribute to biogeochemical cycling – the ecology of low abundance sulfate reducers in the hidden sulfur cycle of a model peatland B. Hausmann (Wien/AT), K.-H. Knorr (Münster), S. Malfatti, S. Tringe T. Glavina del Rio (Walnut Creek, CA/US), M. Albertsen, P. H. Nielsen (Aalborg/DK), U. Stingl (Thuwal/SA) A. Loy (Wien/AT), **M. Pester** (Konstanz; Wien/AT)
14^{45} DEV24	Temporal and depth-related variability of microbial communities in soils along an ecosystem development gradient **S. Turner**, M. Blöthe, R. Mikutta, S. Meyer-Stüve, G. Guggenberger, R. Dohrmann, A. Schippers (Hannover)

VAAM-Jahrestagung 2014 / 66. Jahrestagung der DGHM

13^{30}–15^{00} **Working Group Session 8 (DGHM)**
Antibiotic Stewardship und Infektionskontrolle gehören zusammen (FG PR & StAG HY)

Room	Seminar 5+6
Chair	M. Just (Nürnberg), S. Scheithauer (Göttingen)
13^{30}	Antibiotic Stewardship – die Rolle des Mikrobiologen
HYV16	M. Just (Nürnberg)
13^{52}	Antibiotic Stewardship – die Rolle des Apothekers
HYV17	M. Fellhauer (Freiburg)
14^{14}	Antibiotic Stewardship an einem Universitätsklinikum – Universität Regensburg
HYV18	B. Salzberger (Regensburg)
14^{36}	Antibiotic Stewardship und Krankenhaushygiene aus einer Hand
HYV19	N.N.

Oral Presentations

Poster Presentations

Invited Speakers

Industrielle Mikrobiologie (VAAM)

ANAEROBIC METABOLISM

AMV01
A Regulatory Link Between Nitrate and Oxygen Respiration in *Streptomyces coelicolor*

D. Falke*[1], M. Fischer[1], R. G. Sawers[1]
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The ability to use oxygen as terminal electron acceptor is the main characteristic of aerobic as well as facultative aerobic bacteria. As an obligate aerobic soil-dwelling actinobacterium *Streptomyces coelicolor* requires oxygen for growth. Its developmental program is characterized by undergoing a complex life cycle, including three stages: vegetative hyphae, hydrophobic aerial hyphae and spore formation. Recent studies have revealed that, despite being an obligate aerobe, *Streptomyces* is also able to use nitrate as electron acceptor during periods of hypoxia. Reduction of nitrate is catalyzed by respiratory nitrate reductases (Nars), which are multi-subunit, membrane-associated enzymes that couple electron transfer to energy conservation. The genome of *S. coelicolor* has three copies of the *narGHJI* operon. The three Nar enzymes are not redundant but rather appear to have distinct functions in the developmental program of the bacterium [1, 2]. The genome of *S. coelicolor* also includes genes coding for various types of terminal oxidases. By modulating the levels of terminal oxidases and by respiring with nitrate *S. coelicolor* is thus able to conserve energy under a variety of environmental conditions. In this study a comparative analysis of respiratory chain mutants revealed a link between nitrate and oxygen respiration during the complex life cycle of the bacterium. Analysis of a mutant lacking the *bc1* complex as well as the cytochrome *aa3* oxidase showed no measurable activity of the spore-specific Nar1 enzyme, despite the enzyme being detectable as revealed by western blotting. Biochemical and physiological methods demonstrated that resting spores and mycelium harbour active oxidases. Using an exogenous electron donor, enzyme activities of both the cytochrome bc_1 complex and cytochrome aa_3 oxidoreductase components of the respiratory chain were detectable in solubilized membrane fractions of spores and mycelium. Furthermore, cytochrome aa_3 oxidoreductase activity could be visualized by direct staining after native PAGE, which was confirmed by use of defined knockout mutants. Transcription analyses demonstrated that mRNA transcripts of a newly identified and additional ubiquinol:cytochrome *c* oxidase complex were mainly present in resting spores, indicating that *S. coelicolor* harbours spore-specific respiratory chain components for energy conservation. Taken together, these data indicate that *S. coelicolor* retains the ability to respire using either oxygen or nitrate as electron acceptor during its developmental program and suggest a link between the two respiratory routes to govern Nar1 activity in spores.

References

[1] Fischer *et al.* (2010) Microbiology. 156:3166-79
[2] Fischer *et al.* (2013) Mol Microbiol. 89(6):1259-73

AMV02
Comparing benzene degradation under sulphate- and nitrate-reducing conditions

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Anaerobic mineralisation of benzene under sulphate-reducing conditions has been observed *on-site* in groundwater-percolated sand-filled columns at an industrial site 50 km south-west of Leipzig contaminated mainly with benzene. Previous investigations regarding consortia structure and metabolic pathways revealed hints for a syntrophic mechanism of benzene degradation. It is divided in a benzene-metabolising step done by a fermenting key microbe assigned to the genus *Pelotomaculum* and a sulphate-reducing step to consume the generated electrons and acetate by ε- and *δ*-proteobacteria [Herrmann et al., 2010]. At field site, the consortia were shifted successfully to nitrate as terminal electron acceptor by artificially adding it, further supporting the syntrophy model. Sand from the columns was incubated in laboratory microcosms to determine the microbial composition, benzene degradation kinetics and stable carbon and hydrogen isotope fractionation of benzene during degradation. Subsequently, the obtained data were compared with results from benzene-degrading sulphate-reducing cultures of previous studies also cultivated in microcosms. For analysing the phylogenetic composition, T-RFLP fingerprinting was applied using three restriction enzymes (*Bst*UI, *Hha*I, *Hpy*CH4III). Results revealed the absence of the *Pelotomaculum* phylotype during prolonged incubation under nitrate-reducing conditions. Nevertheless, degradation rates (up to 16 $\mu M\ d^{-1}$) were potentially higher compared with sulphate-cultures described in literature [Vogt et al., 2007]. Considering these results together with observed changes in community composition, determining enrichment factors ε for carbon and hydrogen isotope fractionation could give an insight into the fundamental metabolism with new key microbes. Summarised, new consortia based on a nitrate reduction step were successfully established still with the capability to degrade benzene. In perspective, they could be used in bioremediation strategies at the field site.

References

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[2] Vogt, C., Gödeke, S., Treutler, H.-C., Weiß, H., Schirmer, M., Richnow, H.-H., 2007, Benzene oxidation under sulfate-reducing conditions incolumns simulating in situ conditions, Biodegradation, 18: 625 - 636

AMV03
Identification of naphthalene carboxylase subunits of the sulfate-reducing culture N47

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Non-substituted polycyclic aromatic hydrocarbons are very difficult to degrade in the absence of molecular oxygen and the underlying anaerobic biochemical reactions are only poorly understood. Metabolite analyses have indicated that for the model compound naphthalene, carboxylation was the first reaction activating such a chemically stable molecule. Moreover, Mouttaki et al. recently brought biochemical evidence confirming the carboxylation reaction in the sulfate-reducing culture N47. Naphthalene carboxylase converts naphthalene and ^{13}C-labelled bicarbonate to 2-[carboxyl-^{13}C]naphthoic acid at a rate of 0.12 nmol min^{-1} mg^{-1} of protein in crude cell extracts of N47. Additionally, it exhibits an isotopic exchange capability indicating the formation of free reversible intermediates. Previous proteogenomic studies of N47 and the marine naphthalene-degrading strain NaphS2 allowed identification of a gene cluster which products were suggested to be carboxylase-like subunits potentially involved in the initial reaction of naphthalene degradation. So far, the native purification of this enzyme using N47 raw extract was unsuccessful. Therefore, we developed alternative strategies to identify the native naphthalene carboxylase subunits. A differential protein induction analysis on blue native PAGEs led to the identification of potential subunits of the naphthalene carboxylase of N47 in native conformation. Moreover, the identified subunits are encoded in an operon structure within the previously mentioned naphthalene carboxylase gene cluster. Here, we were able to show the interaction of the gene products of this cluster for the first time. Additionally, these *findings* were supported by a pull-down approach revealing *in vitro* interaction partners of a heterologously produced GST-tagged naphthalene carboxylase subunit. Based on these lines of evidence, we propose the naphthalene carboxylase to be a complex of at least 750 kDa.

AMV04
Iron-sulfur/flavoproteins involved in the anaerobic degradation of naphthalene

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The anaerobic degradation of environmentally relevant polycyclic aromatic hydrocarbons has been studied in the sulfate-reducing, naphthalene-degrading cultures N47[1] and NaphS2[2]. The key intermediate 2-naphthoyl-CoA (NCoA) was found to be dearomatized by 2-naphthoyl-CoA reductase (NCR) to 5,6,7,8-tetrahydro-2-NCoA (THNCoA), as evidenced by *in vitro* assays and enriched enzyme preparations[3,4]. NCR is a flavoprotein of the old yellow enzyme family (OYE). Surprisingly, heterologously expressed genes encoding NCR (*ncr)* from both N47 and NaphS2 catalyzed only the two-electron reduction of NCoA to 5,6-dihydro-2-naphthoyl-CoA (DHNCoA). Both NCRs contained FAD, FMN and a FeS cluster; they were not oxygen-

sensitive and not ATP-dependent. A gene encoding a second putative OYE family member adjacent to that coding for NCR was heterologously expressed and characterized as a specific 5,6-dihydro-2-naphthoyl-CoA reductase (DHNCR) catalyzing the two-electron reduction of the substrate to THNCoA. In the same transcriptional unit a gene putatively coding for the Fe/S-flavoprotein components of NADH:quinone oxidoreductases (NuoEFG) was heterologously expressed and characterized as a NADPH:acceptor oxidoreductase. This third FeS/flavoprotein is suggested to serve as natural electron donor for both, NCR and DHNCR probably by coupling the endergonic reduction of NCoA to the exergonic one of DHNCoA in an electron bifurcation process.

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[4] Eberlein et al (2013), Mol Microbiol 88:1032-9

AMV05
Simultaneous involvement of a phenylacetaldehyde dehydrogenase and an aldehyde:ferredoxin oxidoreductase in anaerobic phenylalanine metabolism

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The denitrifying Beta-proteobacterium *Aromatoleum aromaticum* is a degrader of a variety of refractory aromatic compounds and hydrocarbons in anoxic environments (1). Our interest focuses on the anaerobic degradation of phenylalanine (Phe), 2-phenylethylamine and related compounds. Their degradation proceeds via the intermediate phenylacetate to benzoyl-CoA which is the common intermediate in anaerobic metabolism of aromatic compounds. Most enzymes involved have been identified apart from the enzyme(s) catalysing the oxidation of the intermediate phenylacetaldehyde to phenylacetate. Two distinct enzymes were found to catalyse this step when *A. aromaticum* was growing on Phe. We identified and purified a NAD/NADP-dependent phenylacetaldehyde dehydrogenase (PDH) which is highly specific for phenylacetaldehyde and shows substrate-inhibition. Additionally, an aldehyde:ferredoxin oxidoreductase (AOR) was found to catalyse this step with variable rates, albeit this enzyme also oxidises several other aromatic and aliphatic aldehydes with similar rates (2). Therefore we conclude that the PDH is the main enzyme of the metabolic pathway whereas the AOR has mostly detoxifying functions and is active when aldehydes accumulate, dependent on the growth conditions. Further evidence for this hypothesis will be presented. The AOR was enriched and shown to depend on tungsten (W) incorporated into the pterin cofactor whereas the nitrate reductase (NAR) depended on molybdenum (Mo). In conclusion, *A. aromaticum* is one of few organisms which are able to discriminate these chemically similar metals for the specific incorporation into the respective enzymes (2). The genome of *A. aromaticum* (1) contains multiple copies of genes for specific uptake of MoO_4^{2-} or WO_4^{2-} to the cell as well as genes for their incorporation into the cofactor as potential candidates for regulation. We are in the process of elucidating these mechanisms based on the creation and phenotypic characterisation of mutants.

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AMV06
Anaerobic activation of *p*-cymene in denitrifying betaproteobacteria: methyl group hydroxylation vs. addition to fumarate

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The betaproteobacteria "*Aromatoleum aromaticum*" pCyN1 and "*Thauera*" sp. pCyN2 anaerobically degrade the aromatic plant-derived hydrocarbon *p*-cymene (4-isopropyltoluene) under nitrate-reducing conditions. Metabolite analysis of *p*-cymene-adapted "*A. aromaticum*" pCyN1 cells demonstrated the specific formation of 4-isopropylbenzyl alcohol and 4-isopropylbenzaldehyde, whereas with "*Thauera*" sp. pCyN2 exclusively 4-isopropylbenzylsuccinate and tentatively identified (4-isopropylphenyl) itaconate were observed. 4-Isopropylbenzoate on the contrary was detected with both strains. Proteogenomic investigation of *p*-cymene- *versus* succinate-adapted cells of the two strains revealed distinct protein profiles, reflecting the formation of the different metabolites from *p*-cymene. "*A. aromaticum*" pCyN1 specifically formed (i) a putative *p*-cymene dehydrogenase (termed CmdABC) predicted to hydroxylate the methyl group of *p*-cymene, (ii) two dehydrogenases putatively oxidizing 4-isopropylbenzyl alcohol (Idh) and 4-isopropylbenzaldehyde (Iad), and (iii) a putative 4-isopropylbenzoate-CoA ligase (IclA). The *p*-cymene-specific protein profile of "*Thauera*" sp. pCyN2, on the other hand, encompassed proteins homologous to subunits of toluene-activating benzylsuccinate synthase (termed isopropylbenzylsuccinate synthase IbsABCDEF, identified subunits IbsAE) and homologs of the benzylsuccinate β-oxidation (Bbs) pathway (termed BisABCDEFGH, all identified except for BisEF). This study demonstrates that two related denitrifying bacteria employ fundamentally different peripheral degradation routes for one and the same substrate, *p*-cymene, with the two pathways apparently converging at the level of 4-isopropylbenzoyl-CoA. Moreover, the presumptive *p*-cymene dehydrogenase CmdABC could represent the first known oxygen-independent enzyme that hydroxylates a benzylic methyl group of an aromatic hydrocarbon.

AMV07
The crystal structure of the cobamide-containing tetrachloroethene reductive dehalogenase of *Sulfurospirillum multivorans*

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The epsilonproteobacterium *Sulfurospirillum multivorans* is able to grow anaerobically via organohalide respiration using tetrachloroethene (PCE) as terminal electron acceptor. The reductive dechlorination of PCE or trichloroethene (TCE) is mediated by the PCE reductive dehalogenase (PceA), a cobamide-containing iron-sulfur protein. PceA represents the terminal oxidoreductase of a so far uncharacterized respiratory chain. The cobamide cofactor of PceA, which is essential for the reduction of PCE or TCE, is the unusual norpseudo-B_{12}. This cofactor has an adenine as lower ligand base and an ethanolamine as linker moiety in its nucleotide loop [1]. In general, reductive dehalogenases display no sequence homologies to other cobamide-containing proteins, the structures of which are known.
In this study, a mutant strain of *S. multivorans* producing an affinity-tagged PceA was generated. A purification protocol was established allowing for the isolation of highly active enzyme, which was subjected to crystallization and structural analysis. This is the first report on a reductive dehalogenase structure so far. In crystals of PceA (resolution of d_{min}= 1.65Å) the norpseudo-B_{12} is bound in its *base-off* conformation via hydrogen bonds to the polypeptide chain. The halogenated substrate enters the active site via a defined substrate channel. From the topology of the two [4Fe-4S] clusters a

sequential electron transfer to the norpseudo-B_{12} can be deduced. The PceA crystals were found to bind TCE in the active site of the enzyme. Next to chlorinated alkenes, also chlorinated phenols were shown to bind at this position. In order to test for the conversion of aromatic organohalides by PceA, different chlorophenols were applied as substrate in the enzyme assay. Phenols with two or three chlorine substituents were also reductively dechlorinated, although at significantly lower rates than PCE or TCE. This result extends the substrate spectrum of PceA to aromatic organohalides.

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AMV08
Anaerobic transformation of brominated phenolic aromatics by *Dehalococcoides mccartyi* strain CBDB1

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Dehalococcoides mccartyi strains dehalogenate a wide range of halogenated compounds. The bacterium is viewed as a useful and efficient organism to eliminate halogenated pollutants in the environment. However, current anaerobic transformations catalyzed by *Dehalococcoides* strains are only well studied for chlorinated and simply structured compounds such as chlorinated benzenes, ethenes, ethanes and propanes. Transformations of brominated aromatics with several rings and non-halogen substituents are less investigated. Here, we demonstrate organobromine respiration of *Dehalococcoides mccartyi* strain CBDB1 with tetrabromobisphenol A (TBBPA) and bromophenol blue (BPB). Strain CBDB1 completely converted tetrabromobisphenol A to bisphenol A and bromophenol blue to phenol red with a stepwise removal of the bromide substituents. Meanwhile, toxic effects of the two electron acceptors were investigated. No clear debromination activity or cell growth was observed when CBDB1 was cultivated with 10 µM of TBBPA or 20 µM of BPB. However, when the electron acceptor was added stepwise from nM to µM levels, debromination of both compounds was achieved but with different cell growth rates. Different initial concentrations of BPB were applied to strain CBDB1 to investigate toxicity in more detail. Furthermore, shotgun proteomics will be applied to both cultures to investigate the genes induced compared with cultures using chlorinated compounds as electron acceptors. Our results indicate that the toxicity of brominated phenol aromatics on organobromine respiration by strain CBDB1 decreases with higher water solubility. This information can be useful for the application of *Dehalococcoides* strains for *in situ* bioremediation in the future.

AMV09
Elucidation of anaerobic benzene and alkylated aromatic compound degradation pathways and associated proteins by proteomic and metabolomic analyses

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Benzene and alkylated aromatic compounds are amongst the most persistent contaminants in our environment. The aerobic degradation proceeds fast and is well understood whereas the anaerobic degradation is not fully explored yet. Many cultures that are able to degrade these substances anaerobically are of syntrophic nature. Thus, they rely deeply on the relationships between their key players. The anaerobic culture investigated originates from a column system that is percolated with benzene contaminated groundwater from the aquifer below a former hydrogenation plant in Zeitz, Saxony [1]. Protein stable isotope probing (Protein-SIP) is a newly developed method to unveil elemental fluxes in mixed microbial communities [2]. This is achieved with time-resolved metabolic labeling of the cultures with e.g. ^{13}C, ^{15}N or ^{36}S and subsequent analysis of the resulting proteomes with high resolution mass spectrometry. Studies using ^{13}C-Protein-SIP showed that the sulfate-reducing microbial community, originating from the aquifer columns described above, can be divided into three metabolic groups by their isotopic incorporation patterns: Firstly, benzene degraders which are related to the Clostridiales species *Pelotomaculum*. Secondly, organisms closely related to Deltaproteobacteria which thrive on fermentation products of group 1, heterotrophic CO_2 fixation and sulfate reduction and thirdly, putative scavengers belonging to the Bacteroides/Chlorobi group [3]. Additionally, acetate is suspected to be a central intermediate of the culture [4]. Pulsed $^{13}C_2$-acetate Protein-SIP did not only approved the previously determined groups, but also revealed a complex secondary metabolism including Delta- and Epsilonproteobacteria as well as Archaea. Metabolomic analyses, focused on both the first degradation product and the organic acid intermediate, should unveil further insights into the carbon flux of the community. In general, pure cultures in artificial systems are investigated to reveal *in situ* degradation capability. In this study, a prevalent microbial consortium that is able to degrade benzene *in situ* is analyzed in order to understand the underlying pathways of degradation.

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AMV10
2,3-butanediol production using acetogenic bacteria grown on syngas

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A range of sugar-fermenting microbes are able to produce 2,3-butanediol including *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Lactococcus lactis*, *Paenibacillus polymyxa*, and *Serratia marcescens*. Recently, it was shown that the three acetogenic bacteria *Clostridium ljungdahlii*, *Clostridium ragsdalei*, and *Clostridium autoethanogenum* produce small amounts of 2,3-butanediol using steel mill waste gas as carbon source.
Acetogenic bacteria can use CO as carbon source via the Wood-Ljungdahl pathway. One molecule CO can directly enter the carbonyl branch, while another molecule of CO can be oxidized to CO_2 by a carbon monoxide dehydrogenase (CODH). The resulting energy of this reaction is captured as reduced ferredoxin. CO_2 can enter then the methyl branch, but this depends on whether CO serves as carbon and energy source, or if another energy source such as hydrogen is present. If hydrogen is present, additional reducing equivalents are available, which results in a proportional increase in carbon assimilation. CO and/or CO_2 are transformed to the central intermediate acetyl-CoA that can be converted into different products such as acetate and ethanol. Here we show that *C. ljungdahlii* produced 2,3-butanediol with a concentration of 7.77 mM on syngas. *C. ragsdalei* and *C. autoethanogenum* produced less 2,3-butanediol than *C. ljungdahlii*, and it was demonstrated that *Clostridium aceticum* and *Clostridium carboxidivorans* did not produce any 2,3-butanediol, when grown on syngas. Since *C. ljungdahlii* showed the best performance in 2,3-butanediol production on syngas, the genes for 2,3-butanediol synthesis starting from pyruvate *alsS* (acetolactate synthase), *budA* (acetolactate decarboxylase), and *2,3bdh* (2,3-butanediol dehydrogenase) of this organism were chosen to create a 2,3-butanediol overexpression plasmid. The overexpression of the three genes responsible for 2,3-butanediol formation in *C. ljungdahlii* led to slightly increased 2,3-butanediol concentrations.

AMV11
Heme d_1 - Biosynthesis and Insertion into Cytochrome cd_1 Nitrite Reductase NirS in *Pseudomonas aeruginosa*

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Denitrification is a process used by different species of bacteria to generate energy under anaerobic conditions. In this process, nitrate is reduced to nitrogen *via* nitrite, nitric oxide and nitrous oxide. The second step of denitrification is catalyzed by the cytochrome cd_1 nitrite reductase NirS in *Pseudomonas aeruginosa*. Each subunit of the homodimeric NirS contains a

covalently bound heme *c* and a noncovalently bound heme d_1, which is also the active centre for the reduction of nitrite to nitric oxide [1]. The isobacteriochlorin heme d_1 is unique to the NirS and it is synthesized by enzymes encoded in the *nir* operon (*nirSMCFDLGHJEN*). NirFDLGHJE were shown to be essential for heme d_1 biosynthesis [2]. The last gene in the *nir* operon, *nirN*, encodes a periplasmic *c*-type cytochrome with 24 % identity to NirS. NirN was shown to bind heme d_1 and transfer it to the NirS *in vitro*. It is therefore thought to be important for transport and insertion of the isobacteriochlorin into the NirS [3]. Furthermore, NirN was shown to interact with NirF and NirS during the maturation of the cytochrome cd_1 nitrite reductase [4]. However, recent evidence suggests that NirN might also play a role in heme d_1 biosynthesis. The UV-Vis spectra of the periplasmic extract of a *Pseudomonas aeruginosa* Δ*nirN* mutant display distinct differences in the heme d_1 absorption bands compared to the wild type. Furthermore the UV-Vis spectra of NirS purified from the P. aeruginosa Δ*nirN* mutant are not consistent with the presence of the conventional heme d_1. Solvent extraction and MS analysis of the cofactor suggest that a precursor of the isobacteriochlorin is formed in the Δ*nirN* mutant. Thus, NirN might be involved in the last step of heme d_1 biosynthesis before transferring the cofactor to NirS.

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AMV12
Novel insights into FHL, the *Escherichia coli* formate hydrogenlyase complex

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E. coli hydrogen production was observed in 1931 [1]. Later, three [NiFe]-hydrogenases were identified [2], but it was not until modern genetic tools became available that the components for the hydrogen-producing formate hydrogenlyase (FHL) complex could begin to be characterized [3]. At the same time, sequence analysis revealed similarity between FHL and the proton-pumping Complex I (NADH dehydrogenase) of the respiratory chain. In *E. coli* the FHL complex is synthesized under fermentative growth conditions and comprises a formate dehydrogenase component, that catalyses the oxidation of formate to CO_2 and electrons, and a [NiFe] hydrogenase catalytic subunit that reduces protons to water. Electron transfer between the two components is facilitated by a further three [Fe-S] cluster-containing proteins. Additionally, two integral membrane subunits, HycC and HycD, anchor the metalloenzyme part to the cytoplasmic face of the membrane. This membrane domain shares similarity with the proton-pumping membrane domain of Complex I and, interestingly, in the absence of the membrane proteins no H_2 is evolved from cells, suggesting membrane attachment is critical for FHL operation. In this work, the structure and function of the FHL HycC membrane protein has been explored. Conserved lysine, histidine and glutamate residues in HycC were identified and strains were constructed where those were substituted with alanine. Two of the mutant strains, producing HycC H332 and E391, exhibited dramatic reductions in hydrogen production, but surprisingly this effect was found to be due to a complete destabilisation of the FHL complex. The correlating glutamate in Complex I was previously shown to be important for function [4], thus suggesting that a proton translocating mechanism could be possible for the FHL complex, and that loss of this activity leads to enzyme inactivity and degradation. In addition, a novel purification strategy was established to purify all seven FHL subunits and analyze them electrochemically. These experiments revealed that the hydrogenase component of FHL was able to sustain good levels of hydrogen production even in the presence of high amounts of its product H_2. Combined these methods allow a completely new characterization of a long known enzyme.

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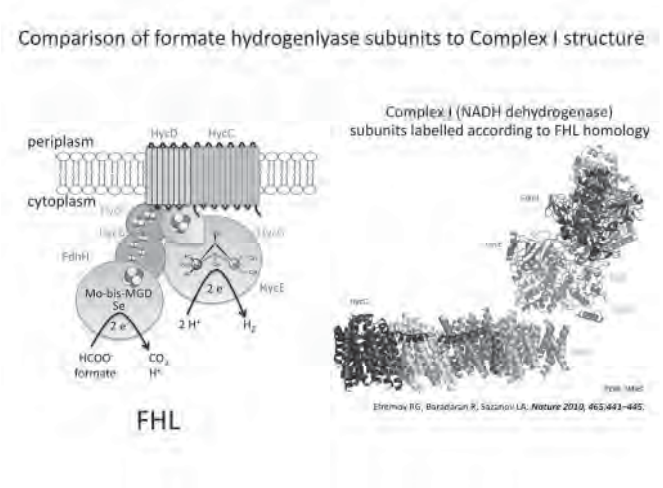
Figure 1



ARCHAEA

ARV01
In-meso crystal structure of a novel membrane-bound nitrite reductase from the crenarchaeum *Ignicoccus hospitalis*

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The Crenarchaeon *Ignicoccus hospitalis* and the Euryacharcheon *Nanoarchaeum equitans* form a unique archaeal association, in which the sulfur-reducing host *I. hospitalis* provides all essential nutrients, lipids, and amino acids to the associated *N. equitans*. The functional role of *N. equitans*, i.e., being a symbiont or a parasite, remains unclear as large parts of the physiology of the host is still poorly understood. *I. hospitalis* comprises a hydrogen:sulfur oxidoreductase to metabolize elemental sulfur but surprisingly also genes that encode for proteins and enzymes required for nitrate ammonification, e.g., a nitrate reductase as well as for nitrification, e.g., two hydroxylamine oxidoreductases (IhHAO1,2). Latter belong to a family of enzymes that in nitrifying bacteria are known to reduce hydroxylamine to nitrite [1]. As the physiological role of such an enzymatic reaction is not obvious in the exclusively sulfur reducing *I. hospitalis* we have functionally and spectroscopically investigated the most abundant IhHAO1 showing that this enzyme is able to efficiently reduce nitrite *in vitro*. To understand the structural basis of this functional inversion of the HAO reaction we have solved the structure of IhHAO1 by lipid-cubic phase to a resolution of 1.65 Å. The trimeric IhHAO1 exhibits significant differences in secondary structure to previously crystallized homologues found in nitrifying bacteria and anaerobic ammonium-oxidizing bacteria, respectively [1,2]. Although having the positions of the eight hemes highly conserved, it shows an altered heme coordination pattern that moreover does not involve an interaction between adjacent subunits. We speculate that by decoupling the individual subunits, IhHAO1 switches its function to a highly activated nitrite reductase.

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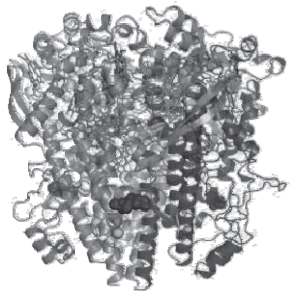
Figure 1



ARV02

Unique metabolic and morphological features of the ammonia-oxidizing archaeon *Nitrososphaera viennensis* sp. nov.

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Introduction: Ammonia-oxidizing archaea are widespread and abundant in Nature and are thus considered to impact the global nitrogen cycle. However, only few organisms have so far been cultivated or enriched. Among these, *Nitrososphaera viennensis* represents the first organism from a soil habitat that is amenable to detailed investigations because it can be grown in pure laboratory cultures. Since bacterial ammonia oxidizers are known to contribute to emissions of nitrous oxide (N_2O) in particular from farmlands, we have investigated the potential and the possible mechanism of *N. viennensis* to produce this potent greenhouse gas. Furthermore, we studied growth requirements and the ultrastructure and S-layer of *N. viennensis*, to obtain general information on this first type strain of the novel class *Nitrososphaeria* within the novel phylum Thaumarchaeota of the domain Archaea (Stieglmeier et al., manuscript accepted in IJSEM).
Materials and Methods: Growth of *N. viennensis* under various conditions was studied in small closed batch cultures. Stable isotope tracing methods combined with Isotope Ratio Mass Spectrometry (IRMS) using ^{15}N-labeled N-compounds were employed to study N_2O production. Ultra-thin sections of cells were prepared and analyzed by TEM to get insights into the ultrastructure. Negative stained S-Layer proteins and freeze-etching were used for the S-layer analyses and were analyzed with the ANIMETRA CRYSTALS software package.
Results: *N. viennensis* is an aerobic, mesophilic and neutrophilic organism, which produces energy by oxidizing ammonia to nitrite at a generation time of 28 h. It predominantly grows autotrophically by fixing CO_2 to build up biomass. However, the organism is strongly dependent on the addition of carboxylic acids, which is different to the marine strain *Nitrosopumilus maritimus*. It produces the potent greenhouse gas N_2O probably by a hybrid formation mechanism (from ammonia and nitrite) and is not capable of nitrifier-denitrification like its bacterial counterparts. The small, irregular coccoid cells are flagellated and have distinct intracellular features. *N. viennensis* has an S-layer with p3-symmetry, which was until now thought to be specific to *Sulfolobales*.
Discussion: The characterization of *N. viennensis* extends the knowledge about the morphology and metabolic capacities of Thaumarchaeota and sheds light on commonalities and differences between ammonia-oxidizing archaea and bacteria. Its deposition in two international culture collections now makes a representative of Thaumarchaeota publically available to the research community.

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ARV03

Ammonia-oxidizing archaea use an energy efficient variant of the hydroxypropionate/hydroxybutyrate cycle for CO_2 fixation

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Ammonia-oxidizing archaea are among the most abundant prokaryotes on Earth and are widely distributed in marine, terrestrial, and geothermal environments. All characterized members of this group belong to the recently proposed phylum *Thaumarchaeota* and couple the oxidation of ammonia at extremely low concentrations with carbon fixation. By representing the predominant nitrifiers in the ocean and in various soils, ammonia-oxidizing archaea contribute significantly to the global nitrogen and carbon cycles. The present contribution provides biochemical evidence that thaumarchaeal ammonia oxidizers assimilate inorganic carbon via a modified version of the autotrophic hydroxypropionate/ hydroxybutyrate cycle previously found in distinct *Crenarchaeota*. Biochemical analysis on cell extract of *Nitrosopumilus maritimus* revealed that this novel thaumarchaeal variant is far more energy efficient than any other aerobic autotrophic pathway. The corresponding genes of this cycle were found in the genomes of all sequenced representatives of the phylum *Thaumarchaeota*, indicating the environmental significance of this efficient CO_2 fixation pathway. In addition, comparative phylogenetic analysis of proteins of the hydroxypropionate/ hydroxybutyrate cycle points to a convergent evolution of this pathway and supports the hypothesis of an early evolutionary separation of the *Cren*- and *Thaumarchaeota*. In conclusion, the high efficiency of anabolism exemplified by this autotrophic cycle perfectly suits the lifestyle of ammonia-oxidizing archaea, thriving at constantly low energy supply, thus offering a biochemical explanation for their ecological success in nutrient limited environments.Taking into account that ammonia-oxidizing archaea represent a significant fraction of the planktonic microorganism community in the ocean, we suggest that the thaumarchaeal variant of the HP/HB cycle probably represents the most important CO_2 fixation pathways below the photic zone in the meso- and bathypelagic realm.

ARV04

Adaptation of *Natrialba* species towards ecological habitats of distinct features

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Members of the family *Halobacteriaceae*, (halophilic *Archaea*), are found in habitats with NaCl concentrations ranging from 15% NaCl to saturation (~32%). These habitats may be divided into two distinct categories, the alkaline (pH >8) and neutral lakes/salterns (pH ~7.0). We investigated the genomic features of the six currently described type strains of the genus *Natrialba*, with the aim of extending our insights into their adaptation strategies. The alkaliphilic species *Nab. chahannaoensis*, *Nab. hulunbeirensis* and *Nab. magadii* were isolated from soda lakes, have an optimum pH at 9 and an optimal growth temperature between 40 °C and 50 °C, an orange-red pigmentation, a G+C content of 63.0 - 65.4%, and phospholipids as the sole polar lipids. The neutrophilic species *Nab. aegyptia*, *Nab. asiatica* and *Nab. taiwanensis* were isolated from neutral marine salterns, have an optimum pH at 7, an optimal growth temperature between 37 °C and 40 °C, a G+C content of 60.3 - 63.1%, are not pigmented, and in addition to phospholipids the glycolipid S2-DGD-1 is also present. 16S rRNA gene based phylogeny, Genome BLAST Distance Phylogeny (GBDP), Average Nucleotide Identity (ANI), Digital DNA-DNA Hybridization (dDDH), and tetranucleotide composition revealed two clusters of either neutral or alkaliphilic strains. The neutrophilic strains contained a significant greater number of glycoside hydrolases (EC:3.2.1.-) encoded on their genomes, 14-16 vs. 4-5 in alkaliphilic strains, and were able to decompose polysaccharides such as cellulose. These results suggest, that the currently known *Natrialba* species have undergone contrasting adaptations to cope in either neutral or alkaline habitats. Neutrophilic *Natrialba* may adapted to marine salterns in which decomposition of algae derived polysaccharides might be an advantage.

ARV05

Protein phosphorylation in *Sulfolobus acidocaldarius*: What is the role of the atypical protein kinases?

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Posttranslational modifications are of major interest for the regulation of cellular processes. Reversible protein phosphorylation is the main mechanism to control the functional properties of proteins in response to environmental stimuli [1]. In the 80's protein phosphorylation has been demonstrated in the third domain of life, the Archaea [2]. So far only few archaeal protein kinases (PKs) and protein phosphatases were investigated and information's regarding signal transduction cascades are very limited. Bioinformatic analysis revealed that bacterial-type two- and one-component systems are present in the euryarchaeota (e.g. CheA/CheY in *Halobacterium salinarium*) [3], but are absent in the creanarchaeota [1]. In contrast eukaryal-type PKs and PPs are found in all archaeal kingdoms indicating that the eukaryal-type protein (de)phosphorylation system plays a major role in Archaea. Investigations of archaeal phosphoproteomes are scares and so far only the phosphoproteomes from *S. solfataricus* [4], *S. acidocaldarius* [5] and *H. salinarium* [6] have been analyzed.Model organism of this study is the thermoacidophilic Creanarchaeon *Sulfolobus acidocaldarius* [7]. Bioinformatic investigation revealed that *S. acidocaldarius* harbors twelve PKs and two PPs [1] and the investigation of the phosphoproteome showed that proteins involved in all cellular processes are targeted via protein phosphorylation [5]. Among the twelve PKs are five PKs which belong to the family of the atypical PKs (aPKs). The general role of aPKs is still unknown in all three domains of life. We will present a first investigated of the ABC1, AQ578, piD261, RIO 1, RIO2 PKs from *S. acidocaldarius*, highlighting their biochemical properties, investigation of gene deletion strains and phylogenetic investigation.

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ARV06

The intestinal archaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* activate human immune responses

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The methanoarchaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* are known to be part of the indigenous human gut microbiota. Although the immunomodulatory effects of bacterial gut commensals have been studied extensively, the impact of methanoarchaea was not examined. Hence, we studied and report here on the response of human epithelial as well as immune cells due to the exposure of *M. stadtmanae* and *M. smithii*. Whereas exposure to *M. stadtmanae* leads to substantial release of pro-inflammatory cytokines in peripheral blood mononuclear cells (PBMCs) and monocyte-derived dendritic cells (moDCs), only weak activation was detected after incubation with *M. smithii*. Phagocytosis of *M. stadtmanae* by PBMCs and moDCs was demonstrated by confocal microscopy and transmission electronic microscopy, and shown to be crucial for cellular activation. Extensive search for the innate immune receptor responsible for recognition of methanoarchaea revealed that none of the common pattern recognition receptors appears to be involved. Activation of MAP-kinases ERK and p38 was demonstrated in moDCs by Western-Blot analysis after stimulation with *M. stadtmanae*, but not with *M. smithii*. However, both strains initiate a maturation program in moDCs though to different extents as revealed by up-regulation of CD86 and CD197. Further, changes in expression levels of genes encoding several antimicrobial peptides have been found in moDCs in response to *M. stadtmanae* and *M. smithii*.
Taken together, these findings strongly argue that the archaeal gut inhabitants *M. stadtmanae* and *M. smithii* are specifically recognized by the human innate immune system. Since both strains are capable of inducing an inflammatory cytokine response to strikingly different extents, both or primarily *M. stadtmanae* may represent so far overlooked contributors in pathological conditions involving the gut microbiota. Consequently, this study indicates that the impact of methanoarchaea as a part of the human gut microbiota has been underestimated until now.

BIOENERGETICS

BEV01

Characterization of the full set of cation/proton antiporters in the soil bacterium *Corynebacterium glutamicum*

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Besides protons, Na^+ and K^+ ions are the most important monovalent cations for bacterial cells. Sodium ions are not only used for sodium-coupled energy conservation and energy transduction but also for solute uptake, pH homeostasis and activation of intracellular enzymes. Potassium represents the most abundant cation in the prokaryotic cytoplasm and plays a role in the control of the membrane potential, regulation of the internal pH value, activation of enzymes and osmotic stress response [1-3].
Corynebacterium glutamicum is a soil bacterium and the most important microbial production organism for amino acids in modern biotechnology. In *C. glutamicum* Na^+ ions are mainly taken up by Na^+/solute symport. For K^+ ions there is only one functional uptake system present, the channel CglK [3]. The export of both cations has not been investigated in *C. glutamicum* so far. We have identified four putative cation/proton antiporters encoded in the genome: ChaA, NhaP, Mrp1, and Mrp2. The latter two transporters belong to the CPA-3 (cation:proton antiporter-3) class that comprises multiple resistance and pH-related antiporter systems (mrp) [4]. The encoding genes are organized in operons and their products form hetero-oligomeric complexes in the membrane. The physiological characterization of *mrp1* and *mrp2* deletion mutants resulted in increased sensitivity to elevated sodium and potassium concentrations, indicating roles in Na^+/H^+- and K^+/H^+-antiport, respectively. The deletion of *chaA* or *nhaP* in the *mrp1/mrp2* double deletion mutant indicated a function of these antiporters in Na^+ and K^+ export, respectively. A mutant lacking all four antiporters turned out to be highly sensitive to increased salt concentrations, especially at alkaline pH. In minimal medium at pH 8.5 100 mM NaCl or 200 mM KCl are sufficient to significantly decrease growth compared to the wild type. In complex medium where bacteria are forced to take up sodium ions by Na^+/solute symport, the *C.glutamicum* antiporter-deficient mutant is impaired in growth. We provide evidence that this is most likely due to an extremely high intracellular Na^+ concentration (exceeding 400 mM), which, however, did not completely stop growth. Important consequences of these results for the physiology of *C. glutamicum* are discussed.

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BEV02

Flavin based electron bifurcation : A mechanistic approach

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Electron bifurcation as strategy of energy coupling was proposed by Peter Mitchell in 1976 as the key process to double the efficiency of the proton motive force in the bc_1 complex of mitochondria. Until the last decade, energy conservation in strict anaerobic bacteria was thought to be only mediated by substrate level phosphorylation (SLP). However, their energetics could not be completely understood, until in clostridia a flavin-based electron bifurcation process was discovered in 2008.Its later detection in methanogenic archaea and acetogenic bacteria as well as probably in sulfate reducing bacteria and benzoate degrading anaerobes demonstrates its fundamental importance in bioenergetics since the origin of life[1]. In butyric acid forming bacteria, an electron bifurcating reaction is catalyzed by electron transferring flavoprotein (Etf) and butyryl-CoA dehydrogenase (Bcd); the two electrons of NADH (E'= -340mV) bifurcate to the high potential crotonyl-CoA (E'= -10mV) and the low potential ferredoxin (E'= -

500mV).Reduced ferredoxin, the preferred electron donor of anaerobic bacteria and archaea, generates either H_2 by reducing protons or a sodium gradient $D\mu Na^+$ by reducing NAD^+ catalyzed via the membrane bound ferredoxin-NAD reductase (Rnf). Using crystallographic and UV-visible spectroscopic methods, we gained profound mechanistic insights into electron bifurcating process of Etf and Bcd of *Acidaminococcus fermentans* [2]. The heterodimeric Etf contains two FAD, α-FAD in subunit α and β-FAD in subunit b. The Etf-NAD^+ complex structure revealed b-FAD as acceptor of the hydride of NADH and as bifurcating FAD. α-FAD is able to approachb-$FADH^-$ and takes up one electron yielding a stable anionic semiquinone, α-$FAD^{\bullet-}$, which donates this electron further to D-FAD of Bcd most likely after a large-scale conformational change. The remaining non-stabilized neutral semiquinone, b-$FADH^{\bullet}$, positioned close to the protein surface immediately reduces ferredoxin. Repetition of this process affords a second reduced ferredoxin and D-$FADH^-$ of Bcd that converts crotonyl-CoA to butyryl-CoA [2].

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BIOTECHNOLOGY (INCL. MICROBIAL BIOCATALYSIS)

BTV01
A new reporter protein for Extremophiles

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Recently there has been a growing interest in the use of non-standard (not *E. coli* or Yeast) organisms in biotechnology. While cloning and expression in well-studied heterologous hosts has become common practice in academic and industrial settings world-wide, for some applications non-standard hosts are beneficial (e.g. tolerance towards high or low temperature, pH or salt environments). However, in order to understand and engineer such organisms and to establish them as 'chassis' for synthetic biology and biotechnology, new tools have to be developed in order to monitor processes on the molecular level and inside the cell. Amongst the most pressing methodological issues to be solved is the implementation of a suitable reporter protein, which could potentially work in a number of extremophile organisms. The standard reporter gene GFP and also its more stable derivatives displayed no detectable fluorescence when analyzed *in vivo* at elevated temperatures in our hands. We thus developed a thermostable esterase into a reporter protein. The protein is active at a broad range of environmental conditions, it is monomeric, does not need maturation or cofactors and can be applied as an *in vivo* reporter. Furthermore, it offers the advantage of signal amplification due to its enzymatic activity, which results in a low limit of detection. As prove of concept different promoters were cloned and tested in thermophilic hosts such as *Geobacillus thermoglucosidasius* and the protein production was quantified (Fig. 1). Intravital whole cell measurements now open the way to engineer promoters inside extremophile hosts, since a high-throughput selection of improved mutants can be performed at the single cell level. In general the proposed reporter protein will enable the molecular analysis of fundamental biological questions regarding the lifestyle of thermophiles and other extremophiles and open the way to utilizing these organisms as whole cell catalysts, e.g. in the production of biofuels like isobutanol.(*1*)

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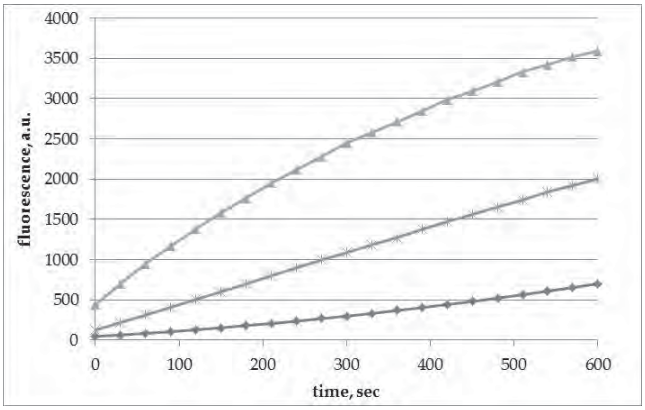
Fig. 1: Conversion of a fluorogenic substrate by *Geobacillus thermoglucosidasius* cells expressing the new reporter esterase under the control of two different promoters (green and blue) and cells expressing no protein (grey)

Figure 1



BTV02
Cage me if you can! Optogenetic bacterial gene regulation using photocaged inducers

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Controlling cellular functions by light has emerged as a sophisticated tool in numerous fields of life sciences. Many biotechnological and synthetic biology applications depend on a precisely controlled gene expression, and light would allow for a non-invasive control with unprecedented spatiotemporal resolution. Such light-mediated control over gene regulation basically relies on two principles, the use of (1) recombinant light-responsive transcription regulators or (2) chemically photocaged inducer molecules that affect transcription factor activities.[1] However, especially for chemical phototriggers, a tight, gradual and homogenous light response has never been truly realized in living cells. Here, we report on the evaluation and optimization of light-responsive bacterial expression systems based on photocaged inducers. A light-responsive *E. coli* expression system was established after validation of different *lac* promoter-controlled and T7 RNA polymerase-dependent expression modules. Microfluidic techniques [2] were applied to identify and circumvent bottlenecks of inducer-dependent gene expression. By implementing a derivative of the synthetic inducer IPTG, which is coupled to the light-sensitive photocaging group 6-nitropiperonal, we assembled a photoswitch that can precisely be controlled by UV-A light.[3] Based on this photoswitch, novel light-responsive expression modules can conveniently be established and used to create synthetic higher-order regulatory circuits.

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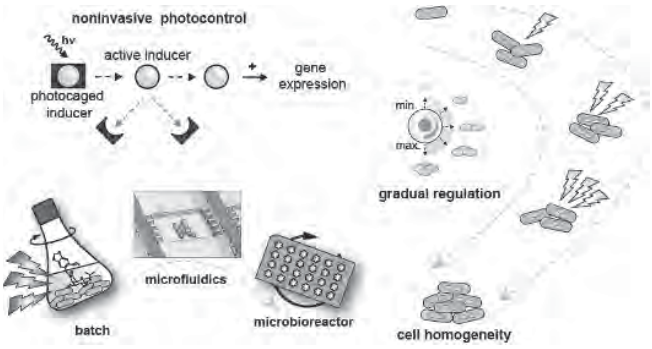
Figure 1



BTV03

A multigene assembly strategy for improved heterologous expression of lanthipeptides in *Escherichia coli* allows for efficient *in vivo* peptide engineering

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Introduction: Peptide natural products have gained increasing industrial importance as they possess manifold pharmacologically relevant bioactivities. Lantibiotics are peptide antibiotics that are ribosomally synthesized by Gram-positive bacteria carrying unusual amino acids as lanthionine (Lan), dehydroalanine (Dha) and dehydrobutyrate (Dhb). These are introduced by post-translational modifications resulting from dehydration of Ser (leading to Dha) or Thr (leading to Dhb) residues and/or cyclisation with a Cys residue. The intramolecular thioether crosslinks provide high structural stability and protect the compound from host resistance mechanisms. Synthetically post-translational modifications are difficult to apply.[1] Therefore *in vivo* expression systems are of urgent need allowing for ample mutagenesis experiments to improve and alter the bioactive properties. Lichenicidin is a class II two-component lantibiotic consisting of the two peptides Bliα and Bliβ acting in a synergistic manner. In our work we aimed to design an inducible heterologous expression system for lichenicidin in *Escherichia coli*.
Materials and Methods: We used the *Bacillus licheniformis* gene cluster for expression of lichenicidin and allocated each gene required for biosynthesis of the lantibiotic with its own strong promoter by intermediate cloning into an expression vector.[2] The promoter-gene cassettes were then amplified and assembled into one high-copy plasmid resulting in pHP_alpha and pHP_beta. Expression yields were analysed by LC-MS experiments.
Results: The reassembly of genes required for lichenicidin expression of either peptide Bliα and Bliβ in *E. coli* resulted in 10 times higher yields than expression in the original host *B. licheniformis* and 60 times higher yields than in an *E. coli* host using the unmodified gene composition from *B. licheniformis*.[3]
Conclusion: Uncoupled expression of each lichenicidin peptide enables extraction and purification of pure α- or β-peptide. The additionally achieved increase in peptide yields by genetic reconstruction of the expression unit and heterologous production in *Escherichia coli* facilitates analysis of the peptides in terms of bioactivity, mode of action and SAR studies. The system we developed in this study is perfectly suited for *in vivo* peptide engineering approaches of either peptide and subsequent analysis of bioactivity by testing pure compound.

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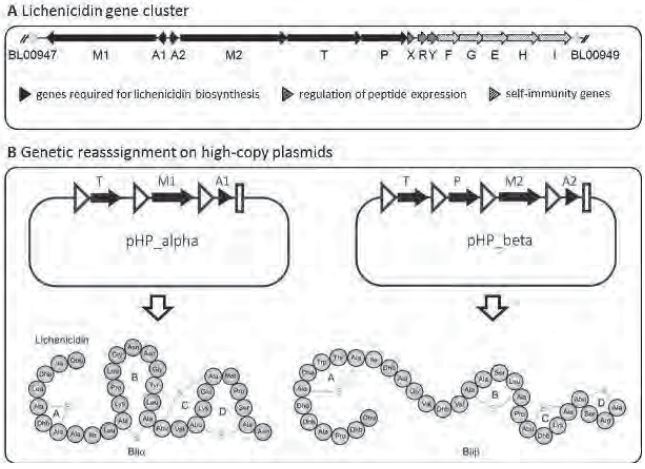
Figure 1



BTV04

Exploiting unconventional secretion in *Ustilago maydis*

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Developing novel protein expression systems is essential to produce the full repertoire of economically relevant proteins. To fill existing gaps, we aim to establish and apply the fungus *Ustilago maydis* as novel protein expression platform. This eukaryotic microorganism is well known as a corn pathogen and a well-described model organism in basic research. However, it also displays some promising features that could be applied in biotechnology. This idea is also supported by the fact, that the fungus is very well suited for genetic manipulation and modified strains can be generated rapidly. Recently, we discovered the unconventional secretion of an endochitinase, Cts1. The secretory mechanism itself is yet unknown. Interestingly, during hyphal growth Cts1 export depends on endosome-coupled mRNA transport indicating an intimate link to post-transcriptional regulation. We are exploiting the Cts1 secretion apparatus for the export of proteins with biotechnological or pharmaceutical interest. The main advantage of using this pathway is the circumvention of N-glycosylation, which usually takes place in ER and Golgi. This can be essential for the production of distinct proteins in an active state or to avoid immune reactions. As a proof-of-principle, we for example expressed a single-chain antibody which was produced and secreted in its active form. As initial yields were low, different steps were conducted to optimize the expression strains. For example, we enhanced protein yields by eliminating harmful proteases. Currently, we are establishing fermentation protocols. Hence, *U. maydis* may constitute a valuable alternative for protein expression in the near future.

BTV05

Gluconobacter factory: Development of a platform strain for oxidative biotransformations

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The strictly aerobic acetic acid bacterium *Gluconobacter oxydans* features a rapid incomplete periplasmic oxidation of a variety of diverse carbohydrates. This property has the consequence that only a small amount of the available carbon source is metabolized in the cytoplasm. Intracellular sugar catabolism can only occur via the pentose phosphate pathway (PPP) or via the Entner-Doudoroff pathway (EDP). Absence of 1-phosphofructokinase renders the Embden-Meyerhof pathway non-functional and a cyclic operation of the citric acid cycle is prevented by absence of succinate dehydrogenase (Sdh) and succinyl-CoA synthetase. Due to these extraordinary metabolic characteristics, *G. oxydans* affords only a very low cell yield compared to other bacteria. *G. oxydans* is used for large-scale production of e.g. vitamin C, ketogluconates and dihydroxyacetone by oxidative biotransformations. To foster the industrial application of *G. oxydans*, it is desirable to develop a platform strain making the cell mass production for biotransformations less cost-intensive. To overcome the problem of low biomass production, we attempt pathway restoration. To this end we introduced succinate dehydrogenase (Sdh) from *Acetobacter pasteurianus* in *G. oxydans* 621H to complete the citric acid cycle as the first step. Heterologous expression of the *sdhCDAB* genes from *A. pasteurianus* using plasmid pBBR1p384_sdh led to a specific Sdh activity of the recombinant strain at the detection limit (≤ 0.01 U (mg membrane protein)$^{-1}$). Co-expression of the *sdhE* gene from *A. pasteurianus*, which encodes an assembly factor required for covalent attachment of FAD to SdhA using the artificial operon *sdhCDABE* resulted in an *in vitro* Sdh activity of 0.43 ± 0.07 U (mg membrane protein)$^{-1}$. Expression of *sdhE* under control of its native promoter, along with *sdhCDAB*, yielded an Sdh activity of 3.99 ± 0.40 U (mg membrane protein)$^{-1}$. The latter expression strain showed an inhibited growth. Future studies aim at a complete restoration of the citric acid cycle by heterologous expression of the succinyl-CoA synthetase genes. Furthermore, the fraction of glucose oxidized in the periplasm has to be minimized by inactivation of the corresponding dehydrogenases.

BTV06

Heterologous production of an electron transport chain and electrode-interaction in microbial electrochemical cells enables *Escherichia coli* to perform unbalanced fermentations

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Microbial electrochemical cells (MEC) are an emerging technology for the realization of unbalanced fermentations. However, the number of exoelectrogenic organisms acting as potential biocatalysts for this type of application is rather limited due to their narrow substrate spectrum. In contrast *Escherichia coli* is metabolically versatile and genetically easy tractable and the best understood microorganism so far. This study describes the process of reprogramming *E. coli* for efficient use of anodes as electron acceptor. Thereby electron transfer into the periplasm of *E. coli* was accelerated by 89% via heterologous expression of the three *c*-type cytochromes CymA, MtrA and STC from the exoelectrogenic organism *Shewanella oneidensis*. STC was identified as a target for heterologous expression by a two stage screening approach. First, mass spectrometric analysis was conducted to identify natively expressed periplasmic cytochromes in *S. oneidensis* under conditions of extracellular respiration. Corresponding genes were cloned and tested for activity in *E. coli* using a novel assay that is based on the continuous quantification of methylene blue reduction in cell suspensions. Periplasmic electron transfer could be extended to a carbon electrode surface using methylene blue as redox shuttle. Results from first MEC experiments revealed a shift in the fermentation product spectrum towards more oxidized end-products. In this context a new reactor setup was designed to optimize the analysis of volatile fermentation products. Previous experiments demonstrated that glycerol fermentation of *E. coli* can be improved by co-cultivation with *Methanobacterium formicicum* [1]. Although methanogens are undesirable in most MEC applications it was shown that the glycerol consumption during the described unbalanced fermentation process could be improved by co-cultivation. Furthermore relevant amounts of current and methane were produced. These results clearly demonstrate that the production of a new electron transport chain enables *E. coli* to perform an unbalanced glycerol fermentation which offers new opportunities for biotechnological applications.

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BTV07

Biogas production from coumarin-rich plants: Impact of coumarin on process parameters and microbial community

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Sweet clover (*Melilotus* spp.) is a pioneer plant and suitable for crop rotation on dry sandy soils. It contains up to 5%$_{TS}$ of the plant secondary metabolite coumarin. Coumarin gives a bitter taste and has bacteriostatic properties. During ensiling of sweet clover coumarin can be converted to dicumarol which acts as poisonous anticoagulant. Hence, coumarin-rich plants are problematic as fodder plant for livestock. An alternative usage as substrate for biogas production is conceivable. Therefore, the aim of the present study was to investigate the applicability and impact of coumarin-rich substrates for biogas production in batch as well as in continuous fermentation processes. Coumarin was added as a chemical to batch digestion of grass silage in two concentrations of 2.5%$_{TS}$ and 5.0%$_{TS}$. Initially, an inhibition was observed which continued until day 37. Thereafter, no inhibition was detected until the end of the experiment (day 54). Finally, the coumarin treatments resulted in an increase of methane yield of 3% for the 2.5%$_{TS}$ and 6% for the 5.0%$_{TS}$ coumarin treatments, respectively, compared to the coumarin-free controls. The surplus of methane yield could be assigned to the conversion of coumarin to methane.

Additionally, coumarin (5%$_{TS}$) was added to co-digestion of grass silage and cow manure in lab-scale continuous stirred tank reactors. Similar to the batch tests, coumarin caused first an inhibition of the biogas process by means of decreasing the biogas production by 19%. Moreover, an increase of metabolites, i.e. volatile fatty acids exceeding a critical level (VOA/TIC value > 0.3 g_{VOA}/g_{CaCO3}), was observed. This might result in a failure of the biogas process in less ideal systems like full-scale biogas plants. However, the process restabilized as an effect of adaptation of the microorganisms which resulted in biogas production and metabolite concentrations on normal levels. Parallel to the analysis of the process parameters, the impact of coumarin on the composition of the microbial community was investigated by terminal restriction fragment length polymorphism (T-RFLP) based on DNA. The methanogenic community composition did not change due to coumarin addition. In contrast, a steady shift of the bacterial community caused by the coumarin addition was observed. As during the stable phase of digestion no coumarin was detected in the digestate its anaerobic degradation is suggested. To the best of our knowledge, the anaerobic degradation of coumarin has not been described yet. Coumarin acts as substrate and as inhibitor in the biogas process and hence, coumarin-rich plants should only be used for biogas production after adaptation of the microbial community to this compound. However, plants containing secondary metabolites like coumarin could be used as alternative substrates compared to common energy plants for biogas production and hence, decrease the food vs. fuel competition.

BTV08

Isolation of *Cellulomonas uda* from biogas reactor sludge

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As partner of the network BioPara, our aim is to investigate limiting factors in the biogas production process. One limiting factor is the degradability of cellulosic and hemicellulosic substrates which remain partially unused in the biogas reactor. Fibre- rich and persistent substrates (e.g. maize or grass silage) should be degraded to sugar monomers as glucose, xylose, and arabinose by direct addition of cellulolytic microorganisms. Methane precursors such as acetate and CO_2 are generated from these sugar monomers. The aim of this work is the isolation and characterization of cellulolytic microorganism in biogas reactors, by using media slightly modified according to Rastogi et al. (2009) and Gupta et al. (2012). Thus, *Cellulomonas uda* was isolated from a sample drawn from an investigated biogas reactor located near Bonn. *C. uda* is a Gram-positive, rod-shaped, and facultative anaerobic bacterium, which was originally described by Kellerman and McBeth (1912) and later by Bergey et al. (1923). *C. uda* was able to utilize whatman paper and the offered carbohydrates such as glucose, cellobiose, and carboxymethylcellulose during aerobic and anaerobic conditions. Major products formed were acetate, lactate, succinate, formate, and ethanol under anaerobic growth conditions. During aerobic growth with cellobiose as carbon source, the strain reached a maximal optical density of around 15, which is suitable for later inoculations of lab-scale biogas reactors. Furthermore, sequencing of the whole genome of *C. uda* was conducted in collaboration with the University of Göttingen (Institute of Microbiology and Genetics). Preliminary data indicate that the genome consists of 3.41 Mbp with a G+C content of 73.85 % and 471 scaffolds.

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BTV09
Continuous Benzene and Ammonium Removal from Contaminated Groundwater using a Microbial Fuel Cell

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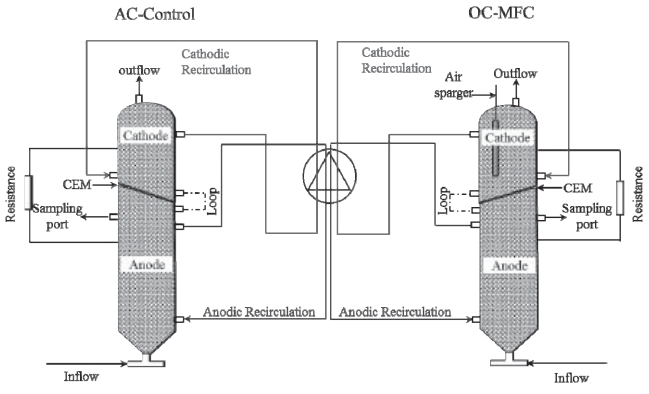
A microbial fuel cell (MFC) with an anoxic anode and oxic cathode (OC-MFC) and an entire anoxic control (AC-Control) were constructed to assess and compare the performance of continuous treatment on groundwater contaminated with benzene and ammonium. In the OC-MFC, benzene and ammonium were removed and electricity was generated during continuous treatment. Best performance of the OC-MFC was determined at a flow rate of 0.3 mL/min, resulting in a retention time of 27 hours. Benzene (15-20 mg/L in the inflow) was completely removed in the OC-MFC; around 80% of the benzene disappeared already in the anodic part. All ammonium (20-25 mg/L in the inflow) was stoichiometrically oxidized to nitrate in the cathodic part of the OC-MFC due to the metabolic activities of nitrifiers. The maximum power density of 318 mW/m^3 net anodic compartment (NAC) was identified at a current of 316 µA. Coulombic and energy efficiencies of 13.9% and 3.9% were obtained based on the anodic benzene degradation at 0.3 mL/min flow rate, respectively. Benzene and ammonium spiking experiments demonstrated that benzene served as electron donor for the anode, whereas ammonium did not have a direct contribution on electricity generation. Oxygen interruption results demonstrated that oxygen was the electron acceptor in the cathode. Pyrosequencing analysis of 16S rRNA genes revealed the dominance of *Chlorobiales* (41%), *Rhodocyclales* (21%), and *Burkholderiales* (6%) in the anode, presumably associated with benzene degradation. The majority of bacteria identified in the cathode of the OC-MFC were affiliated to the *Nitrospirales* (18%), *Burkholderiales* (15%), *Rhodocyclales* (7%), *Nitrosomonadales* (4.5%), and *Rhizobiales* (4%). The results suggest that ammonium oxidation in the cathode of OC-MFC were mainly carried out by phylotypes belonging to the *Nitrosomonadales* and *Nitrospirales*. In the AC-Control, around 60% of benzene was removed in the anodic part and 80% benzene finally disappeared in the outflow, whereas ammonium was not oxidized. The AC-Control failed to recover electricity energy due to lack of available electron acceptors in the cathode. Thus, the AC-Control can be seen as a mesocosm in which granular graphite was colonized by benzene degraders. Two-dimensional compound-specific isotope analysis demonstrated that benzene was activated by monohydroxylation in both OC-MFC and AC-Control. Our study demonstrates the principal feasibility of a microbial fuel cell containing an oxic cathode for treating benzene and ammonium contaminated wastewater.

Figure 1



BTV10
Evaluation of process parameters for the development of a biological treatment concept for sulfate and heavy metal containing mine drainage and waste waters

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Treatment of acidic mine waters with high ferrous iron and sulfate loads represents a major challenge in many areas of the world. A sustainable alternative to the physical and chemical methods currently in use is provided by the microbial dissimilatory sulfate reduction. We developed in this context a moving sand bed bioreactor system that utilises naturally occurring sulfate reducing microorganisms for the elimination of sulfate and of part of the acidity from the acidic mine water. To improve the performance of this process various process parameters, in particular hydraulic retention time (HRT) and frequency of sand bed movement, were evaluated in bioreactor bench-scale experiments. In parallel, the genetic composition, biomass and activity of the microbial community were quantified and correlated to sulfate reduction rates. The results from these analyses demonstrated an influence of the frequency of sand bed movement on the activity of the sulfate reducing microbial community and sulfate reduction rates and a positive correlation between shorter HRT and increasing sulfate reduction rates. The improvement in performance with decreasing HRT was paralleled by an increase of the total enzymatic activity (measured as hydrolase activity) of the microbial community and of the biomass (measured as protein concentration) in the bioreactors. Since the microbial community in the biofilm within the bioreactor changed linearly over the 32-day time period of the incubation it appears likely that reactor performance will further improve during long term running of the reactor system. This experimental approach was accompanied by an extensive literature review of approximately 70 scientific reports on the development of sulfidogenic bioreactor designs with the aim to further reveal important parameters for an optimal operation of the process. The results of the bench scale experiments and the findings from the literature review were finally incorporated into a concept for the biological treatment of sulfate and heavy metal containing mine drainage and waste waters.This concept is currently being realised in form of a larger scale demonstration plant (several hundred litres bioreactor volume) at the east German lignite mining district (Lusatia).

BTV11
Microbial leaching of rare earth elements from fluorescent phosphor

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Rare Earth Elements (REE) are used in mostly all new technologies and until now, there is no environmentally friendly recycling-process for fluorescent phosphor. Furthermore, China has with a worldwide market share of 94 % (2011) [1] a virtual monopoly in the production of REE. Therefore, there is increasing demand for novel recycling technologies to secure the supply of REE. During recycling of energy-saving bulbs fluorescent phosphor containing rare earth elements (REE) is collected as a distinct fraction. In this study hydrometallurgical techniques were investigated to recycle REE from spent technological products. Due to electrochemical restrictions, leaching with organic acids and metal binding molecules is more promising, than oxidation or reduction reactions [2, 3]. On this basis, different hetero- and autotroph aerobe microorganism pure and mixed cultures are selected. Among them are "classical" leaching organisms like *Acidithiobacillus ferrooxidans* and *A. thiooxidans*, as well as the organic acid producing *Corynebacterium collunae* (glutamic acid), the yeast *Yarrowia lipolytica* (citric acid) and the tea fungus kombucha. The investigations regarding their ability to leach the REE from fluorescent phosphor, originating from recycling processes, were performed in fed-batch experiments. It could be shown that complexation of the REE by organic acids produced by the microorganisms lead to considerable higher concentrations of REE in the supernatant than in the control. These results show that the usage of microbial processes for the recovery of REE is possible and could be an eco-friendly alternative to the currently employed methods.

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BTV12
Analysis of different leaching parameters as preliminaries for in-situ leaching

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In the course of industrialization in numerous countries all over the world, the demand on resources steadily increases. Hence, natural deposits of high grade ores become more and more depleted. The greater part of crucial metals and metalloids remains in deposits with lower grades. Conventional extraction methods of these minerals are energy consuming and result in pollution of water, soil or air. To overcome these problems new techniques like bioleaching are in focus of actual research. A very important method in this task field is in-situ leaching, because the extraction of target metals, here indium and zinc, due to the activity of iron-oxidizing bacteria is performed with a minimum of energy consumption local in the mine. Regarding our approaches to bioleach sphalerite (ZnS) parameters like mineral phases, temperatures, leaching media and bacterial communities have to be taken into account. To explore the influence of growth medium composition on the efficiency of the bioleaching process, numerous leaching-tests have been performed in shaking-flasks under laboratory conditions. Enrichment cultures from environmental sites with mining capacity were inoculated in four acidic iron-oxidizer liquid media. The grinded ore applied to bioleaching was won from mine *Reiche Zeche* in Freiberg, Germany, and consists roughly halfway through sulfidic minerals like sphalerite and pyrite. Over the course of the leaching process the pH, redox potential and Zn-extraction rate were monitored at regular intervals. The microbial community structure before and after successful leaching was analysed by terminal restriction fragment length polymorphism (T-RFLP). For future application of in-situ leaching the bacterial diversity of the environmental site "*Reiche Zeche*" is under investigation by moleculargenetic methods. Our results show that the choice of the growth medium influences the composition of the bacterial community and the efficiency of bioleaching as well. For leaching sphalerite from the ore deposit *Reiche Zeche* we unravelled the bacterial key players and determined optimal media conditions. Further efforts are aiming towards the analysis of the bacterial community in the mine *Reiche Zeche* in order to gain knowledge for a future in-situ leaching plant.

BTV13
Process development of glutathione enriched yeast production

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The total production volume of commercial yeast per year was 2009 approximately 4 million tons with an assumed volume of sales per year >3 billion US$. The biggest margin of 33% of total was achieved with autolysates and extracts respectively. Yeast extracts are also applied as a kind of flavor enhancer (uami-taste &kokumi-taste) in food industry. Kokumi-taste is affected by different γ-glutamylcysteine derivatives for instance glutathione (GSH). GSH is also used in pharmaceutical and cosmetic industry as radical catcher in skin care products as well as food industry as baking modifier. This contribution focused the process development of glutathione-enriched yeast, especially the efficient incorporation of GSH inducing compounds. Therefore, a DoE-based milliliter bioreactor technology was applied for the identification of high effective GSH booster in brewing yeasts *Saccharomyces cerevisiae* and *S. baianus* as well as probiotic *S. boulardii*. By means of Plackett-Burman and RSM design cysteine, sodium sulfite and a cysteine-derivate were identified as feasible GSH inducing agents. Thereby, intracellular GSH contents could be improved to values of more than 3% of cell dry weight. For further development of a production process it was necessary to verify the results in 5 l bioreactors. Thereby, high cell density cultivation was optimized via RQ controlled feeding strategy. Furthermore, efficiency of used GSH boosters was increased and critical toxic levels were characterized.
The developed feeding strategy including best performing inducer, optimized yield of Incorporation was transferred in a 150 l pilot scale bioreactor to mimic realistic production conditions. The methodology of DoE in combination with bioprocess technology revealed the high potential for these methods for process improvement. The investigated topic is important from an economical point of view due to the general low margin of yeast products.

BTV14
Alternative pathway for itaconic acid biosynthesis in the basidiomycetous fungus *Ustilago maydis*

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The unsaturated dicarboxylic acid itaconate is a bio-based chemical building block for production of plastics, paints and cosmetics. Currently, itaconic acid is produced by fermentation of *Aspergillus terreus*. In this organism, biosynthesis occurs by decarboxylation of *cis*-aconitate. The reaction is catalyzed by *cis*-aconitate decarboxylase (CAD), which belongs to the PrpD protein family and is similar to methylcitrate dehydratases. Itaconate production has also been observed in *Ustilago maydis* and some related *Pseudozyma* species. However, the biosynthetic route for itaconate production has not yet been characterized in these fungi. Here, we report that *U. maydis* uses an alternative biosynthesis pathway for itaconic acid production. In this fungus, all genes required for itaconate biosynthesis are organized in a gene cluster, which also contains a pathway-specific transcriptional regulator. Within this gene cluster we could identify two genes coding for enzymes critical for itaconate production. Both enzymes were characterized by deletion analysis and their biochemical activity was confirmed *in vitro* with proteins purified from *E. coli*. This allowed us to propose an alternative pathway for itaconate biosynthesis. In *U. maydis,* itaconate is produced by decarboxylation of *trans*-aconitate which is generated by isomerization of *cis*-aconitate. We were able to achieve itaconic acid production in the heterologous host *Saccharomyces cerevisiae* by expression of the respective *U. maydis* genes. The identification of an alternative itaconic acid biosynthetic pathway and the detailed characterization of enzymes will be used to improve biotechnological production of this interesting chemical building block in *U. maydis* and thus highlights the high biotechnological potential of this fungus.

BTV15
Biotechnological application of the ethylmalonyl-CoA pathway: de novo synthesis of mesaconic and methylsuccinic acid from methanol using *Methylobacterium extorquens* AM1

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There is a high need for sustainably producible chemical building blocks being applicable e.g. as monomers for novel bioplastics. The ethylmalonyl-CoA pathway (EMCP) harbors several CoA-esters such as ethylmalonyl-, methylsuccinyl- or mesaconyl-CoA whose free dicarboxylic acid derivatives potentially present promising synthons for chemical industry. The existence of the EMCP in various methylotrophic bacteria such as *Methylobacterium extorquens* AM1 offers the possibility to produce these new building blocks directly from the cheap and non-food competing C-source methanol. Thioesterases are one option to cleave the thioester bond of the EMCP CoA-esters. A test of different thioesterases via *in vitro* enzymatic assays and *in vivo* acid production revealed YciA from *Escherichia coli* as suitable for the production of mesaconic and methylsuccinic acid from methanol in *M. extorquens* AM1. Concentrations of 60-70 mg/L were reached at the end of exponential growth phase with a YciA expression construct containing an optimized ribosome binding site. Observed re-uptake of the released acids was significantly reduced by decreasing the concentration of sodium which may act as symporter ion for dicarboxylic acid transport. Growth under cobalt limiting conditions further increased the amount of released mesaconic and methylsuccinic acid up to 7 fold. In summary, we present the first biotechnological production of unconventional EMCP derived

dicarboxylic acids mesaconate and methylsuccinate. More than 600 mg/L were produced from the alternative C-source methanol by expression of thioesterase YciA in *M. extorquens* AM1 cultivated in a methanol minimal medium.

BTV16

Synthesis of Poly(3HB-*co*-3HV) from unrelated carbon sources in engineered *Rhodospirillum rubrum*

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Polyhydroxyalkanoates (PHAs) are biopolymers, which are accumulated by numerous bacteria and present unique material properties, which make them a promising biodegradable alternative for conventional plastics. A large variety of over 150 different hydroxyalkanoic acids have been detected as constituents of bacterial PHAs. Copolymers of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) are of high commercial interest, as they have a particularly favourable melting point, plasticity and crystallinity (Steinbüchel and Pieper, 1992). However, in most cases the incorporation of 3HV-monomers requires the addition of related carbon sources as propionic or valeric acid to the media. Propionyl coenzyme A (propionyl-CoA), which is a precursor of the C_5 3HV-monomer, is an intermediate of different pathways, as the methylmalonyl-CoA pathway or the synthesis of branched amino acids. We show that the intracellular pool of propionyl-CoA can be increased by altering the balance of the intracellular reducing equivalents NAD^+ /NADH and $NADP^+$ /NADPH. For this purpose, we have genetically engineered the photosynthetic non sulphur purple PHA producer *Rhodospirillum rubrum* to overexpress different genes coding for transhydrogenases (*udhA*, *pntAB*) and acetoacetyl-CoA reductases (*phaB*). A recombinant *R. rubrum* strain harbouring the gene for the membrane bound transhydrogenase PntAB from *Escherichia coli* MG1655 and the *phaB1* gene coding for an NADPH-dependent acetoacetyl-CoA reductase from *Ralstonia eutropha* H16 accumulated a Poly(3HB-*co*-3HV) polymer with a 3HV fraction of up to 13 mol% from the unrelated carbon source fructose. This is, compared to the wildtype strain *R. rubrum* S1, a more than twelvefold increase in 3HV-content. Our study thereby shows a novel approach to alter the monomer composition of bacterial PHA and also provides a promising production strain for Poly(3HB-*co*-3HV), which can utilize varying carbon substrates.

Steinbüchel, A., and U. Pieper. 1992. Production of a copolyester of 3-hydroxybutyric acid and 3-hydroxyvaleric acid from single unrelated carbon sources by a mutant of *Alcaligenes eutrophus*. *Appl Microbiol Biotechnol* **37**:1-6.

BTV17

Genetic manipulation of *Clostridium ljungdahlii*: Reduction of Biomass Intermediates to Tailor-Made Fuels

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The German cluster of excellence "Tailor-made fuels from biomass" (TMFB) at RWTH Aachen University uses an interdisciplinary approach to analyze new and optimize established methods to produce tailor-made fuels from raw biomass. The long-term goal is to develop methods to reduce the addiction to none regenerative fossil energy sources. Our project focuses on strategies to reduce pre-defined biomass intermediates to fuels or fuel components using microbial electroreduction. Therefore, we want to make use of the natural ability of the anaerobic homoacetogen *Clostridium ljungdahlii* to utilize electrons from a cathode in a microbial electrosynthesis cell. We aim to engineer *C. ljungdahlii* as an effective biocatalyst that reduces specific platform chemicals to fuels or fuel precursors by using molecular and synthetic biological approaches. We want to enhance electron utilization by developing a new whole-cell mutagenesis method for Clostridia, based on a controllable mutator-plasmid. The idea is to construct an effective biocatalyst that is able to use an input of electrical energy from renewable resources for different electroreduction and electrosynthetic processes. One of the biomass intermediates specified by the TMFB is itaconic acid (IA). The first step in reduction of IA is the activation with CoA to itaconyl-CoA. Genes for well characterized CoA-ligases/ -transferases with activity towards IA are therefore introduced into *C. ljungdahlii*, and analyzed for activity under electroreductive conditions. In a second step, intrinsic and heterologous reductases will be screened for IA reduction. As an alternative and CoA-independent route for the reduction of IA, ferredoxin-dependent aldehyde oxidoreductases are also under investigation. The proof of principle to tailor *C. ljungdahlii* to reduce a given molecule electroreductively by using synthetic biological approaches will lead to new biotechnological strategies for reductive transformation reactions. In order to get a deeper understanding of the underlying cellular processes of electroreduction in *C. ljungdahlii* and to have additional tools in hand for genetic manipulation of this homoacetogenic organism also interesting for other biotechnological applications in general, we are additionally investigating different knock-out strategies.

BTV18

Development of a Highly Efficient Gene Delivery System for Syngas Fermenting Clostridia

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Several microorganisms belonging to the genus *Clostridia* can convert synthesis gas (syngas), a mixture of carbon monoxide, carbon dioxide and hydrogen, into more complex organic molecules, including biomass, acetate and ethanol. The conversion of syngas into ethanol is particularly relevant in the context of biofuel production, and several start-up companies are already pursuing this technology. The commercial development of syngas-based ethanol fermentation processes is challenging because additional energy is needed to purify the resulting alcohols by distillation. Based on the known metabolic capabilities of different *Clostridium* strains, we can already envision the production of several other chemicals and biofuels, most representing high-value products that cannot be synthesized via established chemical routes such as the Fischer-Tropsch process. However, for the establishment of these biosynthetic pathways (beyond syngas based ethanol production) metabolic engineering approaches involving complex pathways become necessary. Up to today the metabolic engineering of *Clostridia* in general and of syngas fermenting *Clostridia* strains in particular proved rather challenging. We developed a highly efficient gene delivery system capable for introducing complex (large) gene clusters encoding entire biosynthetic pathways into syngas fermenting *Clostidia* such as *C. ljungdahlii* and *C. autoethanogenum*. This development will not only deliver syngas-based fermentation systems for chemicals and biofuels but will also foster the development of further Clostridia fermentation processes using other feed stocks such as cellulose (e.g. by *Clostridium cellulolyticum*).

BTV19

Development of a two-enzyme system comprising of an aryl-alcohol oxidase and a dye-decolorizing peroxidase

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Almost all edible fungi belong taxonomically to basidiomycetes. Besides their value as a food source, they also have a considerable biotechnological potential. One of the major advantages is their capability of using lignin and cellulose from wood as a carbon source by applying a versatile enzymatic system. Lignin degrading enzymes such as laccases and peroxidases produced by basidiomycetes are already used for enzymatic dye degradation or bioconversion of industrial waste materials. In comparison to laccases, which only need molecular oxygen for the redox reaction, peroxidases require H_2O_2 as a cofactor. In nature, fungi secrete H_2O_2 generating enzymes, such as glucose oxidases and aryl-alcohol oxidases [1], providing peroxidases with the mandatory hydrogen peroxide. The white-rot fungus *Pleurotus sapidus* closely related to the oyster mushroom *Pleurotus ostreatus* is known to produce dye-decolorizing peroxidases (DyP) as well as an aryl-alcohol oxidase (AAO). To combine the catalytic properties of both enzymes for biotechnological applications, we established a two-enzyme system comprising a DyP and an AAO for the decolourisation of food materials, such as annatto containing whey effluents from cheese manufacturing. To achieve this, the genes coding for the DyP and AAO were cloned and transformed into the basidiomycete *Coprinopsis cinerea* [2]. Both genes were placed under the control of the constitutive promoter of the glyceraldehyde-3-phosphate dehydrogenase (*gpdII*) from *Agaricus bisporus [3]*. In case of AAO, high yields of active enzyme could be obtained. Batch fermentation of the best producing transformant in a 5 L bioreactor showed AAO enzyme activities of up to 125 U L^{-1}. The purification of the enzyme was carried out with a FPLC using an anion exchange chromatography followed by a size exclusion chromatography. Purity and origin of the acquired *P. sapidus* AAO were tested with SDS-

PAGE and Western Blot. Biochemical properties of the two-enzyme system were analysed and bleaching experiments were performed.

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BTV20
Synthesis of chiral β-amino acids via Lipase/Transaminase reaction cascade

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Optically pure β-amino acids constitute interesting building blocks for peptido-mimetics and a great variety of pharmaceutically important compounds. Their efficient synthesis still poses a major challenge [1]. In earlier studies we detected a transaminase activity transforming chiral β-amino acids into the corresponding β-keto acid which decarboxylates spontaneously [2]. To realize the back reaction from these thermodynamically instable educts we are using β-keto acid esters as precursors. These are hydrolyzed by a lipase resulting in freshly prepared substrates for the subsequent transaminase reaction to finally synthesize chiral β-amino acids (fig. 1). Since the ester precursors are poorly water soluble we are going to perform a cascade reaction in a water-organic solvent two-phase system.

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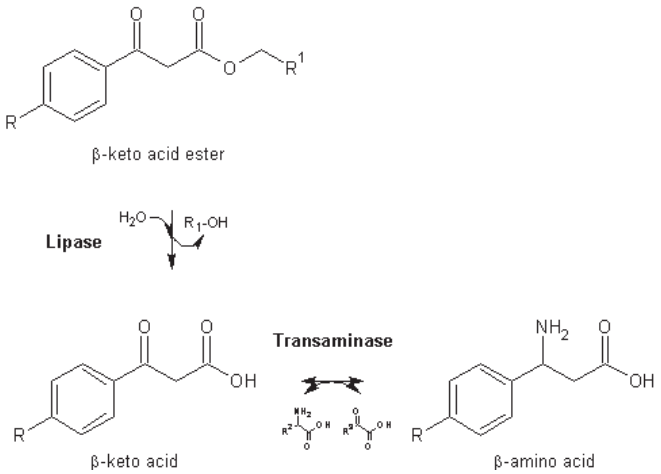
Figure 1



BTV21
Investigation of structural determinants for substrate specificity in zinc dependent alcohol dehydrogenases

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One of the key parameters that characterize a good biocatalyst, independent from its enzyme class, is its capability to accept and convert a large variety of different substrates. In case of zinc-dependent alcohol dehydrogenases (ADH) some representatives, such as *Candida parapsilosis* carbonyl reductase (CPCR2), show a remarkably broad substrate range including bulky hydrophobic carbonyl substrates as well as compounds as small as acetone [1]. Nevertheless, large groups of potentially interesting substrates such as cyclic ketones are rejected or converted with extremely poor activity [2]. In the recent years, several crystal structures of zinc-dependent ADHs were solved, including that of CPCR2, opening the possibility to get insights into the structural features that define substrate acceptance [3]. Based on this structural information, a mutational analysis was performed in order to attribute the observed limitations of the substrate spectrum of CPCR2 to certain structural features in its active site.

Figure 1: Active site of CPCR2 with selected residues for the mutational analysis (red), catalytic Zn2+ Ion (magenta), residues directly involved in catalysis (yellow) and NADH (elements).

The mutational analysis was performed as an exhaustive randomization of all residues that could be assigned to first shell of the active site of CPCR2. The mutant libraries were screened for their acceptance of difficult substrates probing different aspects of limited acceptance in a way that detailed information about its structural origin can be derived. The results suggest a small collection of active site residues as structural determinants which could provide appropriate targets for the rational design of variants with increased substrate scope. Given the fact that the active site structure among the zinc dependent alcohol dehydrogenases is considerably high, the determined hotspots may be transferred to other representatives of that large enzyme family.

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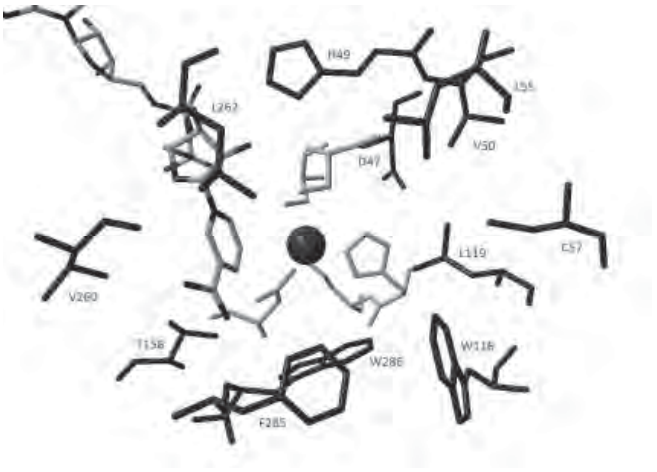
Figure 1



BTV22
Full surfactant resistance landscape of Bacillus subtilis lipase A

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Biocatalysis represents an environmentally friendly alternative to traditional chemical catalysis. However, biocatalysts are enzymes which have not been evolved by nature to operate under chemical process conditions, i.e. in the presence of organic solvents, surfactants, or ionic liquids. These compounds can interfere with the protein water shell, disturb electrostatic interactions and thus lead to unfolding, aggregation, and finally loss of enzymatic activity. Protein stability under these conditions can be increased by means of directed evolution. Current approaches, however, tend to apply semi-rational design with small library sizes to limit the screening effort by targeting sites of a protein known to be important for stability. However, rationales applied to date are derived from results which most likely failed to identify all potential sites for optimization since they are biased in many ways. (I) The experiments are usually designed with only limited coverage along the protein sequence or with respect to the substituted amino acids (e.g. epPCR, Ala-scanning mutagenesis). (II) The results only contain the most prominent variants with multiple mutations, leaving out valuable information about the influence of single mutations and additive effects. (III) The results only cover mutations with the highest positive impact but give no information about sites which only show little, none, or negative effects. We have conducted a full site saturation mutagenesis with complete

coverage to capture the resistance profile of a model enzyme in various non-conventional media. The lipase BSLA (encoded by the gene *lipA* from *Bacillus subtilis*) was chosen as model enzyme [1, 2]. BSLA is a minimal α/β-hydrolase and is biochemically well characterized, its crystal structure is known, and its biotechnological potential has been demonstrated. We have developed a screening method to test each variant for stability against elevated temperatures, changes in the preferred fatty acid chain length, and resistance against different types of surfactants. Here, we present the screening results against four different surfactants, namely anionic SDS, cationic CTAB, non-ionic Tween 80, and zwitterionic SB3-16. The large dataset we have obtained by using a robotic high-throughput screening method can now be used to identify all amino acids of BSLA involved in resistance and thus provide targets for protein engineering strategies. We anticipate that our results hold great potential to advance rational engineering strategies towards the customized design of a given biocatalyst under defined process conditions.

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BTV23

Synthetic biology as means for enzyme optimization: improving *Thermoanaerobacter tengcongensis* esterase by incorporation of non-canonical amino acids

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The incorporation of synthetic amino acids into proteins often leads to new structural and functional characteristics of the resulting protein congeners. This can be applied to improve the properties of industrially relevant lipolytic enzymes [1]. In previous experiments, we were able to identify several non-canonical amino acids (ncAAs) conveying beneficial properties to a thermoactive and thermostable lipase from *Thermoanaerobacter thermohydrosulfuricus* [2]. In the present study, genetic code engineering was used to produce congeners of an esterase (TTE) derived from the thermophilic, anaerobic bacterium *Thermoanaerobacter tengcongensis*. Using the supplementation-based incorporation method (SPI), TTE congeners were obtained by incorporation of (2S,4S)-4-fluoroproline (cFPro) and (2S,4R)-4-fluoroproline (tFPro), respectively. The two resulting variants were produced in shaking flasks using a proline-auxotrophic *Escherichia coli* strain. After purification TTE [cFPro] and TTE [tFPro] were subjected to a semi-automated high-throughput characterization in comparison to the respective wild-type enzyme. The global substitution of proline residues either by cFPro or by tFPro led to significant changes in enzyme properties so that the specific activity of the two obtained TTE congeners towards the synthetic substrate 4-nitrophenyl butyrate was raised more than threefold from 2.7 U/mg to 9.0 U/mg for TTE [cFPro] and 9.9 U/mg for TTE [tFPro].

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BTV24

Dried fungal Enzymes, a Lipase, a Threonine Aldolase, and a Laccase were entrapped in Epoxy Resin to coat a solid Support

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Application of microbial enzymes in technical processes is limited. Two factors are production costs and short half-life. To reduce production costs a cultivation technique for fungi growing under selective conditions minimizing the need for sterile technique was developed [1]. To recycle the enzyme a new immobilisation technique was developed. After drying, e.g. by lyopilisation or solvent-driven precipitation, the protein is mixed with the two components of epoxy resin. The mixture is placed as a second layer on solid supports simply spreading by a spatula or spraying. No treatment, e.g. chemical functionalization of the support surface is needed [2]. Three completely different fungal enzymes, a hydrolase, a lyase and an oxidoreductase were tested. *Thermomyces lanuginosus* lipase, expressed in *Aspergillus oryzae*, was immobilised by spreading with a spatula on a 1cm x1cm steel slide revealed an area specific activity of 170 mU/cm² using rape seed oil as substrate. This data is comparable with literature values where TLL was immobilised in glass fibre membranes [3]. Not satisfying is the result that only 5 to 20% of the added enzyme activity was recovered depending on the method. The second example was *Ashbya gossypii* threonine aldolase, expressed in *Escherichia coli*. It was found to be a tetramer with pyridoxal phosphate as cofactor. Freeze-dried enzyme in the polymeric network placed on steel slides revealed 91 mU/cm² detected by the release of benzaldehyde when phenylserine was used as substrate. *Myceliophthora thermophila* laccase expressed in *A. oryzae* was chosen as a third example for immobilisation. The enzyme is known to be a monomer each chelating four copper ions [4]. Freeze-dried laccase immobilised in epoxy polymer revealed enzymatically active units. The new immobilisation technique was shown to work with different enzyme types. Further investigations are necessary to investigate what is the nature of the method. Two options are possible either an entrapment in the three dimensional network of the polymer or a covalent binding during polymerisation.

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MICROBIAL CELL BIOLOGY

CBV01

The septal cross walls in filamentous cyanobacteria is perforated by a nanopore array enabling cell-cell communication and cell differentiation

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Cell-Cell communication is a basic character of multicellularity. Heterocyst-forming filamentous cyanobacteria show a sophisticated cell differentiation leading to a division of labor between two cell types in one filament. The cells of the filament are in close contact exchanging metabolites and signal molecules. The heterocyst is specialized in N_2 fixation and delivers inhibitors of differentiation and products of N_2 fixation to the neighboring vegetative cells. The vegetative cells perform oxygenic photosynthesis producing an aerobic environment and deliver products of CO_2 fixation to the heterocyst. Conversely the heterocyst protects the highly oxidation sensitive nitrogenase from O_2. Ultrastructure analysis showed that the outer membrane encases all cells of the filament like a skin, not entering the septum between neighboring cells. The individual cells of the filament have their own cytoplasmic membrane and are all surrounded by the peptidoglycan layer. Remarkably, the cross walls are not splitted during cell division and form one single sacculi along the entire filament, as shown by microscopy of isolated PG sacculi. To pass thru this septum, bulky cell-cell joining structures are probably gated thru nanopores of approximately 20 nm, which we could identify in electron micrographs of isolated septal cell walls. Approximately 100-160 nanopores are arranged in a semi regular pattern in the center of the septum forming a nano pore array. A mutant in a cell wall amidase, AmiC1 in *Anabaena* PCC sp. 7120 and AmiC2 in *Nostoc punctiforme*, is not able to form the nanopore array and looses the ability for cell-cell communication and cell differentiation. Hence, the PG forms a novel cell structure, involved in formation of a multi cellular prokaryote.

CBV02
Insights into the molecular basis of cell-cell interactions in phototrophic consortia

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"C*hlorochromatium aggregatum*" is a phototrophic consortium consisting of a central motile chemotrophic betaproteobacterium and its surrounding green sulfur bacterial epibiont *Chlorobium chlorochromatii*. Communication between the partners has been postulated as the central bacterium moves the consortium towards the light enabling the epibiont to conduct anoxygenic photosynthesis and as cell division of the symbiots is highly coordinated. In addition cellular contact sites of epibionts feature specific ultrastructures. To study the molecular basis of this symbiosis the genome of the epibiont was compared with the genomes of free-living relatives, revealing the presence of unique open reading frames. Three constitutively transcribed putative symbiosis genes (Cag_1919, 0616, 0614) were analysed in detail. Cag_1919 contains a RTX domain which is typically found in Gram-negative pathogenic bacteria. Cag_0614 and Cag_0616 represent the largest open reading frames in the prokaryotic world known to date, with lengths of 110418 and 61938 bp, respectively. Cag_0616 and 0614 contain several parallel beta-helices and 0616 harbors two βγ-crystalline Greek key motifs. To facilitate the intracellular localization of these symbiosis factors, recombinant proteins were expressed in *E. coli* and used to produce antibodies for immunogold labelling and fluorescence microscopy. Fluorescence microscopy localized the Cag_1919 protein between the two symbiotic partners. This result is supported through western blotting which detects the protein in the periplasmic fraction of the consortia and the free-living epibionts. High resolution immunogold labelling localized the protein Cag_1919 mainly at the contact site and in the cytoplasmic membranes of the symbiotic partners. Bioinformatic analysis predicted a transmembrane region in the protein. This region may also lead to the insertion of the protein during heterologous expression in *E. coli* cells, which leads to elongated cell types. In contrast to Cag_1919, immunogold labelling localized Cag_0614/0616 in the cytoplasm of the central bacterium. Dot blotting of the cell fractions of the consortium and the free-living epibiont supported these Results:

CBV03
Regulation of amidase-mediated peptidoglycan hydrolysis in *Vibrio cholerae*

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Balance between peptidoglycan synthesis and hydrolysis is crucial for maintenance of cell shape and cell division. Peptidoglycan cleavage at the cell division site is mediated by amidases, hydrolytic enzymes that cleave the bond between the side peptide and the glycan chain. Most gamma-proteobacteria, including *Escherichia coli*, encode several amidases that are functionally redundant. The enteric pathogen *Vibrio cholerae* contains only a single amidase, AmiB. In this report, we show that *V. cholerae* AmiB is crucial for cell division and growth. We further show that AmiB is regulated by two functionally redundant proteins, EnvC and NlpD, which are required for its localization to the cell division site. Transposon insertion sequencing analysis revealed that normal growth in absence of NlpD requires EnvC, FtsE, and FtsX. Importantly, NlpD, but not EnvC, is required for optimal colonization of the intestine in the infant mouse model of cholera. Overproduction of AmiB, EnvC or NlpD leads to sphere formation in the absence of the major peptidoglycan synthase, PBP1A, and PBP1A forms a complex with AmiB, EnvC, NlpD and FtsX. Thus, *V. cholerae* effectively utilizes accessory proteins to regulate peptidoglycan hydrolysis through a single amidase, and to ensure adaption of the cell wall in response to insults specific to the host environment.

CBV04
Another brick in the wall - Evidence for peptidoglycan in the cell walls of planctomycetes

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Planctomycetes are ubiquitous, environmentally important bacteria that comprise a set of unique features. Their cell division for example is based on an asymmetric FtsZ-free mechanism, known as budding. In addition, *Planctomycetes* possess complex life cycles. Previous studies suggested a compartmentalized cell plan and a cell wall that consists of proteinous structures instead of peptidoglycan. However, peptidoglycan is a major cell wall component present in all free-living bacteria but until now has been declared absent in p*lanctomycetes*. Since planctomycetes are generally assumed to lack peptidoglycan, we reinvestigated this issue using our model organism *Planctomyces limnophilus,* employing classical and novel state-of-the-art methods. Using cryo-electron tomography, we found a cell wall layer, being typical in its appearance for peptidoglycan, between two membranes. Furthermore we showed, based on planctomycetal genome data, that *Planctomyces* species indeed have all genes necessary to synthesize peptidoglycan. Employing enzymatic assays, we demonstrated that *P. limnophilus* is susceptible for lysozyme treatment, indicating that target structures for the enzyme are present in the form of peptidoglycan cell wall components. For the first time we were able to identify diaminopimelic acid (DAP) in intact planctomycetal cells and in isolated sacculi. DAP is a non-proteinogenic diamino acid which serves as trifunctional linker in the in the cross-linkage of the of the sugar backbone in peptidoglycan consisting of alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) units. Using modern GC/MS based analysis technology, we demonstrated that planctomycetal sacculi gave higher DAP-signals than entire cells, as expected for this step of enrichment. We also detected the peptidoglycan amino sugars MurNAc and GlcNAc in *P. limnophilus* cells using an enzymatic and biochemical assay for analyses. Consequently, we propose that, against common belief, *Planctomyces* and related genera contain peptidoglycan in their cell walls. This finding is in good agreement with recent studies that point towards a Gram-negative like architecture of *Planctomycetes*.

CBV05
The response of Bacillus pumilus to hydrogen peroxide provoked oxidative stress

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Introduction: The gram positive soil bacterium *Bacillus pumilus* is a candidate for the development of new production strains used as microbial hosts in industrial fermentation processes. Soil bacteria are exposed to changing environmental conditions and have to adapt rapidly to unfavourable starvation and stress conditions, such as oxidative stress. Adaptation to stress conditions is achieved by the induction of general and specific stress proteins. Stress and starvation conditions may not only occur in natural habitats but also during fermentation processes and could influence the growth of the production host as well as product formation and product quality.
Materials and Methods: Transcriptomics and proteomics using 2D-gel electrophoresis were applied to study the response of *B. pumilus* cells subjected to hydrogen peroxide provoked oxidative stress. Low molecular weight thiols were detected and quantified usingion pairing HPLC. Furthermore, catalase activity in cell extracts was analyzed.
Results: Although *B. pumilus* is rather conspicuous, because important proteins involved in detoxification of hydrogen peroxide, like KatA, are missing from its genome sequence, the cells survive significantly higher amounts of hydrogen peroxide when compared to *B. subtilis*. The reprogramming of gene/ protein expression during oxidative stress was investigated as well as a possible influence of low molecular weight thiol compounds. Instead of KatA, a gene annotated as catalase KatX2 was

significantly induced in *B. pumilus* cells at the transcriptional and translational level under these conditions. High intracellular levels of bacillithiol were detected in peroxide stress *B. pumilus* cells. Furthermore, the catalase activity of cell extracts of *B. pumilus* was higher than the activity of cell extracts from other bacilli.
Discussion: We analyzed the response of *B. pumilus* to oxidative stress caused by hydrogen peroxide using transcriptomics, proteomics and metabolomics methods to resolve the reasons why the cells survive such high concentrations of this compound.Since oxidative stress is a crucial topic of high cell density industrial fermentations,the results of our study might be used to construct and optimize production strains which are more resistant to process-related oxidative stress.

CBV06
A dynamin-like protein that reacts to membrane stress in *Bacillus subtilis*

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Membrane fusion and fission are rapid, dynamic processes that occur in eukaryotic and prokaryotic cells to facilitate generation and transport of vesicles, induce membrane trafficking, maintain cell shape and size. Proteins of dynamin superfamily play an important role in maintenance of membrane dynamics. Dynamins are GTPase molecules demonstrating functions such as vesicle scission, division of organelles, cytokinesis and microbial resistance. Recently, a bacterial candidate (DynA) of the eukaryotic dynamin-like proteins (DLPs) involved in membrane remodelling events was identified in *B. subtilis*. DynA is a 136 KDa protein associated with the cell membrane of *B. subtilis*. On account of sequence homology to other bacterial and eukaryotic dynamins, similar biochemical properties such as GTP hydrolysis and membrane fusion, DynA is classified as a member of the dynamin superfamily. Its structure is remarkable as it seems to have developed from a fusion event between two molecules, thus consisting of two separate GTPase and two dynamin-like subunits, namely D1 and D2 respectively. Both fragments, D1 and D2 share 27% similarity and are united in a single-polypeptide. Individually purified D1 and D2 subunits were found to be catalytically inactive, proposing the requirement of both subunits being in close proximity for proper functioning of DynA. Structural resemblance to eukaryotic DLPs, suggest an equivalent functional role of bacterial DLPs (BDLPs), in membrane remodelling. However, no such definite membrane-associated function in vivo has been identified for BDPLs. Our study focuses on biochemical and cell biological characterisation of DynA to unravel the function of BDLPs. We could show *in vitro* tethering and fusion of liposomes in the presence of DynA. Also, we could observe division-septa deformations upon salt-stress in cells lacking *dynA*. DynA-GFP responded to membrane-stress (nisin treatment) by localising to membrane as distinct foci and not spreading through out the membrane. Another major observation was that cells lacking *dynA* showed higher sensitivity (about 40% more plaques) to bacillus phages. All our results suggest a protective role of dynA upon membrane stress in bacteria.

CBV07
Analysis of the alkaline shock protein 23 (Asp23) in *Staphylococcus aureus*

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DUF322 domain proteins are present in multiple copies in every genome of Gram-positive bacteria sequenced so far. Contrasting the wide distribution of these proteins nothing is known about their function. In the genome of the human pathogen *Staphylococcus aureus* four proteins that belong to the DUF322 protein family can be identified. Two of these proteins, namely the alkaline shock protein 23 (Asp23) and SAOUHSC_02443 are encoded in a tetracistronic operon. With a copy number of 25.000 molecules per cell Asp23 is one of the most abundant proteins in the soluble protein fraction of stationary *S. aureus* cells. Using a combination of fluorescence microscopy, electron microscopy and the bacterial two-hybrid system (BACTH), we showed that Asp23 is a membrane associated protein. Interestingly, membrane association of Asp23 is dependent on SAOUHSC_02443, the second DUF322 protein encoded in the *asp23*-operon, which we termed Asp23 membrane anchoring protein (AmaP). Furthermore, using fluorescence microscopy and the BACTH we identified domains and residues critical for Asp23: SAOUHSC_02443 interaction and membrane localization. The BACTH analyses also indicated that Asp23 forms oligomers and indeed purified strep-tagged Asp23 protein formed large flexible helical polymers *in vitro* as visualized by electron microscopy of negatively stained protein samples. Using DNA-microarray analyses, we showed that a deletion of the *asp23* gene was followed by increased transcription of genes with a function in the cell wall stress response of *S. aureus*. Deletion of the Asp23 anchor, *amaP*, resulted in a very similar transcriptional response emphasizing the importance of Asp23 membrane association for its function. In summary we could show that Asp23 is membrane associated and may have a function related to cell wall stress in *S. aureus*.

CBV08
The zinc-buffering system of *Cupriavidus metallidurans* retards efficient zinc allocation in a Δ*zupT* mutant

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In *Cupriavidus metallidurans,* the zinc importer ZupT is required at low zinc concentration, its synthesis is up-regulated by zinc starvation, and its absence leads to a complicated mélange of pleiotropic effects (Herzberg, Bauer and Nies, 2014). To understand the molecular reasons behind these effects, a top-down quantitative proteomic approach was used a High-Definition-MS-System, a multiplex mass spectrometry method coupled with ion mobility separation (HD-MS^E) for the identification and quantification of proteins in the bacteria cell. Protein extracts of the megaplasmid-free *C. metallidurans* parent strain AE104 and its Δ*zupT* mutant were analyses with this method. Of 6120 predicted proteins for strain AE104, 2928 proteins (48%) identified in the proteome. From the initial quantitative results in fmol quantities, protein concentration and amount of cells, the number of proteins per cell could estimate. We are able to sort 90% from the quantity of detected proteins to KEGG pathways, but only 35% of different proteins. The Data gives a deep view into the proteome of *Cupriavidus metallidurans* and shows several differences between wildtyp and mutant strains. This lead to an estimate of the overall number of predicted zinc-binding proteins in both strains in comparison the zinc content of a cell. Thus, the zinc pool of *C. metallidurans* is probably composed of the overall number of zinc binding sites of the cellular proteome. Parts of the zinc pool are rather unspecific and can transiently also accept other divalent transition metal cations.

CBV09
PPK1 and PPK2-independent formation of polyphosphate in *Ralstonia eutropha*

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Polyphosphate (PP) is a linear polymer consisting of 10 to several 100 phosphate residues and is present in all living organisms. PP has more functions than that of a mere phosphate storage compound [1]. In bacteria, the polymer often forms dense particles, known as volutin or PP granules. *Ralstonia eutropha* H16 is famous for its ability to accumulate large amounts of PHB. However, *R. eutropha* also has PP granules, though they are much smaller in size than PHB granules. Polyphosphate kinases (PPKs) catalyse the formation of PP from ATP or GTP. Two different PPKs are annotated in the *R. eutropha* genome (PPK1 and PPK2). Fluorescence microscopical analysis of fusions with eYFP revealed that PPK1-eYFP co-localized with PP granules whereas PPK2-eYFP was located near the cell poles and did not co-localize with PP granules. Expression of PPK1 in *E. coli* but not of PPK2 resulted in the formation of PP granules and confirmed that PPK1 is a PP-synthesizing enzyme. The function of PPK2 in *R.*

eutropha is unknown. To investigate the physiological roles of *ppk1* and *ppk2* both genes were deleted from the chromosome. Surprisingly, the double mutant Δ*ppk1* + Δ*ppk2* still was able to form PP although in less numbers. Obviously, *Ralstonia* has at least one additional PP synthesizing enzyme. Results of *in silico* screening, proteome analysis and biochemical investigations aiming at the identification of novel proteins that are involved in PP granule formation will be shown.

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CBV10
Bacterial membrane domains in super resolution

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The bacterial membrane represents a barrier as well as an interaction platform between the dynamic yet ordered cytoplasm and a changing environment. Growing evidence suggests that the lipids of this bilayer are not randomly distributed but are located to regions with specific physical properties e. g. the highly curved cell poles, or are associated with proteins attracting certain lipids. The family of flotillin/reggie proteins serves as a model to understand this protein-lipid specificity as they exclusively localize to membrane subregions. We have started to characterize the two flotillin homologs of *B. subtilis,* FloA and FloT, that assemble into individual structures within the membrane *in vivo*. We show that FloA accumulates without any additional factor into higher order structures of more than 10 subunits *in vitro* regardless of the presence of a transmembrane domain. Furthermore, we examined flotilllin assemblies *in situ* by super resolution STED microscopy. By visualizing FloT and FloA as well as further microdomain-associated proteins like KinC and NfeD2 we observed a characteristic microdomain diameter of about 80 nm. Despite a conserved cluster size, the distinct flotillin assemblies of the two homologs show differences in associated proteins and in the speed of movement through the lipid bilayer, with FloA diffusing much faster than FloT. We have also investigated the size of flotillin clusters in different eukaryotic cells, and found that the diameter of the assemblies is similar to that of the bacterial orthologs, and thus that microdomain size be an intrinsic property within this protein family and is therefore conserved over all domains of life.

CBV11
The structure of the halobacterial gas vesicle by cryo-electron tomography

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Halobacterium salinarum, an extreme halophilic archaeon found in high salt environments such as hypersaline lakes and salterns, produces air-filled protein shells to increase its buoyancy and help it float near the water's surface. These gas vesicles are formed by the 8 kDa structural protein GvpA, which arranges into a riblike helical tube of 100 - 200 nm in diameter and up to 1.5 µm in length with characteristic conical caps. The alpha-helical protein GvpC attaches to the outside of the gas vesicle hull [1], very likely additionally stabilizing the vesicle wall against pressure variations [2]. Currently, a crystal structure of GvpA is not available; however, prediction models suggest a stable dimer with hydrophilic alpha-helices on the convex side and strongly hydrophobic beta sheets facing inwards [3,4,5]. The building blocks are thought to form regular arrays and the helical ribs of gas vesicles with a characteristic distance of 4.6 nm. However, the architecture of the gas vesicle wall and cones is still speculative. In our approach to study the molecular structure of GvpA, we applied cryo electron tomography, using a 300 keV Titan Krios transmission electron microscope equipped with an energy filter and a particularly sensitive direct electron detector for image recording. Imaging conditions were further improved by a newly developed phase plate that enabled us to exploit the full range of structural information (spatial frequencies) at enhanced contrast. We investigated gas vesicles possessing a point mutation resulting in particularly thin tubes that are advantageous for optimizing the resolution of 3D reconstructions. We obtained the 3D structure of GvpA arrangements from the cylindrical parts of the vesicles and analyzed the conical tips and structural transition areas to get insight into the arrangement of GvpA in less regular regions of the vesicles.

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CBV12
Analyses of chromosome and replisome dynamics in *Myxococcus xanthus* reveal a novel chromosome arrangement

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Bacteria organize their chromosome spatially in a highly reproducible pattern to ensure that both daughter cells receive a correct chromosome complement during cell division. Bacterial chromosome organizations include some with the origin of replication and terminus at midcell or origin and terminus at or close to opposite cell poles. Also significantly different replisome dynamics have been reported for different bacterial species.
Here, we analyzed the spatial organization and temporal dynamics of the 9.1 Mb circular chromosome in the rod-shaped cells of *Myxococcus xanthus*. For chromosome segregation, *M. xanthus* uses a *parABS* system, which is essential, and lack of the *parS*-binding protein ParB results in chromosome segregation defects as well as cell divisions over nucleoids and the formation of anucleate cells and cells with an aberrant chromosomal content. From the determination of the dynamic subcellular location of six genetic loci, we conclude that in newborn cells *ori*, as monitored following the ParB/*parS* complex, and *ter* regions are localized in the subpolar regions of the old and new cell pole, respectively and each separated from the nearest pole by approximately 1µm. The bulk of the chromosome is organized between the two subpolar regions, thus leaving the two large subpolar regions devoid of DNA. Upon replication, one *ori* region remains in the original subpolar region while the second copy segregates unidirectionally to the opposite subpolar region followed by the rest of the chromosome. In parallel, the *ter* region of the mother chromosome relocates, most likely passively, to midcell, where it is replicated. Consequently, after completion of replication and segregation, the two chromosomes show an *ori-ter-ter-ori* organization with mirror symmetry about a transverse axis at midcell. Upon completion of segregation of the ParB/*parS* complex, ParA localizes in large patches in the DNA-free subpolar regions. Using an Ssb-YFP fusion as a proxy for replisome localization, we observed that the two replisomes assemble in the subpolar region of the old pole and then move towards *ter* at midcell reversibly splitting and merging to form two foci or one focus suggesting that the two replisomes move independently of each other on the two replichores. We conclude that *M. xanthus* chromosome organization and dynamics combine features from previously described systems with novel features leading to a novel spatiotemporal organization.

CBV13
The role of the rhomboid protease in virulence factor secretion by Listeria monocytogenes.

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Introduction: The Gram-positive bacterium Listeria monocytogenes is a facultative intracellular pathogen that parasites cells of different types. A number of surface and secreted proteins were established as virulence factors required for L. monocytogenes virulence. The supplementary secretion system SecA2 was shown to be responsible for secretion of a number of virulence factors. Previously, we revealed presence additional regulatory elements that control virulence factor secretion into the medium by processing C-terminal transmembrane domains (Sapenko et al., 2011). Implementation of such a function can be carried out only with the intramembranous type of protease activity. The analysis “in silico” and experiments showed that in the genome of Listeria only three classes of proteases from this type of activity: the proteases I and II is responsible for the processing of the signal peptide, as well as rhomboid and S2P proteases. Rhomboid proteases form a small family of serine proteases with substrates

that are usually cleaved within a membrane-spanning segment. Rhomboid is an intramembrane serine protease responsible for the proteolytic activation of *Drosophila* epidermal growth factor receptor (EGFR) ligands. Rhomboid peptidases (proteases) play key roles in signaling events at the membrane bilayer. Understanding the regulation of rhomboid function is crucial for insight into its mechanism of action. However, the common function of prokaryotic rhomboids is unknown.

Materials and Methods: *Bacterial strains* The mutant strain *GIMins1337 with insertion in the rhomboid encoding lmo1337 gene and the revertant without insertion were obtained in the EGDe strain background. L. monocytogenes was* grown in BHI supplemented with 0,2 % activated charcoal. Membrane proteins were prepared by bacterial cell boiling in 1xLaemmli buffer. Secreted proteins were precipitated with TCA. Proteins were separated onto a 12,5% SDS-PAGE gel. Protein band of interest was excised from the gel and was subjected to proteolysis with trypsin and analyzed with MALDI-TOF mass spectrometry. Peptides were identified using a search engine Mascot.

Results and Discussion: The mutation in the gene lmo1337, encoding rhomboid peptidase, leads to a change in profiles of membrane-associated and secreted proteins *L.monocytogenes* including affecting the representation in the membrane fraction of proteins associated with virulence. Some proteins were identified as follows elongation factors Tu and G, glyceraldehyde -3 - phosphate dehydrogenase and digidrolipoamid acetyltransferase. These data suggest that rhomboid peptidase may participate in processing of membrane proteins of Gram- positive bacterium *L. monocytogenes*.

Figure 1

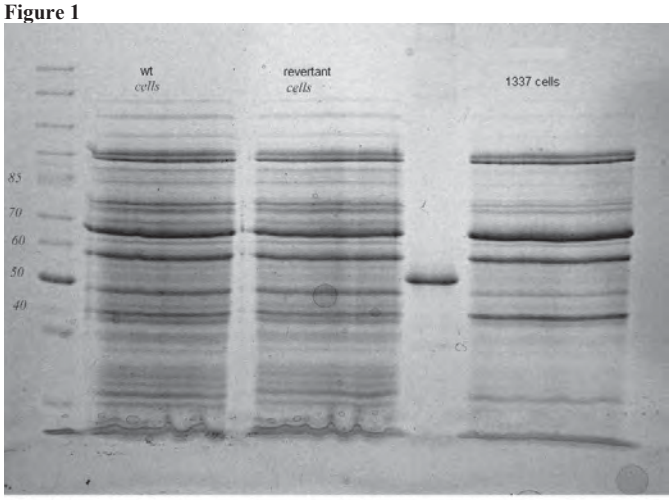

Fig.1. SDS-PAGE of proteins membrane fraction of bacteria strains lmo1337, revertant and EGDe(wt).

Figure 2

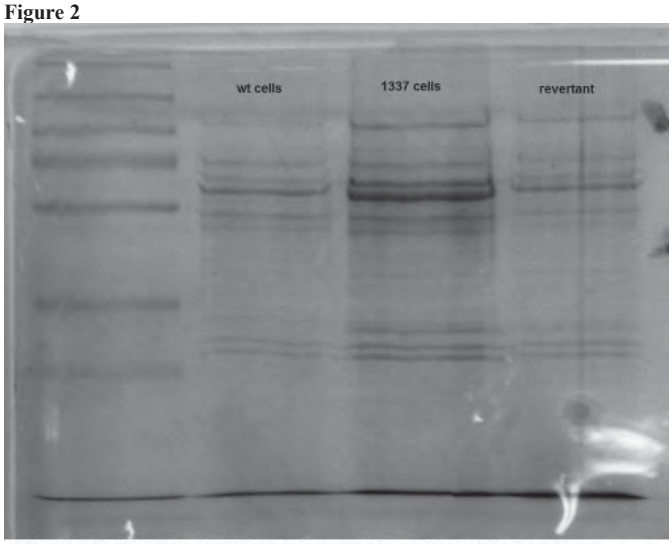

Fig.2. SDS-PAGe of proteins secreted fraction of bacteria strains lmo1337, revertant and EGDe(wt).

CBV14

Swarming Bacteria as Freight Haulage Systems - how *Paenibacillus vortex* physically transports antibiotic resistant cargo bacteria to the mutual advantage of both species

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Swarming bacteria are challenged by the need to invade hostile environments. Swarms of the flagellate, pattern-forming, Gram-positive bacterium ***Paenibacillus vortex*** can collectively transport (i.e. physically move over tens of cm) non-motile microorganisms. These cargo are captured within the swarming colonies that can translocate an otherwise non-motile microorganism at > 1 cm/h. Transport is mediated by a specialized, phenotypic subpopulation of ***P. vortex***. ***P. vortex*** can invade toxic environments by carrying antibiotic-degrading bacteria. Swarms of beta-lactam antibiotic (BLA)-sensitive ***P. vortex*** can move beta-lactamase-producing, resistant, cargo bacteria to detoxify BLAs in their path. In the presence of BLAs, both transporter and cargo bacteria gained from this temporary cooperation; cargo bacteria with higher BLA resistance promoted faster spread. ***P. vortex*** transported only the most beneficial antibiotic-resistant cargo (including clinical isolates) in a sustained way. ***P. vortex*** displayed a bet-hedging strategy that promotes the colonization of non-toxic niches by ***P. vortex*** alone; when detoxifying cargo bacteria were not needed they were lost. These observations have implications for the dispersal of antibiotic-resistant microorganisms and strategies for asymmetric cooperation. Swarming bacteria are generally studied as single species swarms - perhaps they should be considered more as a highly dynamic ecosystem.....? Complex multispecies colonies cooperatively moving over agar plates containing ampicillin. Panel a shows a periodically expanding colony 14 cm across, panels (**b** and **c**) moving microcolonies (hundreds of microns across). In all cases the combination of antibiotic resistant cargo (indistinguishable from transporter in **a**; green in **b** and **c**) and sensitive transporter bacteria (red in **b** and **c**) achieved tasks in terms of dispersal and survival neither could individually.

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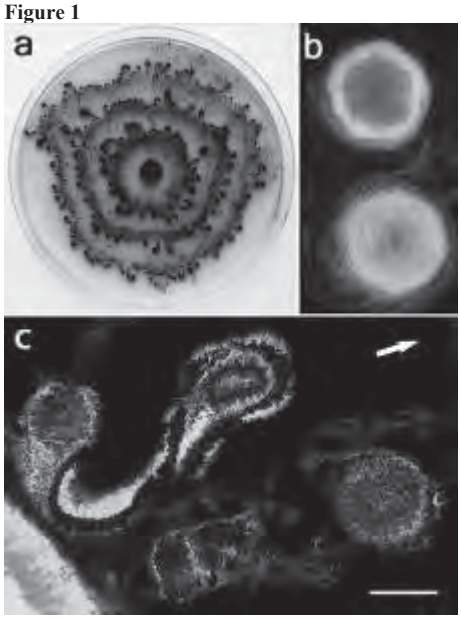
Ariel G, Shklash A, Kalisman O, Ingham CJ, Ben Jacob EBJ (2013) From organized internal traffic to collective navigation of bacterial swarms. New J Physics. **15** 125019.

Figure 1



MICROBIAL DIVERSITY AND ECOLOGY (INCL. SOIL AND WATER MICROBIOLOGY)

DEV01
High activity of acteogenic bacteria in anaerobic biogas fementation plants using complex lignin degradation derivatives

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Many acetogenic bacteria are able to grow heterotrophically on a variaty of organic compounds like sugars, alcohols, and aldehyd- or methylgroup containing aromates, but some can also grow lithoautotrophically using $H_2 + CO_2$. The produced acetate is an improtant substrate in methanogenic anaerobic habitats. Such an anaerobic food web leading to methane production is in operation at biogas plants. However, the role (if such) of acetogenic bacteria has not been studied in detail. We use a multidisciplinary approach to assess the acetogenic population in a biogas plant producing methane from silaged crops, cow and chicken manure. Our approach includes measurments of the catalytic activity of characteristic enzymes and a throughout monitoring of the microbial acetate production rates dependent on various carbon sources. Further classic cultivation techniques are applied to enrich acetogenic bacteria from the sludge. For the first time the activities of formate dehyrdogenase, carbon monoxide dehydrogenase and methylene-tetrahydrofolate reductase were monitored in sludge samples of a biogas plant. Furthermore, these enzyme activies were also assessed in the set-up quantifing the actetate production rate based on lignin degradtion derivatives. Our approace revealed a pronounced actetate production rate (incubated under $H_2 + CO_2$ atmosphere) of about 3.5 µmol acetate g_{sludge}^{-1} h^{-1} which equaled the acetoclastic methane production rate of the system. The produced acetate from phenylacrylated aromates and hydroxybenzoeic acid stoichiometrically equals the available methylgroups present in the substrate. Furthermore, we were able to enrich and purify cultures of acetogens from the sludge samples and verified their phylogenetic relationship to the order *Clostridiales*. However, none of the cultivated acetogens was closely related to cultivated acetogenic species possibly indicating a yet unknown phylogenetic diversity of acetogenic bacteria in biogas plants. In summary, our detailed study of the physiology and biochemistry of the acetogenic population will help to increase productivity and optimize the effective mineralization within biogas plants. Further, it will increase our knowledge of the function and interaction of complex microbial communities present in anaerobic habitats.

DEV02
Investigation of the archaeal diversity during anaerobic digestion of maize silage and municipal waste water

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In the last years, the interest in renewable energy sources has increased considerably. To replace the commonly used fossil fuels, a variety of strategies have been developed. One possible method that can combine waste treatment with the recovery of energy is the anaerobic digestion. This process involves diverse microorganisms with different requirements to their environment. Understanding the microbial composition during anaerobic digestion is essential to run an effective process and avoid perturbations. Therefore, the aim of this study is to gain a detailed knowledge about the diversity and abundance of microorganisms in biogas reactors. The microbial community, especially methanogenic archaea, in samples of a one stage anaerobic digester fed with maize silage and chicken manure (A) as well as a digester using municipal waste water as substrate (B) were analyzed with cultivation independent methods, such as Denaturing Gradient Gel Electrophoresis (DGGE) and sequencing of the DNA of single bands. Additionally, a 16S rDNA clone library was generated and Catalyzed Reporter Deposition Fluorescence *in situ* Hybridisation (CARD-FISH) was used to quantify and differentiate the microbial community within the anaerobic digester by the use of different specific probes. The samples revealed only limited methanogenic diversity by DGGE and sequencing analysis. The genera *Methanoculleus* and *Methanosarcina* could be identified as the most abundant methanogenic archaea in digester A. Additionally, the genera *Methanosphaera* and *Methanobacterium* could be detected. As expected, alterations of the chemical conditions in the digester, like increasing ammonia concentrations, influenced the microbial community and lead to an increase in signal intensity of bands corresponding to the genus *Methanoculleus*. By CARD-FISH a bacteria to archaea ratio of around 10:1 was derived and *Methanoculleus* was shown to be the most abundant archaeal subgroup. Samples originating from digester B differed in microbial composition, as a distinct archaeal fingerprint was visualized. Since a larger number of *Methanobacteriales*-specific DGGE signals was detected, a greater diversity of this subgroup in the anaerobic digestion of municipal waste is assumed.

DEV03
Investigation of H_2-dependent methanogenesis in biogas plants

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Production of biogas in an anaerobic digester is a microbiological multi-step process. In this process organic biomass is degraded to methane and carbon dioxide, the main components of biogas. The very complex microbial community involved can be divided into hydrolyzing, fermenting, syntrophic and methanogenic. Only methanogenic archaea are able to generate methane using H_2+CO_2 (hydrogenotrophic pathway), acetate (acetoclastic pathway) or methylated substances (methylotrophic pathway). Therefore, the methane forming archaea are key players of the biogas process. To learn more about the organisms involved in this process, 13 methanogenic strains were isolated from sludge of a biogas plant. Sequence analysis of the 16S-rDNA and the *mcrA*-gene, as well as physiological characterizations suggests that two of the isolates are so far undescribed species of the genera *Methanosarcina* and *Methanobacterium*. As molecular hydrogen is an important intermediate in the biogas process, we also investigated H_2-dependend methanogenesis *is situ*. Supplementing fresh biogas sludge with various amounts of H_2 revealed that under normal conditions this pathway is operating below its maximal capacity. By relating the activity of methanoarchaeal F_{420}-dependend hydrogenase and total (viologen-dependent) hydrogenase activity in sludge samples we aim at establishing new parameters for monitoring of anaerobic digestion.

DEV04
Population dynamics in thin stillage based biogas reactors

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Sustainable biorefinery concepts focus on efficient conversion of thin stillage, which is a byproduct of bioethanol production, to biogas. In order to gain knowledge about the composition of microbial consortia that are able to degrade thin stillage at high temperatures, a microbial consortium was analyzed in a laboratory scale reactor. Based on 17,400 16S rRNA gene fragments pyrosequencing revealed a dominant cluster of bacteria associated with the phylum *Thermotogae*. Alignment analyses using representative sequences identified *Defluviitoga tunisiensis* as its closest relative. Additional abundant clusters were taxonomically classified as *Elusimicrobia* and *Firmicutes*, and those of methanogenic archaea belonged to *Methanosaetaceae* and *Methanobacteriaceae*. Representing 65 % of the methanogenic population, acetate-utilizing *Methanosaetaceae* was shown to be the most dominant family. Using reactor digestate for enrichment experiments, pure cultures of mainly Firmicutes representing species were isolated. Additionally, *Methanothermobacter thermautotrophicus* and a yet-uncultured species of the genus *Methanosaeta* were enriched and characterized. Population dynamics were analyzed during reactor acidification using FISH technique based on probes specific for 16S rRNA. While bacterial abundance decreased from 77 to 49 %, methanogenic *Euryarchaeota* remained stable at 14 to 17 % of the overall population. However, within the methanogenic community successive dominance was observed between *Methanobacteriales* and *Methanosaetaceae*. Furthermore, an increase of bacterial diversity was detected by DGGE analysis if treatment time was reduced. In order to investigate the adaptation phase of the microbial community that degrades thin stillage under mesophilic conditions, DGGE analysis was performed. *Methanosaeta* and an

unclassified member of hydrogenotrophic *Methanobacteriaceae* appeared as the dominant organisms of the methanogenic population within ten weeks; the latter was sensitive to substrate deficit. The data obtained in this research project enhance our understanding of microbial digestion of industrial waste material rich in proteins and will help establish optimal process conditions in operations beyond laboratory scale.

DEV05
Potential Interactions of Anaerobes in Fen Rhizospheres

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Natural wetlands are the single most important source of methane. The emission of methane increases with increasing availability of organic carbon and can be highest where plant roots occur. Although methanogens have been detected in rhizosphere soil of peatland plants, little is known about the trophic links of methanogens to other anaerobic processes in such habitats. The objective of this study was to identify potential interactions between methanogens and other anaerobes in the rhizosphere of plants that grow in a methane-emitting fen. This objective was addressed with cultivation methods and molecular analyses of *mcrA*, 16S rRNA genes, and genes that encode for different hydrogenases. Formate-H_2-lyase-containing taxa, acetogens, and methanogens competed for formate in anoxic microcosms with roots and soils of *Carex rostrata* and roots of *Molinia caerulea*, but not in anoxic microcosms with intact or decaying roots of *Eriophorum vaginatum* or soils of *M. caerulea*. Methanogens outcompeted acetogens in root-free soil microcosms, but acetogens outcompeted methanogens in soil-free root microcosms. Facultative aerobes and an acetogenic enrichment culture were obtained from the root surface of fen-derived plants. Facultative aerobes were closely related to *Citrobacter* and *Hafnia* species and produced H_2 from formate. The acetogenic enrichment culture utilized formate and H_2, and contained the acetogen-containing taxa *Clostridiaceae* and *Veillonellaceae*. *McrA* sequences related to 9 species-level *mcrA* phylotypes and functionally diverse hydrogenase gene sequences were detected from roots of *C. rostrata*. Most *mcrA* sequences were affiliated with *Methanobacteriaceae*, *Methanoregulaceae*, and *Methanosarcinaceae*, indicating the association of hydrogenotrophic and acetoclastic methanogens with roots. The relative abundance of *mcrA* genes affiliated with *Methanosarcinaceae* was higher in enrichments with formate than without. *Methanosarcinaceae* were likely stimulated by H_2 that was transiently present during the incubation. Detected [FeFe]-and [NiFe]Group 4 hydrogenase gene sequences were related to *Proteobacteria* and *Firmicutes*, suggesting a potential contribution to H_2 production. The relative abundance of [FeFe]-hydrogenase genes related to *Proteobacteria* were more abundant in enrichments with formate than without, suggesting that formate-dependent H_2 production is dominated by *Proteobacteria*. [NiFe]Group 1 uptake hydrogenase gene sequences were related to *Acidobacteria*, *Deferribacteres*, and *Proteobacteria*, suggesting that such taxa might compete for H_2. The collective results reinforce the likelihood (a) that hydrogenase-containing taxa, acetogens, and methanogens are trophically linked via formate and H_2 in the rhizosphere of *C. rostrata* and (b) that the occurrence and trophic links of anaerobes vary in the rhizosphere of different plant species.

DEV06
Isolation and environmental distribution of type Ib methane oxidizing bacteria

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Methane oxidizing microorganisms perform an important function by preventing emissions of the greenhouse gas methane to the atmosphere. To date known bacterial methanotrophs belong to the proteobacteria, verrucomicrobia and the NC10 phylum. Within the proteobacteria, they can be divided into type Ia, type Ib and type II methanotrophs. Type Ia and type II are well represented by isolates, however, the vast amount of type Ib methanotrophs could not be cultivated so far. Based on the *pmoA* gene (encoding the key enzyme of the methane oxidation pathway), 10 major type Ib lineages could be defined, of which only 3 are represented by isolates. In this study, we compared the distribution of the type Ib lineages in different environments. Whereas the cultivated methanotrophs (Methylococcus and Methylocaldum) are found in landfill and upland soils, lineages that are not represented by isolates are dominant in freshwater environments such as paddy fields and lake sediments. Thus, we observed a clear niche differentiation within type Ib methanotrophs indicating that this subgroup is highly diverse. Our subsequent isolation attempts resulted in one type Ib pure culture and two enrichments. The pure culture was further characterized and showed to be an obligate methanotroph. It contains C16:1w9c as major phospholipid fatty acid in its membrane which has not been found in other methanotrophs. The currently ongoing genome sequencing and analysis will give further insights into the lifestyle of this exciting new type Ib methanotrophic isolate.

DEV07
Bacterial metabolism of isoprene: A much neglected atmospheric trace gas

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Isoprene (2-methyl, 1,3 butadiene) is a much neglected climate-active atmospheric trace gas that is released into the atmosphere in similar quantities to that of methane, making it one of the most abundant trace gases. Large amounts of isoprene are produced by trees but also substantial amounts are released by microorganisms. The consequences on climate are complex. Isoprene can indirectly act as a global warming gas but in the marine environment it is also thought to promote aerosol formation, thus promoting cooling through increased cloud formation. We have been studying bacteria that grow on isoprene. These aerobic bacteria appear to be widespread in the terrestrial and marine environment. *Rhodococcus* AD45, our model organism, oxidizes isoprene using a soluble diiron centre monooxygenase which is similar to soluble methane monooxygenase. The physiology, biochemistry and molecular biology of *Rhodococcus* AD45 will be outlined, together with genome analysis, transcriptome analysis and regulatory mechanisms of isoprene degradation by bacteria. The ecology of isoprene degraders in both the terrestrial and marine environment will be described, together with DNA-Stable Isotope Probing experiments which have enabled us to identify active isoprene degraders in the environment.

DEV08
Targeting methanotrophs in environmental samples using stable isotope probing combined with metatranscriptomics

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Aerobic methanotrophs mitigate CH_4 release from environments such as wetlands, lakes and landfills. Stable isotope probing (SIP) can be used to identify methanotrophs in environmental samples by incubating with $^{13}CH_4$ and recovering ^{13}C-labeled nucleic acids. We have combined RNA-SIP and metatranscriptomics to obtain targeted metatranscriptomes of methanotrophs in lake sediment and in rice field soil. Methanotrophs in lake sediment were labeled using $^{13}CH_4$, and both labeled and unlabeled-RNA were isolated and sequenced by pyrosequencing. The unlabeled metatranscriptome had a large diversity of bacterial, archaeal, eukaryotic and viral sequences as expected from a diverse sediment community. The labeled-RNA metatranscriptome was dominated by methanotroph sequences, particularly from *Methylococcaceae*. Transcripts of the *pmoCAB* genes were the most abundant in labeled metatranscriptome, and metabolic pathways of CH_4 oxidation and carbon assimilation could be inferred based on the detection of transcripts. A similar approach was used to investigate the response of methanotrophs in rice field soil to nitrogen addition, which is known to rapidly stimulate CH_4 oxidation by an unidentified mechanism. Replicate rice field soil samples were incubated with added ammonium, nitrate and without added nitrogen as a control. RNA was recovered after 50 h incubation, followed by IIumina sequencing of ^{13}C-RNA. PCA analysis of contigs annotated with KEGG or SEED indicated reproducible differences in transcription profiles between the treatments. Transcripts of genes encoding enzymes associated with assimilation of nitrate, ammonia and urea were significantly overrepresented in the control, consistent with nitrogen limitation. There was no indication of elevated expression of nitrogenase-associated genes in the control, suggesting that a diversion of energy to nitrogen fixation was not the mechanism behind lower CH_4 oxidation rates. In summary, SIP-metatranscriptomics enables a focused analysis of the response of methanotrophs to environmental conditions and should be widely applicable in microbial ecology studies.

DEV09
Unveiling the Microbial Sink of Methanol in Terrestrial Ecosystems

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Methanol is the second most abundant organic molecule in the atmosphere. The main source of atmospheric methanol is plant material. Methanol oxidation by aerobic microorganisms in soils is a not well evaluated global sink in the methanol cycle. Such methanol consumers may reduce methanol emission by its consumption in the rhizosphere and phyllosphere, and recent reports suggest that soil communities are also be capable of uptake of methanol from the atmosphere. Aerobic methylotrophs use methanol as a source of carbon and energy by diverse set of enzymes and enzymatic pathways. Previous own work revealed that GL1 and GL2 (genotypes affiliated with *Alphaproteobacteria*) responded to methanol concentrations that that would explain methanol deposition from the atmosphere into soil. In six grassland and six forest soils, environmental factors were identified that influenced the methylotroph community structure based on *mxaF*, *mch* and *fae*. Thus, vegetation type (forest or grassland), soil pH, the availability of nitrogen and substrate concentration likely determine which methylotroph taxa are involved in the flux of methanol from aerated soils of temperate ecosystems. Molecular tools, i.e. potential gene markers, that will facilitate comprehensive assessment in future are evaluated and need to be extended. Beyond gram negative methylotrophy gram positives such as *Bacilli* and *Actinobacteria* should be considered in upcoming research. Another potential divers group of methanol consumers in terrestrial environments are Ascomycota and yeast - strategies to asses them will be discussed.

DEV10
A metaproteogenomic approach for functional investigation of carbon cycling by marine methylotrophs

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One-carbon compounds, i.e. compounds that only contain one carbon atom, and compounds containing only carbon atoms without carbon-carbon bonds, play key roles in earth's major biogeochemical cycles, and are surprisingly abundant in the world's oceans. Some of these compounds, like methane and dimethylsulfide (DMS), have climatic activity in the atmosphere (1), and thus are of direct relevance for the processes of global climate change. Marine habitats, covering the majority of earth's surface, consequently have a huge impact on atmospheric concentrations of carbon-one compounds, with the potential to act both as sink and source. Microorganism that can metabolise one-carbon compounds are ubiquitous in the environment. Thus, these so called methylotrophs potentially play a major role in modulation of transfer processes between ocean and atmosphere. In our research, we use methylotrophs as model systems for the development of functional metaproteogenomic approaches. A combination of metagenomic and metaproteomic techniques with stable isotope probing (SIP), employing ^{13}C labeled substrates (methane, methanol, methylamine) is used to follow nutrient fluxes through microbial communities. In-depth analysis of abundance and diversity of key functional genes of methyltrophy is done by amplicon pyrosequencing. Furthermore, the metaproteomics approach is used to investigate the prevalent metabolic pathways to complement the study and to connect the general overview provided by metagenomics data to the molecular level.
This methodological approach is used to perform a focussed, functional multi-level analysis in different marine environments. The investigation enables us to identify active methylotrophic bacteria involved in metabolism of methane, methanol and methylamines, and to assess their contribution to the marine food web. The presence of functional genes of interest has been confirmed along with the identification of the respective proteins, enabling conclusions about the active biochemical pathways.
We were able to show that the applied approach provides valuable opportunities to answer key questions about microbial carbon cycling in marine ecosystems. Analogous to this study, we will also test the method's capability to use ^{15}N labeled substrates in the near future, to investigate microbial nitrogen cycling.

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DEV11
Novel syntrophic species link fermentation and methanogenesis in peat

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The syntrophic oxidation of primary fermentation products like ethanol, butyrate, or propionate to methanogenic substrates (H_2/CO_2, formate, acetate) is a thermodynamic bottle-neck during the mineralization of organic matter in anoxic habitats like peatlands. Despite the importance of syntrophic oxidations to the emission of methane, little is known about the microbial community that catalyzes these syntrophic processes in peat. Ethanol, butyrate, or propionate was pulsed at low in situ relevant concentrations into peat slurries incubated anoxically at 5°C or 15°C. Methane and CO_2 were the sole accumulating products during consumption of ethanol, butyrate, or propionate, indicating that the methanogenic food web was not uncoupled. Methane production was stimulated in all supplemented treatments compared to unsupplemented controls. The rate of ethanol, butyrate, or propionate oxidation increased with time, probably due to the enrichment of syntrophs. The microbial community of ethanol or butyrate oxidizers at 15°C was analyzed by 16S rRNA ^{13}C-based stable isotope probing. *Pelobacter*-related 16S rRNA sequences (98% identity to the ethanol oxidizer *P. propionicus*) were highly abundant in heavy fractions but almost absent in light fractions of [^{13}C]ethanol-supplemented treatments at 15°C. *Syntrophomonas*-related 16S rRNA sequences (95% identity to the butyrate oxidizer *S. zehnderi*) were highly abundant in heavy fractions but absent in light fractions of [^{13}C]butyrate-supplemented treatments at 15°C. This indicates that *Pelobacter* was the sole ethanol- and *Syntrophomonas* the sole butyrate-oxidizing genus. Relative abundances of *Syntrophobacter*-(95% identity to the propionate oxidizer *S. wolinii*) and *Smithella*-related 16S rRNA sequences (97% identity to the propionate oxidizer *S. propionica*) increased in clone libraries from 2% and 0% in field fresh soil to 5% and 5%, respectively, in [^{12}C]propionate-supplemented treatments but did not increase in unsupplemented controls at 15°C. Relative abundances of *Pelobacter* increased from 0% in field fresh soil to 5% in [^{12}C]ethanol-supplemented treatments but not in unsupplemented controls at 5°C. *Methanosarcina* (98% identity to *M. vacuolata*) and *Methanosaeta* (95% to *M. concilii*) were dominant detected acetoclastic methanogens, and *Methanocella* (95% to *M. paludicola*) and *Methanoregula* (98% to *M. boonei*) were dominant detected hydrogenotrophic methanogens. The findings indicate that (i) ethanol, butyrate, and propionate are converted to methane and CO_2 by new species of known syntrophic and methanogenic genera in peat slurries at 15°C, (ii) ethanol can be syntrophically oxidized by peatland syntrophs at temperatures as low as 5°C, (iii) syntrophs are specialized to the use of a specific substrate and (iv) the phylogenetic diversity of the syntrophs is limited to a few genera.

DEV12
Nitrous oxide consumption by a remarkably diverse fen denitrifier community

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Peatlands store more than 30% of total soil carbon and nitrogen even though they cover only about 3% of the terrestrial surface. Fens are peatlands that receive ground water input and might act as temporary sources or sinks of nitrous oxide (N_2O). However, key players associated with N_2O fluxes and their regulation remain to be analyzed. N_2O consumption in a pH-neutral Finnish fen was observed in situ by gas chamber measurements. Nitrate rather than ammonium fertilization stimulated initial N_2O production. N_2O in gas chambers was subsequently consumed, indicating complete denitrification. Michaelis-Menten like kinetics were determined in anoxic soil slurries. Apparent maximal reaction velocities for nitrate dependent denitrification and N_2O consumption were 18 and -36 nmol N_2O h^{-1} g_{DW}^{-1}, respectively. Apparent Michaelis-Menten constants were 29 and 0.43 µM for nitrate and N_2O, respectively. Barcoded amplicon pyrosequencing of *narG*, *nirK/S*, and *nosZ* (encoding nitrate, nitrite and N_2O reductases, respectively) yielded 28000 sequences. Up to 360 operational taxonomic units (OTUs) were detected per gene at 97% similarity, suggesting diverse denitrifiers. Phylogenetic analyses revealed clusters distantly related to

publicly available sequences, suggesting hitherto unknown denitrifiers. Representatives of species-level OTUs were affiliated with sequences of unknown soil bacteria and Actinobacterial, Alpha-, Beta-, Gamma-, and Delta-Proteobacterial sequences. 308 OTUs were estimated for *nosZ*, highlighting diverse microbes capable of N_2O consumption. Thus, the data indicates diverse and new denitrifiers, as well as fens as potential denitrification-dependent N_2O sink.

DEV13

Transcriptomics reveals the regulatory response to light mediated stationary phase transition in the ubiquitous freshwater bacterium *Polynucleobacter necessarius*

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Polynucleobacter species are ubiquitous in freshwater lakes and often dominate freshwater bacterial communities. Cultured representatives became available only during the last 5 to 10 years. The genomes of *Polynucleobacter* species encode ~2000 genes and indicate a high specialization to a planktonic life-style. *P. necessarius* now provides the first bacterial model systems representing these typical and abundant planktonic freshwater bacteria. Diurnal changes in light intensity strongly inhibited *P. necessarius* in the illuminated water layers of humic matter rich Lake Grosse Fuchskuhle. Light exposure decreased the uptake of radiolabelled substrates, hampered growth and increased the fraction of membrane damaged cells. These observations may be explained by the photo-reactivity of humic substances leading to the formation of inhibitory reactive oxygen species. The underlying molecular response was investigated by growing *P. necessarius* cultures in dialysis bag cultures in the lake surface water layer. Illumina sequencing of total RNA extracts revealed that mRNA levels of genes encoding central cellular functions as the energy metabolism, anabolic processes and genes involved in cell division were strongly decreased. Together with a low physiological activity this observations suggests a transition to stationary phase of *P. necessarius* during high solar radiation. This interpretation is supported by the observation that pRNA is lacking in light incubated cultures, but not in dark controls. Transcription is very likely blocked by 6S RNA when *P. necessarius* is exposed to light in its natural environment. Decreased mRNA levels were also found for *mazE*, encoding the Antitoxin in the Toxin-Antitoxin system MazE/F, which is typical for stationary phase *E. coli* cultures. In contrast, increased mRNA levels were observed for several genes encoding oxidative stress response mechanisms. We conclude that unfavorable conditions caused by photochemical reactions in illuminated water layers force *P. necessarius* cells to enter stationary-phase. The transition to stationary phase may represent a unique adaptive response of *P. necessarius* to overcome diurnal periods of unfavorable conditions caused by photochemical reactions in aquatic environments.

DEV14

Influence of photosynthesis an its adaption strategy in the aerobic anoxygenic phototrophic bacteria *Dinoroseobacter shibae*

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Dinoroseobacter shibae is a member of the important and abundant Rosebacter clade. This group of bacteria is known for its variety of metabolic processes which allows the occurence in different habitats. The majority of this clade are so-called aerobic anoxygenic photosythetic bacteria (AAP), which means that in the presence of oxygen light is used as supplementary energy source to metabolize organic matter [1]. The main light harvesting pigments are spheroidenone and bacteriochlorophyll *a*. The AAP bacteria *D. shibae* serves as a model organism and is also able to synthesize bacteriochlorophyll. According to the genome annotation of *D. shibae* in 2009 bacteriochlorophyll biosynthesis is regulated in the presence of either oxygen or light. Changing light or oxygen conditions result in different expression patterns of photosynthetic genes[2,3].
In order to unravel the light-dependent signalling pathway for the bacteriochlorophyll biosynthesis in *D. shibae* transposon mutants defective in receptors, photosynthetic genes and bacteriochlophyll synthesis were characterized concerning their growth behaviour. For example, the *acsF* mutant defective in the aerobic Mg-Protoporypherin IX cyclase shows a clear growth defect. In the *acsF* mutant the content of photosynthetic pigments was measured via UV-Vis-spectroscopy. No bacteriochlorophyll *a* was detected even under anaerobic conditions. These results demonstrate the importance of light-harvesting pigments for optimal growth. Further, this may confirm the hypothesis, that bacteriochlorophyll is used for capturing reactive oxygen species and is therefore synthesized only under aerobic conditions. Additionally, single-cell analyses with *D. shibae* have shown a clear decrease of bacteriochlophyll through an active degradation inpresence oflight.
Future analyses using light imaging methods on single-cell level as well as on populational level will determine the bacteriochlorophyll content under different light conditions, expecially for wavelength and intensity. These results will lead to new insights into the use and function of bacteriochlorophyll and the influence of photosynthesis as an adaption strategy for aerobic anoxygenic photoheterotrophs.

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DEV15

Development of a labeling system for microorganisms based on antimicrobial peptides

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Complex biogeochemical processes are essential in various subsurface ecosystems (like in soils and rock formations), and are relevant for e.g. effective bioleaching of metals from various ores. For an improved mechanistic understanding of biogeochemical processes a non-invasive detection method of present microorganisms in a given geological sample is crucial. Ideally, microorganisms from all kingdoms should be determined in a single step (and may be later differentiated from DNA or cell degradation). Furthermore, microbial responses to changes of the physical or chemical conditions of the system such as flow regime, pH, and nutrient concentrations could be addressed non-invasively.
Our strategy for in-situ identification of microorganism in geological samples is to use labeled antimicrobial peptides (AMPs) as selectively binding agents. In the nuclear medical sciences, this strategy is successfully applied for the identification and visualization of bacterial infections in humans [1]. We aim at tagging the AMPs first with fluorescent dyes, in a later step with the radionuclide ^{18}F for imaging with positron emission tomography (PET).
For our study, AMPs are selected based on their ability to bind to the cells of the tested bacterial strains in sub-lethal concentrations while their sorption to matrix compounds is minimal. We show that AMPs readily interact with microorganisms commonly found in soils such as *Pseudomonas fluorescence* and *Lysinibacillus sphaericus.* We aim at labeling the AMPs by means of established, commercial crosslinkers.
In combination with our GeoPET method [2-4]^{18}F-radiolabeled AMPs were an extremely useful agent for the in-situ visualization of microorganisms also in opaque geological environments. Radiolabeled AMPs could be used for the visualization of growth and dispersal of microbial communities in such environments.

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DEV16
From monitoring to steering microbiomes using single cell analysis

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Microbiomes are composed of complex microbial communities providing a multi-facetted metabolic network. Whereas microbiomes can only be optimized by unrevealing and steering the underlying structure-function relationships most often, however, bioreactors are run on empirical experience and the microbial community is only analyzed sporadically. This led to, so far, limited insights into the microbiome structure-function relationships in managed microbial systems like anaerobic digestion, wastewater treatment or bioelectrochemical systems. In this contribution it will be demonstrated that cytometric fingerprinting is an excellent method to monitor microbiomes with high resolution and in a fast and cheap manner. A sample specific cytometric fingerprint (typically based on the flow-cytometric measurements of 250 000 cells in 3 minutes) is demonstrated to be representative for a microbial community structure and thus can mirror functional community changes. Successful functional monitoring of microbial communities is illustrated on the examples of i) long term monitoring of a biogas reactor including phases of functional stability and process failure, ii) community dynamics and speciation in "bioelectrochemically steered" anaerobic digesters as well as iii) anodic enrichment of electroactive microorganisms In conclusion, it will be shown that cytometric fingerprinting allows revealing structure-function relationships of microbiomes in managed microbial systems and thus its (continuous) monitoring will allow a steering based on complex microbial communities in future.

DEV17
Hunting for active degraders: Novel screening approach combining substrate-specific radiolabelling of cells and their separation in microcompartments

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The identification of specific prokaryotes as drivers of organic compound turnover in environmental systems continues to remain a great challenge. Classical identification approaches such as laboratory cultivation may fail due to lack of success in obtaining the relevant microbes, while detection of phylogenetic markers or catabolic genes may not necessarily provide information of *in situ* functionality, and current approaches involving isotope labelling may be problematic due to environmental complexities, high costs, or low sensitivity. With the intention of circumventingthese limitations, we are developing a novel screening methodology for microbes with *in situ* activity that is independent from cultivation as well as prior nucleic acid-based information. Here we show proof-of-principle results of a novel Radio Isotope Probing method which is geared towards single cell genomics of environmentally relevant cells. It encompasses a short incubation of a microbial sample with a ^{14}C labelled substrate followed by encapsulation of cells in microbeads with subsequent microautoradiography (MAR). Thus compound-transforming microbes are spatially isolated and might be separated based on optical differences caused by MAR. DNA can be successfully amplified from encapsulated cells to achieve identification. The main principle of the overall approach has been proven with a model system of the benzene-degrading *Pseudomonas veronii* and *Escherichia coli*. Advantages of the procedure are: I) non-destructive selection of cells solely based on their *in situ* activity; II) high sensitivity, i.e. very low incorporation rates of organic carbon are required per cell; III) general applicability for organic compounds used as carbon- and energy source.

DEV18
Plasmid curing and the loss of grip - RepA-I type replicons of the *Roseobacter* group are essential for biofilm formation, motility and the colonization of marine algae

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Surface colonization is characteristic for a broad range of marine roseobacters and many strains have been isolated from biofilms, microbial mats and macroalgae. A sessile lifestyle provides permanent access to nutrient-rich habitats, but recent studies showed a reversible transition to motile life stages triggered by intracellular signals or extracellular inducers. We analyzed surface attachment and swimming motility of 33 completely sequenced roseobacters. The sampling site of a strain does surprisingly not allow to reliably predict its stickiness, but our results largely support the former observation that biofilm forming representatives are motile. Many roseobacters contain RepA-I type plasmids with a conspicuous accumulation of genes for polysaccharide metabolism including a typical rhamnose operon, which is essential for O-antigen formation in *E. coli*. We started our genetic experiments based on the working hypothesis that the representative 65-kb RepA-I replicon of *Phaeobacter inhibens*, an effective colonizer of marine surfaces, is essential for surface attachment (Petersen et al. 2013). Plasmid curing was performed with the homologous, but distantly related, RepA-I replication system of *Dinoroseobacter shibae*, thereby providing experimental evidence for the proposed incompatibility of these replicons (Petersen 2011). The Δ65-kb mutant of *P. inhibens* completely lost its stickiness and could neither attach to artificial (glass, polystyrene) nor to natural surfaces like e.g., the green alga *Ulva lactuca*. Astonishingly, the mutant also lost the capacity for swimming motility that is required for surface colonization and the dispersal of biofilms. The comparison of wild type and curing mutant via scanning electron microscopy documented the absence of typical rosettes and the loss of the capacity to form biofilms. The results validate our working hypothesis and demonstrated that the 65-kb replicon of *P. inhibens* is a genuine biofilm plasmid. Comparable results were obtained from curing experiments of *P. gallaeciensis*, *P. arcticus* and *Marinovum algicola* hence documenting that biofilm formation is frequently linked to extrachromosomal elements within the *Roseobacter* group. Finally, phylogenomic analyses revealed the wide distribution of RepA-I replicons among Rhodobacterales and plasmid phylogenies documented horizontal transfer.

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DEV19
Water-uptake by desiccated terrestrial *Nostoc commune* colonies from a saturated NaCl solution - consequences for the recultivation of associated heterotrophic bacteria

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Introduction: Heterotrophic bacteria are known to be associated with EPS-producing cyanobacteria in dry environments [1]. Deliquescence of halite (NaCl) can provide a habitable niche for such communities as reported for the hyper-arid Atacama Desert [2, 3]. Accompanying bacteria of highly desiccation resistant *N. commune* biofilms were investigated with respect to reculturability after long-time desiccation and short-time incubation over and in a saturated aqueous NaCl solution. To prove the possibility of water uptake by *N. commune* biofilm and montmorillonite from halite, H_2O sorption properties were determined.
Materials and Methods: Biofilms (dry natural colonies of *N. commune*, stored for 4 years, national park 'Unteres Odertal', Germany) were recultivated on R2A after different treatments: equilibration (14-19 d) over silica gel (relative humidity (RH)=30-40%), over (RH=75%) or in sterile saturated NaCl solution. R was used for statistical analysis (Kruskal-Wallis). Associated bacteria were identified by amplification of 16s rRNA genes and

subsequent cloning and sequencing of plasmids. Sorption isotherms of biofilms, Atacama halite (Chile) and Ca-montmorillonite (USA) were obtained by gravimetrical measurement at 273 K and different RH.
Results: Recultivation: water uptake at RH=75% (14.13 ± 0.76 wt%) did not lead to a significant change in CFU number compared to the silica gel dry controls. A 24-fold decrease of CFU was observed for samples incubated in brine. Cloning: 90% of the sequences were identified as alphaproteobacteria, 10% consisted of beta-, gammaproteobacteria and bacteriodetes. H_2O sorption: Hydration and dehydration characteristics of *N. commune* are formally similar to those of montmorillionite (hysteresis). Halite shows deliquescence at RH=75-80% indicated by a suddenly upraise of the isotherm.
Discussion: Heterotrophic bacteria were recultivated after 4 years of desiccation, a fraction of 4% remains reculturable after incubation in saturated brine. Water uptake next to or from the brine did not induce growth. Hence these bacteria might be adapted to long-time desiccation but not to short-time activity under water-limitation. High proportions of alphaproteobacteria might be common to temporarily dry surfaces as reported for the lichen *Lobaria pulmonaria* [4] and plant leaves [5]. *N. commune* tends to store water whereas the clay shows structural changes between hydration/dehydration [6]. Thus halite can provide liquid water to both because of deliquescence at RH=75-80%.

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DEV20
Solutions to the public goods dilemma in bacterial biofilms
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Bacteria frequently live in densely populated surface-bound communities, termed biofilms. Biofilm-dwelling cells rely on secretion of extracellular substances to construct their communities and to capture nutrients from the environment. Some secreted factors behave as cooperative public goods: they can be exploited by nonproducing cells. The means by which public-good-producing bacteria avert exploitation in biofilm environments are largely unknown. Using experiments with *Vibrio cholerae*, which secretes extracellular enzymes to digest its primary food source, the solid polymer chitin, we show that the public goods dilemma may be solved by two very different mechanisms: cells can produce thick biofilms that confine the goods to producers, or fluid flow can remove soluble products of chitin digestion, denying access to nonproducers. Both processes are unified by limiting the distance over which enzyme-secreting cells provide benefits to neighbors, resulting in preferential benefit to nearby clonemates and allowing kin selection to favor public good production. Our results demonstrate new mechanisms by which the physical conditions of natural habitats can interact with bacterial physiology to promote the evolution of cooperation.

DEV21
Assessing the ecological niches of not-yet-cultured *Acidobacteria* in situ
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Acidobacteria are a dominant phylum in soils and can constitute up to 50% of all bacteria present. Yet, their physiology and ecology is hardly understood since only very few representatives (N = 33) could be isolated so far. In order to access the unknown physiology of not-yet-cultured dominant soil *Acidobacteria*, we studied 150 grassland soil samples from the Biodiversity Exploratories project by high-throughput sequencing of the 16S V3 region of extracted and reverse-transcribed RNA. The clustering of more than 25 million reads yielded more than 3000 operational taxonomic units (OTU) at a 97% cutoff value, suggesting the presence of thousands of species. By using co-occurence network analysis we identified 54 different network groups. These could be assigned to distinct combinations of physicochemical soil parameters, in particular pH, soil water content, soil texture, and different types of carbon and nitrogen content. These results suggest the presence of numerous different ecological groups represented each by several ecologically but not necessarily phylogenetically related OTUs. We further used Huisman-Olff-Fresco modeling (hierarchical logistic regression) of the relative abundance of OTUs across the 150 soil samples in order to identify the maximum response and ecological niche width of the OTUs with respect to the soil parameters. The comparison of the pH growth optimum of the available acidobacterial species to the modeled maximum pH response of the closest OTUs yielded a high congruence of the two approaches. This confirms the large potential of high-throughput sequencing for ecophysiological studies of not-yet-cultured bacteria.

DEV22
Mapping the Flow of Carbon and Nitrogen in an Anaerobic, Autotrophic Mixed Culture that Couples the Oxidation of Fe(II) to the Reduction of Nitrate
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We study an anaerobic, autotrophic enrichment culture which couples Fe(II) oxidation to nitrate reduction. The culture has first been described by Straub et al. (1996), but isolation of the dominant iron-oxidizer was so far unsuccessful. It also remains elusive how the culture couples Fe(II) oxidiation to nitrate reduction and which species interactions sustain a stable community composition in the enrichment.
We applied 454 pyrosequencing and fluorescence *in situ* hybridization (FISH) to study the community structure and population dynamics under different growth conditions. Carbon and nitrogen uptake and distribution in the culture was followed using stable isotope tracers. Assimilation and relative enrichment of ^{13}C-carbon and ^{15}-N-nitrogen was quantified with single cell resolution using nanoscale secondary ion mass spectrometry (nanoSIMS). The culture was either grown autotrophically with Fe(II), nitrate, ^{13}C-bicarbonate and ^{15}N-ammonium or heterotrophically with acetate, nitrate, ^{13}C-acetate and ^{15}N-ammonium to follow community shifts, cellular C/N assimilation activities, and cross-feeding among the numerically dominating cell populations. Sequencing and FISH revealed that the enrichment culture is dominated by an iron-oxidizing bacterium related to the microaerophilic iron-oxidizers *Sideroxydans* and *Gallionella* when grown autotrophically. Under heterotrophic growth conditions with nitrate as electron acceptor, the culture is dominated by a *Bradyrhizobium* species. Single cell based nanoSIMS analysis revealed the selective ^{13}C-carbon and ^{15}-N-nitrogen enrichment of the putative iron-oxidizer and the nitrate-reducer under autotrophic and heterotrophic growth, respectively, but also suggests interspecies carbon cross-feeding to sustain Fe(II)/nitrate-redox coupling and community composition in the mixed culture. Quantification of Fe(II), nitrate, and cell numbers allowed insights into the tight coupling of iron oxidation with nitrate reduction under autotrophic conditions.
This novel microbial community-mediated, chemolithoautotrophic process of coupled iron oxidation and nitrate reduction may significantly contribute to ferric iron formation in the suboxic zone of aquatic environments.

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DEV23

How rare biosphere members contribute to biogeochemical cycling: The ecology of low abundance sulfate reducers in the hidden sulfur cycle of a model peatland

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Freshwater environments such as peatlands, rice paddies, or lake sediments jointly constitute the dominating source of biogenic methane on Earth. They are also characterized by a recently proposed hidden sulfur cycle, which exerts a control function on the production of the greenhouse gas methane. In these environments, the typically low sulfate concentrations are contrasted by very high sulfate reduction rates that are as high as in sulfate-rich marine surface sediments. Since dissimilatory sulfate reduction is thermodynamically favorable compared to methanogenic processes, the responsible microorganisms contribute to balance methane production in these environments. Our previous work revealed that *Desulfosporosinus* species belonging to the rare biosphere have the potential to contribute substantially to sulfate reduction in an acidic model peatland despite their low abundance. To deepen our understanding of sulfate reducer ecophysiology in peatlands, anoxic microcosms were supplemented with typical degradation intermediates of organic matter at *in situ* concentrations and incubated with or without externally supplied sulfate. Responses of the overall microbial community were monitored by highly parallel 16S rRNA gene and cDNA amplicon sequencing, verified by qPCR analysis, and correlated to substrate and sulfate turnover. Periodical small amendments of sulfate (µM range) resulted in rapid sulfate turnover and drastically reduced methane production in comparison to microcosms without external sulfate addition. In parallel microcosms, numerically dominating phylotypes, e.g., belonging to the *Acidobacteria*, showed no significant correlation with sulfate turnover. In contrast, low abundance phylotypes related to known sulfate reducing *Firmicutes* and *Deltaproteobacteria* correlated positively with sulfate turnover. Interestingly, they markedly increased their 16S rRNA and thus ribosome content under sulfate reducing conditions but stayed at low abundance throughout the incubation period. This likely mirrors their ecological strategy also in the natural peat soil. OTU-based analysis indicated different ecophysiological strategies among different *Desulfosporosinus* phylotypes, with some being specialized to butyrate as sole substrate while the most dominant *Desulfosporosinus* phylotype showed a generalist lifestyle being active under butyrate, propionate, and lactate amendments. Parallel sequencing of the peatland metagenome enriched by DNA-stable isotope probing allowed almost complete reconstruction of the *Desulfosporosinus* population pan-genome and confirmed functional properties of this novel acidic fen sulfate reducer, as inferred from the microcosm studies. In conclusion, our results show that the activity of rare biosphere members can have a profound effect on biogeochemical cycling and control of greenhouse gas production.

DEV24

Temporal and depth-related variability of microbial communities in soils along an ecosystem development gradient

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During long-term ecosystem development, soil nutrient contents and mineralogical properties change, therefore probably altering microbial community composition. Vice versa, microbial communities play a key role in soil formation processes and nutrient cycling. While many studies focus on topsoil environments, patterns in subsoil communities remain poorly understood despite the importance of subsoils in soil ecosystem functioning, e.g. as carbon storage. The aim of this study was to analyze the variability of microbial communities along soil development and depth gradients and further to identify the physicochemical and mineralogical factors that shape the community composition. To study microbial long-term patterns we sampled whole soil profiles up to one meter depth along the 120,000 year old Franz Josef chronosequence in New Zealand. Microbial community composition was determined for selected samples from mainly subsoil horizons by tag-encoded pyrosequencing of bacterial and archaeal 16S rRNA genes. Along the ecosystem development gradient, microbial diversity was highest at young to intermediate-aged soils (500 - 12,000 years) and declined at the oldest stage (120,000 years). We found distinct depth-related archaeal communities in organic and mineral horizons and a clear shift in community composition with soil age. Temporal changes were mainly linked to factors associated with soil development such as nitrogen and phosphorus content as well as mineralogical properties. In contrast to the *Archaea*, bacterial community profiling revealed no overall consistent trend along the ecosystem development gradient and showed only shifts for particular taxa. However, changes in bacterial community composition seem to be related to soil horizons.
Our results indicate that also subsoil microbial communities, especially archaeal ones, show a compositional shift along an ecosystem development gradient, analogously to previously described changes for the vegetation and topsoil microbial communities.

DIAGNOSTIC MICROBIOLOGY

DVV01

Endotoxin Masking in Common Formulations of Bio-Pharmaceuticals

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Endotoxin masking is a widely discussed hot topic in the field of Bacterial Endotoxin Detection (BET). In recent presentations and publications, the issue of inadequate or Low Endotoxin Recovery (LER) has been discussed, especially in modern bio-pharmaceuticals, like monoclonal antibodies. To solubilize the antibodies, certain formulations are applied, which consist of surfactants and buffer components. Obviously, there is a complex relationship between the formulation components, Active Pharmaceutical Ingredient (API) and the potential endotoxin contamination. The interaction of these components change activity of endotoxin in Limulus-based detection systems. Hence, we show a detailed overview, about the driving forces, responsible for endotoxin masking.
Additionally, we studied the application of Naturally Occurring Endotoxin (NOE) in affected formulations. NOEs are assumed to be more stable against masking effects than ordinary used standard Endotoxins. Our studies indicate, that NOE can be affected by endotoxin masking as well as purified LPS. Understanding the mechanism of endotoxin masking enabled us to develop dedicated sample preparation protocols for de-masking. Thereby, an essential prerequisite for de-masking is the re-arrangement of a detectable Endotoxin aggregation state. Here, we demonstrate the applicability of these protocols and give an insight into the process of de-masking.

DVV02

Microbial Diagnostics in Pharmaceutical Quality management: Is Molecular Biology improving or replacing Microbial Diagnostics in quality management?

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Introduction: The scope of microbial quality control in the pharmaceutical environment is about generating a full microbial state of control of a production facility. Albeit microbial diagnostic has its origin in the clinical environment, its principles and technologies are applied to pharmaceutical quality management. This deviating practice in line with a different scope of quality management is, especially in the fields of microbial identification and strain typing, increasing variability and thus risk.
Materials and Methods: The study presented here compares different microbial identification approaches (phenotypic vs. proteotypic vs. genotypic) using 16S rDNA sequencing as a reference and evaluates the potential operational impact on a production environment.
Results: Phenotypic systems failed to identify or identified incorrectly 30, 5% of samples due to database limitations, outdated taxonomy or incorrect gram staining interpretations. An under- or overestimation of risk due to incorrect species level identifications would have occurred in 7, 6 % of cases. As expected, almost all clinical relevant samples which have been

collected in an animal facility, have been identified correctly to the species level by Vitek 2.
MALDI - TOF failed to identify correctly to the species level 5, 3%% of isolates due to library limitations. 10, 5% of samples did not generate spectra or resulted in no ID, but have been identified using 16S rDNA sequencing. 16S rDNA sequencing identified 88, 6% to the species, 10, 5% to the genus level and 0, 95% to the family level. 14, 3% of isolates identified to the species level have very similar or identical 16S rDNA sequences. These sequences do not allow a valid discrimination between certain species on species level. 44, 2% of the molecular biologically to the species level identified samples (93) or 32,9% of to the pharmaceutical industry relevant species (73) have not been described in regards to risk. 22, 5% (11) different species are assigned to a certain risk level, but were not included in at least one library of the phenotypic systems. These species are part of the Accugenix libraries.
Conclusion: The data shows that phenotypic systems are capable of species level identifications when applied to clinically relevant environmental isolates. The dependence on subjective decisions and limited libraries are increasing risk and thus lowering the state of control of any environment, when applied non - clinical environments. As MALDI - TOF like 16S rDNA sequencing is dealing with ribosomal information, it is recommended to back up MALDI - TOF by 16S rDNA sequencing in order to reduce the impact of library limitations.
Molecular biological methods should not fully replace microbial diagnostics, as the plausibility control of molecular biological data requires classical microbiological expertise. Overall, molecular biological approaches are capable of improving significantly quality management and thus the state of control in the pharmaceutical production environment.

DVV03
Lower MALDI Biotyper Threshold Values are Appropriate for Nontuberculous Mycobacteria

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Introduction: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is widely used for identification of microorganisms. The MALDI Biotyper system uses log(score) values to indicate the reliability of the result. To date, for usual bacterial isolates threshold values of 2.0 and 1.7 were used to indicate probable species or probable genus identification, respectively. These categories will be changed by the manufacturer to high and low confidence level intervals.
Mycobacteria are more demanding for MALDI-TOF MS analysis and require a special extraction method with a corresponding database. It appeared that for the genus *Mycobacterium* identification results with lower log(score) values were still reliable. Therefore we investigated the applicability of lower threshold values for *Mycobacterium* spp.
Materials and Methods: Mycobacterial isolates (n = 844) were inoculated on solid Löwenstein-Jensen medium or in liquid BD BACTEC™ MGIT™ tubes (BD, Heidelberg) and incubated according to standard procedures. In addition, patient material was inoculated in BD BACTEC™ MGIT™ tubes (n = 50). Mass spectra were recorded with a microflex LT instrument and compared to Mycobacteria Library 2.0 reference spectra using MALDI Biotyper 3.1 software (Bruker Daltonik, Bremen). Reference method for study isolates was GenoType® Mycobacterium CM (Hain Lifescience, Nehren). Sequencing of 16S rRNA gene or ITS sequence was performed for a few isolates.
Results: Out of 844 analyses of pure cultures 710 (85%) resulted in high log(score) identification values ≥ 2.0. The remaining 134 results with log(score) values < 2.0 were further investigated with regard to optimized thresholds. Threshold values lowered to 1.8 and 1.6 did not result in false identifications for 128 samples and seem to be valuable as high and low confidence levels, respectively. Only one isolate matched at best to a closely related species with a log(score) value ≥ 1.8, identity of this isolate still has to be ascertained. The decrease of threshold allowed an additional 76 results to be regarded as high confidence and further 17 as low confidence identifications.
In addition, 34 out of 50 enrichment cultures of directly inoculated clinical specimens resulted in log(score) values ≥ 2.0. Of those below 2.0 14 values were ≥ 1.8 and 2 were < 1.8 and ≥ 1.6. All species identifications were correct including the 16 ones with log(score) values < 2.0.
Discussion: The MALDI Biotyper system was successfully used for identification of *Mycobacterium* spp. In spite of a special extraction method these analyses sometimes yield too low log(score) values although the identifications are still reliable. Therefore the current rigid threshold values may lead to unnecessary time-consuming follow up analyses. The presented investigation suggests the applicability of adapted, lowered limits for identification scores.

DVV04
Microbead-Chip Read for Next Generation Quantitative Multiplex Real-Time PCR

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Multiplex quantitative real-time PCRs (mqPCR) are highly desirable tools for diagnostic applications. mqPCRs depend likewise on a parallel read-out and a multiplex detection probe concept. Conventional mqPCR cycler monitor theoretically up to seven targets simultaneously by using specific fluorescent labeled detection probes. Higher multiplexing is challenging due to spectral overlapping of reporter dyes and technical limitation of the detection systems [1].
To break this limit we developed the first homogeneous probe-based microbead-chip with a real-time image read-out, designated Microbead Hydrolysis Probe Assay (MHA). The microbead-chips are designed for batch production with low technical requirements. Our assay was integrated in an array for eight independently controllable microbead-chips in our previously published VideoScan platform [2]. Only two encoding dyes for thermostable microbead populations and one reporter dye for microbead bound capture probes are needed allow a discrimination of at least 15 targets (15-plex). We detached the amplification reaction in solution and detection reaction on the microbead-surface by short hydrolysable detection probes. The detection probes interact with the microbead bound capture probes and the gene-specific region in the target sequence. Decreasing detection probe quantities are directly reported by the change of the hybridization efficiency on the microbead bound capture probes. The microbead-chip was used for the detection of the genes *aggR*, *eaeA*, *ipaH*, *stx1*, *stx2* and *stlB* from genomic DNA of human pathogenic *Escherichia coli*. Gene patterns were reliably found. The novel method is part on studies of the adhesion of human *E. coli* strains in association with their virulence-associated genes [3]. Our assay is amplicon size independent, requires no alteration of amplicons and has no background fluorescence. The amplifications efficiency is similar to reactions in solution. We anticipate that the MHA is a valuable contribution to next generation diagnostic applications.

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DVV05
The German NAK, the local branch of the EUCAST.

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In 2012 the German NAK (Nationales Antibiotika-Sensitivitätstest-Komitee, NAC) has been founded. The General Committee comprising representatives of national scientific societies and organizations in the fields of infectious diseases and patient safety decides on recommendations proposed by the Steering Committee. The Steering Committee currently consists of 14 experts having a background in clinical microbiology, infectious diseases or regulatory affairs. Both boards will meet at least once a year. Industry has an observational status only.
The major objectives of the German NAC are I) to establish EUCAST breakpoints and technical aspects of *in vitro* antimicrobial susceptibility testing in German laboratories, II) to adapt EUCAST breakpoint to local requirements, and (III) to evaluate breakpoints for antimicrobial agents that have not yet been considered by EUCAST.

The NAC has met several times since 2012 and has reviewed all the EUCAST Rationale Documents on antimicrobials. Due to German dosing practices and regulatory requirements for Ampicillin and Cefuroxime the NAC has decided to follow the EUCAST suggestion for Enterobacteriaceae to set variant susceptible breakpoints for Aminopenicillins and Cefuroxime. This decision endorses the previous ones (Rationale documents on Amoxicillin v. 1.0 No 8 and Cefuroxime v. 1.0 No 10 and DIN) but lowers the susceptible breakpoint to avoid splitting the wild type distribution. In conclusion, there are no susceptible breakpoints for Enterobacteriaceae and Cefuroxime and Ampicillin/Amoxicillin, respectively, since setting such breakpoints would imply that systemic infections by these bacteria were amenable to therapy by the lowest approved doses, which is clearly not the case.
In addition, the German NAC set breakpoints for the urinary therapeutic Nitroxolin, since this substance represents an option for urinary tract infections caused by multidrug-resistant organisms. The EUCAST steering committee will consider setting a breakpoint for this antibiotic in a future revision of its breakpoint table. Also, the committee has approved a recommendation for reporting of fluoroquinolones in chronic bacterial prostatitis.

It is suggested to frequently visit the web site of the German NAK (www.nak-Germany.org) to stay informed on the current decisions and recommendations. The website also offers the opportunity to pose questions to the German steering committee.

DVV06
Development of a protein microarray based test for the rapid and multiplex detection of *Burkholderia pseudomallei* -specific antibodies

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Introduction: The Gram-negative bacterium *Burkholderia pseudomallei* is a natural inhabitant of soil and surface water in tropical and subtropical regions, causing the emerging infectious disease melioidosis in man and animals. Melioidosis has attracted interest outside its known distribution areas by cases in travelers and soldiers returning from *B. pseudomallei* endemic regions and by the recent classification as a tier 1 select agent. The worldwide distribution is still unclear and the reported cases are still likely to be the 'Tip of the Iceberg'. So far the serological diagnosis of melioidosis has been hampered by the fact that available tests such as the indirect hemagglutination assay (IHA) lack sensitivity, specificity and standardization. Modern protein microarray technology might have the potential to improve the serodiagnosis of melioidosis in a clinical setting but also for epidemiological studies. Furthermore, it might translate obtained results into other economic and fast point of care (POC) devices.
Materials and Methods: In this study a protein microarray was developed containing 20 proteins of *B. pseudomallei,* previously shown to be highly specific for the detection of antibodies in melioidosis patients. The proteins were cloned in pPR-IBA1/pPR-IBA33+, expressed in *Eschericha coli* BL21DE3 pLys and purified via Strep-tag and/or His-tag. The protein array was designed and manufactured by Alere Technologies GmbH in Jena. Sera of melioidosis patients and sera from experimentally infected mice together with respective negative controls were used in different dilutions to validate the protein microarray based assay.
Results*:* Our experiments show, that the protein array can clearly discriminate between sera from melioidosis patients and non-infected controls. Moreover, sera of *B. pseudomallei*-infected mice gave rise to specific signals, but not the controls. We could also detect differences in the *B. pseudomallei*-specific antibody pattern between positive human sera and also between positive mice sera.
Conclusion: The described assay is a rapid and easy to perform method for the detection of patterns of *B. pseudomallei*-specific antibodies in sera of humans and animals. This method can also be applied for the analysis of *B. pseudomallei* protein expression *in vivo*. Future clinical studies will aim on the determination of sensitivity and specificity of this protein array as a diagnostic tool.

DVV07
Chip-based Isolation of Pathogens for Subsequent Raman Spectroscopic Identification

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Many fields of application require fast and sensitive detection methods for pathogens. Nowadays, identification of bacterial species is often accomplished using culture based techniques. Even though this approach is reliable, it is also very time consuming. Raman microspectroscopy is a promising alternative, because it allows fast identification of bacteria on single cell level due to the specific spectroscopic fingerprint of each bacterial species. We have developed a Raman compatible chip for isolation of microorganisms from complex media. [1] The Raman measurements for identifying the bacteria can be performed directly on the chip. The chip was designed to be integrated into a microfluidic chamber for future automatization of the system. The isolation of the bacteria is achieved by using antibodies as capture molecules. We chose antibodies against common cell wall surface structures of Gram-positive and Gram-negative bacteria, which enable isolating a broad range of bacterial species. Our system was tested successfully for several Gram-positive and Gram-negative species, among them also sepsis relevant pathogens. All investigated bacteria could be isolated with the chip and Raman measurements of the isolated bacteria cells gave typical spectra exhibiting characteristic features from typical cell components like nucleic acids, lipids, protein and water. We further demonstrated that the acquired data is suitable for chemometric analysis. Using 10-fold cross validation a model was built which enables the identification of five different bacteria species.
An innovative chip-based method for isolation and enrichment of microorganisms has been introduced. With a set of only two antibodies a broad variety of bacteria can be captured on the chip. Since our chip is fully Raman compatible, identification of the isolated cells can be achieved via subsequent Raman measurements.

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Acknowledgement
Funding of the research project 'FastDiagnosis'and the young research group 'Jenaer Biochip Initiative 2.0' within the framework 'Unternehmen Region - InnoProfile Transfer'from the Federal Ministry of Education and Research, Germany (BMBF) is gratefully acknowledged.

DVV08
FISH in medical biofilms: closing the gap between bench and bedside

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Introduction: Biofilms have a major impact on human health being present on a wide range of interfaces on medical devices or the body itself. Whereas biofilms have been studied extensively *in vitro*, we are just beginning to learn about their structure and function in vivo because many models fail to display the high complexity of the microenvironments in the human body.
Materials and Methods: We used Fluorescence in situ hybridization (FISH) to visualize, identify, and quantify amount, composition, and interactions of in vivo grown biofilms in clinical samples.
Results: Pathogen identification: FISH using species-specific probes in tissue sections of heart valves can prove infection and identify the causative microorganisms in endocarditis patients. This is particularly helpful in culture-negative cases that pose a significant diagnostic problem. Activity of biofilms: Within the heart valve sections, we detected highly organized biofilms that were showing stratification with zones of elevated rRNA levels alternating with layers of only DAPI positive cells that were presumably dead. We also found bacteria with a strong FISH signal in culture negative samples and samples from patients under antibiotic therapy. The high signal intensity of FISH correlates to a high ribosomal content of the bacteria indicating metabolic activity at the time of surgery.

To detect the activity of single bacterial cells more precisely we developed FISH probes for the 16S-23S internal transcribed spacer that is only present in actively transcribing cells. Using this 'spacer FISH' we detected positive cells in heart valves of patients under adequate therapy. Localization of biofilms: In a 5 year old boy a pigtail catheter was identified as the focus of *S. aureus* sepsis. FISH allowed visualization, identification and localisation of a homogenous and thick *S. aureus* biofilm within the lumen of the catheter. Architecture of multispecies biofilms was analyzed in subgingival plaque of periodontitis patients. These highly complex biofilms comprise several hundred bacterial species including periodontal species and yet uncultured bacteria. By digital image analysis FISH allowed us to quantify the i*n vivo* co-localization of two pairs of microorganisms on a single cell level.

Conclusion: In summary, these findings show the versatility and impact of FISH for diagnosis of biofilm associated infections in the clinical setting.Spacer-FISH allows visualizing the effect of antimicrobial therapy on in vivo grown biofilms and thus, translate laboratory research results into the situation in the patient.

EUKARYOTIC PATHOGENS

EKV01

In vivo imaging of disseminated murine *Candida albicans* infection reveals unexpected host sites of fungal persistence during antifungal therapy

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Objectives: *Candida albicans* is an important fungal pathogen that can cause life-threatening disseminated infections. To determine therapy efficacy in murine models, determination of renal fungal burden as colony-forming units is commonly used. However, this approach provides only a snapshot of the current situation in an individual animal and cryptic sites of infection easily may be missed. Thus, we aimed to develop real-time non-invasive imaging to monitor infection *in vivo*.
Materials and Methods: Bioluminescent *C. albicans* reporter strains were developed based on a bioinformatical approach for codon optimization. The reporter strains were analysed *in vitro* and *in vivo* in the murine model of systemic candidiasis.
Results: Reporter strains allowed *in vivo* monitoring of infection and determination of fungal burden with high correlation between bioluminescence and cfu. We confirmed the kidney as main target organ but additionally observed translocation of *C. albicans* to the urinary bladder. Treatment of infected mice with caspofungin and fluconazole significantly improved clinical outcome and clearance of *C. albicans* from kidneys; however, unexpectedly, viable fungal cells persisted in the gall bladder. Fungi were secreted with bile and detected in faeces, implying the gall bladder as a reservoir for colonisation by *C. albicans* after antifungal therapy. Bile extracts significantly decreased susceptibility of *C. albicans* to various antifungals *in vitro*, thereby likely contributing to persistence.
Conclusions: Using *in vivo* imaging, we identified cryptic sites of infection and persistence of *C. albicans* in the gall bladder during otherwise effective antifungal treatment. Bile appears to directly interfere with antifungal activity.

EKV02

The transcriptional regulators Nrg1 and Tup1 have a different impact on the morphology of the human fungal pathogen *Candida albicans*

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Introduction: The transcriptional repressors Tup1 and Nrg1 are major antagonists of filamentous growth in the polymorphic fungus *Candida albicans*. As formation of hyphae is major contributor to virulence of this human fungal pathogen, it is important to understand the mechanism which support or repress this morphological change. Mutants lacking either Tup1 or Nrg1 or both are known for their filamentous nature, however they are also attenuated in systemic murine infections. Based on these observations, we analyzed single functions of Tup1 and Nrg1 during the morphological dynamics as well as their influence on the transcriptional core filamentation response.
Materials and Methods: *C. albicans* wild type strain SC5314, *nrg1*Δ, *tup1*Δ as well as a *tup1*Δ/*nrg1*Δ double mutant were studied in minimal media with or without human serum. All strains were analyzed by microscopy and consequently, total RNA was isolated for the gene expression studies. For this purpose, we have a used a set of previously defined core filamentation response (CFR) genes including *ALS3*, *ECE1* and *HWP1*. Additionally, overexpression mutants of *NRG1* or *TUP1* were constructed by using an inducible *TET* promoter system.
Results: In response to stimulation of filamentous growth by the addition of human serum, both wild type and *nrg1*Δ formed long and branching hyphae. In consistence, the expression levels of core filamentation genes in the mutant's filaments were at the level of wild type hyphae. Interestingly, this was not the case for strains lacking the *TUP1* gene. Here, CFR genes like *ALS3* and *ECE1* remained at an intermediate level. For *ALS3*, this was not sufficient for localization of this protein on the surface of the filaments of *tup1*Δ and *tup1*Δ/*nrg1*Δ as it was seen for wild type and *nrg1*Δ hyphae. Consequently, only the latter two strains were able to use ferritin as iron source, which is known to be mediated by Als3.
Induced overexpression of Nrg1 but not Tup1 induced yeast like growth in wild type even under serum- inducing conditions. Interestingly, this Nrg1-driven yeast formation was independent from Tup1 as it also occurred in *tup1*Δ/*nrg1*Δ overexpressing *NRG1*. This induced budding phenotype also lead to a lesser expression of some, but not all CFR genes.
Discussion: In contrast to current models, our data suggest that Nrg1 and Tup1 could act differently on the morphology of *C. albicans*. While Tup1 might have dual roles in both growth forms and is obviously required for full expression of some core filamentation response genes, Nrg1 functions much more as the major repressor of hyphal growth and associated gene expression. Our results also indicate that Nrg1 can induce yeast cell formation in a Tup1-independent manner although the detailed mechanisms remained unclear.

EKV03

Fitness costs of drug resistance in *Candida albicans*

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The pathogenic yeast *Candida albicans* can develop resistance to the widely used antifungal agent fluconazole, which inhibits ergosterol biosynthesis. Resistance is often caused by gain-of-function mutations in the transcription factors Mrr1, Tac1, and Upc2, which result in constitutive overexpression of multidrug efflux pumps and ergosterol biosynthesis genes, respectively. It is not known how the permanently changed gene expression program in resistant strains affects their fitness in the absence of drug selection pressure. We have systematically investigated the effects of activating mutations in

Mrr1, Tac1, and Upc2, individually and in all possible combinations, on the degree of fluconazole resistance and on the fitness of *C. albicans* in an isogenic strain background. All combinations of different resistance mechanisms resulted in a stepwise increase in drug resistance, culminating in 500-fold increased fluconazole resistance in strains possessing mutations in the three transcription factors and an additional resistance mutation in the drug target enzyme Erg11. The acquisition of resistance mutations was associated with reduced fitness under nonselective conditions *in vitro* as well as *in vivo* during colonization of a mammalian host. Therefore, the inability to appropriately regulate gene expression results in a loss of competitive fitness of drug-resistant *C. albicans* strains. Most fluconazole-resistant clinical *C. albicans* isolates containing gain-of-function mutations in these transcription factors were also outcompeted by drug-susceptible isolates from the same patients. However, a few drug-resistant isolates did not exhibit reduced fitness in competition experiments with matched susceptible isolates, indicating that *C. albicans* may also overcome the fitness costs of drug resistance.

EKV04

Critical impact of the negative host cell cycle regulator Tspyl2 on stage differentiation of the protozoan parasite *Toxoplasma gondii*

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The eukaryote *Toxoplasma gondii* is one of the most prevalent intracellular parasites throughout the world and an important human pathogen. A key to the transmission to new hosts and the pathogenesis of reactivated toxoplasmosis is the differentiation from fast replicating tachyzoites to dormant bradyzoites which are contained in intracellular tissue cysts. Tissue cysts predominantly persist in brain and muscle tissues; however, the reason for this tissue preference is unknown. In this study, we have used C2C12 murine skeletal muscle cells (SkMCs) to identify host cell factors that critically regulate bradyzoite development. We show that differentiation of SkMCs from proliferating myoblasts to cell cycle-arrested polynucleated myotubes facilitated expression of the bradyzoite-specific antigen 1 and tissue cyst formation by *T. gondii*. Parasite stage differentiation correlated with the up-regulation of muscle-specific marker proteins including MyoD and myosin heavy chain (MyHC), increased expression of the negative cell cycle regulators testis-specific Y-encoded like protein 2 (Tspyl2) and $p21^{Waf1/Cip1}$, down-regulation of the cell cycle activator cyclin B1 and absence of DNA synthesis. Importantly, infection of mature myotubes with *T. gondii* led to a further increase in Tspyl2 mRNA whereas p21 levels did not further increase. Surprisingly, cyclin B1 mRNA was also further increased after infection but this did not suffice to resume cell cycle progression in infected SkMCs. RNA interference was subsequently used to efficiently inhibit Tspyl2 mRNA and downstream p21 and MyHC protein expression. Remarkably, RNAi-mediated Tspyl2 knockdown in SkMC enabled *T. gondii* to vigorously proliferate while stage differentiation towards the bradyzoite was significantly reduced. We have thus identified the negative host cell cycle regulator Tspyl2 as being crucial for *T. gondii* to differentiate in SkMCs. Since Tspyl2 is also highly expressed in the brain of mice, we hypothesize that Tspyl2 or a down-stream effector thereof functions as physiological regulator of *T. gondii* stage differentiation in brain and muscle tissues.

EKV05

Mechanisms of complement evasion by malaria parasites

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Introduction: The complement system represents a crucial component of the innate immune response against invading pathogens. However, a variety of pathogens evade complement attack by binding to host complement regulators such as factor H, C4b-binding protein (C4BP) or vitronectin. In a recent study we showed that in the mosquito vector, the sexual stages of the malaria parasite *Plasmodium falciparum* bind factor H to inactivate complement factor C3b, thereby protecting the emerging gametes from complement-induced destruction by the blood meal. In consequence, factor H-binding promotes parasite transmission from human to human by the mosquito. While these data provide vital information on a highly complex complement evasion mechanism used by malaria parasites to avoid complement attack in the mosquito midgut, it is hitherto not known, if intra-erythrocytic parasites in the human host also bind complement regulators for protection.
Materials and Methods: In order to determine if factor H also binds to the intraerythrocytic *P. falciparum* stages, we carried out cell-based binding assays, enzyme-linked immunosorbent assays and indirect immunofluorescence assays using asexual blood stage parasites following incubation with either human serum or factor H. Similar approaches were used to investigate the potential binding of C4BP by blood and sexual stage parasites.
Results: We here show that the intra-erythrocytic schizonts acquire factor H and factor H-like protein 1 from human serum as a means to evade complement attack in the human host. In contrast no prominent C4BP-binding by schizonts or gametes was detected.
Conclusion: The combined data indicate that the acquisition of factor H is an important mechanism of the *P. falciparum* parasites to protect themselves from attack by the alternative pathway of human complement. Ongoing work investigates C3b inactivation by the schizonts and aims to identify the blood stage-specific receptor(s) of factor H.

EKV06

Manganese Superoxide Dismutase Mimics Inhibit the Proliferation of *Leishmania* Parasites

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Introduction: The generation of reactive oxygen (ROS) or nitrogen species (RNS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide ($^{\cdot}NO$), and peroxynitrite ($ONOO^-$) can have host-protective or -damaging effects. Numerous infectious, autoimmune or degenerative diseases are linked to oxidative and nitrosative stress. On the other hand, ROS and RNS are important immunoregulatory molecules and are critical for the defense against a variety of infectious pathogens. Superoxide dismutases (SODs) are able to detoxify $O_2^{\cdot-}$ by converting the radical into H_2O_2, which is subsequently cleaved by catalase. The application of SOD mimics in inflammatory or infectious diseases might show beneficial effects due to (1) the generation of H_2O_2 (2) or the consumption of $O_2^{\cdot-}$, which prevents the formation of peroxynitrite and the consecutive tyrosine nitration of host cell components, but also increases the availability of NO.
Objective: In preparation of future applications of various manganese (Mn) SOD mimics (M40403, MnPyane) and their SOD-inactive counterparts (M40404, MnPydiene, $MnCl_2$) in chronic infections such as cutaneous and visceral leishmaniasis, we analyzed the effect of these compounds on extracellular *Leishmania* parasites.
Materials and Methods: The survival and mitrochondrial activity of *Leishmania* and host cells in the presence of the MnSOD mimics was evaluated by MTT-assays. The viability of parasites was assessed by FACS staining with propidium iodide and annexinV. The proliferation of the parasites was analyzed by FACS after labeling with the cell proliferation dye eFluor670. Cell cycle analyses were performed by FACS analysis of propidium iodide-stained parasites.
Results: A striking direct anti-leishmanial effect on *Leishmania* promastigote of various species was observed with the SOD-active compounds MnPyane and M40403 (IC_{50} <2 or <50 mM, respectively), but also with the SOD-inactive compound M40404 (IC_{50} 30-70 mM). We obtained no evidence that the activity of the MnSOD mimics was dependent on the generation of H_2O_2. Propidium iodide and eFluor670 stainings revealed that the anti-leishmanial effect of the MnSOD mimics did not result from increased parasite cell death, but rather from a blockade of parasite proliferation.
Conclusion: Our results document a novel anti-leishmanial effect of MnSOD mimics. It is tempting to speculate that this SOD-independent anti-parasitic activity might complement the previously proposed immunoregulatory activities of MnSOD mimics during inflammatory processes such as chronic leishmaniasis.

EKV07

Pneumocystis jirovecii can be productively cultured in differentiated CuFi-8 airway cells

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Introduction: Despite being a long known and serious pathogen all attempts to isolate, cultivate, and propagate the fungus *Pneumocystis jirovecii* failed so far. This serious gap in Microbiology is closed by the present study.
Materials and Methods: Based on a permanent three-dimensional air-liquid interface culture system of differentiated pseudostratified airway epithelia we successfully isolated and propagated *P. jirovecii* from bronchoalveolar lavages positive for *P. jirovecii* by qPCR. We provide evidence that P. jirovecci induces cytopathic effects in lung epithelia and is even invasive in cell culture.
Results: Using established qPCR protocols we show that P. jirovecii can be cultured in human epithelial cell cultures and can successfully be propagated after passage. The results are confirmed by sequencing and immunofluorescence staining with monoclonal antibody and detection systems available for IVD detection of P. jirovecii.
Discussion: This is the first report of successful productive cultivation and propagation of *Pneumocystis jirovecii*, a human pathogenic fungus of major clinical significance. The established cell culture system offers the chance to perform molecular analyses of the pathogen's life cycle and further *in vitro* studies on *P. jirovecii* such as drug sensitivity and resistance tests as well as tenacity studies, whilst replacing animal models that rely on the rodent pathogen P. carinii. The study is groundbreaking as it will influence the field of diagnostic microbiology, antibiotic testing against Pneumocystis jirovecii, and will allow tenacity studies required for optimized hygiene concepts. Taking into account that productive isolation of *Pneumocystis jirovecii* failed for decades the study is a breakthrough for the field.

EKV08

Survival and proliferation of human pathogenic *Candida* species within macrophages

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Candida albicans and *C. glabrata* are important fungal pathogens, which can cause life-threatening systemic infections in immunocompromised patients. Immune evasion strategies likely play a key role during infection, as both fungi not only survive phagocytosis by macrophages, but even proliferate intracellularly and escape. We are therefore interested in fungal strategies that mediate survival in macrophages.
By analyzing phagosome maturation markers, we observed that phagosomes containing *C. glabrata* remain in a late endosomal stage and do not proceed to a phagolysosome (1, 2). While viable *C. glabrata*-containing compartments remain negative for staining with the acidotropic dye LysoTracker and show low phagosomal hydrolase activity, heat killed yeasts are delivered to an acidic phagolysosome. This altered phagosome maturation is likely not triggered by initial recognition events via MAPK or NFkB signaling, as such pathways were not differentially activated by viable as compared to heat-killed *C. glabrata*. However, Syk activation decayed faster in macrophages containing viable yeasts, suggesting a role of Syk-mediated signaling in phagosome maturation.
Active pH modulation is one possible fungal strategy to change phagosome pH. We discovered that, similar to *C. albicans*, *C. glabrata* is able to alkalinize its extracellular environment, when growing on amino acids as sole carbon source *in vitro*. By screening a *C. glabrata* mutant library we identified genes important for environmental alkalinization that were further tested for their impact on phagosome pH. We found that the lack of fungal mannosyltransferases resulted in severely reduced alkalinization *in vitro* and in the delivery of *C. glabrata* to acidified phagosomes (2). These data suggest a key role of protein mannosylation in alteration of phagosomal properties caused by *C. glabrata*.
C. albicans and *C. glabrata* differ in their escape from macrophages: while *C. glabrata* replicates as yeast cells until a critical mass is reached and host cells burst (1), *C. albicans* escapes via filamentation and hypha-associated activities. We discovered a hypha-associated polypeptide of *C. albicans*, Ece1, which is potentially processed into eight peptides. One of these peptides has the capacity to efficiently produce pores in host membranes and lyses host cells. A deletion mutant lacking *ECE1* was significantly reduced in its ability to damage macrophages. Pore formation by Ece1 in macrophage membranes may therefore play a key role on fungus macrophage interaction, with possible effects on transmembrane molecule fluxes, macrophage activation, membrane lysis, and escape.

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EKV09

Ece1 - a *Candida albicans* pore-forming toxin

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Candida albicans is one of the most commonly isolated opportunistic fungal pathogens in humans. When host immunity is impaired, it can readily cause a multitude of diseases, ranging from superficial infections of the skin and mucosa to life-threatening systemic infections. A pathogenicity mechanism of great importance in this fungus is its ability to switch morphology from single yeast cells to a filamentous hyphal growth, which is involved in tissue penetration and damage. During hyphal growth, *ECE1* -a gene of yet unknown function - is highly expressed. In a screen of over 120 mutants, a strain lacking *ECE1* was the only strain found which - although still being able to form hyphae - was unable to activate the epithelial damage response pathway. We have shown that *ece1*Δ cells are able to adhere to and invade host cells like the wild type, but they are not capable of causing cell damage to either oral, vaginal or gastrointestinal epithelial cells. In this perspective, the aim of this study is to elucidate the function of the Ece1 protein and to unravel the mechanism underlying its damaging effect on epithelial cells. Ece1 is a protein of 271 amino acids, containing a signal peptide and eight tandem repeats of a degenerate 34 amino acid sequence separated by KR (lysine-arginine) motifs. These KR motifs represent potential cleavage sites for the Kex2 protease, which - *in vitro* - processes Ece1 into eight peptides. An *E. coli* clone heterologously expressing Ece1, as well as a *Pichia pastoris* clone expressing Kex2, have been produced. This allowed us to purify recombinant Ece1 and Kex2 to test the effect of unprocessed or *in vitro* digested Ece1 on epithelial cells. Using a complementary approach, the eight peptides predicted to result from complete cleavage by Kex2, were synthesised. We are assessing the cytolytic activity of Ece1 by using cell culture models (oral, vaginal and gastrointestinal epithelia), as well as an *in vitro* haemolysis assay. While we could already show that one of the peptides resulting from Ece1 digestion does have pore-forming activity, the current experiments are aiming at identifying the subcellular localisation of Ece1 during *C. albicans* interaction with human epithelial cells, and whether a processing of the full length protein into short peptides may be essential for Ece1 to reach its full cytolytic potential. Unraveling the function of Ece1 will significantly advance our understanding of candidiasis and help elucidate the mechanisms of *C. albicans* - mediated epithelial cell damage.

BIOLOGY OF FILAMENTOUS FUNGI

FUV01

The role of the pore-forming toxin Ece1 of *Candida albicans* during translocation through the intestinal epithelial barrier

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The opportunistic pathogen *Candida albicans* is a common inhabitant of the human gastrointestinal tract. Although *C. albicans* is mostly a harmless commensal, the fungus can cause life-threatening blood stream infections in immunocompromised patients by translocating the intestinal barrier. To date, the molecular mechanisms of this translocation are poorly understood. Our group recently discovered that the well-known hyphae-associated gene *ECE1* encodes a polypeptide with multiple processing sites, which seems to be processed into eight peptides via an unknown protease. One of the proposed peptides, Ece1-PIII, acts as a pore-forming toxin.
To elucidate the role of Ece1-PIII in the translocation process, we created an *ece1*Δ deletion mutant, a revertant *ece1*Δ *+ECE1* and a mutant that lacks only the PIII-encoding sequence. We used these strains to assess the influence of Ece1 in all possible steps of the translocation process, namely filamentation, adhesion, invasion, interepithelial dissemination, barrier function integrity (measured with TEER), damage and translocation itself. The latter was investigated with a translocation assay and all analyses were carried out using intestinal cell monolayers.
The results indicate that Ece1 has only a minor influence on the *in vitro* translocation process itself, but plays a key role in epithelial damage. Our data show that Ece1 is dispensable for filamentation, adhesion, invasion and even interepithelial dissemination of the fungus, but is essential for epithelial damage.

FUV02

Scrutinizing the *Aspergillus fumigatus* Mating-Type Idiomorphs

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Sexual reproduction of the human pathogen *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008. The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing presence of mating type idiomorphs (*MAT1-1* and *MAT1-2*) and several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-2* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which reveals the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding factors presumably involved in this process. Transcriptional profiling studies demonstrate the depth of the mating-type factor-directed transcriptomes. Given the narrow conditions that favour sexual development in *A. fumigatus* accompanied by the strict need for the opposite mating type, we became interested in the creation of a presumed homothallic strain expressing both mating-type idiomorphs and therefore being capable of progression through the initial stages of sexual development and fruiting body formation. Also, a congenic pair of strains differing only in their mating-type identity is currently analysed with the aim to systematically scrutinize any phenotypical differences based on the *MAT1* genotype. Our studies aim at a comprehensive analysis of the newly discovered sexuality of the human-pathogenic mould *A. fumigatus* and its relevance for virulence.

FUV03

The transcription factor TaSte12 mediates the regulatory role of the Tmk1 MAP kinase in mycoparasitism and vegetative hyphal fusion in *Trichoderma atroviride*

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Mycoparasitic species of the fungal genus *Trichoderma* are potent antagonists able to combat plant pathogenic fungi by direct parasitism. An essential step in this mycoparasitic fungus-fungus interaction is the detection of the fungal host followed by activation of the molecular weapons in the mycoparasite by host-derived signals. The *Trichoderma atroviride* MAP kinase Tmk1, a homolog of yeast Fus3/Kss1, plays an essential role in regulating the mycoparasitic host attack, aerial hyphae formation and conidiation. However, the transcription factors acting downstream of Tmk1 are hitherto unknown. Here we analyzed the functions of the *T. atroviride* Ste12 family transcription factor TaSte12 whose orthologue in yeast is targeted by the Fus3 and Kss1 MAP kinases. Deletion of the *Taste12* gene in *T. atroviride* not only resulted in reduced mycoparasitic overgrowth and lysis of host fungi but also led to loss of hyphal avoidance in the colony periphery and a severe reduction in conidial anastomosis tube formation and vegetative hyphal fusions. While the transcription of several orthologues of *Neurospora crassa* hyphal fusion genes was reduced upon *Taste12* deletion, the Δ*Taste12* mutant showed enhanced expression of mycoparasitism-relevant chitinolytic and proteolytic enzymes and of the cell wall integrity MAP kinase Tmk2. Based on the comparative analyses of Δ*Taste12* and Δ*tmk1* mutants, an essential role of the TaSte12 transcriptional regulator in mediating outcomes of the Tmk1 MAPK pathway such as regulation of the mycoparasitic activity, hyphal fusion and carbon source-dependent vegetative growth is suggested.

FUV04

Velvet components in the industrial penicillin producer *Penicillium chrysogenum*: Regulation of secondary metabolism and morphology

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. It is supposed that subunits of the velvet complex, which were recently detected for *P. chrysogenum*, function as such global regulators, although the exact regulatory mechanisms still have to be elucidated. Core components of this complex are PcVelA and PcLaeA, which regulate secondary metabolite production, hyphal morphology, conidiation, and pellet formation. Here we describe the characterization of PcVelB, PcVelC, and PcVosA as novel subunits of this velvet complex. Using yeast two-hybrid analysis and bimolecular fluorescence complementation (BiFC), we demonstrate that all velvet proteins are part of an interaction network. Functional analyses using single and double knockout strains generated by the FLP/*FRT* recombination system clearly indicate that velvet subunits have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation. Most strikingly, a direct interaction of PcVelB with an enzyme of the penicillin biosynthesis pathway, the isopenicillin N synthase was identified during yeast two-hybrid analysis with PcVelB as bait. This surprising interaction was confirmed with BiFC *in vivo*, thereby localizing the interaction in dot-like structures in the cytoplasm. Our discovery of a direct interaction of the isopenicillin N synthase with a subunit of the velvet complex implies a novel regulatory mechanism how enzymes of penicillin biosynthesis are regulated at the molecular level. The results provided here contribute to our fundamental understanding of the function of velvet subunits as part of a regulatory network mediating signals responsible for morphology and secondary metabolism, and will be instrumental in generating mutants with newly derived properties that are relevant to strain improvement programs.

FUV05

Aspergillus niger as Expression Platform for Secondary Metabolite Production

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Introduction: Filamentous fungi can each produce dozens of different secondary metabolites which are attractive to become exploited as therapeuticals, drugs, antimicrobials, flavour compounds, and other high-value chemicals. Although the genomes of filamentous fungi contain numerous gene clusters that encode for enzymes involved in the biosynthesis of so far uncharacterized secondary metabolites (SM), many of these gene clusters are silent under laboratory growth conditions.[1] Where known, SM production is regulated by complex networks and involves intricate multi-step biosynthetic machineries, as well as major reorganization of primary metabolic fluxes to redirect cellular metabolic resources towards their biosynthesis. As SM expression varies considerably with the host and enzymes catalyzing SM are often difficult to express, we aim to establish the filamentous fungus *Aspergillus niger* as heterologous host for SM production.
Materials and Methods: We used the multifunctional non-ribosomal peptide synthetase (NRPS) enniatin synthetase (Esyn) from *Fusarium oxysporum*, catalyzing the formation of the cyclic depsi peptide enniatin and expressed its encoding gene under control of the inducible Tet-on promoter in *A. niger*.[2] Enniatin was isolated and purified from *A. niger* shake flask cultures using EtOAc extraction, liquid chromatography and crystallization. The purity of enniatin B was proven by MS/MS and NMR analyses as well as x-ray crystallography.
Results & Conclusion: The initial yields of 1 mg/L of enniatin B were increased 1000-fold using a DoE approach, which addressed feeding conditions (precursor addition, concentration of carbon source) and the morphology of *A. niger* (reduction of pellet size by addition of micro particles). From a 1-L culture in optimized medium, around 400 mg of pure enniatin B could be isolated and purified. The titre was found to be significantly higher than that reached in bacterial expression hosts, demonstrating that *A. niger* is a promising host for the expression of NRPS of fungal origin.

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Figure 1

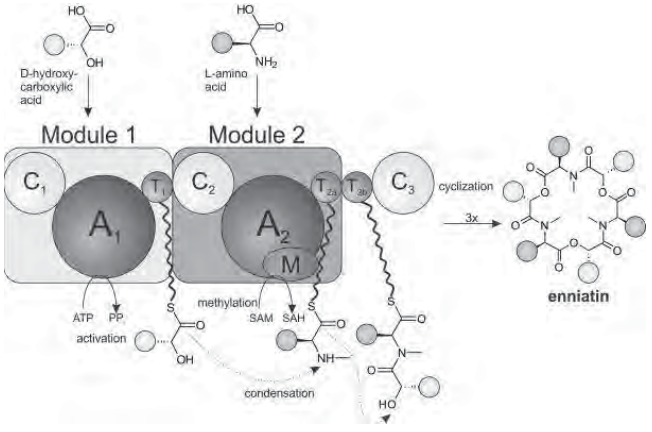
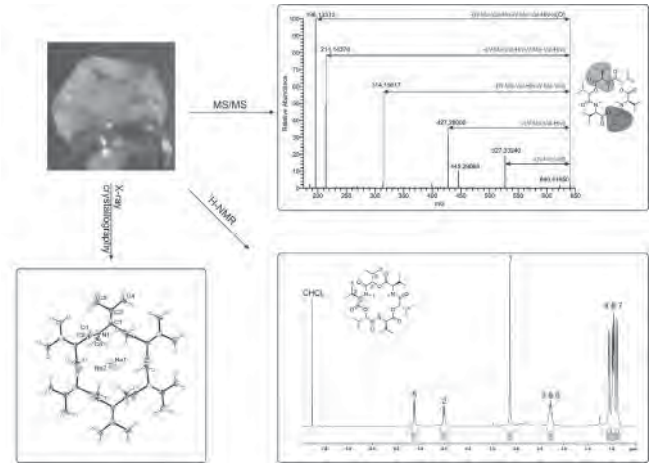


Figure 2



FUV06

Marine Fungi: Understanding the biology of marine fungi for the production of secondary metabolites to establish a natural product library

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Although marine fungi are a prolific group of secondary metabolite producers, they are not well characterised and are underutilised in their biotechnological application. There is very little knowledge of the biology of fungi living in the ocean and the role and regulation of secondary metabolite production in marine fungi. Here, we demonstrate that the application of rationally designed culture conditions for a group of marine fungi enables efficient production of marine natural products in the laboratory and also in large scale cultures. The resulting extracts were screened for new bioactive compounds with the focus on ant-cancer activities and were used to build up a library of purified natural products.
This approach is an outcome of the project "MARINE FUNGI" within the KBBE framework of EU's FP7 (www.marinefungi.eu). We develop a process concept for these compounds providing the technological basis for a sustainable use of marine microbial products as result of "Blue Biotech".

GASTROINTESTINAL PATHOGENS

GIV01

Utilization of host-derived sulphur components enables Campylobacter jejuni to overcome its restricted cysteine metabolism

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Microbial access to nutrients from the host is a fundamental aspect during disease progression. This microbial snatching for food from the host is closely linked to pathogenesis and has recently been described as "nutritional virulence". *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis in industrialized countries. However, despite its clinical relevance, less is known about its virulence mechanisms and about the nutrients contributing to the growth of *C. jejuni* during infection. While it is known that several glucogenic amino acids energize the growth of *C. jejuni* during infection, none alone has been found to be essential for proliferation. Here we demonstrate through phenotypic microarray analysis and growth experiments in defined medium that cysteine is indispensible for *C. jejuni* growth since it can not be synthesized *de novo* from sulfate or methionine. Furthermore we show that *C. jejuni* can overcome its restricted cysteine metabolism by using host-and microbiota-derived substrates like sulfide, thiosulfate, glutathione (GSH, γGlu-Cys-Gly). In addition we show

for the first time that release of cysteine from GSH occurs both in a γ-glutamyltranspeptidase (GGT) dependent and independent manner and that cysteine-containing dipeptides can serve as cysteine-source for *Campylobacter* growth. Strikingly, we identified by growth as well as NMR-spectroscopy analysis the first prokaryotic oligopeptide transporter, named CptA (*Campylobacter* peptide transporter A), which is involved in the uptake of the GSH degradation product Cys-Gly. In total, our study provides new insights into the usage of sulphur-containing amino acids for the proliferation of *C. jejuni* and the utilization of host- and microbiota-derived substrates to circumvent its nutritional dependencies.

GIV02

High throughput mapping of transcription start sites and non-coding RNAs in the pAA plasmid of enterohemorrhagic *Escherichia coli* O104:H4

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) can cause a severe foodborne disease in humans, which is typically characterized by bloody diarrhea and might progress to hemolytic uremic syndrome (HUS) in up to 10% of the cases. EHEC of serotype O104:H4 (EHEC O104:H4) was characterized as the causative agent of the largest German outbreak (May-July 2011) during which more than 3800 people were infected, and of these more than 850 (22%) developed HUS [1]. Besides having a chromosomally integrated Shiga toxin 2a encoding bacteriophage, EHEC O104:H4 carries a pAA plasmid containing the aggregative adherence fimbriae I cluster (AAF/I; a characteristic feature of enteroaggregative *E. coli*) mediating the tight adherence of the outbreak strain to cultured human epithelial cells. This rear combination of virulence factors is believed to contribute to the exceptional pathogenicity of EHEC O104:H4. Our group recently reported that the outbreak strain can lose pAA in the course of illness and that pAA loss was associated with a significantly reduced correlation of HUS development in patients [2].
Materials and Methods: Here we present a thorough investigation into the distribution of transcription start sites (TSS) and non-coding RNAs in the pAA plasmid of the clinical EHEC O104:H4 isolate LB226692. We performed differential RNA-seq, a 5'-end transcriptome sequencing approach designed to selectively identify primary transcripts [3].
Results: In total, we detected 240 TSS and thus much more than expected for a 75 kb plasmid carrying 83 genes. 22% of the TSS were involved in gene expression and TSS for all pAA-encoded virulence associated genes and genes involved in plasmid replication, segregation and maintenance were identified. More than 1/3 of the TSS were mapped antisense to annotated ORFs, therefore demonstrating the existence of extensive asRNA synthesis in pAA. In addition, we detected numerous TSS within operons allowing for transcriptional uncoupling of genes in polycistronic clusters. Last but not least, we mapped 43 primary transcripts (18%) in intergenic regions which could function as regulatory trans-encoded ncRNA.
Discussion: Taken together, our data indicates an unexpected complexity of pAA gene expression and provides the basis for future investigations of pAA importance in host-pathogen interactions and disease severity.

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GIV03

Bidirectional gene exchange between *Helicobacter pylori* strains from a family in Coventry, United Kingdom

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The human gastric pathogen *Helicobacter pylori* is characterised by a high mutation rate and frequent recombination events during mixed infection, which result in extensive genetic diversity and rapid allelic diversification. We have previously used multilocus sequence and core-genome based analyses to study patterns of transmission of *H. pylori* within families.
To better understand the genomic flexibility of *H. pylori* after intra-familial transmission, we used 454 sequencing technology to investigate whole genome sequences of *H. pylori* strains isolated from members of three generations of a family living in Coventry, UK.
The genomes of four *H. pylori* strains isolated from a grandfather, two of his sons and one grandson were sequenced. Three of these genomes showed a high overall sequence similarity, suggesting a recent common ancestor. The genomes differed by 316-336 SNPs, and recombination events (imports) resulted in 170-251 clusters of polymorphisms (CNPs). Imports were particularly frequent in genes encoding *Helicobacter* outer membrane proteins, suggesting an adaptation of the strains to their individual host. The fourth strain differed substantially from these three highly related strains but still shared long fragments of identical sequence, which most likely reflect imports from the highly related family variants. The data show extensive bidirectional exchange of DNA between the strains isolated from the family members. Detailed analysis of the distribution of SNPs and imports permits to draw up a scenario of the most likely transmission pathway. The high frequency of recombination was unexpected for an industrialised country where the prevalence of *H. pylori* infection has strongly declined in recent decades.

GIV04

A small RNA which is regulated by the acid-responsive ArsRS two-component system in *Helicobacter pylori*

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Introduction: The small genome of *Helicobacter pylori* (1.67 Mb) encodes only for a few transcriptional regulators and almost nothing is known about post-transcriptional regulation in this prevalent human pathogen so far. Moreover, *H. pylori* does not carry an apparent homolog of the RNA-chaperone Hfq and was previously thought to completely lack riboregulation. However, based on a RNA-seq approach >60 small RNA (sRNAs) candidates have been identified in *H. pylori* strain 26695 [1].
Materials and Methods: To understand the roles of these sRNAs during stress-response or virulence regulation, we use expression profiling of sRNA candidates under various growth conditions, proteome and transcriptome analyses of sRNA deletion and overexpression mutants, as well as bioinformatics-based target predictions.
Results: Exposure to low pH is one of the main environmental stresses encountered by *H. pylori* during colonization of the human stomach. Using biocomputational analysis, several mRNAs of the regulon of the acid-responsive ArsRS two-component system [2] were predicted as potential targets for a candidate *trans*-acting sRNA, ArsZ. This sRNA is highly conserved among diverse *H. pylori* strains and shows increased expression in a deletion mutant of the ArsS sensor kinase on Northern blots. Initial electrophoretic mobility shifts assays further suggest a direct regulation by the ArsR response regulator on the sRNA promoter. Moreover, we have predicted that a single-stranded, potential anti-Shine-Dalgarno sequence of ArsZ could bind to the ribosome binding site of potential target mRNAs, suggesting a negative post-transcriptional regulation by competing with ribosome binding. *In-vitro* gel shift assays and structure-probing assays confirmed a direct interaction between ArsZ and one of the potential target mRNAs, encoding a histidine-rich protein. We have now constructed *arsZ* deletion and overexpression strains and applied transcriptome and proteome analyses to identify and validate its target genes *in vivo*.

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GIV05

"Drugs from Bugs": The bacterial effector protein YopM is a `self-delivering´ anti-inflammatory agent

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Cell-permeable proteins, also called cell-penetrating peptides (CPP), have the ability to cross cellular membranes, either alone or in association with a bioactive cargo. We identified the *Yersinia* protein YopM as a novel bacterial cell-permeable protein and described the ability of isolated recombinant YopM to enter host cells without a requirement for additional factors. Furthermore, treatment of cells with recombinant YopM leads to the down-regulation of inflammatory cytokines such as TNFa. This might contribute to systemic effects on innate immunity and, furthermore, suggests potential therapeutic applications of YopM e.g. in chronic inflammatory diseases. Hence, we were interested to investigate whether YopM might have potential as a topical applied ´Immune Modulating Molecule´, by exhibiting beneficial effects on inflammation and the production of cytokines that maintain the inflammatory process linked to autoimmune diseases.For this purpose, we employed different murine models of inflammatory disease such as the imiquimod (IMQ)-induced psoriasis model, theT-Cell transfer model of chronic colitis, or $hTNF_{tg}$ mice as a chronic model of rheumatoid arthritis. Our results verified "self-delivering" abilities of the effector protein also *in vivo* and already indicate a remarkable dampening of overt inflammatory reactions in all investigated models. In particular, we could prove the possibility of local application routes of recombinant protein after oral, epicutaneous, or intra-articular treatment and emphasized that YopM is an effective reducer of TNFα-induced production of pro-inflammatory cytokinesvia the NFκB-pathway.
Taken together our results underline the potential of YopM as a novel versatile pathogen-derived immune-modulatorfor the treatment of inflammatory diseases.

GIV06

The role of serine protease HtrA in acute ulcerative enterocolitis and extra-intestinal immune responses during *Campylobacter jejuni* infection of gnotobiotic IL-10 deficient mice

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Introduction: *Campylobacter jejuni* infections have a high prevalence worldwide and represent a significant socioeconomic burden. *C. jejuni* can cross the intestinal epithelial barrier as visualised in biopsies derived from human patients and animal models, however, the underlying molecular mechanisms and associated immunopathology are still not well understood. We have recently shown that the secreted serine protease HtrA plays a key role in *C. jejuni* cellular invasion and transmigration across polarised epithelial cells *in vitro*. In the present *in vivo* study we investigated the role of HtrA during *C. jejuni* infection of mice.
Materials and Methods: We used the gnotobiotic IL-$10^{-/-}$ mouse model to study campylobacteriosis following peroral infection with the *C. jejuni* wild-type strain NCTC11168 and the isogenic, non-polar NCTC11168Δ*htrA* deletion mutant. Six days post infection (p.i.) with either strain mice harboured comparable intestinal *C. jejuni* loads, whereas ulcerative enterocolitis was less pronounced in mice infected with the D*htrA* mutant strain. Moreover, Δ*htrA* mutant infected mice displayed lower apoptotic cell numbers in the large intestinal mucosa, less colonic accumulation of neutrophils, macrophages and monocytes, lower large intestinal nitric oxide, IFN-g and IL-6 as well as lower TNF-α and IL-6 serum concentrations as compared to wild-type strain infected mice at day 6 p.i. Notably, immunopathological responses were not restricted to the intestinal tract given that liver and kidneys exhibited mild histopathological changes six days p.i. with either *C. jejuni* strain. We also found that hepatic and renal nitric oxide levels or renal TNF-a concentrations were lower in the Δ*htrA* mutant as compared to wild-type strain infected mice.
Conclusion: We show here that the *C. jejuni* HtrA protein plays a pivotal role in inducing host cell apoptosis and immunopathology during murine campylobacteriosis in the gut *in vivo*.

GENOMICS AND METAGENOMICS

GMV01

Towards unravelling the origin of the unique natural compound sodorifen of *Serratia plymuthica* by a metagenomic approach

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Microorganisms produce a variety of volatile organic compounds (VOCs). Due to their volatility these molecules can spread over large distances and are ideal infochemicals for inter- and intraspecific communication and cell-cell signals [1]. The volatile compound sodorifen emitted by *Serratia plymuthica* 4Rx13 is a unique feature of this bacterial isolate. Thus, sodorifen is a polymethylated bicyclus with a novel and unusual structure [2]. *S. plymuthica* 4Rx13 as well as the isolates *S. p.* 3Re-4-18 and *S. p.* HRO-C48 emit sodorifen in high amounts while 15 other *Serratia* species and isolates do not have this ability [3]. These three isolates are originated from the rhizosphere of *Brassica napus* or *Solanum tuberosum* grown in and aroundRostock. Until now the biological and ecological function of sodorifen is unknown. Hints about the function of the original sodorifen involved genes can be obtained by elucidation of the original genes.
Via transcriptomic analysis and verification by knock out mutation one gene was found to be involved in the sodorifen biosynthesis of *S. p.* 4Rx13. This gene is co-transcribed with three additional genes imbedded in a cluster. These genes are target genes which can be used as probes to search for bacteria isolated i) from the rhizosphere of *Brassica napus* grown on original fields (Rostock and Schwerin) (=reisolation), ii) from other fields in Mecklenburg-Vorpommern, and iii) from fields elsewhere. Hence, the following questions are addressed: Are the sodorifen producers are still present in regions around Rostock or in other regions? Do more and other bacterial species exist that produce sodorifen? Are the genes transferred by horizontal gene transfer? The following experimental approaches will be taken: i) As a first screen DNA of the isolated rhizobacteria will be analysed with BOX PCR. The generated DNA fingerprints will be compared to the DNA fingerprints of *S. p.* 4Rx13 and the other producers. Additionally, the isolated metagenomic DNA of the rhizobacteria will be tested for the presence of the respective genes via PCR. Finally the newly isolated rhizobacteria will be tested for their ability to produce sodorifen. The second approach (ii) is to screen metagenomic libraries from soil samples of different regions in Germany and worldwide for the genes of the 'sodorifen cluster'. iii) Application of AntiSMASH software a search in published genome sequences detected homologous genes in *Pseudomonas chlororaphis* O6 and *Streptomyces tsukubaensis* NRRL18488. These strains will be obtained and tested for their ability to produce sodorifen.

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GMV02

Identification and characterization of genes coding for enzymes with cellulolytic activities in metagenomes from agricultural soil

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Cellulose degradation takes place in agricultural soil after plant residue incorporation, and it plays an important role in carbon mineralization and sequestration. The process is heavily influenced by the soil chemical and genetic backbone, e.g. soil carbon stock and the presence of cellulase producing microorganisms. The degradation of cellulose is a slow stepwise process and a multitude of cellulase gene families are involved. They include glycoside hydrolase and auxiliary activity families, sometimes possessing carbohydrate binding modules. Low sequence conservation of these genes has made it difficult to quantify the activity and abundance of cellulolytic microorganisms in soil. Here, we took a metagenomic sequencing approach to identify genes coding for cellulases in agricultural soil. Focus was set on the comparison between different tillage strategies

which affect the soil carbon stock. Sequencing yielded a total of 1.1 million clean reads with an average length of 410 basepairs. In addition, enzyme assays were performed to measure potential activities of cellulases. Our results show that soils under reduced tillage contain a higher amount of carbon, microbial biomass and potential cellulase activity than soils under normal tillage. Analysis of the metagenomic data did not reveal prominent differences between tillage treatments. However, a large diversity of cellulase gene families could be predicted based on Hidden Markov Model-logos, including glycoside hydrolase families 1, 3 and 5, auxiliary activity family 3 and carbohydrate binding modules 4, 6 and 32. These genes were harboured by microbial genera belonging to the phyla *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes* and *Verrucomicrobia* in both soils. Interestingly, nucleotide sequence comparison showed that many predicted cellulase gene families also contain enzymes involved in other reactions than cellulose degradation. In conclusion, our data shows that in soil a large diversity of microorganisms with a broad array of cellulase genes are involved in cellulose degradation. To ultimately target specific cellulase gene families and assess their diversity and abundance both on DNA and RNA level, specific primers were designed based on the metagenomic data.

GMV03
Methanotrophic bacteria of the termite gut

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The gut of termites accommodates a great variety of different bacterial symbionts. Over 300 different bacterial genomes were revealed, of which 67 % cannot be addressed to specific bacterial strains and some even cannot be assigned to known bacterial phyla [1,2]. With its rich deposits of CH_4 and even O_2 this habitat also features good conditions for methane oxidizing bacteria (MOB) [3]. However, this is the first study known so far that proves the evidence of MOB in the termite guts of *Incistitermes marginipennis, Mastotermes darwiniensis* and *Neotermes castaneus*. Existence was verified by detecting the *pmoA*, the gene for the particulate methane monooxygenase, which is unique to MOB [4], and supported by fluorescence in situ hybridization (FISH) and quantitative real-time PCR (qPCR) with MOB specific probes and primers, respectively. The MOB cell count was determined to 10^2 to 10^3 per gut. Analyses of the 16S rDNA showed close similarity to the genus *Methylocystis* but, together with various physiological tests and a DNA fingerprint method, no exact match to a known strain, indicating the isolation of a new MOB species. With the unique ability to oxidize CH_4 as a sole source of carbon and energy [5], MOB offer the possibility to be used in biogas upgrading plants in order to decrease the problem of methane leakage. Methane loss occurs when biogas is upgraded to natural gas standards in order to feed it into the natural gas grids. Ongoing studies show a downgrade of methane when the CH_4 containing exhaust emissions are guided over a specially constructed reactor inoculated with MOB and therefore optimizing the economic balance of biogas plants.

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GMV04
Genome analysis of acidophilic sulfate reducing bacteria

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Acid Mine Drainage (AMD) water is caused by anthropogenic mining activities. AMD from brown coal mining for example contains elevated concentrations of sulfate in addition to its acidity and high iron load. In this context, we are studying acidophilic Sulfate Reducing Bacteria (SRB) since microbial dissimilatory sulfate reduction is the only sustainable process for bioremediating high sulfate waters. Our approach is based on the analysis of the genome sequence of acidophilic SRB with the aim to reveal possible metabolic features that could help to improve the performance of the microbial process applied to AMD waters. We sequenced the genomes of three acidophilic SRB: *Candidatus* 'Desulfosporosinus acididurans' strain M1, *Candidatus* 'Desulfobacillus acidavidus' strain CL4 and *Peptococcaceae bacterium* strain CEB3. All of them belong to the bacterial phylum *Firmicutes* and are mesophilic, anaerobic, motile and rod-shaped bacteria that form endospores. Apart from these, there are only two other genome sequences from acidophilic representatives among the approximately 80 partially or completely sequenced genomes of SRB that are freely available in online databases. The comparative analyses of the genomes of acidophilic SRB and those of neutrophilic and alkalophilic species could therefore contribute greatly to our understanding of the genetic basis for the physiological and biochemical adaptation to the acidic environment. For example, the whole genome comparison of the now five available genomes of acidophilic SRB with the genomes of five close neutrophilic relatives has highlighted a set of 33 genes that appear to be specific to the acidophilic strains. The main fraction of these genes code for transporters (12) with a possible role in ion homeostasis and the transport of heavy metals. Moreover, the comparison of the protein sequence of one of these transporters (a NhaC-like antiporter) to genomes of all sequenced SRB indicates a clear correlation of the protein sequence to the acidophilic and neutrophilic life style. Experiments are currently under way to confirm these Results: The approach taken hereby involves both an overall analysis of the cellular response to acidic conditions using techniques in transcriptomics (RNA-seq) and a quantitative expression analysis targeted at the gene encoding the NhaC-like antiporter using quantitative reverse transcription PCR (RT-qPCR).

GMV05
The genome of *Beggiatoa alba* B18LD^T, a large sulfur-oxidizing Gammaproteobacterium

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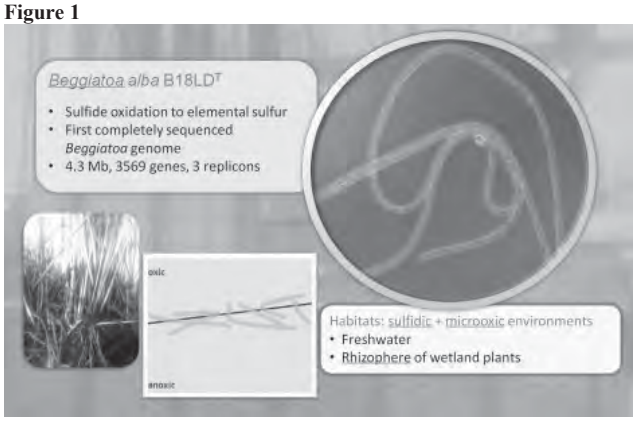
Members of the genus *Beggiatoa* are conspicuous Gammaproteobacteria which thrive at redox interfaces of freshwater or marine environments. There they may couple the oxidation of sulfide to sulfur or sulfate with oxygen or nitrate respiration. The capability to fix molecular nitrogen is widespread among this genus, and at least marine *Beggiatoa* are apparently autotrophs. In contrast to their biogeochemical importance and interesting biology, there is thus far only fragmented information available on the genomic basis for their manifold features.
Here we present the first complete genome sequence of a *Beggiatoa* species, *B. alba* B18LD^T. It was sequenced at the Joint Genome Institute within the Community Science Program and analyzed using standard bioinformatics tools. The genome of this freshwater strain consists of one chromosome of 4.3 Mb and two small plasmids of around 10 kb. In total there are 3569 annotated genes. About 18% of the genes originated from cyanobacteria, anoxygenic phototrophic bacteria, and sulfate-reducing bacteria. Strain B18LD^T has complete gene sets for oxidizing sulfide and thiosulfate to elemental sulfur but only an incomplete system for oxidizing sulfur further to sulfate. Distinct from marine *Beggiatoa*, key genes involved in autotrophic carbon fixation pathways are absent and dissimilatory nitrate reduction may not be possible. Under anoxic conditions, sulfur can be respired coupled to the oxidation of hydrogen and maybe formate. We found genes involved in motility, redox and light sensing, as well as a complete system responsible for polyphosphate synthesis and release.
In conclusion, the identified genomic features of *B. alba* B18LD^T highlight the microbe's niche specialization to dynamic redox interfaces and provide the basis for postgenomic investigations of this environmentally important genus.

Figure 1



GMV06

The genome of *Variovorax paradoxus* strain TBEA6 provides new insights for the 3,3´-thiodipropionic acid catabolism and hence the biotechnical production of polythioesters

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The Gram-negative, aerobic bacterium *V. paradoxus* strain TBEA6 possesses the extraordinary capacity to use 3,3´-thiodipropionic acid (TDP) as sole carbon and energy source for growth [1]. The thioether TDP is employed for several industrial applications and can be applied as non-toxic precursor for biotechnical production of polythioesters (PTE), which represent persistent bioplastics [2]. A comprehensive understanding of the microbial catabolism, including the regulation of cellular activities in the presence of TDP and its degradation intermediates besides the transport, will be crucial for applications of biochemical and genetic methods to influence the microbial metabolic networks. Consequently, the genome of *V. paradoxus* strain TBEA6 was sequenced using the Illumina sequencing technology, and the exceptional features are presented here. The draft genome was annotated and is now available for further studies: The existing genome sequence comprises about 7,2 Mbp and is composed of approximately 6,900 predicted open reading frames. The average GC-content amounts to 67.1%, as common for this species.
The proposed metabolic genes participating in the TDP catabolism were identified: TDP is most probably transported into the cell via the tripartite tricarboxylate transport system and afterwards cleaved by the FAD-dependent oxidoreductase Fox into 3-hydroxypropionic acid (3HP) and the toxic 3-mercaptopropionic acid (3MP), which is the precursor substrate for PTE synthesis. 3HP is presumably further catabolized via malonate semialdehyde and fed in the central metabolism, whereas 3MP is oxygenated by the 3MP-dioxygenase Mdo yielding 3-sulfinopropionic acid [1], which is activated to the corresponding CoA thioester, most probably by the CoA ligase SucCD. The subsequent step is the abstraction of sulfite from 3-sulfinopropionyl-CoA by a novel desulfinase [3], and the resulting propionyl-CoA enters the central metabolism. If the flow of 3MP into the central metabolism could be redirected towards an efficient microbial PTE synthesis, then *V. paradoxus* would be able to utilize the 3HP moiety of TDP for growth and 3MP as building block for production of PTE.

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GMV07

Characterization of the Campylobacter coli Methylome

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DNA methylation plays an important role in the regulation of bacterial protein expression hence influencing the pathogenicity and survival traits of a bacterial cell. Studies on DNA methylation revealed so called epigenetic switch mechanisms enabling the expression of different forms of particular proteins. Both N^6-methyladenine and C^5-methylcytosine have been observed in particular *Campylobacter coli* strains. Hence, the objective of this study was to examine the distribution of adenine and cytosine methylation sites in the *C. coli* genome. Therefore, 71 *C. coli* isolates from a wide variety of sources were examined by 5'-G^{m}ATC-3' and 5'-C^{m}cwgg-3' specific isoschizomer digestion assays. In addition, the gene presence of all DNA adenine methylases and the one DNA cytosine methylase was assessed using PCR. The phylogenetic relatedness of all *C. coli* isolates was examined by MLST-analysis. The genome of one particular isolate tested positive for N^6-adenine methylation was sequenced using pyro-sequencing using the 5'-G^{m}ATC-3' specific restriction enzyme *Dpn*I for library preparation in order to localize 5'-G^{m}ATC-3' motifs. A second isolate tested positive for cytosine methylation is currently being analyzed using bisulfite sequencing to determine the positions of 5'-C^{m}cwgg-3' motifs in the *C. coli* genome. At all 11 isolates were identified showing DNA-adenine methylase (dam) activity and 6 isolates showing DNA cytosine methylase (dcm) activity under laboratory culture conditions (Columbia sheep blood agar, microaerophlie atmosphere at 42°C). These results contrast the presence of methylase genes in all strains. This indicates that DNA methylation may be induced under specific situations. On the other hand, methyladenin sensitive genome sequencing showed a cluster of 29 5'-G^{m}ATC-3' sites in the *C. coli* genome ranging form 4500 bp until 10500 bp (reference genome *C. coli* Z156). In this region are some genes e.g. flagellar hook subunit protein FlgE and the MotA proton channel for which an epigenetic switch mechanism can be postulated.

GMV08

Whole-genome sequencing of *Coxiella burnetii* without prior culture or amplification

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Whole-genome sequencing and analysis is becoming available a routine tool in veterinary and human infectious disease studies. Although routinely used on cultivated materials, the direct application of this method on clinical samples is getting more and more interesting in the recent years. Not only to reduce the time of preparation and cultivation but also to gain genomic information from non-culturable or fastidious organisms. This possibility enhances the quality of diagnostics and could also provide information on pathogenicity and virulence/resistance mechanisms.
Here we present for the first time the successful whole-genome sequencing of *Coxiella burnetii* directly from a clinical sample taken from the nose of a goat. The DNA from the swab was extracted with the Nucleospin Tissue Kit (Macherey-Nagel) and a final concentration of $5x10^7$ genome equivalents per µl (in total 1.7µg) was used for sequencing. Unfortunately, a large portion of the DNA was highly degraded. Therefore we used a combination of two next generation sequencing techniques (Illumina HiSeq and Pacific Biosciences) and different assembly tools. We got 115 contigs with an average coverage of 55 fold. After filtering of host and contaminant DNA 28 *Coxiella*-specific contigs remain. These contigs were filtered again for redundancies and further scaffolded using PBJelly. Finally, the resulting two contigs were polished and circularized. Compared to seven *C. burnetii* references genomes, strain RSA331 (Henzerling) showed the highest similarity to our new genome.
In summary we produced a finished *C. burnetii* genome in best quality within five weeks. This was only possible because we applied for the first time a two-step strategy: the combination of cheap next generation sequencing with third generation long read sequencing and a multi-pass assembly and scaffolding strategy tuned for this special situation of low starting and degraded DNA. This strategy can be used as a standard to get almost complete *C. burnetii* genomes directly from clinical samples without prior cultivation or amplification. It is also suitable for sequencing of other fastidious organisms from clinical material.

GMV09

Diversity of resistance plasmids in bacteria from the Warsaw wastewater treatment plant

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Introduction: Disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. The antibiotic resistance (AR) phenotypes are often transmitted among bacteria by plasmids, which are major natural vectors of horizontal gene transfer. Although they are thought as not essential for bacteria survival, their presence may provide many features which influence the fitness of their hosts. In this study, genomic analyses of a pool of resistance plasmids isolated from activated sludge bacteria of the "Czajka" wastewater treatment plant (Warsaw, Poland) were performed.
Materials and Methods: Resistance plasmids were identified by exogenous isolation. Plasmid DNA extracted from activated sludge bacterial community was introduced into *Escherichia coli* recipient cells. Complete nucleotide sequences of selected plasmids were determined with a combination of 454 and Sanger sequencing. Bioinformatic analyses were performed with commonly accessible software.
Results: Antibiotics of different classes (e.g. ampicillin and kanamycin) were used for selection of *E. coli* transformants. The most numerous group of the identified replicons conferred resistance to penicillins and cephalosporins. *In silico* analyses of determined nucleotide sequences revealed that many of these plasmids are multiresistance replicons. The plasmid-encoded AR determinants (e.g. *blaCTX-M3*, *aac(6')-Ib*) are usually linked with transposable elements (e.g. transposable module generated by IS*Ecp1*) or integrons (e.g. class 3). Sequence comparisons indicated that not all identified plasmids can be assigned to known incompatibility groups. Functional analyses revealed that some of the plasmids are broad host range mobilizable or conjugative replicons, which may play an important role in dissemination of the AR genes in the environment.
Conclusion: The obtained results showed that bacteria residing beyond nosocomial environment constitute a rich reservoir of the AR genes. These genes are usually components of mobile genetic elements, which participate in DNA shuffling between microorganisms and affect genetic diversity.

GMV10

Genome analyses of human pathogenic *Lichtheimia* species

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Infections with mucoralean fungi (Mucormycosis, Zygomycosis) are uncommon fungal infections in humans and animals. However, the number of cases increased during the last decades. *Rhizopus oryzae* represents the most common cause of Mucormycosis worldwide. However, *Lichtheimia* species are the second-most important mucoralean pathogen. In addition, they are associated with Farmer's lung disease (FLD). To get broader insights the molecular basis of pathogenicity of basal mucoralean pathogens, we sequenced the genome of *Lichtheimia corymbifera* using Illumina and 454 sequencing. Evidence-based gene prediction resulted in 12,379 protein-coding genes in *L. corymbifera*. The genome was compared to other fungal genomes. This analysis revealed a high dissimilarity of the *L. corymbifera* genome from genomes of other mucoralean fungi. However, comparable to the situation in *R. oryzae* an elevated number of duplicated genes compared to other fungal genomes was found which do not result from recent whole genome duplication like in *R. oryzae*. Despite the relatively high number of introns in *L. corymbifera* genes, alternative splicing was only found in 2.3% of the genes. Based on transcriptome analyses under infection-associated conditions potential virulence factors were identified including iron-uptake genes, hydrolytic enzymes and transcription factors. Analyses of additional genomes of clinical and non-clinical *Lichtheimia* species revealed that the majority of potential virulence factors are conserved in *Lichtheimia* spp. Our results provide the first insights into the genome of basal mucoralean pathogens and provide a working basis for further research on the pathogenicity mechanisms of these fungi.

Schwartze et al. (2014) PLOS Genetics: accepted.

GMV11

Comparative genome sequencing reveals within-host evolution of *Neisseria meningitidis* during invasive disease

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The mechanism of pathogenesis of invasive meningococcal disease (IMD) and the genetic factors on the side of the causative agent *Neisseria meningitidis* (Nme) are still elusive. Why these commensal bacteria cause IMD is even more puzzling since disease is a dead end for these bacteria. Consequently, it has been hypothesized that virulence and thus IMD is a consequence of short-sighted within-host evolution that provides no benefit to the pathogen beyond the host, and that in particular rapid phase-shifting at phase variable genes (PVGs) is expected to increase the likelihood that colonizing bacteria will cause IMD. To put this hypothesis to an experimental test we sequenced the genomes of throat-blood isolate pairs from four patients. Whole-genome sequences were obtained for one sequence type (ST)-42 serogroup B, two ST-11 serogroup C and one ST-23 serogroup Y throat isolate by combining sequencing with the Roche GS FLX Titanium Series Chemistry and short-read sequencing on a Illumina Genome Analyzer IIx. The genomes of the corresponding blood isolates were re-sequenced at over 1000-fold coverage on a Illumina Genome Analyzer IIx and mapped onto the reference genomes of the throat isolates. All sequence differences between the genomes of the throat and blood isolates were finally verified using standard Sanger sequencing.
Computational analysis of a set of 18 complete genomes showed that Nme contains about 250 potential PVGs per genome with a putative "core PVGenome" of 90 genes. Genome comparisons of throat-blood isolate pairs further revealed that three of the four pairs differed in the sequence of at least one PVG. In addition, in two genome pairs we identified (additional) differences due to gene conversion and non-homologous recombination events, respectively. Altogether, we could between one and three detect genetic differences in all four isolate pairs. Therefore, our data indicate that in addition to phase-variation at PVGs also recombination contributes to within-host genetic diversity, and by considering recombination, our data support the hypothesis of within-host evolution of Nme during acute infection. The phenotypic consequences if any await further experimental investigation yet.

GMV12

Recent changes in the MG-RAST metagenome analysis portal

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Over 15,000 researchers world wide are using the MG-RAST portal. It is home to over 120,000 shotgun metagenomic, metatranscriptomic and amplicon metagenomic data sets.
The widely used portal for metagenomic analysis and comparison has gained a host of novel features and significant improvements in the last year.
The novel sequence pathology features allow early detection of sequencing or library construction issues as well as improving the design of sequencing studies. The addition of an R interface significantly enhances the capabilities.

HOST-MICROBE INTERACTIONS

HMV01

Analysis of the localization of glycolytic enzymes in *Mycoplasma pneumoniae*

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Introduction: The cell wall-less bacterium *Mycoplasma pneumoniae* (M.p.) is a common agent of respiratory infections in humans. The reduced genome results in a limited metabolism of mycoplasmas in which the major pathway for ATP production is the glycolysis. Many studies reported for various microorganisms, that glycolytic enzymes, like the glyceraldehyde-3-phosphate-dehydrogenase (GapA), occurring not only intracellulary. They can be transported to the surface of the microorganisms, where an interaction with human extracellular matrix proteins (ECM) is possible. These interactions could influence the virulence of the microorganisms. So, in previous investigations we confirmed that the subunit B of the pyruvate dehydrogenase complex (Pdh) of M.p. interacts with human plasminogen. It can be assumed that further enzymes of the glycolysis contribute to the pathogenesis of infections.
Materials and Methods: In the present study, complete genes coding for all glycolytic enzymes of M.p. (n=19) were amplified and TGA codons were exchanged by multiple mutation PCR. The expression of recombinant proteins was carried out in *E. coli* BL21(DE3) and polyclonal antisera were produced. For analysis of localization of the glycolytic enzymes, different separation methods of total M.p. proteins into membrane and cytosolic fraction were used. In addition, immunological experiments (Western blot, ELISA, immunofluorescence and colony blot) were performed.
Results: All glycolytic enzymes could be expressed successfully and the reaction of derived polyclonal antisera with whole antigen of M.p. confirmed the specificity of the antigens. The results of the localization studies demonstrated, that all proteins can be found in the cytosolic fraction of M.p. proteins. In addition, lactate dehydrogenase (LDH), transketolase (TKT), PdhA - D, glyceraldehyde-3-phosphate-dehydrogenase (GapA), phosphoglycerate mutase (PGM), pyruvate kinase (PYK) and phosphotransacetylase (PTA) were also detected in the membrane fraction of M.p. proteins as prerequisite for surface localization. The results of further experiments indicated that TKT, PTA and PdhD occurred not extracellulary, whereas PdhA, PdhB, PdhC, LDH, PYK, PGM and GapA seems associated with the surface of M.p. cells.
Discussion: This is the first investigation of the complete set of glycolytic enzymes in mycoplasma. The results of the study confirmed that a high proportion of enzymes could be detected in the membrane fraction of M.p and seven proteins seems surface-localized. This is the precondition for an interaction with human ECM proteins as described for GapA and PdhB. In further investigations we will study these potential interactions in detail.

HMV02

Characterization of the metal ion-inducible autocleavage (MIIA) domain conserved in a small set of pathogenic and symbiotic bacteria

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The metal ion-inducible autocleavage (MIIA) domain (formerly DUF1521) was first identified in proteins from the soybean symbiont *Bradyrhizobium japonicum* USDA110 [1]. Subsequently, the domain was found also in a small set of proteins from α-, b-, γ- and δ-proteobacteria, e.g. the coral *pathogen Vibrio coralliilyticus* ATCC-BAA450, the endophyte *Burkholderia phytofirmans* PsJN, *Photobacterium* sp. AK15 and *Myxococcus xanthus DK1622*. Proteins containing the MIIA domain of *V. coralliilyticus, Photobacterium* sp. AK15, and *B. phytofirmans* are encoded in genomic regions related to type III secretion [2,3,4]. The MIIA domains span approximately 160 amino acids and share between 30 and 42 % identity. The function of this conserved domain in the interaction of *Bradyrhizobium japonicum* and its host plants is currently investigated. Two homologous proteins, NopE1 and NopE2 were shown to be type III-secreted effectors and contain each two MIIA domains [1]. Heterologously expressed NopE1 shows autocatalytic cleavage activity at a conserved GD'PH motif. The cleavage of the peptide bond between aspartate and proline is inducible by calcium. Symbiosis of *B. japonicum* with some host plants is negatively affected in the presence of NopE1 and NopE2. The wild type strain nodulates *Vigna radiata* cv. KPS2 very poorly, while a nopE1/nopE2 double mutant shows high nodulation efficiency. Only one MIIA domain is sufficient to complement the mutant phenotype on *V. radiata*. A protein variant with a modified cleavage site is not able to complement the phenotype [1].
The cleavage site motif GDPH is highly conserved in all MIIA domains [1,2]. Several MIIA domains were expressed in *Escherichia coli* and characterized. They show self-cleavage activity *in vitro*. The results suggest a similar function for the conserved metal ion-inducible autocleavage domain in proteobacteria.

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HMV03

The gut microbiome of phytopathogenic root fly larvae: insights into the detoxification of plant secondary metabolites by insect-associated microbes

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Brassica plants produce various toxic compounds such as isothiocyanates in response to herbivore damage. Despite their toxicity, some insects cope well with these compounds. One example is the larva of the cabbage root fly (*Delia radicum*) which is a serious agricultural pest. The mechanism by which these root feeding insects detoxify isothiocyanates has not yet been explored. Our hypothesis is that microorganisms residing in the gut of *D. radicum* contain enzymes that break down the isothiocyanates and are thus crucial for survival and phytopathogenicity of the root fly larvae.
We substantiated this hypothesis by isolating 15 representative microbial species from the *D. radicum* gut that were highly resistant to the root volatile 2-phenylethyl isothiocyanate. A subset of these microorganisms belonging to the genera *Acinetobacter*, *Serratia*, *Pectobacterium* and *Providencia* was also able to break down isothiocyanates to a variety of different volatile compounds. We are currently characterizing the isolates physiologically and genetically to unravel their isothiocyanate breakdown mechanism. Furthermore, we performed a metagenomic survey of the microbial gut content of *D. radicum* larvae to assess the total genetic content of the insect gut microbiome. The analysis of the 16S rRNA gene reads revealed a moderate diversity of gut microbes in *D. radicum* larvae with the majority of bacterial lineages falling into the class of Gammaproteobacteria. Sequencing reads mapping to the 16S rRNA genes from the genera *Providencia*, *Morganella*, *Acinetobacter* and *Pseudomonas* showed that these genera dominate this fraction of the metagenome. This is dissimilar to many other investigated insect metagenomes that are dominated by members of the Firmicutes which are well known for their fermentative capabilities. Additionally, the metagenome was analysed with respect to functional gene content. One aspect of these analyses is that roots where *D. radicum* larvae feed on have a low nitrogen content; microorganisms residing in the gut may have beneficial effects on the nitrogen supply to the larvae. Surprisingly, the number of bacterial nitrogenases retrieved was low indicating that probably not nitrogen gas is the main biological nitrogen source. However, physiological studies of one of the isolates indicated that it was able to use 2-phenylethyl isothiocyanate as a nitrogen source. Taken together, the results of this study provide for the first time an in-depth analysis of the microbial content of the root fly larval gut. Both the metagenome analyses and the isolation experiments indicate clearly that the *D. radicum* gut contains a highly specialized microbiota that helps the root fly larvae to survive on their toxic host plants. This work was supported by a fellowship within the Postdoc-Program of the German Academic Exchange Service (DAAD) and the Soehngen Institute of Anaerobic Microbiology (OCW 024002002).

HMV04
Colonization pattern of the beneficial endomycotic bacterium *Rhizobium radiobacter* in plant roots

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Rhizobium radiobacter F4 (*Rr*F4) was originally isolated from the growth-promoting fungus *Piriformospora indica* that forms a tripartite Sebacinalean symbiosis with wide range of host plants (Sharma et al., 2008). The genome of *Rr*F4 was fully sequenced using 454 pyrosequencing and the draft genome showed very high synteny with the fully annotated genome of *Agrobacterium tumefaciens* C58; the sequence of the single plasmid of each strain showed however deletions and other differences. Here, we show that plants colonized by *Rr*F4 increased biomass and enhanced systemic resistance against the bacterial pathogens *Pseudomonas syringae* pv. *tomato* DC3000 in Arabidopsis and *Xanthomonas translucens* pv. *translucens* in wheat, respectively. Quantitative real-time PCR analyses confirmed the proliferation of *Rr*F4 in roots of axenically grown barley, wheat and Arabidopsis. GUS- and GFP-tagged *Rr*F4 were used to study the colonization pattern of *Rr*F4 in roots using light, confocal laser scanning microscopy and raster and transmission electron microscopy. *Rr*F4 mainly colonized the root hair zone forming dense biofilms at the root surface. The emergency side of root hairs and lateral root protrusions were identified as distinct entry sides into the root tissue. Unlike its fungal host, *Rr*F4 colonized not only rhizodermis and cortex tissue but progressed beyond endodermis into the stele. This results show for the first time a detailed insight into the localization of a Sebacinales derived plant-beneficial bacterium inside the roots of mono- and dicotyledonous host plants.

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HMV05
Deciphering the plant microbial network

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The importance of microbial root inhabitants for plant growth and health has been recognized already 100 years ago. Since that time, much has been learned about microorganisms and their close symbiotic relationship with plants (3). Comparable to humans and other eukaryotic hosts, plants also may be realized as meta-organism that harbors a “second genome”. These advances in knowledge were driven by both “omics”-technologies guided by next-generation sequencing and microscopic insights. Collectively known as the plant microbiome, plant-associated microbes can help plants fend off disease, stimulate growth, occupy space that would otherwise be taken up by pathogens, promote stress resistance, and influence crop yield and quality. Therefore, the plant microbiome is a key determinant of plant health and productivity. Plant microbiome discoveries could fuel progress in sustainable agriculture, such as the development of microbial inoculants as biofertilizers, biocontrol, or stress protection products (2). Although we recognize a growing market for these bio-products, they still have their problems, e.g., short shelf-life, inconsistent effects under field conditions, and risk predictions. The application of “omics”-technologies has allowed for an enormous progression in the development of so-called next-generation bio-products (2). New tools may have an impact on (i) the detection of new bio-resources for biocontrol and plant growth promoting agents (4,5), (ii) the optimization of fermentation and formulation processes for biologicals, (iii) stabilization of the biocontrol effect under field conditions and (iv) risk assessment studies for biotechnological applications (1). Advances in these aspects could open new perspectives for sustainable agriculture by the development of high impact next-generation bio-products.

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HMV06
Isolation and characterization of endophytic bacteria associated with root-nodules *Medicago sativa* in Al-Ahasa region

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Medicago sativa (Alfalfa), is an important forage crop legume worldwide including Saudia Arabia, due to its high nutritive value.In spite of alfalfa and to somewhat the bacteria associated with its root have been studied extensively, little studies have been focused on bacterial endophytes associated with root-nodules of alfalfa Al-Ahsa region, Eastern province, Kingdom of Saudi Arabia. Therefore, the aim of the current study was to isolate and characterize the endophytic bacteria inhabiting the root-nodules alfalfa growing in Al-Ahsa, Kingdom of Saudi Arabia. Furthermore, the effects of inoculation growth of three economically-important crop legumes upon inoculation with the endophytic bacteria were also investigated.
Materials and Methods: Alfalfa plants were collected from the street sides of AlAhsaa city, Saudi Arabia. Endophytic bacteria were isolated from surface-sterilized root- nodules of alfalfa according the method described by (Vincent, 1970). Strains were characterized phenotypically and genotypically. Furthermore, the effects of the endophytic bacteria on the growth of three economically important crop legumes; *Lens esculentus*, *Phaseolus vulgaris* and *Pisum sativum* were assessed.
Results: A total of 60 rhizobial and non-rhizobial strains were isolated from alfalfa nodules. The strains exhibited phenotypic and genotypic diversity. Some strains were identified as *Sinorhizobium meliloti* while others belonged to different *Bacillus* spp. Non-rhizobial strains showed plant-growth promoting traits such as production of IAA, phosphate solubility. Inoculation of three important grain legumes with non-rhizobial strains resulted in significant increase in their root dry weights and number of lateral roots.
Conclusions: The results confirmed that the occupancy of alfalfa nodules with diverse endophytic bacterial species, in addition to the natural microsymbionts Sinorhizobia. Furthermore, the strains belonged to plant growth promoting rhizobacteria (PGPB) and could have significant agricultural applications.

HMV07
Effects of bacterial inoculation on plant fitness under salt stress

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Salinization of agricultural soils is a major concern worldwide, due to negative effects on both crops health and yield as effect of the plant response to salt stress. Therefore, environmental friendly solutions are required to face this new threat. Certain microorganisms can alleviate the salt stress of plants, and their modes of action range from the production of osmolites to the modification of Na^+ transport in plant. In our work, we selected two environmental bacteria isolated from the rhizosphere of wild barley in a salt meadow in Germany (Münzenberg) for their ability to increase plant fitness of barley cultivar Propino) under salt stress. The two selected inoculants, named strain E108 and E110, belonging to the genera *Curtobacterium* and *Ensifer*, respectively, favoured the germination of barley seeds in soils. Inoculation experiments in the greenhouse showed that both bacteria increased the biomass, especially in the barley stems, and the water content especially in the barley leaves, therefore enhancing the general fitness of the plant. Content of Na^+, Ca^+, Mg^+ and K^+ was measured to investigate the mechanisms of actions. Co-inoculation tests in salinated soil showed the synergy/antagonism of strains E108 and E110, and indicated them as promising candidates for inoculation of barley under natural conditions in salty agricultural soils.

HMV08

Biochemical and *in planta* characterization of the compartment-specific enzymatic activities of the extracelluar exzymes, LscB and LscC, of the plant pathogen *Pseudomonas syringae*

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Pseudomonas syringae pv. glycinea PG4180, the causative agent of bacterial blight of soy-bean plants, possesses several virulence factors, one of them being the synthesis of exopolysaccharides. One of them, levan, is a polymer of fructose, which is synthesized from sucrose by two highly similar enzymes (LscB and LscC) levansucrases. Due to the remarkably different sub-cellular localization of the two enzymes in *P. syringae*, and an in-depth enzymatic characterization of LscB and LscC is needed. We hypothesize that both enzymes might conduct different compartment-specific reactions. This assumption is substantiated by the finding that Lsc generally is known to catalyze three divergent reactions: a) cleavage of sucrose into glucose and fructose; b) polymerization of fructosyl residues to form levan; and c) depolymerization of levan to yield free fructosyl residues. To date it is unclear whether both, LscB and LscC, conduct all three reactions at similar rates and efficiencies. LscB (M5) and LscC (M3) mutants have been generated by homolgous recombination in order to study the role of LscB and LscC enzymes individually, both in vivo and *in plant*. Filter-sterilized supernatants of the wild type PG4180, M5 and M3 cultures were used for enzyme purification by gel-filteration chromatography (SEC). The km value of each of the enzymes is determined under different conditions i.e pH and temperature. In addition, the levan degrading activities of both the enzymes are tested. Furthermore, *in planta* growth of wild type PG4180, M5 and M3 mutants was evaluated on soybeans (*Glycine max* (L.) Merr.). Soybean seedlings were germinated and grown in the greenhouse for three to four weeks prior to the growth assays. For spray inoculation, the cells were adjusted to an OD_{600} of 0.1 (corresponding to approximately 10^7 CFU/ml) and applied to the leaves with an airbrush (~8 psi) until the leaf surfaces were uniformly wet. Subsequently, inoculated plants were grown in the greenhouse (19-21°C), and survival and growth of bacterial strains was monitored by removing random leaf samples at 1-14 days post inoculation. Bacterial counts (CFU/g fresh weight) were determined by plating dilutions of leaf homogenate onto MG agar and counting of fluorescent colonies after incubation for 96 h.

HMV09

Conserved and Divergent Transcriptional Responses of Two Plant Species on PGPB *Kosakonia radicincitans* Colonisation

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Endophytic plant growth promoting bacteria (PGPB) may have significant impact on both the plant physiology and the composition of the plant microbiome. However, the regulatory pathways of the beneficial effects are still questionable. Here we report on the transcriptional analysis of the model plant *Arabidopsis thaliana* and the crop *Solanum lycopersicum* in resonse to endophytic growth of the PGPB *Kosakonia radicincitans* DSM 16656 (formerly *Enterobacter radicincitans*). A total of 826 and 243 significantly different expressed genes were detected in Arabidopsis and tomato, respectively. Gene ontology (GO) enrichment analysis of these transcripts revealed an involvement of GO terms related to development, transport, nutrition, primary and secondary metabolism in the *Arabidopsis* response to *K. radicincitans*, while only two categories, transcriptional regulation and RNA metabolic process, were found in tomato, probably due to the limited gene annotation. Candidate gene analysis revealed that both plant species adapted to endophytic growth by modifying the cell wall and by hormone modulation. The bacterial mediated atmospheric nitrogen supply to the plant was reflected by reduced expression of plant nitrate and ammonium uptake transporters in roots, while total plant nitrogen content was not affected. Quantitative PCR of selected genes confirmed microarray data. By comparative analysis of transcript abundance, we propose a model on how plants benefit from endophytic bacteria.

HMV10

Face-to-face RNA-Seq analysis of the Candida-neutrophil interaction

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Neutrophils are essential in the defense against fungal pathogens. This is reflected in the increased susceptibility of neutropenic patients towards fungal infectants. It is a widely spread misconception that neutrophils have low transcriptional activity and for this reason, little is known about their responsiveness upon microbial encounter on a genetic level. Amongst all fungal pathogens, *Candida albicans* is currently the most abundant. It causes infections with broad-ranged severity, from superficial to systemic. *C. albicans* is isolated from blood as frequent as prominent bacterial agents like *S. aureus* or *E.coli* [1]. Given the constant risk of obtaining a fungal infection - in combination with a growing number of immuno-compromised individuals and antifungal drug resistance [2] - it is of high interest to understand this host-pathogen interaction in detail.
In the present study, we analyzed the early transcriptional response of human neutrophils and *C. albicans* towards each other by RNA-Sequencing. To meet the special needs of a Candida infection, we analyzed the interplay for both fungal morphotypes, yeasts and hyphae. In addition to Candida encountering intact neutrophils, we also included neutrophil extracellular traps (NETs) as a challenge, since the exact antifungal mode of action of NETs is still not fully understood.
Most interestingly, we found human neutrophils to actually have a strong transcriptional response towards *C. albicans* - most of which occurred after 60 minutes. The neutrophil transcriptome indicates re-modelling of the cell shape and extracellular receptors, orchestration of other cells by cytokines, as well as apoptosis delay. We also identified miRNAs yet undescribed in neutrophils. Besides some minor differences, the neutrophil response to *C. albians* yeast and hyphae was found to be very much alike. In contrast, the transcriptional response of *C. albicans* towards intact human neutrophils showed slight morphotype specificity - even when taking the expected hyphal formation of yeasts under the given *in vitro* conditions into account. Finally, our analysis revealed that *C. albicans* reacts towards NETs stress by inducing sugar metabolism and oxidative stress response and restricts fatty acid and ribosome synthesis.
In summary, our novel and unbiased RNA-Seq approach to decipher the Candida-neutrophil interplay gives new insights into the transcriptional capacities of human neutrophils and the response of *C. albicans* towards those innate immune cells in all their facets.

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HMV11

Activation of Ran GTPase by a Legionella effector promotes microtubule stabilization, pathogen vacuole motility and infection

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The causative agent of Legionnaires` disease, *Legionella pneumophila*, uses the Icm/Dot type IV secretion system (T4SS) to establish a distinct "*Legionella*-containing vacuole" (LCV) in phagocytes. LCVs intercept endosomal, retrograde and secretory vesicle trafficking, but do not fuse with lysosomes. Proteomics of intact purified LCVs revealed the presence of the small GTPase Ran and its effector RanBP1 (*1*). Using fluorescence microscopy and RNA interference, Ran and RanBP1 were found to localize on LCVs and to promote intracellular growth of *L. pneumophila*, respectively (*2*). The *L. pneumophila* protein LegG1, which contains RCC1 Ran guanine nucleotide exchange factor (GEF) domains, accumulated on LCVs in an Icm/Dot-dependent manner. *L. pneumophila* wild-type bacteria, but not strains lacking LegG1 or a functional Icm/Dot T4SS, activated Ran on LCVs, while purified LegG1 produced active Ran(GTP) in cell lysates. *L. pneumophila* lacking *legG1* was compromised for intracellular growth in macrophages and amoebae. Furthermore, microtubule stabilization was found to be a downstream effect of LegG1 activation, as revealed by conventional fluorescence and STED (stimulated emission depletion) microscopy, subcellular fractionation and Western blot, or by "microbial microinjection" of LegG1 through the T3SS of a *Yersinia* strain lacking endogenous effectors. Real-time fluorescence imaging indicated that LCVs

harboring wild-type *L. pneumophila* rapidly move along microtubules, whereas LCVs harboring Δ*legG1* mutant bacteria are stalled (*2*). Finally, LegG1 ectopically produced in *D. discoideum* localized to LCVs and other membrane compartments, while LegG1 lacking the C-terminal CAAX prenylation motif did not show a specific cellular localization. Together, our results demonstrate that Ran GTPase activation and RanBP1 promote LCV formation. Furthermore, the Icm/Dot substrate LegG1 functions as a bacterial Ran activator, which upon prenylation by the host cell localizes to LCVs. LegG1 promotes microtubule stabilization, LCV motility and intracellular replication of *L. pneumophila*.

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HMV12

Targeting of host cell autophagy by *Yersinia enterocolitica*

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Autophagy is a major degradative pathway of eukaryotic cells, which delivers cytoplasmic constituents to the lysosome for degradation. Autophagic processes are also implicated in the host immune defense against invading microbes. Previous studies demonstrated that enteropathogenic *Y.enterocolitica* activates cell invasion and autophagy in macrophages through the engagement of beta-1-integrins by the *Yersinia* adhesins invasin. Here, we investigate the effects of *Y. enterocolitica* on autophagy in epithelial cells as a model for *Yersinia*-mediated invasion of intestinal cells. Our results showed that autophagy-related events followed the uptake of *Yersinia* into epithelial cells mediated by beta-1-integrins. Accordingly, the autophagic marker protein LC3-I was converted to LC3-II, which was detected by immunoblotting. The accumulation of LC3-II correlated with the recruitment of GFP-LC3 to intracellular vacuoles containing ingested bacteria. This seems to be an active, bacteria-triggered process because the induction of autophagic responses required viable, vital yersiniae. Invasive *E. coli* was unable to trigger a comparable event. At the ultrastructural level, the *Yersinia*-containing vesicles were trapped by double-membranes characteristic for autophagic vacuoles. The LC3-positive bacteria co-localized with the late endosomal / lysosomal marker protein LAMP-1 which was recruited earlier to the ingested bacteria than LC3. Interestingly, experiments using markers for acidification and maturation of the internalized bacteria showed that the *Yersinia*-comprising compartments did not acidify. From these experiments, it appeared that fusion of the *Yersinia*-containing, autophagic compartment with lysosomes is prevented. In line, *Yersinia* could survive inside cells and might eventually multiply in autophagosomes. Thus, *Yersinia*-related autophagy may play role in enabling intracellular survival of the bacteria. This could be a potential virulence strategy that may support the invasion process by the enteropathogenic bacterium.

HMV13

Neisseria meningitidis infection induces a cyclin independent S-phase arrest in human brain microvascular endothelial cells.

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Introduction: *N. meningitidis* (Nm) is a human commensal which colonises the pharynx and infection can lead to severe septicaemia or meningitis. Several studies have indicated that pathogens can affect the host cell cycle. Published transcriptomic data from our group showed that *N. meningitidis* is capable of altering host cell cycle genes. Numerous molecules are involved in the regulation of the cell cycle, of which the cyclins and their respective kinases have been well described.
Materials and Methods: Cell cycle alteration was studied using the immortalised cell line HBMEC/ciβ and primary HBMECs. Propidium iodide stained DNA content was measured by FACS analyses to identify cell cycle alteration and investigate (1) mutant defective of adhesins and invasins, (2) the effect of live, heat-killed and Nm supernatant on the cell cycle, and (3) *E. coli* recombinantly expressing the Nm opacity proteins. Western blotting was used to investigate the effect of Nm infection on the host cell cyclins.
Results: Infection with the serogroup B strain Nm MC58 and the unencapsulated mutant MC58Δ*siaD* resulted in a S-phase arrest 2 h and 24 h p.i. (HBMEC/ciβ) and was confirmed 24 h p.i. by Edu incorporation, and 3 h p.i. (pHBMECs). Infection with Nm mutants defective of Opc and NadA had no effect on the cell cycle. Only live Nm and Nm supernatant induced a phase arrest. Infection with *E. coli* BL21 recombinantly expressing Opc or Opa1-4 resulted in a phase arrest 2 h and 24 h p.i. (HBMEC/ciβ) and 3 h p.i. (pHBMECs). We identified no effect of meningococcal infection on cyclins (HBMEC/ciβ).
Discussion and Conclusion: We identified a S-phase arrest 2 h and 24 h p.i. (HBMEC/ciβ) and 3 h p.i (pHBMECs) for Nm MC58 and the unencapsulated mutant MC58Δ*siaD.* We identified that both live and Nm supernatant induce the arrest at 24h p.i. (HBMEC/ciβ) and 3 h p.i. (pHBMECs). DNA content measurement of selected meningococcal mutant deficient of pili (required for adherence) or invasins (*nadA* and *opc*) indicated that invasion is required for the arrest. DNA content measurement of cells infected with *E. coli* BL21 recombinantly expressing the meningococcal opacity proteins (Opc, Opa1-4) identified a S-phase arrest for all opacity protein at 2h and 24h p.i. (HBMEC/ciβ) and 3 h p.i., indicating that the Nm opacity proteins are acting as the proposed cyclomodulins. Immunoblotting indicated Nm infection has no effect on the host cell cyclins, which are core regulators of the cell cycle. In conclusion, we found that meningococcal infection induces a S-phase arrest for which invasion is required and is mediated by the opacity proteins and is independent of cyclins.

HMV14

Restriction of *Salmonella* replication in the intestinal mucosa by NAIP/NLRC4 inflammasome-driven expulsion of infected enterocytes

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The intestinal epithelium separates the sterile organs of the body from the intestinal microbes. Some enteropathogens, such as *Salmonella* Typhimurium (*S.* Tm), can invade into and cross this barrier. It remains incompletely understood how the host defends itself against such acute infection. In a mouse model for *Salmonella* diarrhea, we have studied mechanisms limiting mucosal pathogen loads. Microscopy and intravital imaging discovered an initial phase where *S.* Tm invades and grows within the absorptive epithelium. This replicative niche is restricted by NAIP1-6, NLRC4 and caspase-1/-11specific expulsion of infected epithelial cells into the lumen. At 18h post-infection, this reduces the intraepithelial pathogen loads by as much as ~100-fold. IL-1αβ and IL-18 are dispensable for this initial restriction of the intraepithelial *S.* Tm load. *Nlrc4*$^{-/-}$ bone-marrow chimeras and epithelium-specific NAIP1-6 ablation establishes that epithelium-intrinsic inflammasomes drive the expulsion of infected cells. The identified response restricts the pathogen's intraepithelial proliferation and may represent a generic defense against infections of the intestinal epithelium.

HMV15

Inhibition of host immune responses by an effector protease of enteropathogenic *E. coli*

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Enteropathogenic Escherichia coli (EPEC) rapidly inhibit host innate immune responses upon infection of gastrointestinal epithelial cells. Several effector proteins translocated by the bacterial type III secretion system have been indicated in this process. While the bacterial zinc metalloproteases NleC and NleD specifically cleave NF-κB subunits or the MAPKs JNK and p38, another effector, NleE, inhibits activation of the TAB-TAK1 complex by transferring a methyl-group to a conserved cysteine residue in the zinc finger domains of TAB2 and 3. Recently it was shown that the effector protein NleB attaches a single GlcNAc to a conserved arginine residue in the

death domain of cell signalling proteins including FADD, interfering with the formation of death receptor complexes, thereby inhibiting apoptosis in infected cells. Recently, we identified another effector protein to play a role in the inhibition of host innate immune responses. When expressed ectopically or translocated into host cells by the bacteria, the effector induced the degradation of the receptor interacting protein kinases (RIPK) 1 and 3 by a process that is independent of the proteasome or caspase-8 cleavage. Secondary structure analysis suggests that the protein is similar to the family of clan CA cysteine proteases, which also includes the bacterial effector protein YopT of Yersinia. Mutation of the amino acids of the catalytic triad abolished its ability to degrade its target proteins. Furthermore, in these mutants, RIPK1 or 3 can be co-immunoprecipitated, suggesting an interaction between the proteins. As a downstream effect of the degradation of both RIPK1 and 3, the effector protein inhibits caspase-independent cell death (necroptosis) in addition to the host inflammatory response, making EPEC the first pathogen ever described to inhibit inflammation, apoptosis and necroptosis.

HMV16

Tissue specific colonization pattern as consequence of metabolic diversity between *C.jejuni* and *C.coli*

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Campylobacteriosis is the most frequent bacterial gastroenteritis worldwide. This infection is primarily caused by the food-borne pathogen *Campylobacter jejuni* and to a lesser extent by the closely related *C. coli*. Both *Campylobacter* species are commensal residents in numerous animals, but display strikingly different host preferences. While *C. jejuni* is primarily associated with poultry, it was less frequently found in pigs, the main reservoir of *C. coli*. In addition, our and other infection experiments of juvenile pigs revealed that both *Campylobacter* species exhibit a different distribution along the anterior-posterior axis of the intestine: *C. jejuni* colonizes predominantly the small intestine, whereas *C. coli* is more abundant in the large intestine. We hypothesize that distinct metabolic properties of both species are the reason for this tissue tropism. Metabolome analysis from different porcine gut sections revealed pronounced differences in the spatial distribution of nutrients in the upper and lower gut. Phenotype microarray analysis, extensive *in vitro* growth experiments combined with whole genome sequencing and pig infection experiments allowed us to identify distinct substrate utilization pattern that enable *C. coli* to persist in the large intestine more efficiently than *C. jejuni*.
Interestingly, *C. coli* but not *C. jejuni* is able to utilize various catabolic end products from the colonic microbiota. These observations suggest that *C. coli* might benefit through cross-feeding more from the metabolic activities of the colonic microflora than *C. jejuni*. Moreover, our study revealed new insights how different physiological properties of closely related *Campylobacter* species could influence a distinct tissue tropism in the same host.

HMV17

The immune system of the carpenter ant *Camponotus floridanus* is involved in the surveillance of its bacterial endosymbiont

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The enormous evolutionary success of insects is, at least in part, facilitated by their symbiotic interactions with microorganisms.[1] The carpenter ant *Camponotus floridanus* also relies on the obligate association with the γ-Proteobacterium *Blochmannia floridanus* which resides within specialized midgut cells, the bacteriocytes, and provides essential nutrients to its host.[2], [3] This symbiosis appears to be particularly relevant during pupation when the bacterial population experiences a massive expansion transforming the entire midgut into a symbiotic organ. In adult ants the symbiosis slowly degenerates.[4] The basic question behind this project is how the ants are able to tolerate this chronic symbiotic infection, while they efficiently combat pathogenic infections. Primary analysis of the ant's immune system using SSH and qRT revealed that *B. floridanus* is still recognized as non-self by the host and that the immune system might be involved in controlling the symbiont's population. In fact, a strong up-regulation of the amidase pattern recognition receptor PGRP-LB occurs exclusively in the midgut and only during pupation thus correlating with endosymbiont (ES) replication. Such amidase PRRs are known negative regulators of the immune system which function by cleaving peptidoglycan, a molecular pattern stimulating an immune response. Hence, PGRP-LB appears to be a key factor involved in ES tolerance.[5], [6] To get further insight with respect to the role of the immune system in ES control we started a comprehensive characterization of the insect's immune system. A transcriptome analysis of immune-challenged and untreated animals allowed us to identify a large variety of genes possibly involved in immune functions including pathogen recognition receptors, immune signaling factors and several antimicrobial peptides (AMPs) like defensins and hymenoptaecin. Currently, the immune inventory of *C. floridanus* is analyzed with regard to the ongoing debate about whether social insects contrary to solitary insects might have reduced their costly immune repertoire due to hygiene measures on the social level, a phenomenon termed "social immunity".[7] Additionally we have started an analysis of the immune proteome in the ant's hemolymph in different developmental stages. The combination of these data will provide a quite comprehensive picture of the ant's immune system which will be the basis to identify and functionally characterize additional factors relevant for the control and tolerance of the ES.

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HMV18

Interplay of *Streptococcus suis* und swine influenza virus during co-infection in a porcine *ex vivo* precision-cut lung slice model

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Streptococcus (S.) suis is a swine pathogen of the upper respiratory tract causing meningitis, pneumonia, arthritis, and septicaemia. Moreover, *S. suis* is a zoonotic agent associated with meningitis and *streptococcal toxic shock-like syndrome* (STSS) in humans. Respiratory infections are a major problem in swine husbandry. These are often multifactorial diseases caused by interactions of co-infecting pathogens as well as environmental and management factors. Secondary pulmonary infection by bacteria including *S. suis* following primary swine influenza virus (SIV) infection increase the severity of the disease. Mechanisms underlying these synergistic effects are poorly understood. The objective of this study was to analyse the interaction of *S. suis* and SIV in an *ex vivo* precision-cut lung slice (PCLS) infection model consisting of well differentiated primary porcine respiratory epithelial cells maintained in their original setting. Infection of PCLS with a virulent

S. suis serotype 2 wild type strain and its unencapsulated mutant showed that streptococci adhered to ciliated cells of the luminal surface as well as to mucus producing cells of bronchioli. Infection caused decreased ciliary activity and cytotoxicity. Pre-infection of PCLS with SIV increased bacterial adhesion and further reduced ciliary activity. Immunofluorescent microscopy revealed the preference of *S. suis* to adhere to primary virus-infected cells. Furthermore, secondary infection with the *S. suis* wild type strain reduced viral replication. Notably, the unencapsulated mutant strain had no effect on viral growth, suggesting that the capsule sialic acid is a key-component involved bacteria-virus interplay. Co-sedimention of bacteria and virus showed a direct binding of SIV to *S. suis*. Taken together, this study provides new insights in mechanisms underlying interactions of SIV and *S. suis* during co-infection of respiratory cells.

HMV19

Functional high-throughput screening identifies the miR-15 microRNA family as cellular restriction factors for *Salmonella* infection

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In recent years it has become clear that microRNAs, in addition to their pervasive and well-established functions in physiological and pathological processes, also play a crucial role during infection by different pathogens. However, apart from a small number of microRNAs involved in the host inflammatory response, no microRNAs have been shown to directly modulate infection by bacterial pathogens. We focused on the facultative intracellular bacterium *Salmonella enterica* serovar Typhimurium, which is one of the most important causative agents of lethal food-borne diseases.
To systematically identify microRNAs that regulate *Salmonella* infection, we performed a high-throughput, fluorescence microscopy-based screening using a library of microRNA mimics (988 mature sequences). Using this unbiased approach, we identified 17 microRNAs that decrease *Salmonella* infection by at least 2-fold, as well as microRNAs able to increase *Salmonella* infection (11 microRNAs by at least 2-fold). Detailed time-course infection experiments showed that the identified microRNAs affect *Salmonella* infection at different stages of the infection cycle (e.g. invasion, maturation of the *Salmonella* containing vacuole, replication). Among the microRNAs that inhibit *Salmonella* infection more efficiently, we have identified the members of the miR-15 microRNA family (miR-15a-5p, miR-15b-5p, miR-16-5p, miR-195-5p, miR-424-5p, miR-497-5p and miR-503). These microRNAs do not affect *Salmonella* invasion and internalization, but rather processes linked to later steps of the infection cycle. Deep-sequencing analysis of microRNA expression demonstrated that the abundance of miR-15 family microRNAs is decreased upon *Salmonella* infection, indicating an active role of the bacteria in downregulating these host microRNAs that counteract infection. In addition, we show that downregulation of miR-15 family microRNAs occurs through the inhibition of the transcription factor E2F1, and is dependent on *Salmonella* internalization. Analysis of miR-15 family targets playing relevant roles in *Salmonella* infection revealed that derepression of cyclin D1 and the consequent promotion of host cell cycle G1/S transition are crucial events for Salmonella intracellular proliferation. Additionally, we show that Salmonella induces G2/M cell cycle arrest in infected cells, further promoting its replication. Overall, these findings uncover a novel mechanism whereby Salmonella renders host cells more susceptible to infection by controlling cell cycle progression through the active modulation of host microRNAs.

HMV20

Novel immunostimulatory flagellin-like protein FlaC in unsheathed *Helicobacter* and *Campylobacter* species

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Introduction: Bacterial microorganisms which colonize the intestinal tract have to deal with several unique characteristics specific for this habitat: a high density of resident microbiota of various species and an immunological environment primed by the resident microbiota. Little is known about how *Helicobacter* and *Campylobacter* ssp. interact with the innate immune systems of their hosts and with the major pattern recognition receptors (PRR) such as TLR and NOD receptors. It has been reported that *C jejuni* or the closely related gastric pathogen *H. pylori* are restricted in their abilities to activate the innate immune system via TLR5 and also TLR4.
Methods and Results: In addition to the classical flagellin molecules, we found the unusual flagellin-like protein FlaC and potential orthologues to be conserved in nine different *Campylobacter*, three intestinal *Helicobacter* and one *Wolinella* species. FlaC is a secreted protein, not involved in motility. Its amino acid sequences appear to be chimeras with amino acid similarities to both, TLR5-stimulating and non-stimulating flagellins. We hypothesised that FlaC might be involved in host immune modulation. For characterising this hypothetical function, we exploited *Campylobacter* FlaC as a model. Coincubation experiments of highly purified FlaC with chicken and human cell lines were performed. FlaC was able to activate different cell-types, and preincubation with FlaC reduced the responsiveness of chicken and human macrophages towards bacterial LPS. Additionally, FlaC was shown to directly interact with TLR5 and appeared to be immunogenic in chicken.
Conclusion: We propose that intestinal pathogens, which possess flagella without a sheath, including various *Helicobacter* and *Campylobacter* spp., have evolved the novel host stimulatory chimeric flagellin-like molecule FlaC in order to specifically modulate host responses, particularly towards other bacterial PRR ligands, and to act predominantly as a homeostatic or tolerogenic signal in the intestinal tract in the presence of the resident microbiota.

HMV21

Identification by TnSeq of a novel *Staphylococcus aureus* virulence regulator orchestrating leukocyte cytotoxicity

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Staphylococcus aureus is an important human pathogen causing an array of hospital and community-acquired infections. Staphylococcal cytotoxicity is attributed to a remarkable collection of virulence factors, which have been shown to efficiently kill their host cells from within upon phagocytosis.
We thus aimed to identify bacterial factors that contribute to this intracellular cytotoxicity. We generated a high density *S. aureus* transposon mutant library in the strongly cytotoxic *S. aureus* 6850. The library was used to infect human epithelial cells and was applied in a murine pneumonia model. The frequency of transposon insertion sites (TIS) within the genomes of recovered bacteria was compared to that of the inoculum by deep sequencing of TIS (TnSeq). Thus, among others, we identified an AraC-type transcriptional regulator that upon deletion led to significant reduction in hemolysis, cytotoxicity, and virulence, in strain 6850 as well as the MRSA strain LAC. The phenotype was complemented in trans. Further, differential RNAseq of wild-type and mutant demonstrated a virulence regulon which suggests that the identified virulence regulator orchestrates cytotoxicity against leukocytes and is placed upstream of a known global staphylococcal accessory regulator, SarR.

General and Hospital Hygiene

HYV01

Problems in the pre-analytic stages of blood culture diagnostics in German hospitals - a qualitative study using focus groups

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Introduction: Early and appropriate blood culture diagnostics is associated with both, a reduction of sepsis mortality and a reduction of duration of antibiotic treatment. Recent studies have shown that blood culture incidence rates and positivity rates in German hospitals are considerably lower than recommended. This indicates shortcomings in both, the initiation and the practical implementation of blood culture diagnostics. The aim of this study was to identify potential problems in pre-analytic stages of blood culture diagnostics in order to find targets for future intervention concepts.
Methods: Based on a literature review and a pilot study in two hospitals, a topic guide for a qualitative research study was developed. Three focus groups were performed with a total of 20 participants (doctors in different training levels and final year medical students) until a saturation could be achieved. Following the verbatim transcription the material was analyzed by three researchers independently using the qualitative content analysis developed by Philipp Mayring. Analysis of focus groups was first conducted group by group; then results were merged.
Results: We identified a total of eight categories representing three different domains (institutional/organizational, individual, test-specific) that might contribute to shortcomings in blood culture diagnostics in Germany. From an institutional point of view, a lack of standardization, technical difficulties, transport issues, financial necessities and the lack of time were named as problems. With respect to the individual being responsible for blood culture diagnostics, deficits in knowledge as well as a low intrinsic and extrinsic motivation were identified as the major categories. A test-specific problem mentioned frequently in the focus groups was the duration of blood culture testing and the lack of immediate consequences following taking a blood culture. Experienced doctors were more likely to identify problems in the knowledge about the value of blood cultures in sepsis diagnostics, while students and doctors in their first years of training put a special focus on the technical and organizational difficulties.
Conclusions: Within the present study, we identified problems in the pre-analytic stages of blood culture diagnostics that might be responsible for shortcomings in the initiation and implementation of blood culture diagnostics. In the next step, a quantitative survey will be performed in order to quantify the role of the identified problems in clinical practice. Based on the qualitative and quantitative results a multidimensional intervention concept will be developed.

HYV02

The positive association between self-reported cooperation on one's ward and hand hygiene compliance among physicians on intensive care units: Is it based on superiors, colleagues, or patients' relatives?

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Team- and leadership-directed strategies focusing on social influences have been shown to be cost-effective in promoting hand hygiene compliance among nurses [1,2]. Also, perceived good cooperation on one's ward is associated with self-reported compliance among physicians [3]. However, it is unclear whether this is based on superiors, colleagues, or patients or patients' relatives. This study investigates differences in self-reported hygienic hand disinfection by different social influences.
Data come from the research project PSYGIENE (PSYchologically optimised hand hyGIENE promotion) funded by the German Federal Ministry of Health. On 10 ICU and two hematopoietic stem cell transplantation units, 307 physicians and 348 nurses completed a questionnaire (response: 70.9% and 63.4%). This included items for self-perceived cooperation following occupational psychology recommendations. Logistic regression analyses were performed to estimate associations of perceived cooperation with superiors, colleagues, and patients' relatives (trichotomized Likert-scales) with self-reported compliance.
Of all respondents indicating to disinfect their hands (99.2%), 72.4% of physicians and 69.4% of nurses indicated to do so "always" (i.e. compliantly). Bi-variately, perceived good (vs. poor) cooperation was associated with higher odds of compliance among physicians (not nurses) in terms of both superiors (84.6% vs. 65.4%, OR=2.9, p=.003), colleagues (82.5% vs. 63.5%, OR=2.7, p=.004), and patients' relatives (88.2% vs. 62.6%, OR=4,5, p<.001). In multiple regression including all three social influences, only cooperation with relatives retained its association (OR=3,2, p=.033). Further analysis suggested that its effect was most pronounced when team cooperation was good (see Figure 1).
In sum, improved cooperation with and participation of patients' relatives in tertiary intensive care may represent mechanisms relevant to coping with current relapses in hand hygiene compliance in physicians [4]

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Figure 1

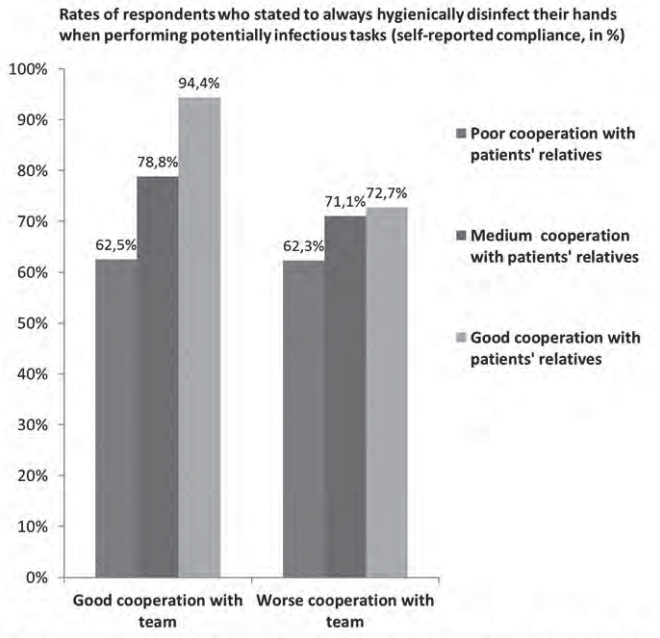

Fig. 1: Self-reported hygienic hand disinfection compliance of intensive care physicians (in %) by perceived cooperation with team (superiors and colleagues) and patients' relatives

HYV03

Hand hygiene compliance rates in 27 ICUs: Does hospital size, profession, working shifts or indication specific opportunities has an effect?

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Introduction: Hand hygiene is the single most effective measure in preventing health care associated infections. Especially patients on intensive care units (ICU) are at high risk and can benefit from a high compliance rate (CR) with hand hygiene (HH).

Materials and Methods: The prospective study was conducted at 27 intensive care units at different hospitals all over Germany. The aim of this multicenter study was to collect data on HH CRs by direct observation, stratified to hospital size, profession, shift- and indication specific opportunities. The standardized observation time was divided in two times 4 hours per shift in every hospital.
Results: 5.792 HH opportunities have been observed. In a multivariate regression analysis (Poisson) the HH CRs are significantly better in ICUs in primary and secondary care centers than in tertiary care centers (p=0,006). Furthermore HH CRs were significantly higher during the night shift compared with the early shift (p=0,004) but no difference between early and late shift. CRs in HH of the medical doctors were lower, but not significantly, compared to the nursing staff (p 0,064). Regarding the HH indications, the indication before an aseptic task was significantly lower compared to other indications (p=0,001). Hence the newly introduced indication after glove use showed a significantly higher CR compared to the remaining HH indications.
Discussion This study provides the first known multicenter prospective trial on CRs in HH during 3 different shifts, within ICUs of different hospital sizes and 6 HH indications. The significantly best CRs are during the night shift, and interestingly in ICUs in primary and secondary care centers. Well known are better CRs by the nursing staff compared to the medical doctors. Unfortunatly the HH indication before aseptic tasks, which may have a great impact preventing health care associated infections, seems to be rarely used. It is in contrast to the newly introduced indication after glove use which has a high CR for HH.

HYV04

Implementing Link Nurses for Infection Control in German Hospitals - Difficulties and Shortcomings during the process: interim report from the HYGPFLEG-Project

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Introduction: Two years ago, the HYGPFLEG-Project started a training program for infection control nurses (ICN), providing them with psychological and didactic skills, as well as technical content for the education of link nurses (LN) in their hospitals. Currently, 92 participants from 72 hospitals all over Germany completed the 4-day course. Aim of this investigation was to analyse the status quo of the implementation of a LN system in accordance with KRINKO requirements in the participants' institutions prior to participating in the training.
Materials and Methods: Participants were surveyed using a standardized questionnaire containing the following elements: first, institutional pre-conditions (positive decision for implementation of LN system; definitions of responsibilities for LN; selection of candidates; granted leave of absence for LN education), second, educational progress (date set for basic course; basic course completed; date set for individualised advanced course, e.g. neonatal care, psychiatric ward; advanced course completed), and third, introduction of structure changes to ensure long term sustainability of LN system (e.g. refresher courses; newsletter; ward-based education).
Results: 62 participants (69%) from 55 different hospitals completed the questionnaire. In 82% (n=51/62) of the participants' hospitals the introduction of a LN system had been concluded; in 61% (n=38/62) specific responsibilities for LNs had been defined. 66% (n=41/62) granted leave of absence for LN education. 56% (n=35/62) had set a date for basic training, 50% (n=31/62) had completed basic training in infection control. 31% (n=19/62) had set a date for advanced individualised training, but only 23% (n=14/62) had completed a comprehensive LN course. Regular meetings between infection control staff and LNs had been established in 65% (n=40), further means to ensure sustainability had been introduced in about one third of the institutions [newsletter 29% (n=18), ward-based education 37% (n=23)].
Conclusion: Our findings indicate a discrepancy between the 2009 KRINKO-requirements for the implementation of LNs and the current situation in the surveyed German hospitals. Institution based pre-conditions are fundamental to comply with KRINKO-requirements, yet 18% of the institutions had not concluded the implementation of LNs, 34% of the employers did not grant leave of absence for LN education, and only 23% had completed LN education. Strategies to ensure sustainability are implemented partly. ICN play a central role in the education of LNs. Providing them with psychological and didactic skills to successfully transfer knowledge may lead to better clinical outcomes.
Limitations: Participation in the training was voluntary. Institutions without a LN system might be overrepresented. At the same time results might suggest that only few institutions at all are concerned about implementing a LN system.

HYV05

Chlorhexidine-containing dressings for external ventricular drainages in order to reduce meningoventriculitis rates: a before after trial.

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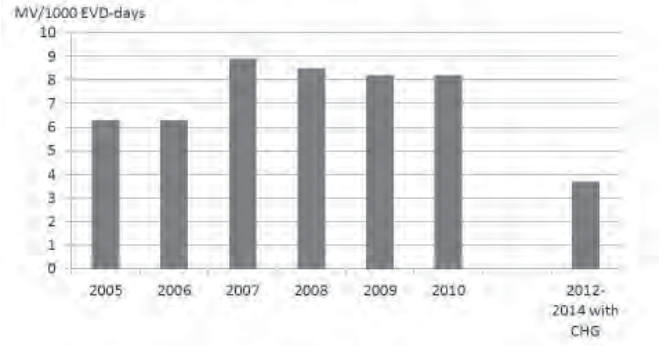
Introduction: External ventricular drainage (EVD)- associated meningoventriculitis (MV) represents a major infection prevention challenge. For central line associated bloodstream infections a marked reduction potential has been shown for the use of chlorhexidine (CHG)-containing dressings.
Aim: The aim of this study was to assess the influence of CHG containing dressings on the MV-rate.
Methods and Patients: We performed a before after trial in a neurosurgical intensive care unit at a University Hospital. Data on MV-rates using standard dressings were available from 2005-2010 (before). After a safety evaluation in 2012 (Infection 2014; 42) the use of CHG containing dressings for EVDs was introduced in 10/2012. During the following 18 month all patients with EVD were enrolled and evaluated for MV according the modified (JNNP 2010) CDC/KISS definition (after). Detailed statistical analysis will be provided.
Results: During 2005-2011 the EVD-MV rate remained stable at about 6 to 8 MV/1000 EVD-days (JNNP 2009; 80 and Infection 2010; 38; Figure 1). A total of 1349 EVD-days during 6084 patients-days were included after the introduction of chlorhexidine containing dressings. A total of 5 MV cases occurred. Thus, the MV-rate with CHG dressings resulted in 3.7 MV/1000 EVD-days compared to 6-8 MV/1000 11.3 MV/EVD-days when standard dressings were used. The duration for CHG dressing usage was about 6 to 7 days. It is of note, that there seems to be a shift in causative pathogens in MV with the use of CHG-containing dressings. Causative pathogens in the 5 MV cases were as follows: gram negative rods (*E. coli*; *P. aeruginosa*; *Moraxella sp.*; N=3), *S. aureus* (N=1) and *Candida sp.* (N=1) compared to a predominance of Coagulase-negative staphylococci before changing the regimen. No relevant adverse events occurred.
Conclusion: CHG containing dressings seem to represent a safe tool in order to reduce the rate of EVD-associated MV. A possible shift in causative pathogens should be confirmed and possibly taken into consideration for empiric therapy decisions.

Figure 1

Fig. 1: Development of meningoventricultis rates



MV: meningoventriculitis; EVD: external ventriular drainage

HYV06

Reduction of Nosocomial Blood Stream Infections (BSI) and Nosocomial Vancomycin-Resistant *Enterococcus faecium* (VRE) Colonisation on an Intensive Care Unit (ICU) after the Introduction of Antiseptic (Octenidine-based) Bathing: An Interrupted Time Series Analysis

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Introduction: On a 32 bed operative ICU at a university nosocomial VRE cases increased despite enforcement of hand hygiene and environmental disinfection. An intervention consisting in antiseptic bathing with octenidine (Octenisan®, Schülke) was started for control.
Materials and Methods: Between 1/2012 and 4/2014 ICU patients were screened for VRE at admission and twice weekly. Patients with a negative admission screening and a subsequent detection of VRE were defined as nosocomial cases. Intervention started 5/2013 and was implemented 8/2013. Octenidine based body washes were standardised by the use of new wash clothes for each body region and engaged hand disinfection before contacts at aseptic sites. Active surveillance for BSI and VRE infection and colonisation was performed, and VRE infections were determined according to the Centers for Disease Control and Prevention (CDC) criteria. Positive blood cultures taken after 3 days of admission were defined as nosocomial BSI. In case of skin commensals only the repeated detection in two independent blood cultures was taken as BSI. VRE were typed by PFGE. One-sided Permutation test was used to test the pre- and the post-intervention periods for significance (open source program "R" used).
Results: During the pre-intervention period 100 admitted (61% vanA, 39% vanB) and 113 nosocomial (60% vanA, 40% vanB) VRE cases were detected resulting in mean incidence densities (ID) of admitted and nosocomial cases of 6.6 and 7.53/1000 patient days, respectively. PFGE analysis revealed three vanA and four vanB clusters with partially differing hyl and esp profiles, as well as unique strains. Post-interventionally, 30 admitted (65% vanA, 35% vanB) and 19 nosocomial (63 % vanA, 37% vanB) cases occurred resulting in mean IDs of 4.13 and 2.61 ($p<0.001$), respectively. PFGE analysis showed two vanA and one vanB cluster, as well as unique strains. Nosocomial VRE infections were 10 in the pre- and one in the post-intervention period. Incidence densities of BSI pre- and post-intervention were 2.98 and 2.06, respectively ($p = 0.147$).
Conclusion: At admission to surgical ICU a high VRE prevalence was detected. The implementation of universal decolonisation using octenidine in combination with a standardised washing regimen led to a significant reduction of nosocomial VRE and a trend in reduction of BSI.

HYV07

Quantitative microbial risk assessment in the „safe Ruhr" project

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The aim of the BMBF-funded project "Safe Ruhr" is the evaluation of a temporary use of the Baldeneysee as bathing water. In the EU Bathing Water Directive, the quality of the bathing water is defined in terms of the concentrations of E. coli and enterococci.In addition to the bacteria, theconcentrationof human pathogenicvirusesinthe Baldeneysee has beendeterminedin thisproject.To assess theimportanceof these pathogensto human health,aQMRA(quantitativemicrobialrisk assessment)has been performed. The virological data in Baldeneysee were obtained during an intensive 12-month investigation phase. Atsevenselected sitesabout 80samples were collected frommid-May tomid-Septemberand analyzed by Real Time PCR. For QMRA the main factors requiredareviralconcentration in surface water,apathogen-specificdose-response relationship(derivedfrom the literature)andthe swallowed amount of water.Mathematically, the dose-response relationship for Rota- and Enterovirus can be described by a beta Poisson model.

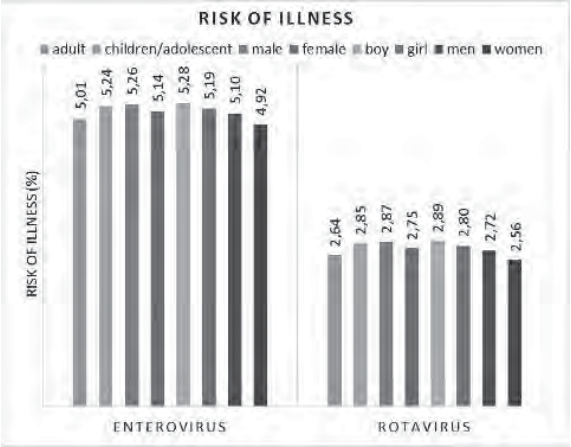
Fig. 1
α - constant; N_{50} - Dose with a 50% likelihood of infection; d - dose; P_{inf} - probability of infection
As known from literature, an adult ingests about 21 ml of water during 60 minutes bath time. This is an average value, which can be significantly larger due to increased activity in the water, but also by a more frequent dive with the head. It has been calculated that the probability of illness for rota- or enteroviruses is 2.7% and 5.1% by a 60 min swim, respectively. In order to evaluate the results, it is important to mention that enteroviruses are a group of viruses including e.g. echovirus or poliovirus. A subsequent Monte Carlo simulation calculates uncertainties of the QMRA.

Fig. 2
The QMRA method is now a common practice to estimate the risk of infection or disease caused by microorganisms, for which no limit value has been defined yet. At each evaluation, however, it should be taken into account that in addition to the pathogens evaluated a variety of microorganisms can occur in surface water, which can neither be quantified nor evaluated by QMRA.

Figure 1

$$Pinf = 1 - \left[1 + \frac{d}{N50}\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha}$$

Figure 2



HYV12

Nosocomial transmission of MDR-*Pseudomonas aeruginosa* among patients isolated in a stem cell transplantation unit is linked to bathroom reservoirs

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Introduction: *Pseudomonas aeruginosa* (P), and especially multidrug-resistant (MDR) strains pose a serious infection control problem in hospitals since they cause severe nosocomial infections like pneumonia and septicemia. Because of the ability to form biofilm the pathogen persists in wet environmental reservoirs, including tap water which was already reported to be a possible source of nosocomial transmission. We describe a transmission chain with a MDR-P strain in a hematology unit where six oncologic patients were colonized with a MDR-P (susceptible only to colistin).
Materials and Methods: The outbreak was investigated by epidemiology, inspection and microbiological sampling of the environment and molecular strain typing using semi-automated REP-PCR (DiversiLab, BioMériex) in order to determine possible sources and reservoirs of the pathogen.
Results: Through a well-established admission-MDR-screening in the risk units, all six cases were identified as being nosocomial-acquired by the

actual or recent stay at the hematology unit. In three of six cases there was no overlap in the patients' residence time; direct patient to patient transmission could therefore be excluded. However, these patients were housed in the same 2 rooms, after terminal room-disinfection and subsequently with an interval of 7 days, 1 month and 2 months, respectively (see figure 1). Patients' MDR-P samples were tested for genetic relatedness by REP-PCR technique and yielded to be closely related (similarity index ≥95%) suggesting clonality. Further, environmental samples were taken in the two rooms in the absence of pathogen carriers. The MDR-P was identified in the two sink waste-traps, toilet pans, shower drains and was found to be genetically identical to the outbreak strain. All samples of incoming waters, cleaning equipment, soaps, and multiple samples of near-patient surfaces were negative. For the preservation of evidence all en-suite wet rooms (n=26) in the hematology unit were investigated (7 of 26 MDR-P-positive) and sanitary-renovation measures were carried out by installation of thermo-disinfecting sink siphons, rimless toilet pans, mechanically cleaning and disinfection of shower drains. In addition, a regular environmental screening program was implemented (every month and after each MDR-P-positive patient).
Conclusions: No further cases of nosocomial transmission were monitored in 6 months of observation after establishing the described sanitary with renovation measures. Direct person-to-person spread seemed not to play a major role in the outbreak (since 3 from 6 cases occurred without overlap in patients' residence time) suggesting that the waste trap system was the possible source of the MDR-P-transmission.

Figure 1

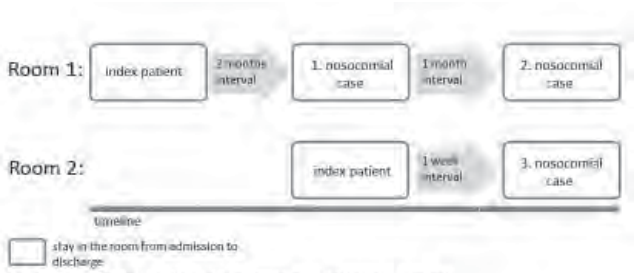

Fig. 1: stay of index-patients and nosocomial-colonized patients

HYV13
Occurence and genetic relationship of *Klebsiella pneumoniae* isolates from two neonatal intensive-care units of a university medical center

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Introduction: Preterm infants are at great risk for developing complications such as bacterial infections. In most cases invasive infection is preceded by asymptomatic colonization with the respective pathogen, which is acquired after birth from the maternal flora or the hospital environment (health care staff or inanimate surfaces). German infection control guidelines therefore recommend weekly screenings for potential pathogens in patients on neonatal intensive-care units (NICU).
In autumn 2013 screening results from two NICUs in our hospital indicated a sudden increase of colonization with *Klebsiella (K.) pneumoniae*. At one time point, 12 out of 14 patients on one ward were found to be colonized. The isolates did not exhibit ESBL production or any other unusual resistance pattern. To assess the clonality of the strains and to gain insight into distribution patterns on the NICUs, all K. pneumoniae isolates were collected and analyzed over a 6 month time period.
Materials and Methods: Rep-PCR from 50 isolates (at least one isolate per child) with consecutive cluster analysis was performed. Strains showing >95% similarity in PCR patterns were grouped in one cluster. Stable colonization with one strain was presumed, and patients were assigned to the clusters according to their colonizing isolate.
Results: *K. pneumoniae* strains could be grouped into 15 clusters, each of which consisted of 1 to 9 isolates from different patients. Patients mostly exhibited stable colonization with one particular strain, 3 patients were colonized with isolates from different clusters. Clusters appeared chronologically in a staggered order and were usually terminated by discharge of the last colonized patient. One isolate, which was recovered from the hands of a nurse, belonged to the cluster that was present in patients at this time point.
Conclusion: Rep-PCR analysis of *K. pneumoniae* isolates on two NICUs over 6 months did not reveal a monoclonal outbreak, but showed repeated entry of new strains into the colonizing population. Subsequent spread to other patients suggests that nosocomial transmission, possibly via the hands of the health care staff, plays an important role for the distribution of the strains on the ward.

HYV14
Outbreak of multiresistant Escherichia coli in a neonatal intensive care unit (NICU)

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Introduction: Outbreaks with multiresistant E. coli in neonatal intensive care units have rarely been reported. We here describe a recent outbreak among premature infants in a level 3 NICU adjacent to the delivery unit and a perinatal care NICU located in the main building of the University children´s hospital.
Materials and Methods: The case definition was colonization or infection of infants with E. coli (resistance to ampicillin, cephalosporins, aminoglycosides and trimethoprim/sulfamethoxazole, adk-53, fum-40 and icd-13). Infants were screened for E. coli at birth and consecutively on a weekly basis. The strain of the primary case was typed as sequence type ST-131.
Results: Time from detection of the primary case to the last case was 40 days. 13 of 13 cases had intestinal colonization, there were three positive blood cultures, and one positive urine sample. 12 of 13 transmission events were associated with a stay at the level 3 NICU. Time from presumed contact event to positivity of anal swabs was few hours to 2 weeks. Outbreak control was achieved by reducing overcrowding by opening a temporary new NICU within 48h and by isolation measures. Voluntary parental or staff screening was offered but not requested for. Environmental screening did not reveal the outbreak strain. Vertical transmission from the mother cannot be excluded for the first case. A maternal screening program was consecutively implemented yielding 4 positive samples among 84 anal swabs taken in a period of 5 months.
Conclusion and Discussion: The outbreak confirms transmissibility and virulence of multiresistant E. coli strains. Interestingly, ST-131 was found, which was also reported in a recent outbreak in Italy (1). The resistance profile left carbapenems as the only, but effective treatment option preventing any adverse outcome. Overcrowding due to increasing birth rates at the hospital and limited space due to accommodation of the unit in an old building was likely to have promoted the outbreak. Repetitive screening of infants turned out to be crucial due to delayed time to positivity of anal swabs. Environmental samples were negative for the outbreak strain suggesting that transmission events were exclusively triggered by indirect contact.

References
(1) Giuffrè et al. Antimicrob Resist Infect Control. 2013

HYV15
Carbapenemase Producing And Not Producing Enterobacteriaceae (4-MR-GNE): Differences in clinical outcomes?

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Objectives: Carbapenem resistance in Enterobacteriaceae (CRE) is emerging in German hospitals. The aim of this study was to investigate outcomes of patients with CRE by using routine CRE surveillance data in one university hospital (752 beds).
Materials and Methods: From 1/2013 to 12/2013 all laboratory detections of Enterobacteriaceae in clinical or screening samples suspicious for Carbapenemase production were sent to the national reference laboratory for multidrug resistant gram negative organisms for confirmation and differentiation of Carbapenemases. Active surveillance for CRE infection and colonisation was performed throughout the whole study period. Infections were determined according to the CDC criteria. Admission

screening for CRE was performed in patients with former hospitalization abroad, known former colonisation with CRE or contact to a CRE positive patient. Screening sites were rectum, urine, wounds and tracheal secretion.
Results: We identified 19 patients with CRE (0.06/100 patient cases; 0.08/1000 patient days), 10 with carbapenemase-producing (CPE) (6 from Germany, 1 Russia, 3 Libya), 9 with not carbapenemase-producing enterobacteriaceae (N-CPE) (8 Germany, 1 Turkey). *Enterobacter cloacae, Enterobacter aerogenes, K. penumoniae, E. coli, C. freundii, P. vulgaris* were detected. In both groups 4 infections occurred. In the CPE group one urinary tract infection, two wound infections and one pneumonia was recorded, in the N-CPE group one intraabdominal infection, one wound infection and two pneumonias. In the CPE-group three infection were mixed infections, one with MRSA and two with a MR-*A. baumannii*. In the N-CPE-group also three mixed infections occurred (VRE, MR-*P. aeruginosa, E. faecium* and *E. faecalis*). The distribution of Carbapenemase-encoding genes was as follows: 6 *Klebsiella pneumoniae* carried OXA-48,1 *K. pneumoniae* carried KPC-2, 2 *Enterobacter cloacae* and 1 *Citrobacter freudii* carried VIM-1. All patients were suffering from serious diseases like acute traumata or burn injury. No positive blood culture was found. All patients in the CPE group survived, in the N-CPE group 1 patient died.
Conclusions: Even though CRE are more common abroad they are also of relevance in Germany. However, special attention needs to be paid to patients coming from countries with a high prevalence of CRE. Interestingly, only 50% of the patients developed an infection and only in the case of N-CPE (one pneumonia) death was associated to CRE. It is important to distinguish between infection and colonisation to avoid unnecessary treatment and to prevent the development of further resistances against the few antibiotics left available. CPE und N-CPE seem to develop a comparable outcome and the concentration of infection control measures on only CPE patients may be inadequate.

INFECTION IMMUNOLOGY

IIV01
Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) treatment ameliorates *Toxoplasma gondii*- induced encephalitis in mice

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Introduction: The intracellular parasite *Toxoplasma (T.) gondii* infects up to one third of the world population. The latent infection can reactivate in case of immunosuppression resulting in life-threatening encephalomyelitis. Current therapeutic regimens, however, exert major adverse effects. Furthermore, to date no current agent is able to completely eliminate the latent stage of the parasites, namely cysts, from the central nervous system (CNS). Hence, the demand for novel therapeutic drugs that reduce inflammation, repair neuronal degeneration and eliminate the chronic stage of parasites is urgent. Pituitary adenylate cyclase-activating polypeptide (PACAP) is well known to play crucial roles in immunity and inflammation. For the first time, we investigated the potential anti-inflammatory and immune-modulatory properties of PACAP in a murine parasite-induced encephalitis model.
Materials and Methods: Encephalitis was induced following intraperitoneal *T. gondii* infection (3 cysts, ME49 strain) four weeks before analysis. Synthetic PACAP38 was administered intraperitoneally (1.5 mg / kg body weight) every other day for 10 days starting at day 18 post infection (p.i.). On day 28 p.i., PACAP treated animals displayed reduced signs of intracerebral inflammation when compared to placebo treated controls as indicated by less inflammatory foci and fewer $CD3^+$, $F4/80^+$ and $Caspase3^+$ cell numbers within the brain parenchyma. Importantly, PACAP treated mice exhibited significantly lower intracerebral IFN-g, IL-6 and IL-10 mRNA expression levels as compared to placebo controls. Moreover, a trend towards reduced *T. gondii* loads in the CNS upon PACAP treatment could be observed. Interestingly, intracerebral p75NTR mRNA expression levels were reduced in PACAP-treated group, suggesting involvement of the neurotrophin signaling pathway.
Conclusion: PACAP treatment ameliorates *T. gondii* induced inflammation in a murine model. These findings might provide new treatment options for *Toxoplasma* encephalitis.

IIV02
Analysis Of Gene Defects In Severe Congenital Neutropenia

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Severe congenital neutropenia (SCN) is characterised by a differentiation block in the bone marrow and low neutrophil numbers in the peripheral blood, which correlates with increased risk of bacterial infections. Several underlying gene defects have been identified, including mutations in the genes of G6PC3 and Hax1. However, the molecular mechanisms leading to SCN still remain unclear. To address the molecular basis of G6PC3- and Hax1-deficiency, we used the ER-Hoxb8 system of neutrophil differentiation from immortalised progenitor lines by conditional expression of Hoxb8. Upon estrogen withdrawal, cells differentiated into mature neutrophils highly similar to primary cells *in vitro* and in vivo. Using this system, we previously identified the BH3-only protein Noxa as important regulator of neutrophil apoptosis on top of Bim and Mcl-1. To study the role of G6PC3 and Hax1 gene defects, G6PC3-/- and Hax1-/- progenitor lines were generated and analysed for survival and maturation during differentiation. In G6PC3-/- cells, apoptotic cell death linked to enhanced ER stress and upregulation of Bim was observed early in differentiation. Overexpression of antiapoptotic Bcl-xL rescued survival, but not maturation and function either *in vitro* or in vivo in a mouse transplantation model. Hax1-/- progenitors underwent normal differentiation *in vitro* with respect to morphology and maturation markers, but cytokine secretion upon proinflammatory stimuli was impaired. Transplantation experiments pointed to a defect in the release of Hax1-/- cells from the bone marrow. The Hoxb8 system is a suitable model to study differentiation and survival of the neutrophil lineage and can contribute to a better understanding of the pathogenesis of SCNs.

IIV03
Novel Role for Pro-Coagulant Microvesicles in the Early Host Defense against Streptococcus pyogenes

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Our previous studies have shown that stimulation of whole blood or peripheral blood mononuclear cells with bacterial virulence factors results in the liberation of pro-coagulant microvesicles (MVs). Though it is generally appreciated that the concentration of MVs is significantly increased in the circulation of septic patients, it is still not completely understood whether this is part of the innate immune response or contributes to systemic complications. Here we describe that the interaction of pro-coagulant MVs with bacteria of the species *Streptococcus pyogenes* is part of the early immune response to the invading pathogen. As shown by negative staining electron microscopy and clotting assays, pro-coagulant MVs bind in the presence of plasma to the bacterial surface. Surface plasmon resonance analysis revealed a strong interaction between pro-coagulant MVs and fibrinogen with a KD value in the nanomolar range, identifying fibrinogen as a linker that allows the opsonization of the bacteria by MVs. When performing a mass-spectrometry-based strategy to determine the protein quantity, a significant up-regulation of the fibrinogen-binding integrins CD18 and CD11b on pro-coagulant MVs was recorded. Further on we show that plasma clots - induced by pro-coagulant MVs - are able to prevent bacterial dissemination and possess antimicrobial activity. Our findings were confirmed in a murine streptococcal sepsis model, where we found that local application of pro-coagulant MVs not only dampened bacterial spreading into varies organs, but it also caused a significant increase in survival. Taken together, our data support the concept that MVs have a protective function when acting locally in the early response of the innate immune system in infectious diseases.

IIV04

A new function of CD11b/CD18: Complement Receptor 3 (CR3) is critical in late defense against intracellular *Chlamydia psittaci* in mouse lung infection

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Introduction: The intracellular bacterium *Chlamydia psittaci* (Cps) causes psittacosis in birds and life-threatening pneumonia in humans. In mouse lung infection, the receptor for the anaphylatoxin C3a (C3aR) is essential in defense against Cps and required for antibody and optimal T cell responses (Dutow *et al.* JID 2014). However, $C3^{-/-}$ mice are even more susceptible against Cps (Bode *et al.* PLosOne 2012) indicating the involvement of additional complement effector functions downstream of C3. The $\alpha_{M\beta}2$ integrin CR3 (CD11b/CD18) interacts with ligands such as iC3b, ICAM-1, -2, 4 or CD14. CR3 acts as primary phagocytic receptor on granulocytes, monocytes/macrophages or dendritic cells. 'Inside-out signalling' by costimulatory pathways such as those of IC/FcγR, LPS/CD14 or fMLP/fMLP-R improves interaction of CR3 with its ligands leading to cellular signalling and responses such as increased PMN adhesion or antimicrobial functions.
Aim: Elucidation of CR3-dependent protection against intracellular Cps and the underlying adaptive immune mechanism.
Materials and Methods: Survival, body weight, and clinical score in primary mouse infection were monitored for two weeks after intranasal infection of C57BL/6J wild-type and $CD11b^{-/-}$ mice with Cps DC 15. Bacterial load, histology, cellular distribution, the granulocyte marker MPO, cytokines, Cps-directed antibodies, and lymphocytes in draining lymph nodes were analyzed on day 9 and 14.
Results: During the first days of Cps infection, there was no difference between infected $CR3^{-/-}$ and corresponding wild-type mice. However, $CR3^{-/-}$ mice showed prolonged pneumonia with drastically decreased survival, lower weight, and higher clinical score during the second week of infection. In absence of CR3 bacterial clearance was impaired, and in accordance to that inflammatory parameters stayed increased. Surprisingly, in contrast to $C3aR^{-/-}$ mice, $CR3^{-/-}$ mice could raise Chlamydia-specific antibodies, and the number of B and T cells in draining lung-lymph nodes was not reduced upon infection.
Conclusions: CR3, most likely stimulated by iC3b bound to chlamydial antigens, is cooperating with C3a/C3aR in the defense against Cps in mouse lung infection. Thereby, C3a/C3aR might provide costimulatory inside-out signals for CR3. These new cooperative functions of the innate complement system strongly influencing the adaptive immune response might be of general immunological importance.

IIV05

The quorum sensing molecule farnesol as a modulator of dendritic cell function

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Introduction: Farnesol was the first eukaryotic quorum sensing (QS) molecule identified in the polymorphic yeast *Candida albicans*. The mechanism by which farnesol blocks the *C. albicans* yeast to hyphae transition is well studied but its role in interaction with innate immune cells remains only partially elucidated. The aim of our study is to determine the impact of farnesol on the dendritic cells (DC).
Materials and Methods: Human monocytes were isolated from buffy coats of healthy volunteers and differentiated into monocyte-derived dendritic cells (moDC) in the presence of GM-CSF and IL-4. The immunophenotype of moDCs was defined by differential FACS analysis. The transcriptome of DC in response to farnesol was performed by a whole-genome expression direct hybridization assay on a bead chip array. Cytokines were detected in supernatants using Milliplex®MAP Kits (Millipore). Cell spreading was performed on fibronectin coated plates and analyzed via microscopy. To determine antigen presentation (AP) function of mDC, CFSE fluorescence in proliferating T cells was measured by flow cytometry.
Results: Farnesol treated immature DC (iDC) showed an increased expression of the activation surface markers CD86 and HLA-DR. However, after stimulating iDC with LPS in addition to farnesol we could observe a significant reduction in the secretion of IL-12. While the phenotype of mature DC (mDC) seems to be unaffected by farnesol treatment, we observed a significant change in surface marker expression of mDC generated in the presence of farnesol. CD1a is no longer expressed on the cell surface, the maturation marker CD83 and costimulatory molecules (CD80, CD86) are significantly reduced. To fully understand the impact of farnesol on DC we performed transcriptomic profiling. Pathway analysis showed that a high number of differentially regulated genes are involved in cytokine-cytokine receptor interaction, antigen processing and presentation and cell adhesion molecules. An increased expression of genes encoding the chemokines (MCP-1, MIP4α, Eotaxin 2) and reduced expression levels of genes encoding proteins with chemotactic activity for neutrophils (CXCL1), monocytes (CCL1) or activated T cells (CXCL9) were determined. To validate the micro-array experiments we investigated the release of cytokines. We detected increased levels of pro-inflammatory cytokines (TNFα, MIP-1α, MIP-1β, MCP-1) and reduced levels of IL-12 in the supernatants of farnesol treated DC. In addition we could observe that these cells show an attenuated cell spreading on fibronectin coated plates. Furthermore farnesol treated mDC showed a defect to induce proper T cell responses.
Conclusions: The fungal QS molecule farnesol influences the ability of dendritic cells to promote inflammation and mitigates the T_H1 response which is important for fungal clearence.

IIV06

Epithelial cells directly contribute to attraction and activation of neutrophils during infection with *Chlamydia trachomatis L2*

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Genital infection with the obligate intracellular bacterium *Chlamydia trachomatis* can cause chronic inflammation of genital tract and pelvis with the potential consequence of tubal scarring and eventually infertility. The mechanisms that lead to genital tract tissue damage are still unclear but previous studies suggest a contribution from neutrophil granulocytes. Although infected epithelial cells may secrete various mediators it is not clear how these cells are recognized by and stimulate neutrophils. We here rebuild this situation *in vitro* with the goal of understanding the factors involved on the sides of *C. trachomatis*, infected epithelial cell and responding neutrophil. Mouse neutrophils are generated from progenitors 'conditionally immortalized' with regulable Hoxb8. Human or mouse epithelial cells are infected with *C. trachomatis*; to test for the role of the chlamydial cryptic plasmid known to contribute to tissue inflammation we also used a bacterial strain cured of the plasmid. Supernatants of infected cells were collected. In migration assays through a transwell-system, neutrophils showed considerably higher migration towards supernatants of *Chlamydia* infected HeLa cells than to supernatants of uninfected cells. When wt neutrophils were incubated in supernatants of infected HeLa cells, neutrophil apoptosis was significantly reduced. This activity was neutrophil MyD88/TRIF independent and was absent from supernatants of HeLa cells infected with the plasmid-cured strain. Surprisingly, supernatants from both strains induced similar levels of TNF in wt but not MyD88-/-TRIF-/- neutrophils. These findings suggest that neutrophil-activation by infected epithelial cells at least partly depends on the chlamydial plasmid and occurs both TLR-dependently and -independently. Our system illustrates that the interaction of infected epithelial cells with neutrophils alone may entertain inflammation and will permit more in-depth study of neutrophil activation by *Chlamydia* infected epithelial cells.

IIV07

Development and Evaluation of an mRNA-based Vaccine Against *Staphylococcus aureus*

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Rapid spread of methicillin-resistant *Staphylococcus aureus* (MRSA) and limited therapeutic options define the need for alternative therapeutic approaches such as vaccine development. Albeit an antibody response is present in nearly all individuals little is known about the protectivity of T

cell responses. In this study we present a novel technique to assess antigen-specific T cell responses to *S. aureus*.
Here we show that using an *in vitro* transcribed (ivT) mRNA of specific staphylococcal proteins we are able to induce T cell responses to staphylococcal antigens in T cells from healthy donors. This is shown for protein A (*spa*), encoded by a core variable gene, and PBP2a (*mecA*), encoded by an accessory gene responsible for methicillin resistance. Notably, donor-dependency of the ivT mRNA-induced recall responses indicates that this method could be used to quantify specific memory T cell responses in the absence of other bacterial components with adjuvant effects, i.e. Toll-like receptor-2 ligands. Our findings further reveal differential reactivity of T cell responses to the sequence-specific differences in the variable domain of protein A, indicating that this technique might be useful in the development of a strain-specific vaccine. In views of the relevance of T cells in immune defense against intracellular persisting pathogens we conclude that mRNA-based vaccination might represent a promising novel approach for the induction of T cell-mediated protective responses in patients prone for chronic infections with *S. aureus*.

IIV08
Comprehensive transcriptome and quantitative proteome analyses of a macrophage-intrinsic type I and II IFN-dependent defense pathway that restricts an intracellular bacterium in the lung

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The innate defense mechanisms that control infections with intracellular bacteria are still incompletely understood. Here we show that type I and II IFNs are key regulators of the early gene expression and the host-protective innate immune response during *Legionella pneumophila*-induced pneumonia. Using mixed bone marrow chimeric mice and isolated cells we indicate that both IFNs protect against *L. pneumophila* by activating an alveolar macrophage-intrinsic antibacterial defense pathway. Quantitative mass spectrometry analysis of purified *Legionella*-containing vacuoles reveals that both IFNs markedly alter their protein composition, and integrated network analysis in comparison with the transcriptome data defines distinct subsets of IFN-regulated proteins. A siRNA-based screening among candidate proteins uncovers *Immune responsive gene 1* (Irg1) as a pivotal effector molecule restricting bacterial growth in a cell-autonomous manner. Collectively, our study provides a comprehensive analysis of IFN-mediated effects on gene expression and protein abundance at a bacterial vacuole, and identifies Irg1 as an IFN-dependent effector molecule that restricts *L. pneumophila* infection.

IIV09
Live cell imaging of autophagy in *Leishmania* infected human primary macrophages

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Human monocyte derived macrophages (hMDMs) are the preferred host-cell for the obligate intracellular parasite *Leishmania (L.) major*. Inside hMDMs different maturating compartments can contribute to either parasite survival or control. In this project we focus on the biogenesis and maturation of autophagy compartments in hMDMs and their role in parasite control. First we co-incubated hMDMs with either the virulent inoculum, containing viable and apoptotic promastigotes, or with apoptotic promastigotes alone. Using immunofluorescence analysis, both viable and apoptotic parasites can be found in EEA1-positive compartments 15 min and 30 min after infection. Subsequently, apoptotic promastigotes enter a LC3-positive compartment that in time becomes LAMP2 positive. Using lentiviral transduction for overexpression of eGFP-LC3 in hMDMs and Live Cell Imaging we observed the formation of LC3-positive compartments around apoptotic parasites. We found that 3h after infection with the virulent inoculum 50% of the parasites were outside and 50% were inside LC3-positive compartments. In time after infection, LC3 positive compartments disappeared. Interestingly, we found that viable promastigotes can be targeted by eGFP-LC3 proteins and are engulfed into an LC3-positive compartment. Over time we observed that the viable promastigotes are able to escape this compartment. In addition, we generated lentiviral particles containing eGFP-2xFYVE for analysis of early events after parasite uptake and LAMP2-mCherry to visualize compartment maturation. To modulate the autophagy machinery we specifically targeted the protein Unc-51 Like Autophagy Activating Kinase 1 (ULK1) using siRNA knockdown. We successfully knocked down ULK1 on mRNA and protein level in hMDMs. Dynamic imaging of ULK1 knockdown cells transduced with eGFP-LC3 will be used to analyze the role of ULK1 in *L. major* compartment development.
Dynamic and molecular evaluation of different compartment proteins reveals the maturation steps of compartment development and their role in *L. major* infection.

IIV10
Induction of collagen IV autoimmunity by streptococcal M protein is PARF-specific

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M proteins are potent virulence factors of *Streptococcus pyogenes* that have been implicated in the pathogenesis of acute rheumatic fever (ARF). They trigger autoimmunity by molecular mimicry of host proteins with coiled-coil structure such as myosin or laminin. In addition, a collagen-binding (A/T/E)xYLxx(L/F)N sequence motif occurs in the N-terminal type specific part of M proteins that evoke collagen IV autoimmunity. This immune response against collagen IV is observed in ARF patients, but not a cross reaction to M protein as in molecular mimicry. As these observations suggest a link between the collagen binding motif and the autoimmune responses in ARF, the motif is referred to as “peptide associated with rheumatic fever” (PARF). Still, the role of collagen binding and of other properties of the M proteins in triggering the autoimmunity against collagen IV remains elusive. Examining *S. pyogenes* isolates and M proteins of different *emm*-types for their ability to bind collagens I or IV and determining the autoimmune response against collagen I, collagen IV, myosin and laminin caused by the M proteins in mice, we prove that PARF is the trigger of the collagen IV autoimmunity in M proteins. Experiments in mice suggested a causative connection between autoimmunity against collagen IV and laminin. However, no causative connections between collagen specific and coiled-coil specific autoimmune responses were observed in humans with ARF. We conclude that induction of collagen IV autoimmunity is PARF-specific, thus independent of other host responses to M proteins.

IIV11

CD1b restricted T cell antigens of *Mycobacterium tuberculosis* - a particular focus on mannosylated Lipoarabinomannan

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Introduction: The thick and lipid rich cell wall is one of the hallmarks of mycobacteria of the tuberculosis complex. Among these lipids there is a number of known CD1 restricted T cell antigens. Here, we set out to establish a ranking of different lipid antigens with respect to their immunogenicity in mycobacteria exposed humans and compare this to the immune response of mycobacteria sensitized, CD1 competent guinea pigs.
Materials and Methods: PBMCs are obtained from human donors or sensitized guinea pigs and purified by Ficoll gradient centrifugation. CD1 expressing APCs are generated, mixed with responder cells and stained with CFSE. Cells are stimulated with defined lipid antigens, after five to six days cell proliferation is quantified by flowcytometry.
Results: We identify mannosylated Lipoarabinomannan (ManLAM) and Glucosemonomycolate (GMM) as the most immunogenic CD1 restricted T cell antigens. The responding T cells are CD4-positive, inflammatory T helper cells. Notably, when comparing the response between healthy latently infected donors and Tb patients, we find that the proliferative response to MHC restricted protein antigens, such as ESAT-6, is equal or even higher in patients while the reactivity to CD1 restricted antigens is clearly lower. An identical hierarchy of lipid immunogenicity is observed in mycobacteria sensitized guinea pigs.
Discussion: The comparison between infected healthy donors and TB patients indicates that CD1 restricted T cells may be involved in protective mechanisms against virulent mycobacteria. The comparability of our findings in the guinea pig show that the guinea pig is a suitable small animal model to study the protective efficacy of CD1 restricted T cells *in vivo*.

IIV12

Effects of bacterial infection on primary human macrophage subpopulations

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Listeria monocytogenes, is an important human, food-borne pathogen and a model organism for intracellular pathogenesis. Following entry into the human body, *L. monocytogenes* gains access to various intracellular niches due a wide range of virulence factors. Nevertheless, sooner or later *L. monocytogenes* comes into contact with macrophages. Macrophages are a major population of phagocytes of the human innate immune, which play an important role in the defense against bacterial pathogens. In this study, different types of human macrophages were generated *ex vivo* from fresh human blood monocytes. The monocytes were either incubated with M-CSF or GM-CSF to generate to generate an anti- or pro-inflammatory populations of macrophages, respectively. After differentiation, macrophages were infected with *L. monocytogenes* as well as other human pathogenic (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and non-pathogenic (*Lactococcus lactis* and *Escherichia coli*) bacteria. The effects of infection on cell death, killing capacity, T cell stimulation, chemotaxis and cytokine production of macrophages were analyzed using various readouts. M-CSF-derived macrophages (M-Mq) generally showed higher phagocytic activity than GM-CSF-derived macrophages (GM-Mq) for all bacteria tested. However, both types of macrophages were able to efficiently kill intracellular bacteria. GM-Mq infected with *L. monocytogenes* rapidly stained positive in TUNEL assays indicating cell death. High levels of IL-1ß were detected in supernatants of these cells suggesting pyroptosis. By contrast, M-Mq survived infection for prolonged periods of time without any signs of cell death. In response to *L. monocytogenes* infection GM-Mq obtained the ability to activate T- cells and induce proliferation of $CD8^+$ T cells. By contrast, no significant T- cell response was observed in co-culture with infected M-Mq. Also, M-Mq showed no chemotaxis towards *L. monocytogenes*, MCP-1 or fMLP whereas uninfected GM-Mq strongly migrated towards these stimuli. The ability of GM-Mq for chemotaxis was lost upon infection with *L. monocytogenes*. Cytokine profiles of GM-Mq and M-Mq confirmed the pro- and anti-inflammatory phenotype of these cells.

Clinical Microbiology and Infectious Diseases

KMV01

Abandonment of prophylactic antibiotic therapy after kidney transplantation leads to a reduction in urinary tract infection with ESBL producing bacteria

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Introduction: Symptomatic urinary tract infections (UTI), especially caused by ESBL-producing enterobacteria represent a major challenge in renal transplant patients. According to one standard therapeutic concept, all patients receive antibiotics for about the first one to two weeks while ureteric stenting by percutaneous catheters is performed. The hypothesis was to minimize the risk of UTIs by prescribing antibiotics periinterventionally.
Aim: The aim of the study was to evaluate the impact of a system change from percutaneous ureteric stenting with prolonged antibiotic "prophylaxis" to internal double-J-catheter stenting with only single shot prophylaxis concerning the number of UTIs and the proportion of ESBL-producing enterobacteria, respectively.
Materials and Methods: Before after trial at a University Hospital. All patients undergoing renal transplant were consecutively enrolled coming up with

- Phase 1: external catheter and prolonged antibiotic prophylaxis 2006-2008 (N=67 patients)
- Phase 2: internal double-J catheter and single shot prophylaxis 2009-2011 (N= 80 patients)

In all patients cefuroxime was used: for single shot dosed 1,5g, for prolonged prophylaxis adapted according renal function. Primary endpoints were the frequency of UTIs and the frequency of UTIs due to ESBL-producing enterobacteria and the days on antibiotics during the first year after transplant.
Results: A total of 274 UTI occured in 147 patients, thereof 129 UTI in phase 1 patients and 145 UTI in phase 2 patients. Despite omitting the prolonged antibiotic "prophylaxis" the frequency of UTIs did not increase while using internal double J stents coming up with 2.1±2.0 UTI/patients in phase 1 versus s. 1.9±2.7 UTI/patient in phase 2, respectively.
In additional, the days on antibiotics significantly decreased from phase 1 to phase 2 with 34.7±37.5 versus 24.7±46.7 days per patient ($p< 0.05$).
Moreover, the part of UTI due to ESBL-producing enterobacteria significantly decreased from 45% to 23% ($p<0.0001$). This change was in contrary to the hospital-wide time trend with an increase in the part of ESBL-producing enterobacteria in urinary tract infections from 4% at the beginning of the study to 13,5% at the end.
Interestingly, a pathogen shift was seen with a significant increase in UTI caused by *E.coli* (N=37/129; 29% in phase 1 versus 61/145; 42% in phase 2; $p<0.01$) and a significant decrease in UTI caused by *P. aeruginosa* (23/129; 18% versus 12/145; 8%; $p<0.05$). It is of note that the increase in *E.coli* as causative agent in UTI was in-line with a hospital wide trend, respectively.
Conclusion: Omitting the „prolonged „ antibiotic prophylaxis after renal transplant did not lead to an increase in UTIs at least when combined with internal versus external ureteral stenting. Moreover the number of infections due to resistant bacteria significantly decreased. Thus, this approach represents a safe strategy to avoid unnecessary antibiotics.

KMV02

Is the Implementation of a Microbiological Surveillance Screening Beneficial in Neonatal Intensive Care Units?

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Introduction: Gram-positive bacteria, mainly coagulase negative staphylococci (CNS), which colonize the patient's skin, are the most frequently detected pathogens in nosocomial blood stream infections in neonatal intensive care units (NICU). Gram-negative bacteria detected in blood cultures have been found to colonize the patient's gastrointestinal tract previously. In 2012, the German Commission for Hospital Hygiene and Infectious Disease Prevention recommended a microbiological screening of premature infants for multiresistant gram-negative bacteria (MRGN) and for multiresistant gram-positive bacteria that should be accounted for in calculated antibiotic therapy. Little is known about the benefit in clinical practice. The study aimed to identify the number of nosocomial infections caused by pathogens identified by this kind of screening. The second aim was to determine the cases in which the preemptive adaptation of the calculated antibiotic therapy because of the detection of multiresistant pathogens was of proven benefit.
Materials and Methods: The study period was 07/2012 to 01/2014. We analyzed pharyngeal and rectal swabs for MRGN, resistant gram positive bacteria (VRE, MRSA). In addition, pharyngeal swabs were analyzed for bacteria causing pneumonia. The screening was done on a weekly basis. Occurring nosocomial infections were recorded.
Results: 177 patients were included. 37% of all patients had positive screening Results: 40 patients (23%) were colonized with 41 MRGN: *Enterobacter cloacae*, *Klebsiella pneumonia* and *Escherichia coli*. 37 nosocomial infections were recorded in 28 patients. Only 3 of them were caused by bacteria previously identified by the screening: 1 peritonitis due to *Enterobacter cloacae* (2MRGN), 1 sepsis and 1 pneumonia due to *Pseudomonas aeruginosa* (not MRGN). In 10 blood stream infections CNS were detected. 1 blood stream infection was caused by *Staphylococcus aureus*. In 24 nosocomial infections no pathogen was found.
15 of the 28 affected patients were colonized with MRGN. These were treated with Meropenem.
Discussion: The endemic microflora contains a significant percentage of MRGN. CNS were the most frequently detected pathogens in nosocomial infections. Only in 3 of 37 infections, the pathogen was identified by screening. MRGN was detected in 1 only infection. The number of nosocomial septicemia caused by pathogens identified by the screening may be underestimated due to the relatively low sensitivity of blood cultures in case of small sample sizes.
14/15 patients received Meropenem because of the detection of MRGN in the screening without proven infection by MRGN. A benefit of the screening in terms of preemptive relevant adaptation of the calculated antibiotic therapy was proven only for 1/177 patients.

KMV03

(1→3)-β-D-Glucan kinetics in patients with candida blood stream infection

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Objectives: Empirical therapy in febrile ICU-patients with candidemia contains an antifungal drug in only 5.7 % of cases. As a consequence, antifungal therapy is usually started at the time of blood culture positivity, i.e. with a delay of 2 to 3 days after the onset of symptoms. However, a delay in the initiation of adequate treatment is associated with an increased mortality and therefore therapy should be guided by rapid diagnostic tests. A question of debate is how fungal antigen assays should be used. Specifically, it remains unclear whether a prophylactic screening approach (testing all patients 2-5 times per week) or a symptom-driven approach (testing only after the onset of symptoms) should be implemented. It is also unknown, whether and for how long the appearance of fungal antigens in serum precedes the onset of symptoms and for how long these antigens continue to circulate in the blood. Therefore, we initiated a study to determine (1→3)-β-D-Glucan (BDG) kinetics before and after diagnosis of candidemia.
Materials and Methods: We prospectively collected serum samples from patients with culture-proven candidemia at the University Medical Center Freiburg between March, 2013 and May, 2014. The sera were obtained from Clinical Chemistry which stores all samples for 5 days. This enabled us to get access to sera from the 3 to 4 days prior to the blood culture sampling (day 0). For follow-up purposes, serum samples were collected on day 2, 5, 9, 14 and then every 7 days until death or discharge. Furthermore, clinical information and data on antibiotic/antimycotic therapy of these patients was acquired. BDG levels were measured with the Fungitell assay according to the manufacturer's instructions.
Results: In total, 512 serum samples (1-30 per patient) from 52 patients with candidemia were collected. The earliest serum was drawn 10 days before and the latest 120 days after day 0. Using the manufacturer´s cut-off (80 pg/ml) 45 patients tested positive for BDG at day 0 (sensitivity: 86.5 %, 95%-CI: 74.2-94.4). The median BDG level at this time point was 280 pg/ml (IQR 106-634). Thirty of the BDG-positive patients (88 %) had elevated levels prior to day 0, with BDG levels being elevated up to 5 days before. Interestingly, in patients suffering from recurrent candidemia the relapse was accompanied by increasing BDG levels. In 7 out of 20 patients with a fatal outcome (35%) BDG levels increased drastically before death. None of the survivors showed such a kinetic.
Conclusion: Serum BDG proved to be a highly sensitive biomarker for diagnosis of candidemia. Because BDG positivity precedes the day of blood culture sampling, which is normally the day of the first symptoms, a screening approach would allow early diagnosis and initiation of therapy and therefore appears to be preferable over the symptom-driven approach. Strongly increased BDG levels during follow-up are indicating relapse or imminent death.

KMV04

The microbial diversity of the female urogenital tract in the context of STIs and female infertility

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Introduction: A community of commensal bacteria colonizes the female urogenital tract (fUGT) of humans. In most cases, members of the genus lactobacillus are the dominating species, providing an environment that is characterized by a pH below 4.5. This acidic microenvironment is thought to be one of the most important factors in the protection of sexually transmitted infections (STIs). Changes in the composition of the bacterial community are supposed to induce microenvironmental conditions, favoring infections of the fUGT and enhancing the risk for STIs. We aimed to investigate microbiome signatures that might be linked to STIs, infection induced infertility (INFINF) and promiscuity.
Materials and Methods: We sampled cervical swabs of 100 fertile females, 21 females with INFINF and 84 female sex workers (FSW). All swabs were tested for the presence of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Neisseria gonorrhoeae* and 454-pyrosequencing of the V1/V2 region of bacterial 16S rRNA genes was performed to assess the microbiome. We processed raw sequencing data using the software *mother* and statistical analyses were carried out using *R*.
Results: Members of the genus *Lactobacillus* dominated the microbial communities in fertile females as well as in INFINF, while the proportion of *Lactobacilli* was reduced in FSW. Generally, microbial diversity was low in fertile females, significantly enhanced in INFINF ($p<0.05$) and further elevated in FSW (FSW/fertile: $p<0.001$, FSW/INFINF: $p<0.05$). FSW as well differed from other women when addressing differences in the microbial community struture between the samples ($p<0.001$), whereas no significant changes were observed between INFINF and fertile women. We identified several indicator species for the FSW and INFINF. Furthermore, women suffering from *N.gonorrhoeae* and *U. urealyticum* infections

exhibited a microbiomal pattern that differed significantly ($p<0.05$) from women without underlying infections of the fUGT.
Discussion: Our results indicate that a disturbed microbiome could play a role in acquiring both STIs and INFINF. We could also highlight the impact of sexual behaviour on the urogenital microbiome, which points to its role as risk factor for STIs not only by enhanced sexual intercourse itself but also by impared defence through the commensal bacteria colonizing the fUGT. We will further analyse our data using the obtained sequences as proxy for metagenomic analysis, aiming for functional predictions on how the observed microbiomes might influence the microenvironment and use the results for upcoming functional studies on the impact of the microbiome on the pathogenicity of STIs.

KMV05
Characterization of bacterial persistence and phenotypic drift in an acute-chronic murine sepsis model

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Introduction: Intra-abdominal infections are one of the most common causes of death in patients of the intensive care unit. Peritonitis is further complicated by abscess formation, a major cause of morbidity and mortality. It is associated with inconsistency in antibiotic therapy allowing bacterial persistence. Here, we hypothesised that the variation in the antibiotic therapy could result in bacterial persistence and abscess formation in an acute-post-acute sepsis model, and that antibiotic concentration in blood and liver tissue collected using three antibiotic stratagems affects survival and persistence of *E. coli* and *Enterococcus spp.* as well as abscess formation.
Materials and Methods: For this purpose, we have established the Peritoneal Contamination and Infection (PCI) sepsis model to mimic the human situation of diffuse peritonitis with respect to microbe, therapy and outcome. Mice were intraperitoneally injected with a dilution of human faecal slurry. The animals were treated with different concentrations of Meropenem over a period of 10 days and observed over 28 days.
Results and discussion: Primarily, measuring antibiotic concentration, we highlighted variability in concentration in plasma and tissue within the same group. We isolated microorganisms in both the acute (day 3) and post-acute phases (days 10 and 28) of sepsis. At day three, similar to the clinical situation, we registered positive blood cultures only collected pre-antibiotic therapy whereas those collected post-antibiotic therapy came back negative. However, liver cultures were positive at both time points which is of rather great clinical significance. Whereas blood cultures presented with a single microorganism, *E. coli*, in liver tissue 36% also revealed the presence of *Enterococcus spp*. At day ten, although blood cultures were negative, liver cultures remained positive both pre- and post-antibiotic therapy. At day 28, we observed the formation of abscesses with the presence of *E. coli* and/or *Enterococcus*. Interestingly, we also identified the presence of small colony variant *E. coli* with resistance to aminoglycosides. Additionally, with different treatment protocols, we observed a difference in survival where the administration of the dose in a 12-hour interval significantly increase survival compared to animals treated once every 24 hours with a single dose. With the progression of sepsis along with abscess formation, we also registered a shift in bone marrow cellularity indicating a dysbalanced immune response.
Conclusion: We address bacterial survival in tissue during sepsis and stratify the effect of variable antibiotic therapy on bacterial phenotype and animal survival. We describe the limitation of blood cultures with respect to detection of present infection as well as the effectiveness of antibiotics with respect to reaching target organs. We also highlight the challenge in abscess formation; although animals were rescued with the administration of Meropenem, we observed the formation of abscesses with the presence of *E. coli* and *Enterococci* which mimics the clinical situation resulting in recurrent infections and long-term sequelae.

Figure 1

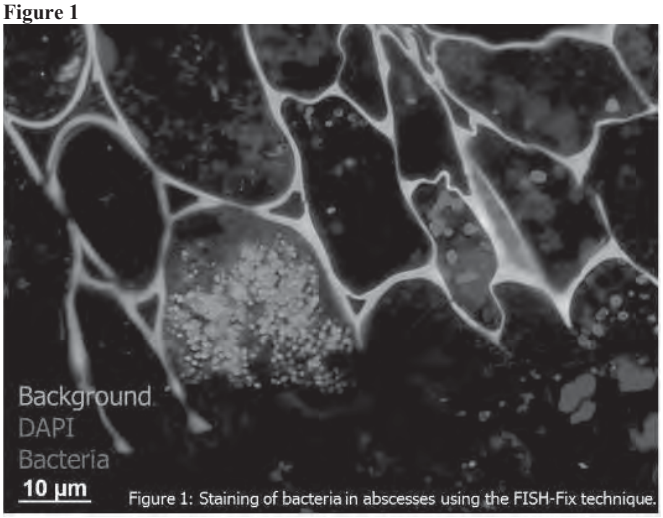

Figure 1: Staining of bacteria in abscesses using the FISH-Fix technique.

KMV06
Molecular genotyping of 1200 community acquired commensal and clinical Staphylococcus aureus isolates of the African-German StaphNet multicenter study

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Objectives: The goal of the African-German StaphNet multicenter study (www.African-German-Staph.net) is the comparison of genotypic and phenotypic patterns of Central European versus Sub-Saharan Staphylococcus aureus isolates of commensal versus invasive origin, in a large prospective community-acquired strain collection.
Materials and Methods: The identity of all 1200 isolates (600 commensal and 600 invasive strains, collected from 2010 till 2013) were confirmed by MALDI-TOF (Biotyper, Bruker). They were genoytyped by *spa*-typing and DNA microarray (Identibac® *S. aureus* Genotyping Microarray, Alere) providing clonal complex (CC) analysis, and dentification of 174 gene loci (encoding for regulatory, virulence factor, metabolic, antimicrobial resistance genes). CC subgroups based on microarray profiling were defined by advanced bioinformatics modelling (affinity propagation).
Results: Geographic specific CC patterns could be found for 38 different clonal complexes comprising five new sequence types. In Africa, the most abundant CCs were found to be CC15 (14%) and CC45 (12%) whereas among German isolates, CC 121 (18%) and CC30 (12%) were most prevalent. Some CC's, i.e. CC20, CC50, or CC182 were exclusively retrieved among German isolates while CC80 and CC88 were only recovered in Africa. Further gene differences associated with geographic provenience were found, e.g. for PVL genes, *tetK* (tetracycline resistance), the beta lactamase operon and *sasG* (adhesion protein), each with high prevalence among African isolates (>50%). Overall, independent of geographical regions the MRSA prevalence in the community was low (
Conclusion: Genotypic analysis of a large prospective, defined isolate collection reveals important differences in *S. aureus* population structure and the virulence/resistance gene repertoire as a function of the geographic region and the commensal versus invasive origin. These findings will provide for enhanced understanding of staphylococcal disease mechanisms in a cross-continental perspective.

FOOD MICROBIOLOGY AND FOOD HYGIENE

LMV01
Live and let die: How the interaction of *Listeria monocytogenes* and *Acanthamoeba* spp. affects growth and distribution of the foodborne pathogen

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Listeria monocytogenes is a facultative intracellular pathogen causing listeriosis of humans and many other vertebrate species. Beyond its intracellular lifestyle the bacteria are ubiquitously found in nature, where they feature a saprophytic life, sharing many environmental habitats with predatory protozoa. *Acanthamoeba* are well known to provide a hideout for *Chlamydia*, *Legionella* and other intracellular pathogens, and had also been proposed as natural reservoirs for *Listeria monocytogenes*.
Thus, the aim of this study was to better understand the microbial ecology of this foodborne pathogen by investigation of the interaction of *Listeria* and *Acanthamoeba*, using co-cultivation experiments and confocal laser scanning microscopy. In contrast to previous studies claiming survival and multiplication of *L. monocytogenes* in *Acanthamoeba,* we could provide clear evidence that the bacteria do not persist or multiply following phagocytosis by the amoebae. Instead, *Listeria* cells are attracted by *Acanthamoeba* trophozoites and accumulate on their surface, forming large aggregates of densely packed bacteria which we termed "backpacks". These backpacks seem to be held together by ultra-thin filaments, which are supposed to stem from the amoebae itself, as suggested by electron microscopy. Subsequently, the immobilized bacteria "hitchhike" on the *Acanthamoeba* trophozoites until they change direction of movement and start to devour their backpacks by phagocytosis. Further characterization of this unusual type of interaction revealed that the formation of backpacks is dependent on bacterial motility, while the mere presence of the flagella is not sufficient.
Another interesting observation was that non-motile *L. monocytogenes* represent a less preferred prey in co-cultures with *A. castellanii*. Moreover, we found that the presence of *Acanthamoeba* strongly promotes growth of *Listeria* in nutrient-poor media. Despite the bacteria representing the prey, co-culture supported increased multiplication rate and higher viable counts of the bacteria. Interestingly, direct contact of the microbes is not required, since the effect also occurred when both microorganisms were cultured separate in transwell plates. This observation suggests that a diffusible substance is released from *Acanthamoeba* which can be utilized by the bacteria. The identification of the growth promoting factors and their chemical composition is under investigation.
In conclusion, the results available from our studies demonstrate that *Acanthamoeba* spp. do not act directly as environmental reservoirs for *L. monocytogenes*. However, both *Listeria monocytogenes* and *Acanthamoeba* seem to profit from this peculiar type of interaction. In particular the lack of motility and the ability to utilize amoebal metabolites may aid the bacteria to avoid eradication by amoebal predation in low-nutrient environments.

LMV02
Comparison of the bacterial community structure of *Listeria monocytogenes* positive and negative food samples from the retail market

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The foodborne pathogen *Listeria monocytogenes* has been frequently found in all sectors along the food chain. Therefore food samples are routinely collected from retail markets in Germany and checked whether they contain this human pathogen. In our study we were interested in *L. monocytogenes* positive food and the effect of the pathogen on the structure and composition of the original bacterial community. For this reason we applied a combined approach of terminal restriction fragment length polymorphism (T-RFLP) analysis and cloning and sequencing of the 16S rRNA gene. Thirty six different *L. monocytogenes* positive and 33 similar but *L. monocytogenes* negative food samples from the retail market were examined. A significant shift in the structure of the bacterial community was only found in 3 *L. monocytogenes* positive food samples. This shift was independent from the respective food type. The bacterial community structure of the other positive food samples were almost similar to those of negative food samples indicating a minor influence of *L. monocytogenes* on the bacterial community composition. As a conclusion *L. monocytogenes* can be introduced as a human pathogen in the food chain with low interaction to the matrix and the orginial bacterial community of the food. Therefore, *L. monocytogenes* is inactively surviving in the food matrix and awaiting better growth conditions after food consumption.

LMV03
Detection and Identification of *Pseudomonas* Species on Oak Leaf Lettuce

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Based on culture-dependent studies, species of the genus *Pseudomonas* belong to the most dominant bacterial taxa in the phyllosphere of leafy vegetables such as lettuce. Some species are important phytopathogens, cause human infections or are associated with food spoilage. On the other hand, some species and strains have beneficial bioremediation and biocontrol activities. Therefore, it is important to investigate the diversity of the predominant *Pseudomonas* species on lettuce.
The aim of this study was the culture-independent identification of the main *Pseudomonas* species on oak leaf lettuce. Lettuce samples were treated with a Pulsifier™ device to remove bacteria from the leaf surfaces. The biomass was harvested and after genomic DNA-isolation a *Pseudomonas*-specific PCR was performed. The 990 bp PCR product was ligated into the pGEM®-T Easy vector (Promega) and transformed into *Escherichia coli* JM109 competent cells. Transformant colonies were selected by blue-white screening. Plasmid-DNA was isolated and the complete insert of each clone was amplified. The amplicons were analyzed by Amplified rDNA Restriction Analysis (ARDRA) with a restriction endonuclease (*AluI*) to look for different patterns indicating species variation. Based on these restriction patterns, the inserts were sequenced. The sequence data were analyzed by BLAST and compared with the sequences of selected *Pseudomonas* reference strains deposited in the GenBank library of the NCBI. A total of 108 clones were randomly selected for the studies based on the genus specific *Pseudomonas* primers. Among the tested clones, 12 different restriction patterns were identified, and three of them occurred commonly. All tested clones were identified at *Pseudomonas* species level by sequencing. Furthermore, all determined species could be associated with plants and vegetables. Interestingly, phylogenetic analysis showed that the identified species could be assigned to diverse branches of the *Pseudomonas* species taxonomic tree, and are not closely related to each other. Further analyses include the combination of culture-dependent and -independent identification of the predominant *Pseudomonas* species on the same sample of oak leaf lettuce.
Thus, the results can be compared and offer valuable information on the actual *Pseudomonas* microbiota of lettuce leaves.

LMV04
Highly sensitive loop-mediated isothermal amplification for rapid phage detection in dairy samples

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In dairies, bacteriophage populations capable of infecting lactic acid bacteria that are used as starter cultures are a persistent challenge for fermentation processes. Early phage identification is a critical requirement for preventing fermentation failures and can thus reduce financial losses and save time. For detection of dairy phages, we therefore adapted loop-mediated isothermal amplification (LAMP) [1], a method for highly sensitive detection of phage genomic DNA allowing rapid and easy performance at low costs. A polymerase with strand displacement activity is required in LAMP, which allows keeping the temperature constantly at 60-65°C during amplification. Besides the polymerase, a set of four primers is needed. A typical LAMP-assay is carried out within one hour and can be performed in laboratories with standard equipments (i.e., heating block or water bath, microcentrifuge tubes). A LAMP-assay was developed for *Lactococcus lactis* phage P680, a

highly thermo-resistant phage of the widespread 936 phage species [2]. Results are presented for detection of pure phage isolates by LAMP compared to a standard PCR-based assay. We further tested LAMP with DNA extracted directly from different whey preparations and compared its sensitivity with that of the standard PCR assay.

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LMV05
Rapid quantification of viable *Legionella*

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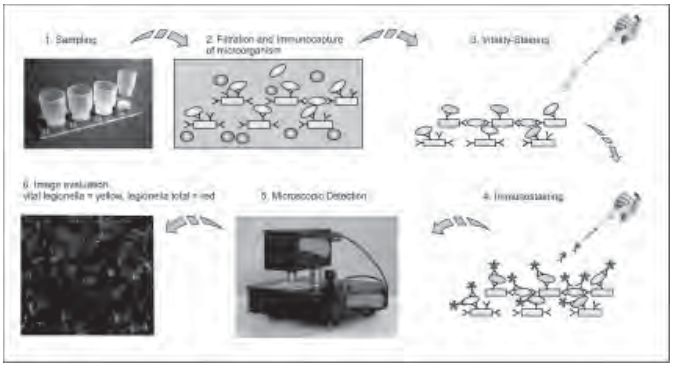
Introduction: We present a new method for rapid detection and viability assessment of *Legionella pneumophila* in water. Culture based methods require 7 to 10 days for this task and are therefore unsuitable for use in emergency situations. Methods like PCR and lateral flow assay allow a more rapid detection of Legionella cells, but do not differentiate between live and dead cells or lack sensitivity for the quantification of low bacterial loads.
Materials and Methods: A transparent macroporous membrane is modified by immobilization of Legionella specific antibodies. Bacteria are selectively captured from a water sample by filtration through the membrane. Captured bacteria are stained by incubating the membrane with an anti-*Legionella pneumophila*-Phycoerythrin conjugate solution and an esterase based viability dye. Microscopic counting of single and double stained cells is used for quantitative determination of total and viable Legionella load.
Results: High resolution pictures of individual fluorescent stained cells captured to the membrane were acquired. The limit of detection for esterase positive Legionella was 10 cells per membrane corresponding to 100 cells per 100 ml of tap water. The method is robust against particulate contaminants up to 10 µm in size. Total time for one analysis was about 1 hour.
Experiments with other microorganisms and sample matrices e.g. Salmonella in meat indicate that the test system can also be used for the quantification of specific bacterial loads in food hygiene and biotechnological applications.
Discussion: The method presented here permits rapid quantification of highly diluted target cells. Living bacteria can be distinguished from dead. The sensitivity for *Legionella pneumophila* is sufficient for detection of contaminations at the federally mandated exposure limit. The short analysis time makes this method useful for finding contamination sources during outbreaks of Legionellosis and for monitoring the success of decontamination efforts. Parallelization of sample and reagent handling and automation of image acquisition appear feasible and would expand the method into high throughput screening. The system requires only minimal, portable equipment and can be used outside laboratory environments.

Figure 1



LMV06
Fast and effective killing of *Bacillus atrophaeus* endospores using a new generation of light-activated vitamin B2 derivatives

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Spore forming bacteria like *Bacillus* or *Clostridium* provoke massive problems in the food and packaging industries as well as in medical and biotechnological processes due to the highly intrinsic resistance of spores against a variety of stress factors. Only strong chemical or physical agents show a satisfactory result in spore decontamination, but these measures exhibit harmful potentials to humans and are often also harmful for the environment. In addition, many chemicals are not allowed to get into contact with food. Alternatively, the photodynamic inactivation (PDI) of microorganisms and spores presents several positive aspects regarding the killing efficacy and the environmental hazard. PDI is based on positively charged and non-toxic dyes (photosensitizers) that attach to the negatively charged spore surface. Upon irradiation the photosensitizers generate reactive oxygen species, especially singlet oxygen, that kill spores via oxidative damage. In a cross-disciplinary approach of physicists, chemists and biologists, we developed new Flavin derivatives (FLASH-01a, FLASH-07a) that are based on naturally occurring Vitamin B2 (Riboflavin). We added one (FLASH-01a) or eight (FLASH-07a) positive charges to Riboflavin that allow the attachment of photosensitizers to the spore surface. The new Flavins convert efficiently visible light energy into singlet oxygen with a quantum yield of 0.75 ± 0.05 and 0.78 ± 0.05, respectively. The absorption spectrum of FLASH-07a matched closely to the emission spectrum of the non-coherent light source. Incubation of FLASH-01a or FLASH-07a with *Bacillus atrophaeus* endospores for 10 seconds and a following irradiation of 10 seconds with 70 J/cm² caused a biologically decrease of spore survival of ≥ 3 $\log_{10}$ orders (≥ 99.9%) *in vitro*. Immobilized spores on food related surfaces like polyethylene terephthalate (PET) were efficiently killed with 7.0 $\log_{10}$ orders (99.99999%) using the same parameters as for *in vitro* experiments. Thus, PDI with this new photosensitizers offer a great potential for a safe and sustainable use in food industry and environmental technologies as well as in medical applications.

LMV07
Inhibitory effect of nitrite on growth and survival of pathogens - molecular analysis of a preservation method

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The use of sodium nitrite in the curing process is a common food preservation technique for meat products, because of its suggested antimicrobial effect. Indeed, the addition of nitrite to raw sausages results in a better elimination of *Listeria monocytogenes* and *Salmonella* Typhimurium. *In vitro* growth analyses of *L. monocytogenes* and *S.* Typhimurium, taking into account various parameters relevant for sausage ripening like pH-value, NaCl-concentration, temperature and oxygen availability, revealed, that the inhibitory effect of nitrite massively increases in combination with lowering the pH. *L. monocytogenes* is more sensitive to the combined inhibitory effect of nitrite and acidification than *S.* Typhimurium. It is assumed, that during sausage ripening not nitrite itself, but rather reactive derivatives are responsible for the observed antimicrobial activity of the curing agent sodium nitrite. One reactive nitrogen species that has often been mentioned in this context is nitric oxide. Interestingly, both pathogenic bacteria, *L. monocytogenes* and *S.* Typhimurium, are exposed to nitric oxide during the infection process. However, whereas *S.* Typhimurium is well equipped with NO-detoxification systems (HmpA, NorV, NrfA), no such systems are described for *L. monocytogenes*. Global transcriptional analysis with microarray analyses or next generation sequencing revealed that acidified nitrite causes massive transcriptional changes in both organisms. Whereas a global general stress response is induced in *L. monocytogenes* (up-regulation of genes encoding for proteins involved in general stress response, down-regulation of genes encoding for proteins

involved in cell division, translation, metabolism of lipids and others) a more directed response can be observed in *S.* Typhimurium, with *hmpA* being one of the strongest induced genes. The pathogen specific nitrite stress response might influence the ability of these organisms to withstand nitrite stress in the food product.

LMV08
Characterization of *Staphylococcus carnosus* Strains for the Application in Raw Ham Production

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Particular *Staphylococcus carnosus* strains are used as starter cultures in fermented meat. The aim of this study was to investigate if *S. carnosus* strains can be identified that improve the organoleptic properties of fermented raw ham. Therefore, bacterial strains must fulfill certain characteristics, *e.g.* an active nitrate reductase, which is important for the development of a stable color, and proteolytic activity, which is important for aroma production. Moreover, strains should be regarded as safe according to the qualified presumption of safety (QPS) system.
More than 70 staphylococcal strains, both commercially available strains and isolates from different raw meat products, were tested. 42 out of those 74 strains were identified as *Staphylococcus carnosus* by 16S rRNA gene sequencing and species-specific PCR (Blaiotta *et al.*, 2005). Strains were differentiated by RAPD-PCR with seven different primers. These strains were tested according to the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for resistance against 17 antibiotics commonly used in animal health and in human medicine. Two strains were found to be resistant against ciprofloxacin and one strain showed resistance against sulfamethoxazole-trimethoprim. Another four strains were found to be intermediate resistant against cefotaxime. Furthermore, the potential of the strains to produce the staphylococcal toxins SEA-SEE and TSST was investigated by PCR analysis. None of the tested strains carried any genes for toxin production.
The strains were also tested *in vitro* for their ability to metabolize proteins, tributyrin and nitrate, as those are the characteristics which are important for aroma- and color development in ham. Three strains stand out with a nitrate reductase activity of more than 0.6 mM $NaNO_2/10^7$ KBE x mL^{-1}. While most of the strains showed a nitrate activity between 0.3 and 0.5 mM $NaNO_2/10^7$ KBE x mL^{-1}, four strains showed no nitrate reduction at all.
At present 13 out of 42 *Staphylococcus carnosus* strains show the potential to be used as a starter culture in raw ham. It would be interesting to investigate their characteristics further in a meat model.

Literature: Blaiotta G, Casaburi A, Villani F. (2005) Identification and differentiation of *Staphylococcus carnosus* and *Staphylococcus simulans* by species-specific PCR assays of *sodA* genes. Systematic and Applied Microbiology. 28: 519-526.

LMV09
Who lives out there? - Molecular monitoring of the smear microbiota composition and diversity of surface-ripened red-smear cheese by high-throughput sequencing

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The surface of red smear cheeses is characterised by the establishment of a multifaceted microbial ecosystem during the ripening period, which is crucial for the development of appropriate sensorial as well as hygienic properties of the final product. However, microbial contaminants or the type of packaging during storage may cause severe smear defects, which often manifest in off-odors and a moist and sticky surface smear [1].
We employed high-throughput sequencing to gain a better insight into the smear microbiota composition and the microbial diversity of different cheese surface smears. The results of the 16S rDNA based metagenome and 16S rRNA based metatranscriptome analysis were in line with culture-dependent analysis and molecular community fingerprinting methods, but offered a much broader interpretation. An unexpected high diversity of microorganisms was present in the community profiles realized by high-throughput sequencing. Surprisingly, not only typical smear microorganisms were detected, but also various bacteria not previously thought to be involved in cheese ripening. Our data also suggest that differences in the relative abundance of 16S rRNA between various smear samples are due to an altered metabolic activity of smear microorganisms.
In conclusion, total 16S rRNA sequencing proved to be a powerful tool for the study of the complex cheese surface microbiota, also providing insights into the metabolic activity of the ecosystem.

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LMV10
Growth behavior of different lactic acid bacteria in lupin flour and lupin protein isolate

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All over the world, fermented foods play a major role in the human diet. Besides food preservation, a number of advantages are linked with the fermentation process like improved flavor and digestibility or reduction of antinutritives[1,2]. In contrast to soy, the fermentation of lupin, a high protein and high nutritive legume[3], has not been studied extensively so far. Especially research information on the interaction of secondary plant metabolites with microorganisms is scare. To contribute to this, the objective of this study is to investigate the fermentation performance of different lactic acid bacteria on lupin containing substrates (*L. angustifolius* cv. *boregine*). The metabolism of *Lactobacillus (Lb.) plantarum*, *Bifidobacterium (B.) animalis* ssp. *lactis*, *Pediococcus (P.) pentosaceus* and *Lactococcus (L.) lactis ssp. lactis* was investigated at their respective optimum growth temperature on a 10% lupin flour (LF) and 10% lupin protein isolate (LPI) suspension. Carbohydrate content (10%) and composition (mainly sucrose, raffinose and stachyose) of the protein suspension was thereby adjusted to that of lupin flour. Growth behavior including the competitiveness of the strains tested by statistical MALDI-TOF MS analysis of microbiota, lactate/acetate production and sugar utilization (analyzed by HPAEC) was evaluated. Fermentation experiments revealed that all selected microorganisms were able to grow and metabolize sugars on LF in the same order of magnitude as on LPI. In comparison, the growth rate of *B. animalis* ssp. *lactis* was slightly higher in LPI suspension and greater amounts of organic acids (+51.1% lactate, +51.2% acetate) were produced. On the contrary, *Lb. plantarum* showed a significantly higher metabolism activity on LF and *L. lactis* ssp. *lactis* was only dominant in the flour sample. Moreover, fermentation performance of *P. pentosaceus* was not significant different ($p>0.05$) on the two substrates.
In conclusion, fermentation behavior of the studied microorganisms was apparently not influenced by the secondary plant metabolites occurring in lupin flour used in this study.

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LMV11
Non-thermal atmospheric plasmas for food decontamination

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Introduction: Gentle sanitation of fresh fruits and vegetables is highly demanded since especially produce that is eaten raw increases the risk of food borne illnesses. Currently used disinfection or sanitation methods for fresh fruits and vegetables lack antimicrobial effectiveness, but are high in costs, water consumption or chemicals. Non-thermal atmospheric pressure

plasma offers a promising opportunity for the preservation of fresh food. The antimicrobial effects of plasma are well-known and investigated. [1] However, the diversity of plasma types and sources as well as the complexity of plasma chemistry and a variety of food (size, surface, and composition), each need is specific and requires individual adaptation.
Materials and Methods: 3 different atmospheric pressure plasma set-ups, constructed by the INP Greifswald, were used with argon and ambient air as working gases for direct and indirect treatment of fresh fruits/ vegetables or seed.
Overnight cultures of human and phytopathogens (e.g. *Escherichia coli* and *Pseudomonas carotovorum*)were used in 10^6 cfu/ ml concentrations for inoculation of specimen. After plasma treatment the surviving bacteria were detected by spread plate count method as proliferation assay.
For detection of food quality chlorophyll fluorescence analysis, germination and sensory examinations were used depending on the specimen.
Results: Depending on the used plasma source, treatment time, microorganism and specimen, reduction rates greater than 6 log were achieved. [2-5] The product safety must be increased without affecting the product quality. Sensory examinations showed only little influences on texture, appearance and odor.
Discussion/ Conclusion: The advantages of plasma and the generated microbicidal compounds (reactive oxygen and nitrogen species) which led to high microbial inactivation on specimens offer a wide rage of possible uses along the whole value chain. Besides the scientific work, networking is essential for this kind of interdisciplinary research in order to include the industrial requirements successfully.

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LMV12
Impact of different measures on virus inactivation in meat products

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Aim: Questions about food safety regarding viral pathogens have become more important from a scientific as wells as from a public point of view. Amongst others they are aimed at the stability of viruses under the impact of different food technologies. In this context data about the influence of different measures concerning processing of meat products on virus infectivity was evaluated during three research projects*.
Materials and Methods: Examinations were carried out by in-vitro studies as well as by experimentally infected meat products. In the studies mainly human norovirus surrogates (murine norovirus, feline calicivirus) and influenza viruses (H5N6, H3N8, H1N1) were included. Course of virus titers, derived by infection of cell cultures and embryonated chicken eggs, was estimated under the influence of salt, nitrite cured salt, D,L-lactic acid, bacterial starter and protective cultures as well as of heating and smoking procedures.
Results and conclusions: In summary, extend of virus inactivation was very different dependend on virus species and technology tested. In general, influenza viruses showed a comparatively low stability. Titer of human norovirus surrogates decreased rapidely during heat treatment at 60 °C, but the non-thermal treatments salting, curing and fermentation/ripening did not or did only slightly effect their infectivity. Apart from D,L-lactic acid bacterial starter cultures did not express any additional antiviral effect on the tested pathogens. In contrast, cold smoke and liquid smoke treatment lead to a marked and fast titer reduction of viruses suspended on stainless steel surfaces.
In the presentation a summary of selected results will be presented and discusssed.

*These research projects were supported by the German Ministry of Economics and Technology (via AiF), the FEI (Research Association of the German Food Industry, Bonn) and the BVDF (Federal Association of the German Meat Industry). Project numbers AiF 15189 BR, AiF 16509 BR, AiF 16479 BR.

METABOLISM AND METABOLIC NETWORKS/ METABOLOMICS

MMV01
Characterization of the *Chlamydia pneumoniae* transcriptome under hypoxic conditions

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Chlamydia pneumoniae is a gram-negative obligate intracellular bacterium infecting the human respiratory tract. Low oxygen concentrations of 5-10% occur in the peribronchial tissue and further decline during inflammatory processes or decreased ventilation. Increased *C. pneumoniae* growth was demonstrated in a low oxygen environment. While on the host cell side these effects were mainly modulated by enhanced stabilization of the hypoxia inducible factor-1α (HIF-1α), little is known about the transcriptional profile of *C. pneumoniae* under hypoxic conditions. We therefore analyzed the effect of hypoxia on chlamydia by performing transcriptome analysis of *C. pneumoniae*. HEp-2 cells were infected with *C. pneumoniae* CWL029 under normoxic (20% O_2) or hypoxic (2% O_2) conditions. Total RNA was isolated 24 hpi and was depleted of human rRNA by the RiboZero rRNA removal Kit. The chlamydial transcriptome was sequenced using Illumina HigSeq technology. High quality reads were mapped to the reference genome and the obtained coverage (counts per gene) was normalized using the RPKM conversion. Differentially expressed genes were identified using the statistical analysis NoiSeq.
The sequencing obtained 4.2 - 4.4 x 10^8 reads per condition whereof 0.8 - 2.8% were mapped to the *C. pneumoniae* reference genome. Analyzing differentially expressed genes between normoxic and hypoxic conditions, a total of 153 genes showed higher and 18 genes showed lower expression under hypoxia. The expression of genes connected to transcription and translation including transcriptional regulators, genes of the nucleotide metabolism as well as ribosomal proteins were increased under hypoxia. The pentose phosphate pathway (PPP) providing NADPH which is important for chlamydial metabolism was upregulated under hypoxic conditions as well. In addition, NADPH is essential for fatty acid metabolism and this metabolism related genes showed higher expression under hypoxic conditions. In contrast, shikimate pathway which is interconnected with PPP and synthesizes aromatic amino acids was downregulated under hypoxia. Our results demonstrate that hypoxia influences chlamydial gene expression that is associated with enhanced growth under hypoxic conditions. Ongoing studies will focus on transcriptional regulators such as sigma-factors to understand these changes in a low oxygen environment.

MMV02
Characterization of mercaptosuccinate dioxygenase from *Variovorax paradoxus* strain B4, a novel cysteine dioxygenase homologue

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Mercaptosuccinate is a versatile thiol compound with a wide range of applications, e.g. in quantum dot research. Its metabolism is intensively investigated in the Gram-negative β-proteobacterium *Variovorax paradoxus* strain B4, which can utilize this compound as sole source of energy, carbon and sulfur. Proteomic studies of strain B4 revealed a putative mercaptosuccinate dioxygenase, which exhibited highly significant up-regulation in cells cultivated with mercaptosuccinate compared to cells grown with succinate or gluconate as carbon source [1]. This putative mercaptosuccinate dioxygenase is a cysteine dioxygenase homologue and represents the key enzyme in the degradation of mercaptosuccinate. Therefore, the enzyme was heterologously expressed, purified and characterized. The results clearly demonstrated that the enzyme utilizes

mercaptosuccinate with concomitant consumption of oxygen. Thus, it is designated as mercaptosuccinate dioxygenase. Succinate and sulfite were verified as the final reaction products. The obtained kinetic data revealed a comparably high turnover rate of the enzyme of 12.5 s^{-1}, which was much higher than the rates of cysteine dioxygenases characterized so far [2]. Furthermore, the enzyme was highly specific for its substrate, while no activity was observed with the thiols cysteine, dithiothreitol, 2-mercaptoethanol, and 3-mercaptopropionate. These structurally related thiols did not have an inhibitory effect, either. Fe(II) could clearly be identified as the required metal cofactor of the mercaptosuccinate dioxygenase. The recently assumed hypothesis for the degradation of mercaptosuccinate based on proteomic analyses could be strengthened: (i) mercaptosuccinate is first converted to sulfinosuccinate by the key enzyme mercaptosuccinate dioxygenase; (ii) subsequently, sulfinosuccinate is spontaneously hydrolyzed yielding succinate and sulfite; and (iii) while succinate can then be utilized as carbon source, sulfite has to be detoxified which is possibly achieved by the previously identified putative molybdopterin oxidoreductase.

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MMV03
The lipidome of *Myxococcus xanthus*

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Myxococcus xanthus is a Gram-negative bacterium mostly abundant in soil. Myxobacteria are well known for their complex life cycle. Amino acid deprivation induces the formation of spore-filled fruiting bodies. Besides the morphological development from vegetative rods to round myxospores significant changes in the lipidome of developing cells have been observed. Ether lipids, usually known from extremophile bacteria accumulate during development and act as signaling molecules during fruiting body and spore formation. HPLC-MS analysis made it possible to identify a wide range of lipid species from lysophospholipids to glycerophospholipids and finally to glycerolipids in one step. By doing so, the complete lipidome of myxobacteria can be observed at various stages of fruiting body development and can support the identification of biomarkers for subsequent experiments.

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MMV04
The first 2D GC/MS non-targeted metabolomic analysis of bacteria and archaea by use of the new version of the automated software MetaboliteDetector

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As metabolic networks in microorganisms contain about 600 different substances, efficient compound separation and identification with conventional chromatography is limited by the physiochemical parameters obtained by use of only one single separation column. By using a second column in 2D GC/MS the separation of chemically similar compounds can be strongly improved. High scan rates and the two dimensional time axis increase size and complexity of data files which can only be handled by few analysis softwares. Up to now, in contrast to 1D GC/MS analysis software, none of them is automatically combining spectra similarity with retention time index matching for more reliable compound identification.
Here we present the first 2D GC/MS measurement of bacterial and archaeal metabolome. We detected several additional metabolites via 2D GC/MS which were overlain by higher concentrated compounds in 1D GC/MS. Although we achieved enhanced separation-power with 2D GC/MS, particular metabolites, e.g. phosphorylated compounds, were shown to be lost due to sample modulation which is necessary for second column operation. To cope with challenging 2D GC/MS data processing, we developed a new version of the MetaboliteDetector software to automatically analyze complete sample batches. Furthermore our software carries out an automated compound identification not only based on spectra similarity but also considering retention time index accuracy.
Our results show the potential and limitations of 2D GC/MS for the analysis of bacterial and archaeal metabolome compared to conventional 1D GC/MS. Together with our software customized for complex 2D GC/MS data, we present a chromatographic application and facilitated data analysis for future metabolomic studies of bacteria and archaea.

MMV05
New insights into methylotrophy of the thermophilic methylotroph *Bacillus methanolicus*

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Bacillus methanolicus represents a unique group of methylotrophic *Bacilli* which possesses a soluble NAD^+-dependent methanol dehydrogenase for methanol oxidation and the ribulose monophosphate pathway (RuMP) for formaldehyde assimilation. *B. methanolicus* is also known for its natural ability to overproduce glutamate from methanol setting a link between methylotrophy and its possible biotechnological application. In this study we aimed to understand the basic metabolic pathways in *B. methanolicus* during growth on methanol as single carbon and energy source in comparison to the multi-carbon based substrate mannitol based on proteome, metabolome and single enzyme activity measurements.
For the proteome approach we used label-free quantitative proteomics generating a reference proteome data for this bacterium and compared the proteome of *B. methanolicus* on two different carbon sources (methanol and mannitol) [1]. For the same conditions a metabolome data set with focus on central metabolites was acquired and analyzed by liquid-chromatography high accuracy mass spectrometry (LC-HR MS). Additionally the activity of essential dehydrogenases participating in key reactions related to substrate oxidation or assimilation in crude lysate fractions from *B. methanolicus* cells grown on both substrates were elucidated and connected to the context of the omics data. In the global proteomic approach, approximately 1200 different proteins were detected whereas 1000 were used for quantification. Out of these, 109 proteins showed differential expression on the different carbon sources. According to this data the utilization of methanol as sole carbon and energy source influences strongly the level of enzymes of the central metabolism especially the assimilatory and dissimilatory RuMP as well as the proteins of the oxidative TCA branch. The performed metabolome analysis reinforced these findings focusing on the possible importance of the oxidative RuMP cycle as key metabolic pathway for formaldehyde oxidation and NADPH generation. The enzyme activities in crude lysate fractions on both carbon sources revealed in detail different cofactor specificities of important oxidation reactions which might play essential role for the differential expression of the corresponding enzymes on the particular substrates. In summary this study revealed detailed view of the carbon substrate specific regulation of central metabolic pathways on proteome, metabolome and single enzyme level in the methylotrophic *B. methanolicus* as a promising host for biotechnological applications.

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MMV06
The essential putative cysteine desulfurase MPN487 from *M. pneumoniae* is a H_2S producing enzyme and involved in virulence

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Mycoplasma pneumoniae is a human pathogenic bacterium that colonizes the lower respiratory tract and constitutes a causative agent of atypical pneumonia. The virulence of *M. pneumoniae* strongly relies on the production of hydrogen peroxide leading to the destruction of the host cell tissue. Here, we report that *M. pneumoniae* is in addition able to form hydrogen sulfide (H_2S) - a toxic gas that is responsible for hemoxidation and lysis of red blood cells. H_2S has been recognized as a virulence factor in a large number of oral pathogens. In those bacteria, H_2S is produced from L-

cysteine by the enzyme L-cysteine desulfhydrase. However, a homolog is not present in *M. pneumoniae*. Instead, we show that the putative L-cysteine desulfurase MPN487, which is supposed to be involved in Fe-S-cluster assembly, is the responsible enzyme for H_2S formation. Using heterologous expression and purification of MPN487 in *E. coli*, we were able to perform *in vitro* measurements of H_2S production. Additionally, we show that *M. pneumoniae* forms hydrogen sulfide from cysteine *in vivo* and that the release of H_2S leads to hemoxidation thus presenting a potentially novel aspect of pathogenicity of this minimal organism.

MMV07
Sulfoglycolysis in *Escherichia coli* K-12 closes a gap in the biogeochemical sulfur cycle

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Sulfoquinovose (SQ, 6-deoxy-6-sulfoglucose) has been known for 50 years as the polar headgroup of the plant sulfolipid in the photosynthetic membranes of all higher plants, mosses, ferns, algae, and most photosynthetic bacteria; it is also found in some non-photosynthetic bacteria, and SQ is part of the surface layer of some *Archaea*. The estimated annual production is 10,000,000,000 tonnes of SQ, thus comprising a major portion of the organo-sulfur in nature, where SQ is degraded by bacteria. However, despite evidence for at least three different degradative pathways in bacteria, no enzymic reaction or gene in any pathway has been defined, though a sulfoglycolytic pathway has been proposed. We showed that *Escherichia coli* K-12, the most widely-studied prokaryotic model organism, performs sulfoglycolysis, in addition to standard glycolysis. SQ is catabolized through four newly discovered reactions that we established using purified, heterologously expressed enzymes: 6-deoxy-6-sulfoglucose (SQ) isomerase, 6-deoxy-6-sulfofructose (SF) kinase, 6-deoxy-6-sulfofructose-1-phosphate (SFP) aldolase, and 3-sulfolactaldehyde (SLA) reductase. The enzymes are encoded in a ten-gene cluster, which probably encodes also regulation, transport and degradation of the whole sulfolipid. The gene cluster is present in almost all (>91%) available *E. coli* genomes, and is widespread in Enterobacteriaceae. The pathway yields dihydroxyacetone phosphate (DHAP), which powers energy conservation and growth of *E. coli*, and the sulfonate product 2,3-dihydroxypropane-1-sulphonate (DHPS), which is excreted. DHPS is mineralized by other bacteria, thus closing the sulphur cycle within a bacterial community.

MMV08
Disinfectants as environmental pollutants: Novel mechanisms of microbial biotransformation and detoxification

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Introduction: Hospital disinfection is very important to prevent nosocomial infections and thus the use of disinfectants is indispensable. Disinfectants are also widely used in the food industry, livestock farming and in private households. This broad range of applications results in increasing input of disinfectants into the environment and an associated accumulation in water and sediments. Studies in 8 hospitals have shown that the average total use of disinfectants - excluding alcohol-based ones - is around 4.4 g per bed per day, corresponding to a wastewater concentration of active ingredients of about 9 mg per liter (Gartiser et al., 2000). In dry marine sediments concentrations up to 20 µg per kilogram chlorocresols have been detected (Kahle and Nöh, 2009). However, little is known about the fate of disinfectants in the environment. We therefore investigated the microbial biotransformation of *p*-tert-amylphenol, *p*-chloro-m-cresol, and 4-chloro-3,5-dimethylphenol by bacterial and yeast strains mainly isolated from polluted environments.
Materials and Methods: The analysis employed high pressure liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance spectroscopy (NMR) analysis for structure elucidation of the products formed.
Results: Microbial attack mainly occurred on the aromatic ring system resulting in hydroxylated products which were then substrates for ring fission. Besides dioic acids, products with newly formed pyran and furan ring structures were detected. In particular, though *p*-tert-amylphenol was toxic to the strains used, one of the ring cleavage products was not (Schlueter et al., 2014). This demonstrates that detoxification of an aromatic disinfectant can be achieved by ring cleavage. The products formed are dead-end-products for the strains used, but (*i*) they are non-toxic and (*ii*) there are many other organisms in natural environments which may be able to use them as growth or transformation substrates.
Discussion: Thus, our investigations throw light on the fate of active ingredients of disinfectants in the environment.

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MICROBIAL PATHOGENESIS

MPV01
Autopenetrating Bacterial Effector Proteins as Biological Therapeutics

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In the last 20 years biologics including anti-TNFα antibodies have been discovered and are constantly further developed as very efficient treatment of auto-immune diseases such as rheumatoid arthritis, inflammatory bowel disease or psoriasis. However, a bottleneck of any therapeutic drug including biologics is their bio-availability, which also involves their capacity to reach also intracellular targets. Cell-penetrating peptides (CPPs) can overcome this problem by passing cellular barriers and even delivering cargos into cells. Therefore, they are investigated as potential drug-delivering agents. *Rüter et al.* could demonstrate that recombinant Yersinia Outer Protein M (YopM) from *Yersinia enterocolitica* has the capacity to overcome cellular barriers autonomously and thus represents a bacterial CPP. Previously, virulence factors and effector proteins have been mostly targeted to counteract infection, however, as these factors have been largely optimized during coevolution of bacteria with their respective hosts, they might be applicable also as tools to modulate and/or reduce detrimental immune responses. Hence, these factors might potentially be employed for therapeutic purposes. In this project the therapeutic capacity of bacterial effector proteins that are known to down-regulate pro-inflammatory cytokines is investigated. Here, we demonstrate the functionality of a recombinant protease, which has cell-penetrating abilities as demonstrated by immunofluorescence and cell fractionation. Furthermore, we show that this CPP is functional as it specifically cleaves a subunit of NFκB in cell lysates as well as in whole cells. This results e.g. in down-regulation of Interleukin expression in LPS-stimulated HL60 macrophages. With these features this protease will be an interesting candidate for further development as a therapeutic for (auto-) inflammatory diseases.

MPV02
Deletion of the *galU* gene abolishes the impaired immune response to uropathogenic *E. coli*.

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Introduction: Uropathogenic *Escherichia coli* (UPEC) are a major cause of morbidity causing about 80% of community acquired urinary tract infections. These exceedingly successful pathogens are equipped with many different virulence factors, such as siderophores, toxins and adhesins. In contrast to other *E. coli*, UPECs have been shown to modulate and impair the host's innate immune response. Recently, a novel virulence factor has been described, named TcpC (Tir-domain containing protein), which suppresses NF-κB signaling and thereby subverts the innate immune system. Interestingly, UPEC isolates lacking TcpC are still capable of suppression the NF-κB mediated proinflammatory cytokine response indicating that beside TcpC other, yet unknown factors contribute to the immune suppressive phenotype of UPECs. Here, we further characterize distinct UPEC mutants to identify UPEC factors that interfere with the host innate immune system.
Materials and Methods: Distinct transposon mutants of UPECs lack the immune suppressive phenotype of the parental wild type strain. Site directed knockout mutants of respective genes were created in the UPEC strain

UTI89 and analyzed in a macrophage infection model (co-incubation with J774.A2 macrophages). TNF-α secreted from macrophages was measured by ELISA and the intracellular survival of UPECs was investigated by means of gentamicin protection assays. Furthermore, cell toxicity assays and analyses of bacterial LPS composition have been performed.
Results and discussion: The *galU* deletion in the UPEC strain UTI89 caused a significant higher cytokine release in J774.A2 macrophages. Transient recomplementation of the mutant restored the original UPEC phenotype. Additionally, we showed that intracellular survival in macrophages is impaired by the mutation. However bacterial or macrophage survival was not significantly affected in these experiments. As the *galU* mutant lacks the O-polysaccharide side chain of the lipopolysaccharide (LPS), we compared it to a *waaL* deletion mutant, which also lacks the O-polysaccharide side chain. With this mutant we obtained similar Results: Therefore we assume that the LPS composition of UPECs contributes mainly to the impaired immune response to UPECs. Our results further emphasize the importance of LPS in the interplay between UPEC and host.

MPV03
SPI-4 mediated adhesion to polarized epithelial cells: is signaling by chemotaxis sensors involved?

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Introduction: *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is a facultative intracellular pathogen with a large variety of virulence factors clustering together on *Salmonella* pathogenicity islands (SPI). *Salmonella* pathogenicity island 4 (SPI-4) encodes a type I secretion system (T1SS), a tripartite complex (SiiCDF), that secretes the giant non-fimbrial adhesin SiiE. This 600 kDa adhesin enables intimate contact of *Salmonella* to the apical membrane of polarized epithelial cells which is a prerequisite for the bacterial invasion process mediated by the SPI-1-encoded type III secretion system (1). Surface localization of SiiE and hence SPI4-dependent adhesion and invasion, but not SiiE secretion, critically depends on the two accessory SPI-4 proteins SiiA and SiiB. Both are integral inner membrane proteins which share significant homology in their transmembrane regions with proton channels of the Exb/Mot family (2).
Materials and Methods: Protein interaction partners of SiiA, SiiB and SiiF were identified using *in vivo* crosslinking with subsequent affinity purification of cross-linked complexes and mass spectrometry (AP-MS). Potential protein-protein interactions were evaluated using a bacterial two hybrid (B2H) system. Knockout strains of interaction partners were tested for SPI-4 dependent adhesion to and invasion of polarized epithelial cells.
Results: Besides known interactions between SPI-4 components (2) we identified several methyl-accepting chemotaxis proteins (MCPs) as potential interaction partners using AP-MS. With B2H we were able to confirm various interactions between SiiAB and different MCPs. Furthermore, the MCP Aer and the ABC protein SiiF formed a complex in B2H. Strains defective for a single MCP were not attenuated for SPI-4 dependent adhesion to polarized epithelial cells. The same was observed for a *cheY* mutant strain which uncouples flagellar motility from MCP signaling.
Discussion: Spatio-temporal localization of SiiE is precisely controlled by the accessory SiiAB proton channel to allow efficient adhesion to polarized epithelial cells when the SPI-1 encoded type III secretion system is active. We think that so far unknown environmental signals provide the switch between SiiE secretion and retention. Here we show first evidence that signals perceived by several methyl-accepting chemotaxis proteins could be involved in that process. Direct protein-protein interactions and dispensability of chemotaxis (CheY) suggested a non-classical signal transduction pathway between SiiAB and MCPs.

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MPV04
The regulation of c-Abl activity in *Helicobacter pylori* infected gastric epithelial cells

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Introduction: Infections with the human pathogen *Helicobacter pylori* (*H. pylori*) can lead to severe gastric diseases ranging from chronic gastritis and ulceration to neoplastic changes in the stomach. *H. pylori* has developed sophisticated mechanisms in order to highjack signaling events in host cells which lead to phenotypic changes and altered cell motility and survival. A key factor driving these events is the bacterial effector protein CagA, which is translocated into the host cell cytoplasm, where it is subject to rapid tyrosine phosphorylation by host kinases of the Src- and c-Abl family. In addition to the activation-associated tyrosine phosphorylations of c-Abl (pY245 and pY412) we have identified threonine 735 as a new phosphorylation site of c-Abl in *H. pylori* infections. This site is supposed to contribute to the nuclear-cytoplasmic distribution of c-Abl. In order to evaluate the effects of T735 phosphorylation to *H. pylori* mediated effects, we analyzed c-Abl activation and signaling as well as cell motility and cell survival in cells expressing c-Abl^{wt} versus c-Abl^{T735A}.
Materials and Methods: We have generated a panel of c-Abl mutations (constitutive active, kinase dead, and T735A, Y245F or Y412F mutants) and stable cell lines expressing physiologic levels of c-Abl^{wt} and c-Abl^{T735A}. In addition to analyses of the phosphorylation and activation status of c-Abl (western blot, *in vitro* kinase assays) and the subcellular localization (subcellular fractionation, immunofluorescence) in overexpression settings, we investigated cell survival, cell motility (wound healing assay, transmigration assays) in the stable cell lines.
Results: The phosphorylation of threonine 735 in c-Abl followed a similar kinetic as the activation associated tyrosine phosphorylations and is independent of CagA. Only a minor reduction of pT735 is observed with *H. pylori* cagPAI and CagL deletion mutants, whereas the tyrosine phosphorylation on Y245 and Y412 was strictly dependent on CagL expression. Although the individual phosphorylations were no precondition to each other, tyrosine phosphorylation appears enhanced in the c-Abl^{T735A} mutant. Apart from the activation status, phosphorylation-resistant c-Abl^{T735A} displayed elevated nuclear levels and as a consequence increased cell death in *H. pylori* infections. Additionally, absent threonine phosphorylation contributed to the increased cell motility and migration.
Conclusions: c-Abl has been shown to be an important kinase in *H. pylori* mediated pathogenesis, however mainly its cytoplasmic kinase activity received attention. c-Abl has been shown, to shuttle between cytoplasm and the nucleus, and is able to induce apoptosis in the latter compartment. Here we propose that *H. pylori* regulates c-Abl phosphorylation to prevent nuclear localization of c-Abl and thus controls cellular responses.

MPV05
Evaluation of *Acinetobacter baumannii* trimeric autotransporter adhesin as an adhesion and virulence factor

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Introduction: *Acinetobacter baumannii* is a Gram-negative gamma-proteobacterium and is recognized as an emerging pathogen causing major nosocomial infections with multidrug and pan-drug resistance isolates being reported. However, little is known about its virulence mechanisms. Trimeric autotransporter adhesins (TAA) have been hypothesized to be important virulence factors. TAA are modularly constructed consisting of an N-terminal head domain, neck/stalk domains and a C-terminal membrane anchor. Our aim was to investigate the TAA of *Acinetobacter baumannii*, Ata, and its impact on adhesion, which is a crucial step during bacterial infections.
Materials and Methods: *Acinetobacter baumannii* ATCC 17978, two isogenic *ata* deficient mutants, ATCC 19606 and five multidrug resistant clinical isolates were used. *ata* expression was determined by qRT-PCR and *ata* gene mutations were analysed by PCR and Sanger sequencing. The daTAA bioinformatic toolkit (MPI Tübingen) was used to analyze TAA domain structures. Adhesion experiments were performed using coverslips which were coated with collagen-1, fibronectin, laminin or VCAM-1 using fluorescence microscopy.

Results: Expression of *ata* was detected in ATCC 17978, ATCC 19606 and clinical isolates but not in the *ata* deficient mutants. Up to 7-fold Ata expression rates were noted in clinical isolates and in ATCC 19606 compared to ATCC 17978. *ata* mutations were present in ATCC 19606 and clinical isolates compared to ATCC 17978 with yet unknown biological consequences (range: from 4-14% nucleotide and 4-19% amino acid sequences). Mutations in Ata domain structure were noted with differences occurring in the head and neck domains. Deletion of *ata* led to a severe adhesion defect of *A. baumannii* under static conditions: all clinical isolates, ATCC 19606 and ATCC 17978 had adhesion numbers up to 24-fold on fibronectin, 12-fold on collagen-1 and laminin, and 9-fold on VCAM-1 as compared to the Ata deficient mutants.
Conclusion: The TAA of *A. baumannii*, Ata, mediates adhesion to extracellular matrix proteins. *ata* gene sequence varied between reference strains and clinical isolates, and expression was upregulated in the clinical isolates. Thus, Ata could play an important role during infection of *A. baumannii*. To verify this, we are currently investigating the impact of Ata on adhesion and invasion of *A. baumannii* to and into eukaryotic cells, as well as virulence in the *Galleria mellonella* infection model.

MPV06
Physical determinants on surface protein deformation and staphylococcal attachment - a cautionary tale

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Introduction: The interaction of microbial pathogens with artificial or natural substrates is the crucial step in the pathogenesis of infectious diseases. Interfacial forces and adhesive protein interactions comprise the functional principles of the bacterial adherence mechanism; yet, this interplay is not fully understood. Previous studies almost completely neglect the influence on adhesion of turgor pressure, cell shape deformation and surface molecule alteration, and only recently a model on contact formation and peptidoglycan deformation in spherical bacteria was published. Here we extend these findings with a quantitative analysis of the interplay of peptidoglycan elasticity, turgor pressure and surface adhesins on bacterial adhesion.
Materials and Methods: We assume that attachment might induce a turgor and cross-linkage dependent peptidoglycan deformation which changes the possible contact area significantly thus enlarging protein-surface availability and adhesive strength. To characterize the interplay and identify major adhesion components we conducted a series of atomic force microscopy (AFM) experiments using single living cells of *Staphylococcus carnosus*, *Staphylococcus aureus* and *S. aureus pbp4* crosslinking mutants. Adhesion was determined on hydrophilic/hydrophobic substrates using AFM. Elasticity of purified and native peptidoglycan layers as well as turgor pressure under various salt concentrations was measured by quantitative nanomechanical mapping (PeakForce QNM).
Results: Comparing hydrophilic and hydrophobic Si wafers, we found strong adhesion of *S. carnosus* to the hydrophobic wafers (up to about 3000 pN) and low adhesion (about 30-50 pN) to the hydrophilic ones. Measurements of purified peptidoglycan and turgor pressure revealed the elastic modulus to be in the range of 5-6 MPa for native and about 10 MPa for purified peptidoglycan sacculi. The turgor pressure was found to be 3-4 MPa under physiological conditions changing significantly when salt concentration changes.
Conclusions: Our results strongly corroborate a model that the unspecific adhesion of *S. carnosus* TM300 is mainly governed by number, properties and arrangement of the bacterial cell wall proteins. The fact that the form of the force/distance curves is (i) characteristic of each of the bacterial probes, (ii) independent of the 'adhesive history' and (iii) independent of the tip velocities probed leads us to the conclusion that cell wall proteins may act as elastic springs. Notably, the latter exhibit a certain degree of freedom (~ 50 nm) which enlarges contact area and thus available adhesion sites for adhesins on the substrate. Additionally, turgor and peptidoglycan cross-linking dependent deformation further influences the real contact area dependent on experimental conditions. Thus, deformation has to be taken into account before comparing adhesion values for different specimen.

MPV07
c-di-AMP signalling in Staphylococcus aureus

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Abstract has not been submitted.

MPV08
Structural analysis of c-di-AMP synthesis by the DAC domain and downstream c-di-AMP recognition

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The bacterial DNA-integrity scanning protein A (DisA) has been shown to form a mobile focus that scans the DNA prior to sporulation and is responsible for delay in sporulation upon possible DNA-damages (1). We analyzed *Thermotoga maritma* DisA in a hybrid methods approach using biochemical and structural methods and could show that DisA synthesizes c-di-AMP. This DAC (diadenylate cyclase) -activity is modulated by the presence of recombination intermediate DNA structures such as Holliday junctions (2). A decrease in c-di-AMP level then probably serves as a signal to delay sporulation to ensure viable spores. The DAC-domain catalyzing the synthesis of c-di-AMP from two molecules of ATP in presence of Mg^{2+} is highly conserved and present in various species. As the presence of a DAC-domain has been shown to be essential in *B.subtilis* (3) and might be a potential antimicrobial drug target we are interested in the DAC-reaction mechanism. Using a combination of X-ray crystallography and biochemical assays we were able to identify important catalytic residues and also pinpoint the metal-coordination site which is essential for the reaction. As we are just at the beginning of c-di-AMP pathway analysis, little is known about the structural basis of c-di-AMP binding to proteins except for a small number of proteins that have been shown to bind the dinucleotide in biochemical assays (4, 5). To also get structural information about the recognition of c-di-AMP by its receptor proteins and the possible discrimination between c-di-AMP and c-di-GMP we are also biophysically and structurally analyzing proteins that have been shown to bind c-di-AMP *in vitro*.

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MPV09
A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization.

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Nasal colonization is a major risk factor for *S. aureus* infections (1, 2). The mechanisms responsible for colonization are still not well understood and involve several factors on the host and the bacterial side (3). One of the key factors is a glycopolymer termed cell wall teichoic acid (WTA) of *S. aureus*, which governs direct interactions with nasal epithelial surfaces (4). We recently identified a WTA receptor on nasal epithelial cells that plays a role

in *S. aureus* adhesion to nasal surfaces (5). The WTA receptor is termed SREC-I and is a type F-scavenger receptor that binds WTA in a charge dependent manner. In flow chamber adhesion assays the WTA/SREC-I interaction facilitates *S. aureus* adhesion to epithelial cells by modulating the initial contact of bacterial- and host cells. The initial interaction then creates the kinetic window for subsequent, protein/protein interaction mediated, tight adhesion. Furthermore, we were able to demonstrate an important role of this WTA-receptor interaction in a "state of the art" cotton rat in vivo model of nasal colonization. Most importantly, inhibition of WTA mediated adhesion abrogated nasal colonization in the animal model, and therefore we propose targeting of this glycopolymer/host-receptor interaction as a novel strategy to prevent or control *S. aureus* nasal colonization. This novel concept could have a considerable impact as it directs the attention to bacterial glycopolymer/host-receptor interactions, a so far neglected field with a huge potential for therapeutic interventions.

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MPV10
Clostridium perfringens Enterotoxin Targeted Pancreatic Cancer Therapy using *Clostridium sporogenes*

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Clostridial Directed Toxin Therapy (CDTT) could be used as a promising non-invasive tool for tumor retardation and regression. The lack of early symptoms and delayed diagnosis of pancreatic cancer makes surgical interference useless and are responsible for the agressivity and low five-year-survival rate of the disease (less than 5%). Claudin-3 and Claudin-4 are overexpressed on the surface membrane of pancreatic cancer cells and are the only natural receptors for *Clostridium perfringens* enterotoxin (CPE). After specific binding to its receptors, CPE triggers tumor lysis. Naturally, CPE is a sporulation-dependent cytotoxic protein that is released through mother cell lysis. In this study, *cpe* was fused to different signal peptide sequences. The whole fusions were cloned downstream of *tetO1* promoter and a constitutive expression and secretion of the cytotoxic protein from *Clostridium sporogenes* has been proven using Western Blot. For therapy, the immunologically inactive recombinant spores could be intravenously injected into patients, and due to the obligatory anaerobic feature of *C. sporogenes*, spores will localize, germinate and secrete CPE only in the anaerobic necrotic areas inside tumors. This can be considered as a highly promising therapy for pancreatic cancer.

MPV11
Crystal structure of pneumococcal carboxypeptidase DacB and the impact of carboxypeptidases on pathogenesis

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Introduction: *Streptococcus pneumoniae* naturally reside in the human oral cavity but cause also serious local and invasive infections including otitis media, pneumonia, septicemia, and meningitis. The peptidoglycan (PGN) is an essential exoskeleton needed to maintain the shape and osmotic stability of Gram-positive and Gram-negative bacteria. The peptidoglycan is a complex macromolecule and composed of two the alternating sugars residues, N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which form oligo-(GlcNAc-MurNAc) glycan strands. These heteropolymers are cross-linked by short peptides to form a complex three-dimensional scaffold (murein). The cell wall hydrolases such as L,D- and D,D-carboxypeptidases DacB and DacA respectively, have been shown to be important for cell division and shape. Here, we were interested in the crystal structure of DacB and the impact of DacA and DacB on pneumococcal pathogenesis.
Materials and Methods: Pneumococcal Δ*dacA* and Δ*dacB* single and double mutants were generated in *S. pneumoniae* by insertion-deletion mutagenesis. The molecular organization of the genetic loci and the mutants was conducted using standard molecular techniques such as PCR, RT-PCR and Northern blot. The mutants were further characterized and compared with the wild-type strain by immunoblot analysis, growth behavior, flow cytometry and virulence studies. The effect of the carboxypeptidases deficiency on phagocytosis was tested using macrophages while the impact on adherence was investigated using A549 epithelial cells. The acute pneumonia mouse infection model and real-time bioimaging was employed to demonstrate whether the loss of function has any impact on colonization and invasive infections. Furthermore, new muropeptide species have been identified in Dac proteins deficient pneumococci by PNG analysis. Importantly, the structure of DacB was solved successfully.
Results: The DacB, a surface-exposed lipoprotein with L,D carboxypeptidase activity was characterized at the atomic level. Importantly, the morphological changes observed in *dac*-mutants are associated with an altered peptidoglycan composition and hence, lower bacterial fitness under infection-related conditions. By employing *in vivo* mouse infections and cell cultured-based adherence and invasion assays, we demonstrate that loss-of-function of DacA and/or DacB impaired full-virulence of pneumococci and accelerated uptake by professional phagocytes, while adherence to epithelial cells is decreased.
Conclusions: In this study, we further characterized the crucial role of the pneumococcal carboxypeptidases DacA and DacB for PGN architecture, bacterial shape and pathogenesis. By applying *in vivo* and *in vitro* approaches, a close relation between peptidoglycan metabolism and bacterial pathogenesis was discovered.

MPV12
Characterization of the protein translocation channel of bacterial type III secretion systems

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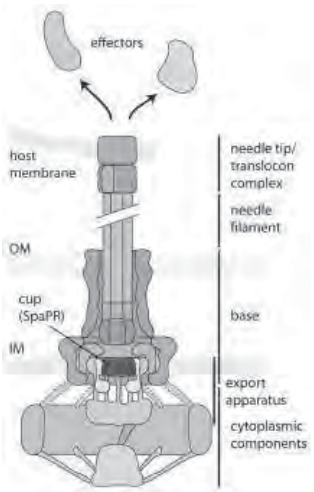
Bacterial type III protein secretion systems secrete effector proteins that can alter host cellular functions in order to promote bacterial survival and colonization. The core unit of type III secretion systems is the so called needle complex that facilitates substrate secretion through an associated export apparatus located in the bacterial inner membrane. Recently, we showed that the central "cup" substructure of the needle complex base is composed of the small hydrophobic export apparatus components SpaP and SpaR in the *Salmonella* secretion system located on pathogenicity island 1. Here we present evidence that this subcomplex forms the protein translocation channel of the type III secretion system. We were able to purify a stable complex formed by SpaP and SpaR and to characterize the membrane topology and stoichiometry of its components. By blue native PAGE, we could show that this central complex is ubiquitously present in flagellar and injectisome-like type III secretion systems. Electron microscopy showed that the SpaPR complex forms a doughnut-shaped structure. Its central pore has a diameter of approximately 15 Å as judged by small molecule conductance measurements.
Bacterial type III secretion systems translocate a biochemically diverse set of substrate proteins at a high rate in one step through at least two membranes. The presented data shed light on the core complex facilitating this process and will help to understand a central mechanism of these virulence associated machines.

Figure 1



MPV13

Identification and functional characterization of plasminogen-binding proteins of *Acinetobacter baumannii*

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Introduction: Multidrug resistant *Acinetobacter baumannii* can cause severe infections in critically ill patients, mainly in intensive care settings. Infections caused by *A. baumannii* include pneumonia and bacteremia. To establish an infection, pathogens must first contend with the innate immune response. Complement represents a major barrier to invading pathogens, and very little is known about factors contributing to complement resistance of *A. baumannii*. Here we describe the identification of plasminogen (Plg)-binding proteins that may contribute to dissemination and complement evasion by *A. baumannii*.
Materials and Methods: Far Western blotting of whole cell *A. baumannii* ATCC 19606 lysates and crude membrane extracts was employed to screen for Plg-binding proteins. Mass spectrometry was used to identify candidate proteins. Following cloning and production in *Escherichia coli*, the recombinant protein was tested for its ability to bind Plg using Western blotting and ELISA. To localize the binding site, C-terminally truncated proteins were also analyzed by ELISA. Furthermore, we analyzed degradation of fibrinogen as well as the key complement component C3b by p41-bound, activated plasmin.
Results: Western blotting revealed at least five potential Plg-binding proteins present in *A. baumannii*. After Triton X-114 phase partitioning, two of the potential Plg binding proteins fractioned into the hydrophobic phase, and were identified, using mass spectrometry, as a heat shock protein of 41 kDa as well as an outer membrane protein, OmpW, of 21 kDa. The 41 kDa protein was shown to be surface exposed and tentatively termed p41. Following purification by affinity chromatography, recombinant p41 was able to bind Plg. Binding of Plg was strongly dependent on lysine residues and could be inhibited by addition of tranexamic acid. The C-terminal lysine residues of p41 seem to be involved in Plg binding, as truncation of the C-terminus significantly diminished binding. Degradation assays showed that Plg bound to p41 was able to degrade both fibrinogen and the central complement component C3b, following activation to plasmin via urokinase-type Plg activator (uPA).
Discussion: Little is known about how *A. baumannii* evades the complement system to establish an infection in the host. Here we show for the first time, that *A. baumannii* is able to bind human Plg through at least one surface exposed Plg binding protein. Plg bound to p41 is accessible to uPA. Following activation, p41-bound plasmin was able to degrade its natural substrate fibrinogen and complement component C3b. One strategy of *A. baumannii* to survive in human serum may thus entail the acquisition of plasminogen, to resist opsonization and complement-mediated killing, and aid in dissemination of the pathogens.

MPV14

Reprogramming of Myeloid Angiogenic Cells by *Bartonella henselae* leads to microenvironmental regulation of pathological angiogenesis

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Bartonella henselae is a facultative intracellular bacterium and the causative agent of bacillary angiomatosis, a vascular-proliferative disease characterized by the tumorous growth of capillary sized vessels in immune suppressed patients. In recent years there has been a growing interest in the contribution of myeloid cells to cancer progression through microenvironmental control of tumor invasion and angiogenesis. In particular, a pro-angiogenic subset of circulating progenitor cells (Myeloid Angiogenic Cells; MACs) have been implicated as important accessory cells in both regenerative and pathological angiogenic conditions. To date, however, knowledge about how *B. henselae* interacts with myeloid cells and how they might contribute to *B. henselae* related pathological angiogenesis is still limited. Our investigations revealed that MACs are readily infected with *B. henselae* and that infection inhibits apoptosis, increases migratory capacity and activates the hypoxia inducible factor 1 (HIF-1) dependent pro-angiogenic program. Infected MACs developed a vascular mimicry phenotype over long term culture and in a 3D spheroid assay of sprouting angiogenesis they incorporated into growing endothelium and increased the rate of sprouting angiogenesis in a paracrine manner. Phenotypic analysis of infected cells (FACS, gene microarray) revealed that this increase in angiogenic activity was associated with the development of a distinct macrophage phenotype including upregulation of angiogenic and matrix remodeling genetic programs and a predominantly M2 anti-inflammatory activation profile. Finally, analysis of cytokine secretion profiles from infected cells revealed that *B. henselae* infected MACs release a diverse array of inflammatory-angiogenic cytokines and matrix remodeling compounds creating a tumor-like paracrine microenvironment with a high potential to promote pathological tissue growth. These results provide new insights into the interaction of *B. henselae* with myeloid cells and highlight their role as paracrine mediators of *B. henselae* induced vascular tumor formation. Furthermore, these findings establish a connection between the manipulation of myeloid cell responses by intracellular bacteria and the creation of stimulatory microenvironments that promote pathological tissue growth in conditions such as cancer.

MPV15

Influence of the virulence factor Mip on the secretome profile of *Legionella pneumophila*

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Legionella pneumophila is a Gram-negative bacterium, which naturally inhabits freshwaters. It replicates intracellularly in amoebae but also in human alveolar macrophages. After inhalation of *Legionella*-containing aerosols, the bacterium can colonize the human lung, destroy lung tissue and cause Legionnaires' disease, a severe form of atypical pneumonia. The macrophage infectivity potentiator (Mip) is a membrane-associated protein with peptidyl-prolyl *cis/trans* isomerase (PPIase) activity. The Mip-negative strain shows a reduced intracellular replication in macrophages or amoebae and is attenuated in the guinea pig infection model. Although Mip was one of the first identified virulence factors, its role during infection and its natural substrate remain unclear. We screened the Mip negative mutant for additional phenotypes and found, that Mip plays a role in temperature

tolerance, sliding motility, serum resistance and oxidative stress. A comparison of *L. pneumophila* Corby (wildtype) and an isogenic *mip* knock-out mutant revealed similar growth rates in liquid medium. Interestingly, the protein concentration of the cell free culture supernatant of the Mip-negative strain was higher. A proteomic approach corroborated Mip-dependent changes in the secretome profiles and revealed the increase of several proteins in the absense of Mip. Among these was the PPIase PpiB and the catalase KatG. We assumed, that PpiB compensates for the lack of Mip-dependent PPIase activity. Hence, we generated isogenic Δ*ppiB* single and Δ*ppiB*Δ*mip* double mutants and tested these strains for intracellular replication, temperature tolerance, sliding motility, serum resistance and oxidative stress. All mutants revealed defects in the tested phenotypes, whereby the double mutant strain was more severly affected. We show for the first time, that PPIases are responsible for various physiological processess in *L. pneumophila* and that PpiB can in part compensate for the lack of the Mip-dependent PPIase activity. Finally, we propose that the Mip-dependent phenotypes are indirect effects of regulatory pathways, where the PPIase is involved.

MPV16

Improved plaque assay identifies a novel anti-*Chlamydia* ceramide derivative with altered intracellular localization

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Chlamydia spp. are obligate intracellular bacterial pathogens found inside the eukaryotic host cell in a membrane-bound compartment, the inclusion. *Chlamydia* spp. lack a number of biosynthetic pathways and are therefore auxotroph for different nutrients including amino acids and lipids. As an example, sphingolipids are acquired from the host and are essential for *Chlamydia* propagation. In this study we have established and validated a novel plaque assay for the titration of *Chlamydia* species. This assay allows for convenient and fast screening and quantification of substances with anti-chlamydial activity. Moreover, using this immunofluorescence-based assay we are able to stain plaques for both bacterial and cellular markers on a single-cell level, enabling us to analyze plaque morphology in great detail and to discriminate potential clonal differences among *Chlamydia* infectious particles and the host cell response. Interestingly, different inhibitors of sphingolipid metabolism displayed species-specific effects on *Chlamydia* plaque formation. We next synthesized and tested several NBD-labeled, nonconvertible analogues of C_{16}-ceramide, the natural precursor of sphingomyelin. 1-*O*-methyl-C_{16}-ceramide was found to strongly reduce plaque numbers comparable to the antibiotic chloramphenicol and also inhibited *Chlamydia* propagation and infectious progeny formation in a single infectious cycle. Additionally, NBD-labeled 1-*O*-methyl-C_{16}-ceramide was not taken up by the chlamydial inclusion in contrast to NBD-labeled C_{16}-ceramide, which accumulates in bacterial membranes. Taken together, with this new plaque assay we were able to discover a novel and highly potent anti-chlamydial compound. Further, with the work presented here we aim to understand sphingolipid acquisition routes and biosynthetic pathways employed by *Chlamydia* spp.

MPV17

Correlative super resolution/atomic force microscopy unravels the localization of two protein secretion systems during invasion of polarized epithelial cells by *Salmonella enterica*

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Salmonella enterica is an important gastrointestinal pathogen of humans and animals with the ability to invade non-phagocytic cells and to persist and proliferate within mammalian cells. Invasion of polarized epithelial cells by *Salmonella* requires the cooperative activity of two protein secretion systems[1]. The type I secretion system (SPI4-T1SS) mediates close contact to the apical side of polarized epithelial cells via the giant non-fimbrial adhesin SiiE[3] binding an apical glycan structure[2,5] and the type III secretion system (SPI1-T3SS) translocates effector proteins inside the host cell leading to actin remodeling and uptake of *Salmonella*. Whereas synchronized activity of both SPI1-T3SS and SPI4-T1SS is known, the localization of these systems and the form of their interaction is ill-defined.

Direct Stochastic Optical Reconstruction Microscopy (dSTORM)[4] is a versatile tool to resolve structures in nanometer range and enables us for the first time to visualize both secretion systems in living bacteria. Local point pattern analysis according to Getis and Franklin[5] allows the calculation of clustering or co-clustering of both secretion systems. To distinguish between non-active and actively translocating SPI1-T3SS, we established a stably transfected MDCK cell line harboring the SPI1 effector SipA and its chaperone InvB fused to eGFP. This allows us to follow the recruitment of InvB to the site of translocation forming distinct foci[6,] while SipA is translocated. In combination with dual color dSTORM, we determined the co-clustering of active SPI1-T3SS with SPI4-T1SS. Combined with correlative atomic force microscopy (AFM) revealing the cellular positioning of the SPI1-T3SS and SPI4-T1SS in relation to the host cell surface, we are able to follow the formation of the microcompartment involving these two systems at the zone of contact between *Salmonella* and its host cells. Changes in the host cell surface structure like microvilli effacement or ruffle formation also indicate SPI1-T3SS activity and therefore reveal the state of invasion of the observed *Salmonella*. Our findings suggest that the cooperation of SPI1-T3SS and SPI4-T1SS is not only based on successive processes but also on the subcellular localization of these secretion systems.

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MPV18

Functional characterization of the small non-coding RNA 26 in *Streptococcus pyogenes*

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Introduction: *Streptococcus pyogenes* (Group A *Streptococcus,* GAS) is an important human pathogen which is associated with superficial infections of the skin and the naso-pharynx as well as with severe toxic and invasive diseases like rheumatic fever. In the past years, small non-coding RNAs (sRNAs) have attracted attention as a new class of gene regulators. The aim of this study was to analyze the impact of the sRNA candidate 26 on the virulence of GAS serotypes M49 and M18.
Materials and Methods: Recombinant plasmids were constructed in *Escherichia coli* DH5α and used to generate a deletion mutant as well as a complementation and an overexpression strain of the respective gene. Afterwards, GAS M49 and M18 were transformed with the plasmids. The resulting mutant strains were analyzed *in vitro* concerning growth, survival in human blood, capsule synthesis, and resistance towards phagocytosis. Furthermore, virulence was determined in *in viv*o employing a mouse infection model.
Results: With regard to its survival skills in human blood, the deletion mutant showed significant differences compared to the wildtype (WT). Growth of GAS M49 Δ26 in human blood was only slightly inhibited. However, in blood samples which inhibited the growth of the WT, growth of the deletion mutant was strongly enhanced compared to the WT. Growth in human plasma was not affected in the mutant and in a phagocytosis experiment, no significant differences between mutant and WT could be detected. In the mouse infection model, the GAS M49 deletion mutant showed an increased virulence compared to the WT. RNAseq transcriptome analysis identified a set of putative target genes of candidate 26. Determination of the hyaluronic acid content revealed a significantly reduced capsule in the GAS M18 deletion mutant.

Discussion: GAS M49 Δ26 showed a conditionally increased growth in human blood and enhanced virulence in a mouse infection model. Due to the mutant's behavior in human serum and its survival of phagocytosis, we propose that a cellular component might be responsible for this phenotype. Capsule production was unaffected in M49, whereas M18 strains showed candidate 26-dependet changes of capsule synthesis. This result hints towards a serotype-specific target repertoire of candidate 26. The next step is to verify and characterize candidate 26 targets.

MPV19

A differentially expressed small regulatory RNA influences metabolism and biofilm matrix production in a hypervariable *Staphylococcus epidermidis* strain

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Staphylococcus epidermidis is primarily a commensal of the healthy human skin and mucosa, but also a common causes of health care-associated infections. Our previous studies aimed at the elucidation of genetic and regulatory factors contributing to the establishment of *S. epidermidis* as a nosocomial pathogen. In this respect, biofilm formation on indwelling medical devices proved to be a crucial pathomechanism. We found that biofilm-forming *S. epidermidis* are extremely versatile microorganisms with an enormous potential to rapidly adapt to a changing environment both by genetic and regulatory mechanisms. These adaptive processes not only affect the virulence potential of the bacteria, but are also accompanied by varying metabolic patterns. Recently, we have focussed on the role of non-coding RNAs (nc-RNAs) in the control of biofilm formation and metabolic adaptation. In *S. aureus*, the nc-RNA RsaE is known to down-regulate key enzymes of the central carbon metabolism[1]. Here, we demonstrate that RsaE is conserved and heterogeneously expressed in *S. epidermidis* clinical isolates, which were sampled during the course of a fatal infection from an immunocompromised patient[2]. The isolates were found to be highly variable in their biofilm matrix composition, and biofilm-negative chromosomal deletion variants occurred spontaneously at high frequencies. Varying transcriptome profiles of the isolates were recorded with significant differences regarding central carbon utilisation, aminosugar synthesis and amino acid metabolism. Interestingly, overexpression of RsaE enhanced polysaccharide-matrix (PIA)-mediated biofilm formation and triggered varying arginine utilisation patterns. Additionally, RsaE overexpression almost completely abolished the occurrence of the spontaneous biofilm-negative deletion variants in those isolates. Currently, there is growing evidence that ncRNAs represent missing links in the complex gene regulation networks of staphylococci. The combined data presented here suggest that RsaE not only controls major metabolic pathways in *S. epidermidis*, but is also involved in the (re-)organisation of the genetic material by an unknown mechanism. The possible impact of these findings for the generation of phenotypic and genetic heterogeneity and, finally, the adaptation power of nosocomial *S. epidermidis* is discussed.

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MPV20

Proteomic profiling of longitudinal *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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Cystic Fibrosis (CF) is one of the most common genetic diseases in Europe and North America. The mutation of a transmembrane chloride channel leads to a defective chloride ion transport which in turn leads to increased viscosity of the mucus and to reduced clearance of inhaled microorganisms. Patients suffering from CF develop chronic lung infections which are often caused by simultaneous infections with various species capable of colonizing the lung epithelium and forming biofilms. The composition of the bacterial community and the prevalence of bacterial species changes during progression of the disease. Here we analyzed the changes of the proteome composition of eight isolates of one of the major CF threats, *Pseudomonas aeruginosa*, isolated from two patients who were intermittently colonized with this pathogen. As a starting point cytosolic proteins were analyzed by mass spectrometry applying the data-independent acquisition mode (MS^E) and leading to the identification and quantification of about 2,500 different proteins. Early adaptation processes of *P. aeruginosa* to the CF lung are characterized by the mutation of global regulators (reviewed in (Folkesson et al., 2012). *P. aeruginosa* strains isolated in the later course of the disease carry mutations in the *retS* sensor kinase and/or the *gacS/A* two-component system. Accordingly, major changes between the isolates on the proteome level were observed for secretion systems T3SS and T6SS. Notably, various other virulence-associated functions, e.g. motility or phenazine biosynthesis, were affected as well. Moreover, various general metabolic pathways were found to be differentially expressed between the early and later isolates indicating that host-adaptation during infection is a complex and dynamic process.

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MPV21

A novel membrane-bound phospholipase B of *Pseudomonas aeruginosa*

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The Gram-negative human pathogen *Pseudomonas aeruginosa* causes a wide range of infections with severe morbidity and mortality cases [1]. Among many virulence factors produced by *P. aeruginosa* are several phospholipases which are classified into four major groups (A, B, C and D) depending on the position of hydrolysis within a phospholipid. They are known to contribute to nutrient utilization, phospholipid homeostasis, damage of host cell membranes and modulation of lipid signaling in eukaryotic cells [2, 3]. Here, we present PlbF, a newly identified phospholipase B of *P. aeruginosa* with broad substrate spectrum. It is able to hydrolyse both acyl chains in phospholipids with bound saturated, unsaturated, short and long fatty acids. Subcellular localisation experiments revealed that PlbF is anchored to the bacterial inner membrane with a single transmembrane (TM) helix located at its N-terminus and its C-terminal domain exposed to the periplasm. Recently, we solved the X-ray structure of dimeric PlbF at 2Å resolution which revealed a unique dimerisation mode through interaction of the TM helices. Immunoblotting and cross-linking experiments indicated that PlbF exists in both monomeric and dimeric form in the bacterial membrane. A PlbF variant with Val residues within the TM helix replaced by Ala showed reduced dimerisation and increased catalytic activity as compared to wild-type PlbF suggesting that dimerization negatively regulates PlbF activity. We further observed that a *P. aeruginosa* Δ*plbF* mutant produced thicker biofilms and its virulence was significantly reduced in a *Drosophila melanogaster* infection model. Interestingly, we could identify in the crystal structure fatty acid ligands bound in the active site; this finding was confirmed with purified PlbF by GC-MS analysis. We assume that these fatty acids may represent lipid messengers which could provide a link to virulence properties of *P. aeruginosa*. Furthermore, the regulatory role of PlbF-dimerization in cellular process triggered by lipid messengers may represent a novel cell-signalling mechanism of *P. aeruginosa*.

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MPV22
Itaconate degradation promotes pathogenesis of *Salmonella* Typhimurium

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Itaconate (methylensuccinate) has recently been identified as one of the antimicrobial compounds produced by macrophages upon activation (*1,2*). This compound is a potent inhibitor of the key enzyme of the glyoxylate cycle, isocitrate lyase, which is important for survival of many pathogens within macrophages (*2-4*). The growth of *Yersinia pestis* in activated macrophages requires the functioning of the *rip* operon (*rip* stays for **r**equired for **i**ntracellular **p**roliferation) (*5*). We have recently shown that *Y. pestis*' *rip* operon encodes three genes involved in itaconate degradation, namely itaconate CoA transferase (RipA), itaconyl-CoA isomerase / mesaconyl-CoA hydratase (RipB), and (*S*)-citramalyl-CoA lyase (RipC) (*6*). The corresponding genes can be found in many other pathogens including *Salmonella* Typhimurium. Now we show that *Salmonella* Typhimurium is capable to grow on itaconate as a sole carbon and energy source using the enzymes encoded by the *rip* operon. Furthermore, we were able to detect the corresponding enzyme activities in *Salmonella* Typhimurium cell extracts. The *ripB* and *ripC* knockout mutants were unable to grow on itaconate, and their growth on acetate in the presence of itaconate was significantly impaired. Interestingly, the *ripA*, *ripB* and *ripC* mutants were severely attenuated in *cybb*$^{-/-}$ *nos2*$^{-/-}$ mice, further confirming the importance of *rip* operon for the pathogenesis. Our study identifies itaconate as an important growth substrate for *Salmonella* Typhimurium during infection.

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Infection Epidemiology and Population Genetics

MSV01
Whole genome sequencing approach to determine the acquisition of *vanB* gene clusters in epidemic strains of *Enterococcus faecium* ST192 in Germany

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Objective: Vancomycin-resistant enterococci (VRE) were isolated with increasing frequencies in recent years. Hospital-associated strains of *E. faecium* become VRE by acquiring mobile *vanA* or *vanB* type gene clusters. Independent acquisition of the *vanB*-encoding Tn*1549* transposon was reported, thus leading to the emergence of clonally distinct VRE populations. Strains of MLST ST192 containing *vanB* were on the rise in Germany, i.a. by causing several outbreaks. To elucidate different aspects of spread of *vanB* type resistance (clonal spread vs. independent acquisition of *vanB*), we set out to characterize a set of clinical ST192 isolates by molecular methods including whole genome sequencing (WGS). We also analyzed *vanB E. faecium* of various MLST types to determine insertion sites and transferability of the *vanB* transposon.
Materials and Methods: Clinical *E. faecium* isolates were collected at the National Reference Center. Isolates were characterized by presence of several marker genes (*esp*, *hyl*, IS*16*, *vanB*), prior to determination of the MLST type for a representative subset of strains (2009 -2012; >20 institutions). Selected isolates were sequenced by Illumina technology. Integration sites of *vanB* were determined by PCR and WGS. Transferability of the mobile *vanB* element was examined by filter-mating experiments with a series of donor and recipient strains.
Results: WGS revealed the integration of the *vanB* transposon at distinct chromosomal loci. Insertion sites differed from those reported for *vanB* enterococci from other countries, thus representing novel hot spots for insertion. Transferability of the resistance cassette to various *E. faecium* strains showed a donor-dependent integration of the *vanB* element. Analyzing surrounding genomic regions before and after transfer of the *vanB* transposon suggests the transposition of a large chromosomal fragment, thereby co-transferring virulence genes if located in close proximity. Transfer of the *vanB* locus to closely related *E. faecalis* strains remained unsuccessful.
Conclusion: WGS and subsequent molecular analyses disclosed genetic requirements necessary for acquisition of *vanB* gene clusters in related clinical isolates of *E. faecium* ST192. Co-transfer of flanking DNA regions could mobilize colonizing and virulence factors. As integration appears to occur in a site-specific manner, we hypothesize that the absence of *vanB* type resistance in *E. faecalis* is due to the lack of certain insertion sites in the genomes of this species.

MSV02
Whole-Genome Sequence Comparision of *Salmonella* Entertidis Strains Isolated from Samples Associated with Almond Outbreaks Suggests a Reduction in Genome Complexity Over Time

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Introduction: Consumption of contaminated raw almonds was the source of three outbreaks of *Salmonella enterica* serovar Enteritidis between 2000 and 2006. The *S.* Enteritidis (SE) strains from these outbreaks had rare phage types (PT), SE PT30 (2000-2001, 2005-06) and SE PT9c (2003-4). In this study, we determined the complete genome sequence for 2 outbreak-related strains, a 2001 SE PT30 strain isolated from almonds and a 2004 SE PT9c clinical strain. The whole genome relatedness through SNP analysis of additional SE PT30 and SE PT9c clinical and environmental strains was determined by employing whole genome shotgun (WGS) sequencing.
Materials and Methods: Both 454 (15-20X coverage) and Illumina MiSeq (>200X coverage) technologies were used to sequence the 2 SE strains to completion. The 454 shotgun and paired-end sequencing reads were assembled into initial contigs using Newbler assembly software (v2.1), and the remaining gaps were closed using Sanger sequencing and Geneious software (v7.0). Final base calls were discerned utilizing Illumina reads. Subsequently, Illumina sequencing reads (~200 X coverage) were employed to identify SNPs in the genomes of additional clinical and environmental isolates by mapping a reference genome using Geneious and Breseq (v0.24) software programs.
Results: Genomic analysis of the complete genomes of SE PT30 and SE PT9c demonstrated 4490 conserved chromosomal genes with 122 unique genes and 99 unique genes in the SE PT30 strain and PT9c strain, respectively. Most unique genes between the two phage types were prophage genes. SNP analysis of non-duplicated regions demonstrated very stable genomes. Among eight SE PT30 environmental and clinical strains collected over a 5-year period, only 15 SNPs were observed, and two SE PT30 strains also possessed unique plasmids. Among four SE PT9c environmental and clinical strains collected in 2004, only 7 SNPs and one unique plasmid were identified.
Conclusion: This study demonstrates that *S.* Enteritids strains associated with almond outbreaks have a highly conserved genetic backbone and distinct prophage genes can distinguish these PT. This study confirms the resolving ability of whole-genome analysis to distinguish clonal isolates, and suggests that *S.* Enteritidis PT30 strains are genomically stable over several years. This stability may be the result of genetic selection for persistence in the almond orchard, since early isolates exhibit more genomic complexity than isolates recovered years later.

MSV03
Population dynamics of *Staphylococcus aureus* recovered from the airways of cystic fibrosis patients during a longitudinal prospective observational multicenter study

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Introduction: *Staphylococcus aureus* is not only the first but also one of the most prevalent and persistent pathogens cultured from the airways of CF-patients.
Objectives: The aim of this prospective longitudinal multicenter study was to dissect colonization from infection in patients with *S. aureus* cultured from the airways by determining a variety of host- and pathogen specific parameters.

Materials and Methods: Inclusion criteria: >6 years, persistent *S. aureus* cultures from airway specimens within the year before recruitment. Specimens were processed at the study laboratory in Muenster. *S. aureus* isolates from primary cultures were distinguished by phenotypical appearance (hemolysis, pigmentation, size). All isolates were analyzed by *spa* sequence typing.
Results: Data were collected for 195 patients from 16 centers in Germany and 1 center in Austria. 75 female (38.5%) with a mean age of 15.7 years (range 5 to 41 y) were recruited; 49% patients were ΔF508 homozygous. Data from 1359 visits were evaluated (mean visits/ patient: 7). 1381 of 1897 specimen (73%) were positive for *S. aureus*. 3963 *S. aureus* isolates were recovered from nasal and throat swabs, sputa or nasal lavage. The isolates could be assigned to 269 different *spa* types. During the observation period up to 12 different S. aureus clones were isolated from individual patients. 174 clones (65%) were unique clones present in individual patients only, while the 4 most prevalent clones (*spa* types t084, t091, t015, t008) were present in 16, 14, 12 and 10 centers and recovered from the airways of 99 patients (52%).
Conclusions: The results of our study revealed that most patients were infected by their individual clone, while 4 clones were present in many CF centers and in many patients. Such distribution indicates that CF patients acquire not only special but also clones, which are prevalent in the community.

MSV04

Establishing a molecular test assay based on genomic analysis of multidrug-resistant *Mycobacterium tuberculosis* outbreak strains from Gabon

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Tuberculosis (TB) is still a fatal infectious disease. The WHO reported an estimated number of 8.6 million new infected people and 1.3 million died from the disease in 2012. Over the last years, the number of people infected with multidrug-resistant (MDR) TB (strains resistant to the first line drugs isoniazid and rifampicin) is increasing. According to WHO the number of notified MDR TB cases in Africa doubled since 2010. Emergence of MDR TB is a challenge for the healthcare system. Especially in Africa with its high rate of HIV co- infected people, the effective transmission of MDR TB is a threatening scenario. To prevent spreading of MDR TB and apply effective treatment to patients we analyzed *Mycobacterium tuberculosis* (M. tb) strains from a rural area in the high incidence country Gabon.
Our analysis revealed a cluster of MDR strains of the Haarlem genotype in Gabon. Establishing a molecular test assay to identify those MDR strains and to stop further spreading is urgently needed. Therefore we performed whole genome sequencing (Illumina MiSeq) on all MDR strains from Gabon to identify a cluster specific Single Nucleotide Polymorphism (SNP) in the genome of the MDR strains. To exclude SNPs specific for strains of the Haarlem genotype as well as SNPs specific for MDR strains, we sequenced further antibiotic susceptible Haarlem strains and MDR Haarlem strains from Sierra Leone, Swaziland, Ghana and Germany.
The genome analysis of the 58 strains showed 30 unique SNPs for the MDR cluster in Gabon. Among these 30 SNPs four are synonymous (genes: Rv0402c, Rv0450c, Rv1384, Rv3391). To confirm these SNPs we investigate these four target genes in additional M. tb strains by Sanger sequencing (ABI 3500xL, life technologies). In future study, we will design and evaluated a real-time PCR assay to identify one of those confirmed cluster specific SNPs to rapidly detect MDR strains in Gabon and prevent further spreading of these strains.

MSV05

Association of meningococcal type with disease outcome

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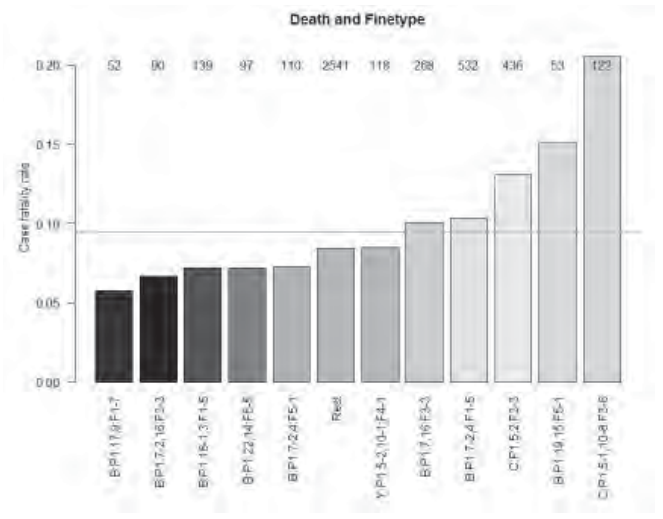
Introduction: We present analyses based on a matched dataset containing 4,590 laboratory confirmed cases of invasive meningococcal disease, processed over a twelve year period (2002 to 2013), and representing 63.9% of notifications to the Robert Koch-Institute. Of 4,590 cases, data on full finetype (i.e. serogroup, PorA, FetA), fatal outcome, and disease manifestation ("meningitis", "sepsis", and "fulminant sepsis") were available in 96.9%, 99.3%, and 79.3%, respectively. We explored whether particular finetypes were more commonly associated with septic disease (i.e. sepsis and fulminant sepsis) or death.
Materials and Methods: Finetypes represented less than 50 times were collectively defined as the reference group in logistic regression models testing the association of finetype with death, and in multinomial logistic regression models analyzing the association of finetype with clinical manifestation. While all models were adjusted for age group and sex, models predicting death were fitted with and without a variable representing clinical manifestation. Of 1,002 finetypes within the dataset only 11 occurred more than 50 times and were thus tested for associations.
Results: Of three finetypes significantly associated with death (B:P1.7-2,4:F1-5; C:P1.5-1,10-8:F3-6; C:P1.5,2:F3-3) only the two serogroup C types were also associated with septic disease. Accordingly, inclusion of manifestation into the model predicting death lowered odds ratios of serogroup C types, but not of B:P1.7-2,4:F1-5; significant associations, however, remained for all three types, suggesting an independent effect on death. In addition, two types (B:P1.17,9:F1-7; B:P1.7,16:F3-3) were associated with septic disease, without, however, causing significantly higher case fatality.
Discussion: These analyses suggest that while disease manifestation remains a strong predictor of death, some finetypes are associated with death independently of clinical manifestation. Also, some types are associated with sepsis without a concomitant association with death. Higher case fatality, but also increased risk of sepsis, might be due to higher bacterial load attained during disease caused by above types. To test this hypothesis, we are currently quantifying meningococcal DNA in a subset of 280 samples (mainly cerebrospinal fluid and serum) collected over the last decade, in which culture-independent typing of meningococcal DNA was successful. Results will be presented and discussed at the conference.

Figure 1



MSV06

Bayesian phylogeography analysis of *Clostridium difficile* in Germany: a critical evaluation

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Objective: Bayesian frameworks are increasingly used for inference of bacterial phylogeographic history and for the reconstruction of microbial spread. The BEAST 2.0 cross-platform program was introduced recently to ease the handling of such analyses. We applied BEAST 2.0 on a set of genome sequences from 61 *C. difficile* ribotype 027 isolates to gain insights into the spatial and temporal dynamics of the spread of this pathogen. By applying extended statistical analyses we tested for limits of the spatial and temporal resolution of the phylogeographic approach.
Materials and Methods: We sequenced the genomes from 61 *C. difficile* ribotype *027* isolates by using the Illumina method on a MiSeq system. Isolates had been collected from hospital patients at 33 locations widely spread over Germany. Single nucleotide polymorphisms (SNPs) were discovered by applying a read mapping approach incorporating BWA for mapping and VarScan for consensus calling, combined in a customized pipeline framework (reference genome sequence, acc. no. NC_013316). The BEAST 2.0 application was used to perform an Markov chain Monte Carlo analysis of the molecular sequences. Several mechanistic models that describe the biological process of building a certain tree topology were tested concerning their statistical reliability at a population level. Due to statistical instability of numerous runs, we performed a parameter reduction by grouping locations based on hierarchical cluster analysis and then projecting them onto 11 points. Bayesian stochastic search variable selection (BSSVS) as implemented in the SPREAD software was used to determine well-supported diffusion rates modelling the spreading process.
Results: Phylogenetic analyses of genome sequences show that the majority of ribotype 027 isolates is related to two internationally disseminated, fluoroquinolone resistant strains, previously designated 'FQR1' and 'FQR2'. While FQR1, to date, appears to be rare in Germany, data from 51 FQR2 isolates were subjected to in-depth analyses. We determined the short-term rate of evolution of FQR2 to 1.9 x 10^{-7} (95 % HPD 1.3 x 10^{-7}, 2.5 x 10^{-7}) substitutions per nucleotide site and year. Our results suggested that FQR1 got imported into Germany already around 1998 (95 % HPD 1993, 2003). Apparently, it then spread from South West Germany to the north-western regions (Rheinland-Pfalz, Nordrhein-Westfalen, Hessen) initially, and reached eastern Germany (Berlin, Thüringen, Sachsen) at the end of the past decade. In conclusion, short-term evolution in *C. difficile* proceeds fast enough to enable monitoring of its regional dispersal based on genome sequences. Depending on the specific dataset, parameter reduction may be useful to enhance statistical support. Even with the new BEAST 2.0 implementation, designed for easier model adjustment via the BEAUTI 2.0 interface, it is still burdensome to validate the results, however. Especially model selection requires extensive computing capacity.

MSV07

Sexual transmission of meningococci may account to an outbreak of meningococcal disease among men who have sex with men

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Introduction: An increase in group C meningococcal disease incidence among men who have sex with men (MSM) was recently observed in Europe and the United States. We aimed to explore bacterial specific factors that might explain this outbreak
Materials and Methods: Invasive meningococcal isolates were extensively analysed using whole genome sequencing (WGS), transcriptomic and proteomic analysis. Experimental infection in transgenic mice was performed to evaluate invasiveness of the isolates.
Results: Meningococcal isolates from MSM were identical by genotyping, and belonged to clonal complex cc11. WGS showed emergence of a new branch (clade) within cc11. While close to the branch of other ET-15 isolates, MSM isolates expressed new functional *fHbp* alleles. Interestingly, group C/cc11 isolates from urethritis cases in men also clustered in the new branch based on WGS. However, all urethritis isolates harboured a non-functional *fHbp* allele with a frame-shift mutation. Experimental infections in transgenic mice expressing human factor H (fH), a complement regulatory protein, suggested higher invasiveness of invasive isolates from MSM compared to urethritis isolates. Moreover, transcriptomic and proteomic analyses showed consistent expression of *aniA* gene in invasive MSM and urethritis isolates when compared to other cc11 isolates. Lack of AniA expression was caused by point mutations as evidenced by genome data. Biochemical and growth tests confirmed the enzymytic activity of AniA and functionality of the anaerobic respiration pathway in MSM isolates, but not from isolates of an adolescent outbreak of group C disease.
Discussion and conclusions: Altered expression of AniA may have conferred a selective advantage on urethral mucosal surfaces and sexual spread. AniA is essential for gonococcal growth under oxygen limiting conditions that may prevail on the genitourinary pathway. The consecutive acquisition of functional fHbp by invasive isolates found in MSM in contrast to urethritis isolates may explain, at least in part, the recovery of invasiveness, as these isolates as shown by enhanced blood stream survival during experimental infection. Our data provide evidence for specific patho-adaptation of meningococcal serogroup C cc11 isolates from MSM and warrant targeted preventive measures (MenC vaccination) for persons closely associated with this community.

MSV08

Sequencing bacterial genomes from clinical samples without cultivation

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Introduction: Genomic data is critical for understanding the relationships between and within bacterial species. In the investigation of novel bacterial pathogens, microbial identification through 16S ribosomal RNA gene sequencing often provides insufficient resolution. Within species, particularly those which undergo recombination, typing schemes cannot truly inform about the diversity and relatedness of strains. Many bacteria of interest are either uncultured, difficult-to-culture, or the material available for analysis is not preserved for culture.
Materials and Methods: Novel methods for DNA manipulation have been developed and trialled, in order to obtain full or partial genomic data from clinical samples without the need for culture. High-throughput Illumina sequencing was used to generate genomic data.
Results: Using discarded clinical samples, complete genomes of the sexually transmitted pathogen *Chlamydia trachomatis* were obtained using antibody targeting and whole genome amplification. This novel protocol of immunomagnetic separation and multiple displacement amplification (IMS-MDA) can be used on samples where the bacteria remain intact.
Recent work identifying and characterising pathogens of commercially important fish from preserved samples has identified two novel pathogens within the proteobacteria. Following microbial identification and fluorescent *in situ* hybridisations, sample manipulation and commercial kits for the depletion of eukaryotic DNA have been employed to generate partial genomes.
Conclusion and Discussion: Using IMS-MDA we can investigate the genomes of bacteria which are uncultured or difficult to culture. Within a few hours, material for genome sequencing was generated from 10-15% of discarded *C. trachomatis* clinical samples. This technique allows access to the genomes of a much greater number of samples than is possible using culturing alone. IMS-MDA has the potential to be used on any bacterium with specific antibodies and intact samples.

Genomes of novel pathogens, even partial data, can be used to confirm and more rigorously identify the bacteria present, provide information for comparison with to better characterised related microbes, and guide the investigations into their lifestyles.
Sequence capture techniques are being used increasingly to generate genomes from low load material based on target reference genomes.

MSV09

Assessment of the microbial diversity in groundwater used for drinking water abstraction by 16S-tag pyrosequencing and physiological analysis of enrichment cultures

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Major problems for the drinking water abstraction from groundwater are caused by hydrogen sulfide and iron ochre formation under anaerobic and aerobic water conditions, respectively. While the latter also occurs abiotically the rate of iron ochre formation is still accelarated by the activity of microbial iron oxidising bacteria. In order to assess the potential impact of these microbial processes on groundwater well performance (iron ochre formation) and well water quality (hydrogen sulfide formation) we monitored the microbial diversity in four groundwater wells used for drinking water supply near Leipzig over a period of 18 months by 16S-tag pyrosequencing. The four water wells differed in the prevailing microbial diversity which, by and large, correlated with water geochemistry. For example, microaerophilic representatives of the iron oxidising *Gallionellaceae* were abundant in the more iron rich groundwater (*ca*. 4 to 10 mg total iron per litre), whereas so far unknown mesophilic representatives of the *Thermodesulfovibrionaceae* seemed to dominate the groundwater samples with low iron loads (*ca*. 0.3 to 0.4 mg/L). Moreover, incubation experiments with enrichment cultures from groundwater water samples of the four wells confirmed the corresponding microbial activities as inferred from the 16S-tag sequence reads.
However, a particularly striking observation was the presence of representatives of the *Acidithiobacillaceae* and of the recently described genus *Ferrovum* in groundwater samples since both groups are known as acidophilic iron oxidising bacteria. These acidophilic strains comprised up to a quarter of sequence reads in some of the samples. Monitoring chemical changes (ion chromatography) and the dynamics of the microbial diversity (TRFLP) during incubation of enrichment cultures at acidic pH (pH 3) demonstrated that at least the *Acidithiobacillaceae* were still viable and able to oxidise ferrous iron to ferric iron under acidic pH. The origin of those acidophilic strains is likely to be found in the middle German lignite coal mining district and their detection might open an avenue for biomonitoring the paths of acid mine waters in aquifers.

MSV10

A next generation sequencing approach to understand the evolution and global success of *Mycobacterium tuberculosis* complex strains of the Beijing lineage

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The transmission of multidrug resistant (MDR, resistance against isoniazid and rifampicin) *Mycobacterium tuberculosis* complex (MTBC) strains constitutes a major threat for global tuberculosis (TB) infection control. While high rates of MDR-TB among new cases, particularly in Eastern Europe and Russia, pointing towards an ongoing transmission of MDR strains, it is unclear if strains of specific MTBC lineages (e.g. genotypes) possess selective advantages compared to other local strain types in MDR-TB high incidence regions. In this view MTBC strains from the Beijing lineage gained increased attention due to numerous reports on hyper-transmissibility and the strong association with drug resistance in many study regions worldwide. Epidemiological studies indicate that the genetic diversity of the Beijing lineage might be by far underestimated and the genetic background therefore may strongly influence the outcome of TB treatment and regional control programs. To address this question, we analyzed a comprehensive dataset of 4,987 clinical Beijing isolates originated from 99 countries worldwide with 24-locus mycobacterial interspersed repetitive unit - variable number tandem repeat (MIRU-VNTR) typing to gain insights into the global population structure. We were able to identify seven clonal complexes which show a biogeographic distribution and were differentially associated with a MDR phenotype. In the following a representative subset of 110 clinical Beijing isolates was subjected to a next generation sequencing (NGS) approach to infer the phylogeny and identify recent genetic changes that occurred in the era of urbanization, globalization and extensive antibiotic treatment. A combination of classical dN/dS analysis, identification of convergent mutations and a Bayesian factor model pointed out genes under positive selection. Identified targets include mechanisms associated with drug efflux pumps, compensatory effects, virulence factors and cell wall processes. Applying a coalescent-based analysis we recognized a two-step expansion of modern Beijing strains starting around 200 years ago, coinciding with the urbanization, industrialization and historical human migration waves. Moreover, the generalization of antibiotic use had a strong negative impact on the overall circulating MTBC population size, including Beijing strains. Out of this genetic bottleneck two modern MDR Beijing clones rapidly emerged and spread nowadays successfully throughout Central Asia and Russia.

Antimicrobial Resistence and Drugs, Infection Prevention

PRV01

Distribution of Methicillin-resistant *Staphylococcus aureus* clonal lineages in bacteraemia isolates from North Rhine-Westphalia, 2011-2013

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is still a major cause of severe nosocomial infections. However, it has been shown that the clonal structure of MRSA differs regionally and locally due to outbreaks, dissemination via patient transfers or divergent spread in the community. This is the first representative study assessing the clonal structure of MRSA isolated from cases of bacteraemia in the largest German federal state (n=17.5 million inhabitants).
Materials and Methods: Microbiological laboratories in North Rhine-Westphalia (NRW) were asked to send all MRSA bacteraemia isolates obtained from patients in NRW between 12/2011 and 10/2013 to a central typing facility together with information on the patient's age, sex and information to which local public health authority (first 2 numbers of postal code) the MRSA bacteraemia case was notified. All bacterial isolates were typed using *S. aureus* protein A (*spa*) sequence-based typing. For cluster formation of *spa* types the Based Upon Repeat Pattern (BURP) algorithm of the StaphType™ software was used; *spa* types <5 repeats were excluded from cluster formation.
Results: Overall, a total of 1,906 MRSA isolates (i.e. 70% of the 2,741 cases of MRSA bacteraemia notified to NRW public health authorities during the study period; http://www3.rki.de/SurvStat/) were collected in 17 postal-code-regions (PoR) in NRW (range: 50-235 isolates per region). 62% of the bacteraemic patients were male; mean age was 72 years (range 0-100 years). *spa* typing identified a total of 178 *spa* types; one isolates (0.05%) were not *spa* typeable. *spa* types belonged to the following *spa* clonal complexes (*spa*-CCs): 003 (overall 47.9%; range between PoR: 34.1-71.8%; t003 was the predominant *spa* type in this CC), 032 (42.3%; 16.5-59.5%; t032), 011/034 (1.7%; 0-10.5%; t034), 008 (1.5%; 0-3.9%; t008), 038 (1.1%; 0-4.0%; t038), 004 (0.8%; 0-3.5%; t004), 012 (0.5%; 0-2.4%; t037), 359 (0.4%; 0-2.4%; t044 and t359), singletons (1.5%; 0-10.5%; t127 and t2807); excluded types (2.1%; 0-4.3%; t535).
spa-CC011/034 including *spa* types indicative for livestock-associated MRSA occurred mainly in PoR "32" (5.8% of all regional isolates), "48"

(10.5%) and "59" (4.8%), respectively. These are regions characterized by a high density of livestock production. Moreover, several regional clusters were observed for single *spa* types (e.g., t2807) indicating local spread or dissemination in healthcare networks.
Discussion: This representative, federal state-wide survey demonstrated substantial geographic variation and regional clustering among MRSA isolated from cases of bacteraemia indicating the impact of transmission within the healthcare system. Clonal lineages (spa-CC011/034), potentially associated with livestock contact, accounted for a substantial proportion (up to 10.5%) of MRSA bacteraemia cases in some parts of NRW.

PRV02
Mupirocin susceptibility in clinical staphylococcal isolates from Germany

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Objectives: Mupicrocin is a topical antibiotic widely used for MRSA decolonization in both, colonized patients and healthcare personnel. Its antibacterial activity is mediated by inhibition of the bacterial isoleucyl-tRNA-tynthetase (IleRS) thus preventing proteinbiosynthesis. Low-level mupirocin resistance is caused by point mutations in the native *ileRS* whereas high-level resistance is mediated by acquisition of *ileS*-2 (mupA) which is located on mobile genetic elements (MGE) and facilitates the expression of an alternative *ileRS*. Wider occurrence of mupirocin resistance is not yet reported in Germany; however, the increased use of mupicorin might result in a continuous increase of resistance thus impairing the efficiency of future decolonizing strategies.
Materials and Methods: We investigated a total of 1428 clinical staphylococcoal isolates sent to the National reference center for staphylococci and enterococci from January to May 2014. This included 1336 *S. aureus* and 92 coagulase negative staphylococcal (CNS) isolates. They were tested for their antimicrobial susceptibility according to EUCAST. The presence of *ileS*-2 and mutations in *ileSR*, respectively, were investigated by PCR and Sanger sequencing. Current work is focusing on the characterization of *ileS*-2 positive isolates with respect to their susceptibility towards several disinfectants as well as the characterization of *ilsS*-2 associated MGEs.
Results: We found 11 *S. aureus* (0.8 % of all *S. aureus*) and 6 CNS isolates (6.5 % of all CNS), which were resistant according to EUCAST guidelines (MIC >256 mg/L; high level (HL) resistance); except of three, all HL-resistant isolates carried *ileS*-2. A total of 105 isolates exhibited intermediate mupirocin resistance according to EUCAST (MIC ≥ 2mg/L, ≤ 256 mg/L; low level (LL) resistance) including 82 *S. aureus* (6.1 %) and 23 CNS isolates (25.0 %). Only one LL-resistant CNS isolate carried *ileS*-2. The remaining isolates contained different patterns of mutations in the native *ileSR*.
Conclusion: Currently, HL-mupirocin-resistance is not widespread in clinical staphylococcal isolates in Germany. Less than 1 % of all *S. aureus* investigated in this study were resistant towards the antibiotic; however, mupirocin HL-resistance was more common in CNS, which share the same ecological niche and might represent a reservoir for mobile genetic elements carrying mupirocin-resistance-associated determinants. Moreover, we found a comparatively higher rate of LL-resistant *S. aureus* isolates, which might also be associated with decolonization failure.

PRV03
Occurrence of carbapenemase producing *Enterobacteriaceae* (CPE) isolated from pig-fattening farms throughout Germany

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Carbapenems belong to the group of broad-spectrum beta-lactam antibiotics and are mostly considered as drugs of last choice for the treatment of serious infections in humans. Therefore, the dissemination of carbapenem resistance among Enterobacteriaceae possesses an increasing threat to public health. Although a wide variety of carbapenemase variants have been isolated from cases of human infections, until now just a few studies have reported their occurrence in livestock and livestock associated surroundings. However, since recently Enterobacteriaceae carrying bla_{VIM-1} genes have been isolated on a pig-farm in Germany (Fischer et al., 2012; 2013) the monitoring of CPE in livestock became a major topic within the European Union (EFSA, 2013). As carbapenem resistance in Enterobacteriaceae is often associated with extended-spectrum-beta-lactamase or AmpC beta-lactamase production (Birgy et al., 2012) we recently started the carbapenemase-screening of bacterial isolates sampled during the first period (2011-2013) of the national RESET project (www.reset-verbund.de). Within this study the occurrence of ESBL-/ AmpC-producing Enterobacteriaceae in livestock farms throughout Germany was investigated. The here described screening is focused on 239 pooled feces and boot swab samples, chosen from a cross-sectional study including 58 pig-fattening farms throughout Germany. Also included were the farms with the recently by Fischer *et al.* described *E. coli* and *S. enterica* carrying bla_{VIM-1} genes. DNA of the previously isolated bacterial samples was used for a real-time PCR based screening for the carbapenemase genes bla_{VIM}, bla_{KPC}, bla_{NDM}, bla_{OXA-48} and bla_{GES}. Out of 58 investigated farms one showed bla_{VIM}-positive boot swap and feces samples, which indicates a prevalence of 1.72%. However, within a simultaneously performed phenotypical screening approach, using MacConkey agar plates containing 0.125 mg/l meropenem (EFSA, 2013), additional Enterobacteriaceae were detected. These isolates are currently further investigated. The so far obtained results are of great interest and the ongoing investigations will be of great importance to get an insight into the spread of carbapenemase genes among bacteria isolated from livestock.

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PRV04
Emerging carbapenemase-producing multi-drug-resistant bacteria in University Hospital Bonn

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Emerging multidrug-resistance is a global health problem. The aim of the study was to investigate the molecular epidemiology and the genetic support of carbapenem resistance in MDR (Multi-Drug Resistant) Gram-negative rods in University Hospital Bonn, Germany.
For the last 2 years we introduced cultural screening methods for MDR Gram-negative rods in all admitted patients. Additionally to the entry screening, the weekly surveillance of all intensive-care patients was performed. Identification and antimicrobial susceptibility testing was performed on Vitek MS and Vitek-2 platform. In our algorithm the enhanced ertapenem-MIC in enterobacteriacea and enhanced imipenem and meropenem MIC in non-fermenting rods was used as selection criterion for possible carbapenemase presence. All suspected isolates were typed by in-house established high resolution melt (HRM) curve analysis utilizing: OXA-48, KPC-1/-2, VIM-1/-2, NDM, GES, IMP and OXA-23 specific primers in a single PCR reaction. During the evaluation period all results were confirmed by the national reference center. From July 2012 until May 2014 a total of three hundred and seventeen (n=316) isolates were identified to be intermediary or resistant to one or more carbapenems tested according to EUCAST breakpoints. Almost one half (n=150, 47.5%) of all isolates were tested positive for carbapenemase by the HRM PCR assay describedby XXX et al. . XXXXXX All other methods used, e.g. Hodge test, MALDI and phenol red, were found to be less reliable. OXA-48 was the most common carbapenemase found in 37% (n=56) of all positively tested isolates, followed by: 27% of VIM-1/-2 (n=40), 17% of OXA-23 (n=25), 3% OXA-24 (n=5), 7% of KPC-1/-2 (n=11), 6% of NDM (n=9), 1% of GES (n=2), 1% and 1% of OXA-48/NDM (n=2) double positive isolates. No IMP-positive isolates were detected during the study period. The most common bacterial species among carbapenemase-positive strains was Klebsiella pneumoniae 56,8% (n=62), followed by: Pseudomonas aeruginosa 37% (n=39), Acinetobacter baumannii 97% (n=36), Escherichia coli 27,6% (n=8) and Enterobacter sp. 19% (n=4). Additionally, some extraordinary carbapenemase-bearing strains were found: VIM-positive Klebsiella oxytoca and Citrobacter youngae, both unique isolates, and an OXA-48-bearing Serratia marcescens. Carbapenemase-positive strains

(n=151) were isolated from 254 different patients of whom 40% were non-German residents. Of note, 7.5% (n=19) of all patients were colonized with 2 or more different carbapenemase-bearing strains and in 7 of these we detected the same carbapenemase in different bacterial species. Due to the high prevelance of carbapenemase-bearing strains at the University Hospital Bonn we need fast and reliable on-site techniques to detect carbapenemase production and enable early adequate infection control measures. We have decided to place a previously published HRM PCR assay directly after susceptibility testing, thus saving the time normally used for less reliable testing with Hodge test or MALDI.

PRV05

Staphylococci living in aquatic environments with inducible resistance to macrolides, lincosamides and streptogramin B antibiotics and the capacity of the metabolite anhydroerythromycin to induce cross-resistance against these antibiotic classes

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Staphylococcus spec. in human and veterinary medicine are well examined, especially for their antibiotic-resistances. However little is known about abundance and species diversity of staphylococci in aquatic environments where they are exposed to antibiotics (AB) and their metabolites in low concentration, due to increased AB consumption in human and veterinary medicine and the use as growth promoters. To fill this gap 1980 staphylococci were isolated from sewage treatment plants and receiving water bodies, identified on species-level by their physiological reactions and screened respective their susceptibility against oxacillin, ciprofloxacin, erythromycin and clindamycin, often used antibiotics to treat infections caused by staphylococci. The abundance and the distribution of *erm*-genes, which encode for inducible or constitutive resistance against macrolides, lincosamides and streptogramin B antibiotics (MLS_B), were analyzed. Furthermore, it was checked if and at which concentration anhydroerythromycin, a metabolite of erythromycin in river water, can act as an inducer for cross-resistance.

Twenty % of the staphylococci isolated from the aquatic environment were resistant at least against one of the four tested antibiotics. Resistance against the macrolide erythromycin was the most often detected one: 19.4 % of all isolates were resistant against this macrolide which is able to induce cross-resistance against lincosamides and streptogramin B antibiotics and 18.3 % of them showed the $iMLS_B$-phenotype. Coinciding with clinical studies the most common MLS_B-resistance gene was *erm*C (55.8 %). However the inventory of *erm*-genes of staphylococci from aquatic environments seems to be more diverse as described for iMLS-staphylococci from humans and animals. Most of our isolates were members of the Saprophyticus- (52.9 %) and the Sciuri-group (21.1 %). Whereas *S. aureus* is the predominant species in clinical samples, only four of the 1980 isolates belonged to the mentioned species indicating its weak survival in river water and sewage. The species diversity of staphylococci in aquatic environment seems to be more diverse than in clinical samples. In total, 20 species were isolated and identified over 2 years of sampling. Traces of erythromycin and anhydroerythromycin (1 ng L^{-1}) can induce resistance against clindamycin within only 10 minutes exposure. Reported concentrations of the metabolite anhydroerythromycin in water bodies, which very often serve for recreation as well as reservoirs for drinking water, are thus high enough for cross-induction of resistance against clindamycin an antibiotic for therapy of *Staphylococcus* infections. These findings as well as the detection of different *erm*-genes in these environments carried by different species of the genus *Staphylococcus* must be considered as a wide-spread risk for human health.

PRV06

The antibiotic roseoflavin from *Streptomyces davawensis*: Mechanism of action and resistance

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Introduction: Roseoflavin (RoF) produced by *Streptomyces davawensis* and *Streptomyces cinnabarinus* is the only known natural riboflavin/vitamin B_2 (RF) analog with antibiotic function and is studied as a model compound in our laboratory (1) in order to pave the way for the structured analyis of other vitamin analogs yet to be discovered. RoF is active against Gram-positive/Gram negative bacteria, fungi and protozoa.

Materials and Methods: Standard procedures of molecular biology, microbiology and biochemistry were used.

Results: RoF is taken up by RF transporters and is converted to the flavin mononucleotide (FMN)/flavin adenine dinucleotide (FAD) analogs RoFMN/RoFAD by flavokinases/FAD-synthetases (1,2). The addition of RoF leads to a decrease of intracellular RF/FMN/FAD levels. This is the consequence of RoFMN mediated blocking of bacterial FMN riboswitches, which leads to a reduced expression of genes involved in RF transport and/or biosynthesis (3). A specialized FMN riboswitch thereby confers roseoflavin resistance to *S. davawensis* and *S. cinnabarinus* (4). Additional cellular targets for RoF are flavoproteins, proteins, which depend on the cofactors FMN and FAD. We could show that 37 out of 38 *Escherichia coli* flavoproteins contained either RoFMN or RoFAD when cells were treated with just toxic doses of RoF (5). FMN-dependent AzoR (EC 1.7.1.6) from *E. coli* exemplarily was analyzed in greater detail with regard to the molecular effect of RoF. RoFMN binds to AzoR apoenzyme with a higher affinity compared to that of FMN, however, AzoR-RoFMN is less acitve (30% of AzoR-FMN activity). Structural analysis (1.07 Å) revealed that RoFMN binding did not affect the overall topology of the enzyme and also did not interfere with dimerization of AzoR (6).

Discussion: Although, until now, only very few natural vitamin analogs with antibiotic function have been identified, we expect that a multitude of yet unknown vitamin analogs can be isolated from microorganisms/ plants/etc. Since most vitamins are active at more than one site, vitamin analogs in principle have multiple cellular targets (as shown for RoF, see Fig. below). Moreover, many microorganisms (target organisms) have efficient vitamin transporters which ensure the delivery of the antibiotic to the target molecules in a cell.

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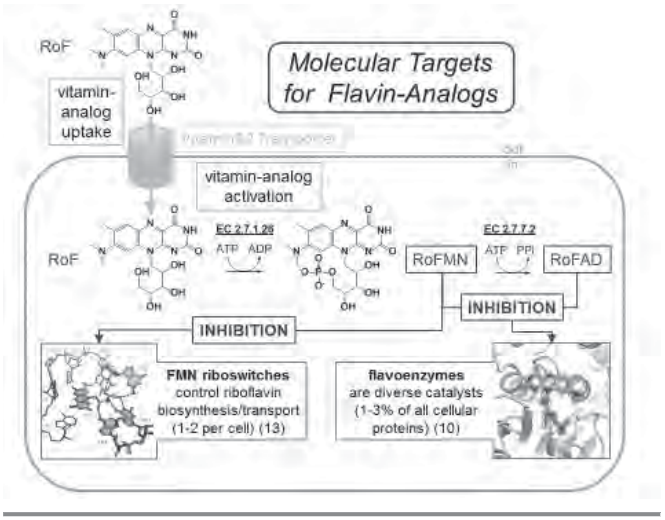
Figure 1



PRV07

Outbreak control of multi-resistant Pseudomonas aeruginosa in a hematology and bone marrow transplant unit

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Introduction: Multiresistent gramnegative bacteria show an increasing incidence and are associated with high rate of mortality especially in hematological patients. We report the management of an outbreak with carbapemenase producing *Pseudomonas aeruginosa* in a hematology and bone marrow transplant unit. A sequence type (ST)-175 strain was first introduced into the University hospital in 2007 (1). The outbreak commenced in December 2011. Decontamination of wastewater systems was the key to outbreak control.
Materials and Methods: Case definition was colonization or infection with VIM-2-carbapemenase positive P. aeruginosa (ppsA-14, trpE-19). The sequence type was ST-175, which has been reported to cause outbreaks worldwide (2). For weekly screening of patients, anal/rectal swabbing turned out to be superior to pharyngeal and urine samples.
Results: The protracted outbreak commenced in December 2011. After the fourth case infection control was initiated. Despite an intensified surveillance the outbreak could officially be declared as terminated in July 2014. A total of 22 cases with vim-2 *P. aeruginosa* was observed. All patients were positive on anal/rectal swabbing. There were 10 confirmed infections (blood stream infection, UTI, wound infection). Genome sequencing confirmed the identity to the 2007 strain from urological patients (1). Ca. 3000 environmental samples were investigated in the outbreak management period. Contamination of wastewater systems (wash basin and shower sinks, toilet installations) was confirmed repeatedly. Outbreak control was achieved by continuous chlorination of toilet flushing water, regular decontamination of sinks using defined cleansing protocols, and installation of thermal disinfection devices for washbasin sinks. As with patient isolates, genome sequencing confirmed the identity of environmental strains to the outbreak strain.
Conclusions: Weekly screening using anal swabs was essential to readily detect patients shedding the outbreak strain. Given the likely persistence of biofilms in the central wastewater drainage system and repeated hospitalization of colonized patients, control measures for waste water systems need to be pursued. The future design of new plumbing units for severely immuno-compromised patients should take into account the emergence of multi-resistant pathogens flexibly adapting to various niches including aqueous habitats.

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PRV08

Antimicrobial peptides delocalize peripheral membrane proteins and elicit a hypoosmotic shock response in *Bacillus subtilis*

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Increasing antibiotic resistance in hand with decreasing antibiotic approvals has created an urgent need for novel antibacterial agents. Antimicrobial peptides are a natural class of compounds that is known to attack the bacterial membrane. Although their interaction with model membranes has been well-studied [1], only limited information is available on their interaction with bacteria *in vivo*. The non-pore forming peptide RWRWRW-NH_2, representing the minimal pharmacophore of Arg-Trp-rich antimicrobial peptides [2], was chosen for in depth analysis of the *in vivo* mechanism of action of this compound class and the bacterial adaptation strategies towards peptide exposure. The stress response of *Bacillus subtilis* to the peptide was investigated by proteomic profiling of both cytosolic and membrane fractions. Proteins involved in membrane and cell wall stress responses, energy metabolism, and amino acid biosynthesis were found upregulated after peptide stress. Differential scanning calorimetry, electron microscopy, and Western blot analyses revealed that the peptide integrates into the cytoplasmic membrane and deforms its structure, which leads to displacement of peripheral membrane proteins. This effect was shown for cytochrome *c* involved in respiration, MurG involved in cell wall biosynthesis, and the cell division regulation protein MinD. We propose that substantial energy limitation and loss of cell-wall integrity resulting from the delocalization of essential peripheral membrane proteins are the major factors that contribute to bacterial cell death. In response to this peptide bacteria adapt by adjusting their membrane and cell wall composition, upregulation of proteins involved in energy metabolism and cell wall biosynthesis, and synthesis of membrane-stabilizing proteins [3]. HPLC analysis provided evidence that *B. subtilis* synthesizes massive amounts of glutamate and aspartate and releases these amino acids into the culture medium, a response that was also observed after hypoosmotic shock. Sensitivity assays under different glutamate and salt concentrations showed that glutamate provides membrane protection by osmotic stabilization. As shown by mutant analysis, amino acid release is mediated by mechanosensitive channels, which are probably activated by membrane deformation due to peptide integration [3].
Our study provides a detailed model for the *in vivo* mechanism of action of RWRWRW-NH_2. These perspectives on the action of small cationic antimicrobial peptides, involving lipids and proteins, are complementary to the lipid-focused pore formation and carpet mechanism models.

References

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PRV09

Artilysins: Combination of a novel antibacterial mode of action, high effectivity against multidrug-resistant strains and persisters of *Pseudomonas aeruginosa* with a low risk of resistance formation

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Multi-resistant Gram-negative bacterial pathogens form an increasing global threat. Especially *P. aeruginosa* is well known for being highly resistant to antibiotics and being responsible for re-occurring infections.
Artilysins constitute a novel class of efficient enzyme-based antibacterials with a new mode of action. They are recombinant fusion proteins consisting of a bacteriophage-encoded endolysin, which degrades the peptidoglycan, combined with a targeting peptide that transfers the endolysin through the outer membrane of Gram-negative bacteria.
In contrast to the basic endolysin, Art-085 and Art-175 pass the outer membrane and kill *Pseudomonas aeruginosa*, including multidrug-resistant strains, in a rapid and efficient (~5 log) manner.
Time-lapse microscopy confirms that Art-175 punctures the peptidoglycan layer within a minute, inducing a bulging membrane and complete lysis. Minimal inhibitory concentration (MIC) experiments show Art-175 to be highly effective on*P. aeruginosa* with a MIC_{90} of 10µg/ml,including many strains that are highly resistant to several antibiotics. Resistance development against Art-085 and Art-175 is not observed within 20 experimental cycles of exposure to subinhibitory concentrations for all strains investigated, whereas a significant resistance development against ciprofloxacin occurred already within 7 cycles.
Due to its novel mode of action Art-175 does not require an active bacterial metabolism for its antibacterial activity, thus it has a superior bactericidal effect against *P. aeruginosa* persisters (up to more than 4 log reduction).
In summary, Artilysin Art-175 is representing a class of novel antibacterials well suited for a broad range of applications and with the unique potential to target persister-driven chronic infections.

PRV10

Molecular typing of Toxic shock syndrome toxin-1- and Enterotoxin A-producing Methicillin-sensitive Staphylococcus aureus isolates from a neonatal intensive care unit

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Objective: *Staphylococcus aureus* is one of the major causes of nosocomial infections in neonatal intensive care units (NICUs). Due to their immature immune system premature and very-low-birth-weight neonates are particularly susceptible to *S. aureus* infections. Strains may be acquired from colonized parents and healthcare-workers. We investigated infections and colonization in a NICU, caused by Toxic shock syndrome toxin-1 (TSST-1) - and Enterotoxin A (EntA) -producing Methicillin-sensitive *Staphylococcus aureus* (MSSA), by different typing methods.
Materials and Methods: The scenario comprised two NICU wards of a tertiary care hospital in Berlin. Depending on an assumed index patient (which was identified after 3 cases of serious infections) this clone spread within the two wards. However, only few infants were clinically affected, most of them were colonized. Infection control measures were commissioned by the local health department and supported by the RKI. The antimicrobial susceptibility, TSST-1- and EntA-production and presence of the respective toxin-genes were analyzed. Molecular typing was done by *spa*-typing and MLST. Initially relatedness of isolates was estimated by DNA microarray analysis and *Sma*I-macrorestriction. Furthermore whole genome sequencing was applied.
Results: From April to November 2012 162 MSSA were recovered from neonates, parents and healthcare-workers. 17 neonates, 4 healthcare-workers and 3 parents were tested positive for MSSA t021/ ST30, TSST-1- and EntA-positive. Based on microarray analysis 20 and on *Sma*I-macrorestriction 21 isolates were closely related. Isolates from 2 (microarray)/ 1 (*Sma*I-macrorestriction) neonates and 2 parents did not match with this cluster while non-related strains added for reasons of comparison clustered with the major clade. Whole genome sequencing indicated a close epidemiological linkage between most of the isolates, whereas the 3 isolates from parents, 1 isolate from a healthcare-worker and 1 isolate from a neonate were unrelated. Furthermore the analysis of genomic data enables us to develop different hypothesis on the transmission route(s) of this clone.
Conclusion: MSSA, exhibiting particular virulence factors, are facultative pathogens in NICUs and parents and healthcare-workers serve as possible reservoirs and transmitters. Discrimination of closely related strains even by a combination of sophisticated, molecular typing techniques might be limited and even misleading. Whole-genome sequencing provides new and more reliable insights into aspects of source-tracking, re-introduction and/or ongoing transmission of identical strain types at a local level.

PRV11

The undiscovered power of vitamins - fast and effective killing of multiresistant bacteria by light activation of Riboflavin derivatives

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The increasing emergence of multiresistant bacteria like Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important clinical challenges. Besides the development of novel antibiotics, other methods for effective killing of pathogenic bacteria have been considered. One of these methods is the photodynamic inactivation of bacteria (PIB).
The mechanism of PIB is based on positively charged, non-toxic dyes (photosensitizers, PS) that attach well to the negatively charged cell wall of bacteria. After excitation by visible light (400-700 nm) PS generate reactive oxygen species that lead to irreversible oxidative damage of bacterial cell wall structures and proteins. Based on vitamin B2 (Riboflavin) we developed new Flavin photosensitizers (FLASH-01a; FLASH-07a) that can eagerly attach to the bacterial surface and that are effective against different types of multiresistant bacteria. MRSA was incubated with different concentrations of FLASH-01a or FLASH-07a for 10 seconds and was subsequently irradiated with 50 mW/cm². A FLASH-01a concentration of 10 µM and a radiant exposure of 1.5 J/cm² resulted in a bacterial killing of 5 $\log_{10}$ orders (≥ 99.999%). Using 50 µM of FLASH-07a and a radiant exposure of 1 J/cm², the number of viable MRSA decreased up to 6 $\log_{10}$ steps (≥ 99.9999%), equivalent to high level disinfection. Similar results were achieved when enterohemorrhagic *Escherichia coli* (EHEC), multiresistant *Pseudomonas aeruginosa* and multiresistant *Acinetobacter baumannii* were irradiated with our new Flavins. The killing effect (≥ 99.9999% reduction of viable bacteria) was independent of the type of bacteria and its antibiotic resistance pattern. Additionally the cell toxicity of FLASH-01a and FLASH-07a was tested against normal human epidermal keratinocytes (NHEKs). NHEK cells were incubated with FLASH-01a or FLASH-07a with concentrations up to 100 µM and irradiated with the same light parameters as used for PIB. The results clearly showed that cell viability was not affected by both photosensitizers for radiant exposures up to 9 J/cm² (FLASH-07a) or 12 J/cm² (FLASH-01a). Multiresistant bacteria can be effectively killed by a clever combination of modified vitamin B2 molecules, visible light and oxygen. PIB with both Flavin derivatives shows a great potential of bacterial killing without harming the adjacent tissue and therefore may be a realistic prospect for future clinical use in humans.

PRV12

Using PhiSigns for rapid identification of bacteriophage isolates with lytic activity against multi-drug resistant pathogens

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Introduction: With the emerging threat of infections caused by multidrug-resistant bacteria and scarce prospects of newly introduced antibiotics in the future, viruses affecting bacteria (bactiophages) are currently being reconsidered as alternative therapeutics. However, despite many advantages over chemotherapy a number of obstacles have prevented phage therapy so far to become established in the Western countries as alternative treatment option. Future research should aim at providing confidence in the efficiency and safety of phages and at identifying and characterizing new phages with improved therapeutic potential.
Materials and Methods: In our study novel phages from hospital sewage samples with lytic activity against six clinical isolates of *Pseudomonas aeruginosa* were obtained with the double-layer plaque technique and subsequent standard isolation procedures. Using the same technique phages with lytic activity against nine clinical isolates of *Staphylococcus aureus* (incl. MRSA) were analyzed from a bacteriophage cocktail obtained from the Eliava Institute of Bacteriophages in Tiflis, Georgia. For further characterization signature genes of *P. aeruginosa* and *S. aureus* phages were identified from publicly available whole genome data using the online tool "PhiSigns" (http://www.phantome.org/phisigns/). Based on identified signature genes six broad-ranged PCR primer pairs where designed with which extracted DNA from the isolated phages was amplified.
Results: Sequence analysis of resulting PCR amplicons showed that phages against *P. aeruginosa* belonged to the phage family *Podoviridae* and were moderately related to LUZ24-like viruses (with 93% to 99% sequence identity depending on phage isolate). Phages against *S. aureus* grouped within the Twort-like viruses of the phage family *Myoviridae* (with sequence identities of 99% to 100% to the *S. aureus* phage ISP).
Discussion: PhiSigns enables a rapid classification of newly isolated phages. Based on this approach a high number of novel phage isolates can be preliminary screened and those of interest can then be selected for whole genome sequencing analysis.

PRV13

The inhibitory effect of a novel NA inhibitor on bacterial growth and biofilm formation of *Streptococcus pneumoniae*

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Introduction: *Streptococcus pneumoniae* is a major causative agent of severe pneumonia and sepsis. Bacterial growth as well as biofilm formation was shown to be influenced by neuraminidases (NAs). Thus, pneumococcal NAs represent a virulence factor which might act as a therapeutic target for novel NA inhibitors.
Materials and Methods: The susceptibility of a pneumococcal strain (serotype 1) against known NA inhibitors (oseltamivir, zanamivir) and a novel synthesized indazoledione was studied. The effect on *in vitro* biofilm formation was evaluated by the crystal violet microtiter plate method. In addition, the formation and vitality of the biofilm was investigated by scanning electron as well as fluorescence microscopy. Furthermore, the NA inhibitory effect of all compounds was analyzed using a newly established peanut lectin-based hemagglutination assay.
Results: Oseltamivir and zanamivir exhibited no inhibitory effect on bacterial growth or biofilm formation up to the maximum tested concentration of 50 µM. However, the indazoledione showed a strong inhibition of planktonic and biofilm-forming cells with a MIC_{90} of 23.0 ± 0.6 µM and a $MBIC_{90}$ of 14.3 ± 5.2 µM, respectively. The latter effect was confirmed by using scanning electron and fluorescence microscopy.
Due to the importance of NAs in biofilm formation described previously, we investigated the compound for its NA inhibitory activity in the lectin-based hemagglutination assay. Like oseltamivir, the indazoledione efficiently inhibited the activity of pneumococcal NA. The mean inhibitory concentrations were 2.1 ± 1.3 µM and 3.2 ± 0.0 µM, respectively. In contrast, zanamivir did not inhibit the bacterial enzyme up to the maximum tested concentration of 100 µM.
Discussion: These results clearly demonstrated that pneumococcal growth, biofilm formation, and NA activity can be inhibited by the studied indazoledione. Hence, this compound might be considered as a potential anti-pneumococcal agent.

PRV14

Estimation of the burden of common sequelae due to healthcare-associated neonatal sepsis

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Introduction: Calculating the burden of healthcare-associated infections (HAI) involves epidemiological challenge related to the attribution of disability to HAI and to the underlying comorbidity. To date, despite global efforts in the field of summary measures of population health, these challenges have prevented the estimation of HAI burden.
Objective: We conducted a systematic review, to describe the natural disease pathway (outcome tree), essential basic step to estimate the burden due to healthcare-associated neonatal sepsis.
Materials and Methods: Two authors searched independently for systematic reviews in EMBASE, Medline and Cochrane library. Cohort-studies from identified reviews were used for further analysis. Sepsis was defined as detection of bacteria in blood culture. As sequelae we considered: Cerebral palsy (CP), vision impairment (VI), hearing impairment (HI) and death. We performed random-effects meta-analysis of risk differences (RD) and compared these to absolute risks for sequelae among septicaemic neonates.
Results: Our systematic search revealed 207 reviews, of which 4 were eligible for full text evaluation and one fulfilled the inclusion criteria. From this, we extracted 8 cohort-studies (1994-2011) including ≈6000 infants. RD of CP was 8% (95%CI 6-10%), of VI 9% (95%CI 7-11%) and HI 4% (95%CI 2-10%). Absolute risks for these sequelae were ≈two-fold higher than RDs. Mortality data available from one cohort-study: absolute mortality was 38% (RD 14%).
Conclusions: It is crucial to address the background rates of sequelae in a healthcare setting, when estimating the burden of disease due to HAIs - RDs are a suitable tool. Using systematic approaches will increase transparency and reproducibility of burden estimates. We aim to compare the burden of HAIs to other infections to allow an evidence based approach for the description of the impact of infectious diseases in Europe.

PRV15

Evaluation of the adjustment of antimicrobial therapy to microbiological results at an intensive care unit in a regional hospital

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Introduction: Appropriate antimicrobial therapy is crucial to improve clinical success, reduce selection pressure and by this prevent development of resistance clones, and diminish the number of administered dosages, costs and side effects caused by inappropriate antibiotics. The aim of the current investigation was to determine the effect of a continuous antibiotic stewardship (ABS) on an intensive care unit (ICU) on the sampling frequency, duration of antimicrobial therapy, choice of substance, and appropriateness/adaption to cultured pathogens in comparison to a matched-cohort.
Materials and Methods: ABS comprised direct and immediate daily counselling of any individual microbiological report to the ICU staff by a specifically-trained pharmacist. In addition, microbiological grand rounds were provided directly on the ICU on a weekly basis. ABS intervention phase was conducted as of 12/2009 through 09/2010 to ensure optimization of antimicrobial therapy to any patient. Furthermore, advice on additional sampling was provided if mandatory. Patients within the intervention group were matched to a historical cohort within the same ICU within the time period from 10/2008 through 09/2009. The choice of antimicrobial compound, individual changes of the antibiotic regimen provided (escalation/de-escalation) and the number of sampled specimens for

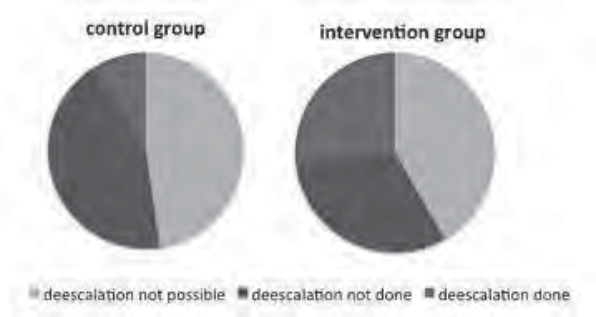
microbiological analyses were evaluated for all patients who were treated on the ICU for at least 5 days.
Results: The number of blood cultures drawn increased from 26% to 41% by implementing ABS. There was also an extended sampling approach to detect bacterial or fungal infections as shown in the table. In addition, the proportion of appropriate de-escalation of antimicrobial therapy increased significantly during the intervention phase (figure). The average duration of anti-infective substances also decreased significantly from 30.5 down to 26.3 days (p=.021). Significant changes in the use of antimicrobial compounds were noticed for two substances: decrease of 1st/2nd cephalosporins from .47 to .32 days of therapy per patient day (p=.047) and of metronidazole from .53 to .32 (p=.027).
Conclusion/Discussion: ABS yielded in both improved approaches to detect infections and rational use of treatment options minimizing the risk of resistance. ABS expert counselling is of pivotal importance in the management of the critically ill with infectious disease and may thus contribute to overall quality of patient care.

Figure 1

	control group (10/2008-09/2009) n=65			intervention group (10/2009-09/2010) n=60			p-value
	# patients	# samples	# samples per patient	# patients	# samples	# samples per patient	
TS / BAL (total)	44	267	6.1	49	218	4.5	p = .055
TS / BAL (non-sterile)	36	218	5.4	45	170	3.8	p = .061
BC (total)	17	25	1.5	25	92	3.7	p = .003
BC (non-sterile)	5	11	2.2	12	31	2.6	p = .267
intra-OP swabs (total)	17	36	2.1	20	38	1.9	p = .837
intra-OP swabs (non-sterile)	17	34	2.0	15	33	2.2	p = .491
CVC (total)	18	26	1.4	20	39	1.9	p = .827
CVC (non-sterile)	16	18	1.1	16	25	1.6	p = .036
thoracic drain (total)	12	16	1.3	11	21	1.9	p = .003
thoracic drain (non-sterile)	8	12	1.5	4	7	1.8	p = .685
wound swabs (total)	20	52	2.6	13	37	2.9	p = .212
wound swabs (non-sterile)	18	46	2.6	10	32	3.2	p = .107
urine (total)	17	32	1.9	25	44	1.8	p = .729
urine (non-sterile)	11	20	1.8	9	15	1.7	p = .115
stool (total)	6	13	2.2	9	16	1.8	p = .142
stool (non-sterile)	2	2	1.0	2	2	1.0	n. c.
other samples (total)	6	15	2.5	5	11	2.2	p = .245
other samples (non-sterile)	4	7	1.8	4	7	1.8	p = .414

TS = tracheal secrete; BAL = bronco alveolar lavage; BC = blood culture;
CVC = central vascular catheter; n. c. = not calculated due to a too small number of events

Figure 2



PRV16

Variability of linezolid concentrations after standard dosing in critically ill patients: a prospective observational study

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Introduction: Severe infections in intensive care patients show high morbidity and mortality rates. Linezolid is an antimicrobial drug frequently used in critically ill patients. Recent data indicate that there might be high variability of linezolid serum concentrations in intensive care patients receiving standard doses. This study was aimed to evaluate, whether standard dosing of linezolid leads to therapeutic serum concentrations in critically ill patients.
Materials and Methods: In this prospective observational study, thirty critically ill adult patients with suspected infections received standard dosing of 600 mg linezolid intravenously twice a day. Over 4 days, multiple serum samples were obtained from each patient, in order to determine the linezolid concentrations by liquid chromatography tandem mass spectrometry.
Results: A high inter-patient variability of serum linezolid concentrations was observed (range of area under the linezolid concentration time curve over 24 hours (AUC_{24}) 50.1 - 453.9 mg/L, median 143.3 mg*h/L; range of trough concentrations (C_{min}) < 0.13 - 14.49 mg/L, median 2.06 mg/L). A high intra-patient variability of C_{min}-values was also observed (range of maximum/minimum C_{min}-values 1 to 36). Furthermore, potentially subtherapeutic linezolid concentrations over 24 hours and at single time points (defined according to the literature and based on MIC_{90}-values of relevant bacteria as AUC_{24} < 200 mg*h/L and C_{min} < 2 mg/L) were observed in 63% and 50% of the patients, respectively. Potentially toxic levels (defined as AUC_{24} > 400 mg*h/L and C_{min} > 10 mg/L) were observed in 7% of the patients. Finally, in each of the different patient subgroups evaluated in this study (patients with and without continuous renal replacement therapy, extracorporeal lung assist, liver or lung transplantation), at least 40% of the patients had linezolid levels outside the therapeutic range.
Conclusions: A high variability of linezolid serum concentrations with a substantial incidence of potentially subtherapeutic levels was observed in intensive care patients receiving standard dosing. These findings suggest that therapeutic drug monitoring of linezolid for critically ill patients might be helpful for individualisation of linezolid dosing leading to optimized antimicrobial therapy for this patient group.

PRV17

S. aureus develops increased resistance against antibiotics by forming dynamic small colony variants during chronic osteomyelitis

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Objectives: *Staphylococcus aureus* is the predominant cause of osteomyelitis that has a high tendency to develop to chronicity despite antimicrobial treatments. As many chronic bone infections are caused by strains that have been tested sensitive to antibiotics in-vitro, the complex infection strategies of *S. aureus*, including host cell invasion and intracellular persistence via the formation of dynamic small colony variant (SCVs) phenotypes, could be responsible for therapy-refractory infection courses.
Materials and Methods: To test the efficacy of antibiotics in the acute and chronic stage of bone infections, we have established long-term in-vitro and in-vivo osteomyelitis models that develop to chronicity and subjected them to different antibiotic treatments. Antibiotics that were tested include b-

lactams, fluoroquinolones, vancomycin, linezolid, daptomycin, fosfomycin, gentamycin, rifampicin and clindamycin.

Results: Cell culture experiments revealed that all tested compounds reduced the bacterial numbers within infected osteoblasts when treatment was started immediately, whereas some antibiotics (particularly antibiotics inhibiting cell wall synthesis and acting in a bacteriostatic manner) lost their activity on intracellular persisting bacteria. Only rifampicin completely cleared infected osteoblasts in the acute and chronic stage. Furthermore, we detected that some compounds (gentamicin, fosfomycin, moxifloxacin, clindamycin) enhanced the formation of SCVs, which could even promote the development of chronic infections. To test defined antibiotics (rifampicin, gentamycin, cefuroxime) in-vivo, we treated a murine osteomyelitis model in the acute and in the chronic stage. Rifampicin and cefuroxim significantly reduced the bacterial load in bone tissue in the acute phase, whereas gentamicin was less effective, but strongly induced SCV-formation. In the chronic phase none of the antimicrobial compounds tested showed a beneficial effect on bone deformation or reduced the numbers of persisting bacteria.

Conclusion: In all infection models rifampicin was most effective to reduce intracellular bacteria. In the chronic stage, particularly in the in vivo model, many tested compounds lost activity against persisting bacteria and some antibiotics even induced SCV-formation. Our results shed light on the versatile and dynamic bacterial adaptation mechanisms that cause therapy-refractory chronic infections.

Figure 1

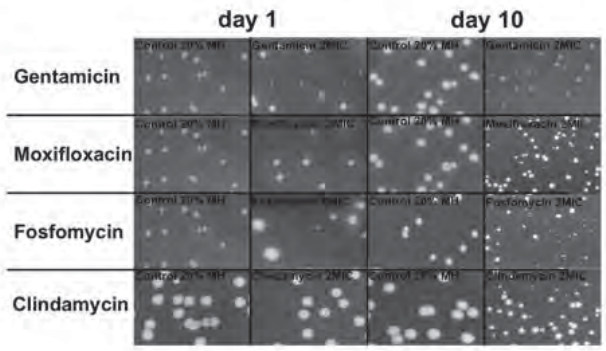

Fig. 1: Testing the effect of antibiotics to induce the SCV-formation in *S. aureus*

Figure 2

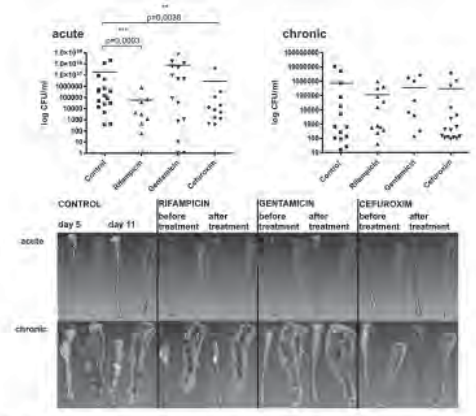

Fig. 2: Testing the efficacy of rifampicin, gentamycin and cefuroxim in treating a murine osteomyelitis model in the acute and chronic stage

PRV18

Emergence of daptomycin non-susceptible, vancomycin resistant *Enterococcus faecium* isolates in colonizing populations during empiric daptomycin therapy in patients after bone marrow transplantation

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Infections due to multi resistant pathogens are a significant cause of morbidity and mortality in aplastic patients after human stem cell transplantation (HSCT). Consequently, fever during neutropenia is aggressively treated, including vancomycin for gram-positive coverage. Given the significant clinical importance of vancomycin resistant enterocci (VRE) in this high-risk population, and with respect to an advantageous side effect profile, VRE-active lipopeptide daptomycin has become an interesting alternative to vancomycin in empiric escalation therapy during neutropenia.The emergence of daptomycin non-susceptible VRE during daptomycin therapy has been described. Here the hypothesis was tested that daptomycin therapy induces the emergence of daptomycin non-susceptible isolates in colonizing VRE populations. 11 vancomycin-resistant *E. faecium* strain pairs recovered from rectal surveillance swabs were available for analysis. Isolates from individual patients were collected with a minimum distance of three weeks. While all initial isolates exhibited daptomycin MICs within the wild type MIC distribution of *E. faecium* (MIC ≤ 4 µg/ml), in six patients a 2 - 3-fold daptomycin MIC increase was detected. All patients carrying daptomycin non-susceptible VRE (DNSE) received daptomycin (14 - 28 days), while only one patient in whom no DNSE emerged was treated with daptomycin for 2 days. Comparative whole genome sequencing identified DNSE-specific single nucleotide polymorphisms (SNP), including mutations in cardiolipin synthase Cls previously reported to contribute to increasing daptomycin MICs. Moreover, additional novel SNP in genes potentially relevant during DNSE development were discriminated, affecting membrane proteins, regulators or the cell wall synthesis machinery. No mutations in the *liaSFR* operon were detected. Mutations found in DNSE were not present in susceptible strains. This is the first report documenting the emergence of DNSE in colonizing VRE during empiric daptomycin treatment in HSCT patients. Genetic analysis not only demonstrated the relevance of Cls for daptomycin resistance, the heterogenous SNP pattern in DNSE underscores the multifactorial character of the DNS-phenotype. Although no endogenous infections caused by DNSE were observed, it is evident that induction of DNSE in colonizing VRE populations primes the spread of DNSE, with the subsequent risk of infections in high risk patients. Thus, the thoughtful use of daptomycin is critical.

PRV19

Impact of sub-inhibitory antibiotic concentrations on *Pseudomonas aeruginosa* physiology

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Introduction: The emergence of antibiotic resistant bacterial pathogens represents a steadily increasing threat to public health. During antimicrobial therapy drug concentrations may vary across a patient's body and microorganisms frequently encounter antibiotics gradients in their environments. As it is already known that, besides direct target site mutations, a variety of secondary mechanisms may contribute to antibiotic resistance, we aimed at investigating the response of bacteria upon sub-inhibitory antibiotic exposure to identify induced mechanisms that aid the evolution of resistances.

Materials and Methods: Whole transcriptome sequencing and stable isotope labelling were applied to elucidate the effects of sub-inhibitory concentrations of five structurally different antibacterial compounds (ciprofloxacin, tobramycin, meropenem, ceftazidime, and piperacillin) on *P.*

aeruginosa cells. Furthermore, intracellular hydroxyl radical formation was monitored by flow cytometry following staining with a radical sensitive dye.
Results: We observed a variety of specific transcriptional responses for the different compounds (DNA repair, cell envelope modification, ribosome regulation). However, there was also a number of common adaptations such as the induction of the SOS response and, interestingly, the induction of denitrification pathways. Both could be connected to global metabolic reactions as a consequence of intracellular reactive oxygen stress. Furthermore, we detected an enhanced biosynthesis of amino acids which are directly derived from tricarboxylic acid cycle-dependent or closely related pathways.
Discussion: Our findings suggest a number of common impacts of low-level bactericidal antimicrobials on bacterial cellular systems, independent of their specific chemical structure. The enhanced biosynthesis of particular amino acids and the transcriptional regulation of energy metabolism pathways imply that there is a connection between sub-inhibitory antibiotic responses and a hyper-activation of the TCA cycle.

MICROBIOTA, PROBIOTICS AND HOST

PWV01
Complete genome sequence of the probiotic Escherichia coli strain Nissle 1917

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In spite of *E. coli* Nissle 1917's (EcN) long-term use in medicine (trade name Mutaflor®), its comprehensive investigations by many research groups, and its proven clinical efficacy, the genomic DNA sequence of this probiotic bacterium has only been published as a draft (Cress et al. 2013). Here we report EcN's complete and annotated genome sequence. Genome sequencing was performed on a HiSeq2000 and a PacBio machine by GenXPro GmbH. In total 75,152 reads were generated. The determined chromosomal genome sequence contains 5,441,200 bp, has a GC-content of 50.6 % and encodes 5,324 ORFs, 117 tRNAs , 53 rRNAs and 3 complete prophages, as annotated by RAST and Interproscan. The closest related *E. coli* strains are uropathogenic strain CFT073 (genome size 5,231,428 bp; Welch et al. 2002) and asymptomatic bacteriuria causing strain ABU83972 (genome size 5,313,397 bp; Zdziarski et al. 2010). Only 190 ORF's are solely present in EcN, but absent in CFT073 and ABU83972. These genes represent about 3,5 % of EcN's genome. However, EcN is more similar to CFT073 with respect to genotype and phenotype than ABU83972 (Vejborg et al., 2010). The now available genome sequence of EcN will be helpful in identifying genes and gene products essential for this strains probiotic properties. One such gene cluster might be the yersiniabactin determinant which is strongly overexpressed under Mutaflor® production conditions in comparison to cultivation in LB broth in our lab as determined by transcriptome analysis.

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PWV02
The oral microbiome as a source of bacterial genes for insights into human population structure

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Introduction: The complexity of the human microbiome has led to an emerging view for the need for interdisciplinary research linking human microbial ecology with various subfields of anthropology. For instance, little is known so far about the microbiome variation among individuals from different geographic or ethnic origins. If parts of the microbiome are geographically structured this lends itself to the reversed and somewhat applied research question: can we deduce human population relationships and migration history based on the genetic variations within their microbiomes? This is a worthwhile question given that there is still controversy over how humans have migrated in prehistoric times, and given that studies on *H. pylori* have helped resolve some of these issues. Owing to the claim for easy and extensive sampling (which does not apply to stomach biopsies) we tested the hypothesis that *Streptococcus oralis* and *S. sanguinis* from human saliva are suitable markers for human population studies.
Materials and Methods: Saliva samples collected from healthy individuals world-wide were analyzed with a combination of Sanger and barcode amplicon sequencing and a focus on the glucosyltransferase gene (*gtf*). Sequence data were cured from erroneous sequences with PyroNoise and collapsed into operational taxonomic units (OTUs). Data were subjected to analysis of molecular variance (AMOVA), as well as UniFrac and haplotype network analysis.
Results: The analysis of roughly 500,000 gtf-sequences revealed that both streptococcus species exhibit a geographic signature of around 10%. Fst-values determined for any pair of countries (n=66) ranged from 0.002 to 0.38 (median 0.11) which exceeds Fst-values described for *H. pylori* ranging from 0.002 to 0.13 (median 0.04, n=210). With few exceptions both the *S. oralis* and *S. sanguinis*-dataset independently supported a tree structure in which European countries clustered together and branched with American countries to the exclusion of Asian and African countries. Haplotype network analysis ruled out homologous recombination as a frequent event and positioned each OTU in a reticulation compatible with its actual geographic origin.
Discussion: The current study provides proof-of-principle that members of the oral microbiome have a biogeographic structure and points towards investigations to establish specific saliva bacteria and their genes as a useful alternative to *H. pylori* for investigating human population structure and migrations.

PWV03
Comparative study of the microbiome of the abomasum in cattle with or without abomasal ulcers

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Abomasal ulcers are an important cause of indigestion in cattle and are usually not diagnosed until slaughter. The microbiome of the abomasum in cattle with and without ulcers has been studied very rarely until now, mostly using culture-dependent methods. The aim of the present study was to characterize the bacterial communities associated with abomasal ulcers of slaughter cows, steers and calves (n=42) in Austria using 16S rRNA gene-targeted pyrosequencing. The sequences were analyzed using the software mothur and classified into operational taxonomic units (OTUs). In total, 63 samples yielded 268,479 reads, of which 58.3% passed the quality control (average read length=202 bp). Sequences were clustered in 10,459 different OTUs (distance level=0.03). Additionally, near full-length 16S rRNA gene sequences of most abundant phylotypes were obtained by cloning and Sanger sequencing (n=88). Pyrosequencing reads affiliated to 28 phyla with *Proteobacteria*, *Firmicutes* and *Bacteroidetes* dominating (96.2% of all reads). The most abundant genera belonged to *Helicobacter*, *Granulibacter, Alloicoccus, Lactobacillus* and *Abiotrophia*-like. Significant differences between the microbial communities of calves compared to cows and steers

could be observed. However, no significant differences between the bacterial communities of healthy and ulcerated abomasal mucosa were found. In conclusion, our results allow the first deep insights into the composition of abomasal mucosal bacterial communities in cattle and describe a hitherto unknown high diversity and species richness of abomasal bacteria in cattle. Our results suggest that bacteria do not seem to be involved in the etiology of abomasal ulcers. Further investigations are required to determine the etiologic agents of abomasal ulcer formation.

PWV04

Proteome analysis of human sebaceous follicles reveals health- and disease-associated proteins of human and microbial origin

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Objective: Unlike the gut microbiota, the contribution of the skin microbiota to human health and disease is largely unknown. The pathobiological events leading to acne vulgaris are in the focus of this study. We analyzed sebaceous follicles of the skin and determined human and microbial proteins present in such follicles isolated from healthy individuals and acne patients.
Materials and Methods: Human follicles were extracted by cyanoacrylate biopsies and the protein content was determined by mass spectrometry (NanoESI-MS/MS).
Results: Health- and acne-associated human proteins were detected. Healthy follicles are enriched in proteins such as prohibitins and peroxiredoxins which are involved in the protection from various stresses, including reactive oxygen species. By contrast, acne-affected follicles specifically contained proteins involved in inflammation, wound healing and tissue remodeling. The most significant biological process among all acne-enriched proteins was 'response to a bacterium'. Identified bacterial proteins were exclusively from *Propionibacterium acnes*, supporting the role of *P. acnes* as an inducer of inflammation. In both diseased and healthy follicles the most abundant *P. acnes* proteins were surface-exposed dermatan sulphate adhesins, CAMP factors, and a so far uncharacterized lipase.
Conclusion: Our study shows that the host inflammatory reaction in acne could be explained as a response to *P. acnes*. Vimentin was exclusively expressed in acne-affected follicles and a new model is presented for vimentin-mediated invasion of *P. acnes* into follicle-associated cells that could account for the long-lasting inflammation and difficulties in antibiotic eradication known in acne treatment.

PWV05

Colicin M biology: Does ColM have a lethal effect on its producing bacteria?

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Colicin production is a common trait among the *Enterobacteriaceae*. Under stress conditions these protein toxins are secreted into the environment and kill close relatives, sensitive to these colicins. Colicin-producing bacteria are usually protected against self-killing by a specific colicin immunity protein. Thereby, colicins can be of advantage for the producer population under competitive situations. We identified an *E. coli* isolate (*E. coli* 252R) which secretes a colicin upon induction of the SOS response which leads to "self-killing". Using defined knock-out mutants we confirmed that the production of Colicin M (ColM; *cma*) is responsible for this "self-killing" effect. ColM is usually encoded with Colicin B (ColB) on a conjugative plasmid. The expression of both colicins is regulated by a common SOS-responsive promoter containing a LexA binding site, which is located upstream of the ColB activity gene (*cba*). The genes for the immunity proteins (*cmi* and *cbi*) are also located at the same locus but encoded in the reverse direction. We demonstrated that the ColM immunity protein (*cmi*) of *E. coli* 252R is expressed and functional. However, expression of the ColM immunity protein, in general, only partially protects against self-killing by ColM. In order to investigate, if ColM-mediated "self-killing" is common to all ColM producers we characterized a collection of 35 different *E. coli* strains, which were originally classified as ColM producers. Out of these 35 strains none showed ColM-mediated "self-killing". One third of the strains produced high amounts of ColM while exhibiting resistance against ColM-mediated "self-killing". Interestingly however, the majority of these strains carry insertion sequences (IS) in the *cba* gene which leads to disruption of the ColB activity gene (*cba*) and concomitant loss of the common *cba cma* promoter region. We show that these mutants do not produce ColM. Based on these results we hypothesize that ColM producers face an immense fitness cost due to high "self-toxicity" of ColM upon first acquisition of a ColBM plasmid by conjugation. Thus, mutations which lead to resistance against ColM (e.g. by disrupting ColM production) are positively selected. This explains the high abundance of environmental *E. coli* isolates which carry non-functional ColBM loci (Christenson JK and Gordon DM, 2009). One third of the strains seem to cope with ColM expression and release (ColM tolerance) by an unknown mechanism. Based on these data, we speculate that *E. coli* 252R might represent an isolate which just had acquired a functional ColBM plasmid and has not yet evolved towards either ColM tolerance or downregulation of ColM production.

PWV06

Quantitative Determination of Neurochemicals and their Derivatives in Dairy Products by HPLC with Amperometric and Fluorometric Detectors

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Introduction: Recently, much evidence has been presented that *neurochemicals* such as biogenic amines and regulatory amino acids are produced by a wide variety of microorganisms including those currently used as *probiotics*. Indesputably, microbial neurochemicals are likely to produce considerable effects on the health state and particularly the nervous system and the psyche of the probiotics' consumers. The goal of this work was to find out whether microbially fermented dairy products as well as milk per se (a "natural prebiotic preparation") contain neurochemically important substances.
Materials and Methods: The contents of biogenic amines (BAs) and regulatory amino acids (RAAs) in dairy products were measured using high-performance liquid chromatography (HPLC) on a reversed-phase column. The BAs were determined amperometrically with a glass-carbon electrode (+0.85 V), and the RAAs were complexated with ortho-phthalic aldehyde and determined fluorometrically (the excitatory and emission wavelengths were 230 and 392 nm, respectively).
Results: We revealed the presence of physiologically significant concentrations of BAs and RAAs in yogurts with lacto- and bifidobacterial cultures: Activia Natural® and BioBalance® STRAWBERRY that contained a fruit filler as well as in 2.5% ultrasterilized milk (Prostokvashino®). The tested samples contained 0.2-2 mM of norepinephrine, dopamine (that was not detected in milk), taurine, and glycin; these concentrations are considerably higher than those contained in body fluids [1]. The contents of the tested neurochemicals (except for glutamate) were 2-15 times higher in the microbially fermented dairy products than in the milk samples. Importantly, the tested dairy products contain micromolar amounts of DOPA, the catecholamine precursor (the highest DOPA concentration was revealed in the strawberry-flavored yogurt sample). DOPA crosses the gut-blood and blood-brain barriers. In the brain, DOPA is converted to dopamine and thereupon to norepinephrine that regulate important brain processes involved in locomotion, affection, sociable and dominant behavior, as well as aggression.
Discussion: The data presented above demonstrate that HPLC with amperometric or fluorometric detectors is an adequate and efficient technique enabling the detection of neurochemicals (BAs and RAAs) in dairy products. Our findings indicate that tested samples contain large amounts of neurochemicals that can significantly influence the health state, mental status, and social behavior of the dairy products' consumers.
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Quality Management in Diagnostic Microbiology

QSV01

Microbiological testing and assessment of cell based medicinal products - an area of conflict between pharmaceutical and clinical microbiology?

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Since the introduction of Directive 2004/23/EC and subsequently the German Tissue Law in 2007, tissue preparations and hematopoietic stems cells are regulated under the German Medicines Act as medicinal products, requiring a national licensing procedure. In ATMPs (advanced therapy medicinal products) national licensing of clinical trials and centralized licensing for marketing authorization is necessary. It was the first time, when challenges regarding the microbial safety in such cell-based preparations came into the focus of the authorities. The situation found in microbiological testing of those products was as heterogeneous as the preparations themselves. The type of sample may vary from a transport or rinsing solution, cell culturing media or cell suspensions to solid tissue samples. Sometimes the sample and the applied method were inadequate. In many cases, a validation of the method in presence of the sample material was missed. Requirements for testing of medicinal products for sterility, bioburden or exclusion of specific micro-organisms are defined in the European Pharmacopoeia (General chapters 2.6.1, 2.6.12, 2.6.13 and 2.6.27). Microbiological testing provides information on the quality of the sample itself, but the sample should be representative for the whole preparation in order to draw conclusions from sample to product quality. Within the scope of assessment of applications for cell-based products, PEI developed recommendations for a basic approach regarding a microbiological concept for those products. Another issue was the selection of appropriate methods and their evaluation for method suitability. Questions were raised, whether an additional testing for micro-organisms not detectable by usual pharmaceutical testing methods are needed for specific preparations. Testing of the source material for quality control and exclusion of objectionable micro-organisms, in-process-testing and final product testing is recommended as the basic microbiological concept. Where testing on the final product itself is not possible, it may be performed on adequate surrogate material. Furthermore, specific manufacturing procedures have to be considered, for instance manufacturing in closed systems or with decontamination steps. A new chapter regarding microbiological testing is compiled for inclusion in the next edition of the EDQM "Tissue and cell guide". Currently, assistance in planning of method suitability studies according to pharmaceutical requirements is one of the main topics. Regulatory requirements should support the manufacturers to stay flexible in the selection of the most appropriate approach and testing methods. Still, questions which should be addressed in this context, include endotoxin and mycoplasma testing and - if relevant - testing for fastidious or viable but not culturable micro-organisms.

QSV02

You will never talk alone - Bacteria on smartphone touchscreens in a German university setting and evaluation of two popular cleaning methods

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Smartphone and tablet touchscreens are well known as pathogen carriers in clinical environments [1]. However, despite a rapidly growing number of smartphones worldwide (1.3 billion in 2013; [2]), little is known about bacterial contamination of smartphone touchscreens in non-clinical settings. Such data are needed to better understand the hygienic relevance of these increasingly popular items. In this study, 60 touchscreens of smartphones provided by randomly chosen adult students of Furtwangen University were sampled by directly touching them with TSA contact agar plates. The average bacterial load of uncleaned touchscreens was 1.37 ± 0.33 CFU cm^{-2} (average ± standard deviation). Touchscreens wiped with commercially available microfiber cloths or alcohol-impregnated lens wipes contained significantly ($p << 0.001$) less bacteria than uncleaned touchscreens, i.e. 0.22 ± 0.10 CFU cm^{-2} and 0.06 ± 0.02 CFU cm^{-2}, respectively. Bacteria isolated from cleaned and uncleaned touchscreens were identified by means of MALDI-Biotyping using the direct smear approach and identification scores as described in [3]. Out of 111 bacterial isolates, 56 isolates (50%) were identified to genus level and 27 (24%) to species level. The vast majority of the identified bacteria were typical human skin, mouth, lung and intestinal commensals, mostly affiliated with the genera *Staphylococcus* and *Micrococcus*. Based on the German TRBA, five out of 10 identified species were opportunistic pathogens (*S. epidermidis*, *S. hominis*, *A. johnsonii*, *P. luteola*, *H. alvei*), suggesting the potential role of touchscreens as pathogen carriers also in non-clinical environments. Ongoing research in our group addresses the correlation of the microbial load of smartphone touchscreens with user data such as sex, age, frequency and method of cleaning, hygiene awareness etc. as well as the prevalence of antibiotic resistant bacteria on these items.

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National Reference Laboratories and Consiliary Laboratories

RKV01

Comparative genome analysis of 9 toxigenic *Corynebacterium ulcerans* strains by NGS reveals zoonotic transmission and rapid acquisition of pathogenic islands

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Introduction: Toxigenic *C. ulcerans* can cause a diphtheria-like illness in humans and was found to outnumber toxigenic *C. diphtheriae* in the last years. *C. ulcerans* can be found in domestic animals, which could serve as reservoirs for a zoonotic infection. To gain deeper insights into this transmission pathway we analyzed nine isolates derived from humans and their domestic animals by comparative genomics / NGS.
Materials and Methods: *C. ulcerans* strains of four patients and their domestic animals were isolated and the genomes were sequenced with a 250bp paired end Nextera (Illumina) library using an Illumina MiSeq next generation sequencer. The reads were assembled after quality control using SOAP *denovo.* Data analysis was carried out using Galaxy, Mauve, MEGA6.0 and BRIG.
Results*:* We obtained high quality genome wide data, which was mapped to the *C. ulcerans 809* reference genome (Trost el al. 2013), with a high median coverage of >50x for the 2.5 Mb genome. We found that the domestic/lifestock animal isolates only differ to a minor extend in the number of single nucleotide polymorphisms (SNPs) to their matched patient isolates, while the patient isolates showed a much higher variety in their SNPs among each other. Moreover, we found that Multi locus sequence typing (MLST) results are similar to NGS analysis, but NGS provides more detailed and robust data, proofing zoonotic transmission of *C. ulcerans* in the 4 analyzed cases. Furthermore, we found in a human isolate a prophage integration which was not present in the matched domestic animal. Interestingly, this prophage carries a putative virulence factor demonstrating that *C. ulcerans* can rapidly acquire pathogenic islands.
Conclusions*:* The genome-wide SNP analysis of the patient isolates and corresponding domestic animal isolates clearly shows a very high degree of similarity indicating the transmission of the *C. ulcerans* strains between the matched animals and humans. The superior resolution of NGS data compared to *e.g.* MLST data provides a new tool which will be helpful for outbreak analysis and which is able to detect pathogenic island acquired rapidly by horizontal gene transfer.

RKV02

Outcome of phenotypic and genotypic resistance guided *Helicobacter pylori* eradication therapy in German individuals

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Objectives: Assessment of the impact of phenotypic and/or genotypic antimicrobial susceptibility testing based therapy recommendations on treatment outcome in *H. pylori* infected individuals.
Patients and methods: From October 2004 to December 2012, a total of 1229 *H. pylori* positive patients with the majority (87%) having undergone at least one unsuccessful eradication therapy in the past were retrospectively enrolled. Antimicrobial susceptibility of *H. pylori* was tested either phenotypically by the Etest method (N=946; 77%) or genotypically straight from stomach biopsy by means of molecular genetic methods (N=283; 23%). Susceptibility testing based therapy recommendations were given, and treatment outcome was assessed by urea breath test, stool-antigen ELISA, histopathology or microbiology testing.
Results: Overall the most successful therapies were combinations of quinolones, rifabutin and proton pump inhibitor (PPI) (77,6% success) or amoxicillin and PPI (73,6%).
In particularly difficult-to-treat patients as defined by three or more therapy failures in the past, the most successful therapy regimens were combinations of amoxicillin, rifabutin and PPI (67,9%) or amoxicillin and PPI (69,0%). The underlying gastric disease did not have any impact on treatment outcome. Genotypic susceptibility testing proved to be far quicker and as reliable when compared with phenotypic testing.
Conclusions: Susceptibility testing guided eradication therapies achieve satisfying eradication rates in difficult-to-treat patients. As genotypic susceptibility testing of *H. pylori* is a quick and reliable method, it is to discuss whether it might replace phenotypic testing in future.

RKV03

Reference Diagnostics and Research at the New National Reference Center for Invasive Fungal Infection NRZMyk

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The German National Reference Center for Invasive Fungal Infections (NRZMyk, www.nrzmyk.de) is situated at the Leibniz-Institute for Natural Product Research and Infection Biology (Hans Knoell Institute) in Jena since 2014. Since then, we provide tools for the diagnosis of invasive mycoses and advise both clinical colleagues and public health services in all questions related to invasive fungal infection. A first overview of clinical cases will be presented. The NRZMyk hosts the *Jena Microbial Ressource Collection* as the major national strain collection for fungi, operating in close collaboration with the *German Collection of Microorganisms and Cell Cultures* (DSMZ). Scientists of the NRZMyk have actively participated in the Fungal Barcoding Consortium, establishing a database of molecular barcodes which serves as a global reference for the molecular identification of Mucorales. All activities of the NRZMyk are closely linked to research on human fungal pathogens. Thereby the NRZMyk contributes to national research initiatives like the collaborative research center FungiNet (SFB/TR124) and the BMBF funded consortium *InfectControl2020* as well as to the Jena research focus „sepsis and sepsis sequelae". Recently we participated in the identification of a novel genetic risk factor for invasive aspergillosis in the gene encoding pentraxin 3, a major antifungal molecule of human neutrophils. We coordinate the first genome-wide study on genetic risk factors for invasive aspergillosis (AspIRS). Based on these activities and in close cooperation with all mycologists and clinicians interested in mycology we hope to contribute to a continuous improvement of the clinical management of invasive fungal infections.

RKV04

Asymptomatic bacterial carriage in the elderly: a multicentre prevalence study of the network Invasive Bacterial Infections (IBI)

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Introduction: The network Invasive Bacterial Infections (IBI) has been constituted as part of the Robert Koch Institute initiative to create a Reference Laboratory Network in Germany. It was formed by the German reference laboratories for meningococci and H. influenzae, for pneumococci and for diphtheria. We report a multicentre study to determine the prevalence and risk factors of asymptomatic carriage of meningococci, pneumococci, *H. influenzae*, toxigenic *C. diphtheria*, and *S. aureus* in the nasopharynx of elderly people.
Materials and Methods: From October 2012 to May 2013, individuals from the regions Aachen, Oberschleißheim and Würzburg living in their own household or in nursing homes were invited to the study. Inclusion criteria comprised age ≥ 65 years and absence of an active infection. Bedridden people, patients with infectious diseases or under current antibiotic treatment were excluded. Potential risk factors for bacterial carriage were recorded in a standardized questionnaire.
Results: 950 volunteers with a median age of 77 were analysed (Aachen n=225; Oberschleißheim n=171; Würzburg: n=554). Preliminary data analysis revealed highest prevalence rates for *S. aureus* (25.2 %). MRSA prevalence was low (<1%). Nursing home was not a risk factor. For *H. influenzae*, only 13 isolates were found (12 unencapsulated [NTHi], one serotype e [Hie]) representing a prevalence of 1.4%. Carriage of meningococci (n=1) and pneumococci (n=0) proved to be a rare event.
Conclusions: This study is one of few examples analysing the prevalence of invasive bacterial pathogen carriage in the elderly. Although sampling methods might have provided some degree of underestimation, the low prevalence is surprising and might contribute to waning immunity events, which might explain increasing invasive infection rate in the very old population. Subsequent statistical analysis of risk factors will be presented.

RKV05

Serotype distribution and burden of pneumococcal disease in adults in Germany.
- Reaching the limit of herd protection? -

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Objectives: *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. In July 2006, vaccination with pneumococcal conjugate vaccine was generally recommended in Germany for all children ≤ 24 months. In this study, we present the burden of disease and serotype distribution among adults with invasive pneumococcal disease (IPD) before and after the start of childhood vaccination.
Materials and Methods: The GNRCS has monitored the epidemiology of IPD in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system, including over 300 laboratories throughout Germany. The present analysis includes cases documented between 1992 and 2013. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.
Results: In the first 14 seasons of our surveillance an average of 341 isolates from IPD among adults were received per season. Due to the introduction of a web-based surveillance system (Pneumoweb), these numbers could be increased to about 2000 in the last 6 seasons. 80% of the burden of disease for IPD in adults occurs at ages older than 50 years.
Before the introduction of childhood vaccination (1992-2006) the most prevalent serotypes among adults with IPD were 14, 3, 7F, 4, 23F, 1 and 9V. In 2013-2014 serotypes 3, 12F and 22F were most prevalent. Serotypes more prevalent in 2013-2014 as compared to the pre-vaccination era were 6C, 10A, 12F, 15A/C, 16F, 23A, 23B (PenR), 24F, 35B and 35F.
Before childhood vaccination 40-45% of IPD cases among adults were caused by PCV7 serotypes. After vaccination this percentage was gradually reduced to 5.7% in 2013-2014. This indicates a herd protection effect among adults. In 2009, higher valent vaccination (PCV10 (April 2009) and PCV13 (December 2009)) was introduced among children (current uptake: PCV10: 5%, PCV13: 95%). Among adults, a reduction of the percentage of IPD caused by the six additional serotypes from 47.1% in 2010-2011 to 31.6% in

2013-2014 was observed. The reduction was observed for serotypes 1, 7F and 19A. The amount of IPD cases caused by serotype 3 appears to be uninfluenced by childhood vaccination. The prevalence has remained between 14 and 15% since 2009.
Conclusions: Although a herd protection effect of PCV7 and PCV13 was observed, the burden of IPD among German adults remains high, underlining the importance of individual vaccination. The herd effect is not the same for all serotypes and is not observed for serotype 3.

RKV06
Diagnostic and epidemiologic aspects on Clostridium difficile infections in Germany - experiences from a multi-centre bi-annual point prevalence study in European countries (EUCLID)

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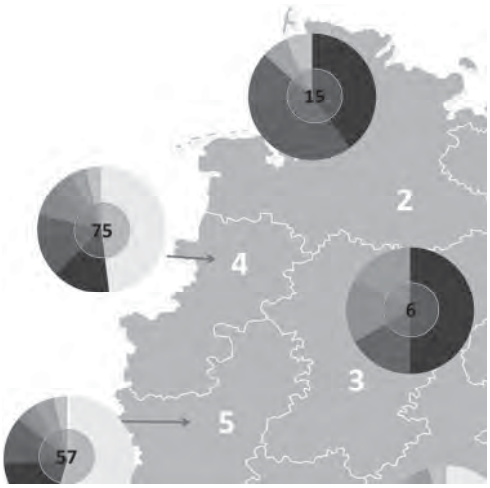
Objectives: The European bi-annual point prevalence study on C. difficile (EUCLID) was designed as a multicenter study for comparison of *C. difficile* epidemiology. Additionally C. difficile diagnostic algorithms of local laboratories were compared with those of global C. difficile infection (CDI) diagnostics of all diarrheal samples (national advisory laboratory).
Materials and Methods: A total number of 88 clinics located at different sites in Germany participated in this prospective cohort study. All diarrheal samples of one day in January and a second day in July 2013 were included. Global screening was performed by initially glutamate dehydrogenase (GDH) and toxin A/B (C.diff Quik Chek Complete) testing. GDH positive samples were confirmed by multiplex PCR (Genotype, HAIN) and anaerobic culture. All isolates were characterized by toxin and toxin gene testing as well as PCR ribotyping.
Results: In most clinics CDI diagnostics is focused on risk groups as e.g. patients hospitalized >3 days and also the diagnostic algorithm are different between local laboratories, most of them using sensitive screening plus conformational testing for toxigenic infections as recommended in current guidelines. Most previously undiagnosed CDI were detected in the advisory laboratory due to unrestricted screening of hospitalized patients with diarrhea and only to a minimal extend due to false negative testing in local laboratories. In total, 2156 stools were available but 99 samples were excluded due to low amount of material. Of 2057 study samples 472 (22.9%) were GDH positive and 255 (12.4%) were toxin A and B positive. 400 (19.5%) were confirmed by stool PCR, of those 368 were toxigenic (92%). Isolates were obtained from 424 samples (20.6%); of those 371 were toxin (87.5%) and 373 toxin gene positive (88%). PCR ribotyping revealed highest rates for ribotype 027 (21.7%), 001 (19.1%), 014 (10.1%), 002 (3.8%), 140 (3.1%), 015 (2.8%) and 078 (2.6%); however, with regional differences. Highest CDI prevalence was detected in older patients but also in children <10 years.
Conclusion: The emergence of ribotype 027 as a high prevalent strain has become a threatening scenario for Germany in recent years. Screening of all diarrheal samples from hospitalized patients for CDI increases detection rates an may help to optimized treatment and hygiene management of CDI in hospitals.

Figure 1



RKV07
Microbiological Investigation during an Outbreak of Legionellosis in Warstein, Germany, August 2013

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Legionella pneumophila causes mostly sporadic infections but also outbreaks in community settings and results in significant morbidity and mortality. An outbreak of Legionella pneumonia was reported from the city of Warstein, federal state of North Rhine-Westphalia, Germany. From August 1st to September 6th 159 cases of pneumonia occurred within the city. After September 6th no further infections were epidemiologically linked to the outbreak. 79 cases were confirmed as Legionella infection either by urinary antigen detection, culture and /or PCR in respiratory samples. 12 patients needed intensive care treatment. Two patients died. During the outbreaks, clinical and environmental isolates were typed. Current typing methods include phenotypic (monoclonal antibody subtyping) and genotypic (7 gene sequence based typing, SBT) methods. Clinical isolates from seven patients were characterized as L. pneumophila, serogroup 1, monoclonal subtype Knoxville (MAb 3-1 positive), sequence type (ST) 345. Direct sequence based typing performed on culture negative samples from three additional patients revealed the same strain as the causative agent. This epidemic strain was detected in two cooling towers of two different companies and several other environmental reservoirs. Of note, this strain was in the minority among the environmental isolates from most water samples. According to the European database (as by 30.05.2014) this ST has been isolated in additional 8 out of 5535 cases of pneumonia (0.14%) worldwide. 6 isolates were obtained from water samples unrelated to clinical cases. Thus, the epidemic strain is rare, but must be considered as a highly virulent one.

RKV08
Bartonella henselae serology - not as easy as commonly believed as

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Bartonella henselae infections are frequent diseases, however, they are often not recognized due to the low awareness of medical doctors and due to the often difficult to interpret laboratory Results: Since *B. henselae* was described to be the causative agent of cat scratch disease and bacillary angiomatosis in 1990, several serological diagnostic tools were developed, yet the diagnosis of a *B. henselae* infection sometimes remains intricate. The commonly used indirect immunofluorescence assay (IFA) consisting of infected Vero-cells for detection of *B. henselae*-IgG-antibody titers has been used for about two decades and has only undergone minor changes since that

time. We evaluated if *B. henselae* grown in a cheap and easy to handle liquid medium (a) could replace the laborious and costly production of cell-culture derived antigen and (b) whether liquid-grown *B. henselae* could replace agar-grown bacteria in the production of cell-culture derived antigen. Furthermore, we wanted to assess whether the use of particular strains and variants affects serological Results: Thus, we produced six cell culture-derived and liquid-grown antigens using *B. henselae Houston-1* (*expressing Bartonella* adhesin A, BadA), *B. henselae Houston-1 variant* (BadA negative) and *B. henselae Marseille* (BadA positive). As reference the commercially available serology kit from Euroimmun was used (IIFT *B. henselae* IgG, no. FI 219B-1010-1G), containing cells infected with *B. henselae Houston-1 variant*. Using these seven antigens we determined the antibody titers of 20 patient sera from a previously generated serum reference library. We found that non-cell culture-based IFAs (whole-cell antigen grown in liquid medium) are not feasible for diagnostics yet as they are hard to interpret and less reliable compared with cell culture derived antigen. Cell culture based IFAs using *B. henselae Houston-1 variant* grown in liquid medium and the commercial serology kit render equal and reliable titers. Yet, our results indicate that the used strain or variant of *B. henselae* strongly influences test Results: We have evidence that the difference between the two variants of *B. henselae Houston*-1 can be explained in part by the presence of BadA, a trimeric autotransporter adhesin on the surface of the bacteria. Our results demonstrate that the formulation and production as well as the used bacterial strain for the production of antigens greatly affect serological results in *Bartonella*-serology resulting in problems when comparing results from one laboratory with that of others.

REGULATION AND SIGNALING (INCL. STRESS RESPONSES)

RSV01
The stringent response and its impact on rRNA regulation in *S. aureus*

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Introduction: The stringent response is one of the most conserved regulatory mechanisms in bacteria, defined by the rapid synthesis of (p)ppGpp. This signal molecule impacts biosynthesis and uptake of amino acids, translation and replication as well as virulence, antibiotic tolerance and intracellular survival. The most conserved feature of the stringent response, namely down-regulation of rRNA synthesis, is vital for survival and adaptation to amino acid starvation. We characterised the properties and regulation of one rRNA operon in the important human pathogen *S. aureus*.
Materials and Methods: For measurement of promoter activity we cloned the original rRNA promoters ($P1^{GTTG}$, $P2^{TTGT}$) as well as altered promoters containing an ATP instead of GTP ($P1^{ATTG}$, $P1^{ATTA}$, $P2^{TTAT}$) in front of a truncated *gfp* gene and integrated these constructs into the chromosome. Promoter activity was assessed in the WT and a $(p)ppGpp^0$ synthetase mutant under different conditions. Corresponding nucleotide pools were measured by MS-HPLC.
Results: Analysis of the single promoters revealed that both, the native P1 and P2, are severely down-regulated under stringent conditions (mupirocin treatment) while the altered constructs remained unaffected. The presence of GTP in the initiation region at position +1 and/or +3 is required for full activity of both promoters. In a $(p)ppGpp^0$ strain, the rRNA promoter expression was not repressed by mupirocin treatment, independent of the GTP composition in the initiation region.
We were able to link this down-regulation with the GTP pool. Therefore we constructed a *guaBA* mutant which is guanosine auxotroph and depends on externally fed guanosine. We transduced the rRNA promoter constructs into the guaBA mutant, grew it in media with various guanosine concentrations and observed a clear correlation between the GTP pool and rRNA regulation in the native, but not the altered rRNA promoter constructs.
However, down-regulation of rRNA transcription during the growth cycle in the late exponential phase was found to be independent of (p)ppGpp and not correlated to the nucleotide pool.
Discussion: Our findings clearly support a model where a critical GTP within the initiation region of rRNA promoters is responsible for precise regulation of transcriptional activity and is highly dependent on the intracellular GTP pool. With this work we contribute to the understanding of rRNA regulating mechanisms in firmicutes, which seem to bee very different from proteobacteria.

RSV02
Oxygen control of alginate production in *Pseudomonas aeruginosa* by a signaling module comprising an oxidoreductase, a hydratase and a cyclic di-GMP synthase

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The majority of *Pseudomonas aeruginosa* strains isolated from chronically infected CF patients displays a mucoid phenotype. Mucoidy is caused by the production of the exopolysaccharide alginate, which is critical to the persistence of the bacteria in the CF lung. The signaling mechanisms responsible for the stimulation of alginate production under oxygen limitation - conditions common to the CF lung - are not well understood. Here we show that the deletion of the GGDEF-class diguanylate cyclase (DGC) gene *sadC* (PA4332) abolishes production of alginate under anaerobic growth conditions. The inhibitory effect appears to be due to the lack of cyclic di-GMP produced by SadC, as two amino acid substitutions in the catalytic DGC domain (G402A and E403A) were sufficient to abrogate alginate synthesis. While DGCs harboring oxygen-sensing activity have been described, sequence analysis of SadC did not reveal similarity to known oxygen sensing domains. However, the *sadC* gene is located in an operon with two other genes encoding proteins predicted to contain an enoyl-CoA hydratase domain (PA4330) and a Fe-S binding ferredoxin reductase (PA4331), respectively, which could be involved in oxygen sensing and signaling. We found that a PA4330 deletion mutant was defective in alginate production, whereas the deletion of PA4331 resulted in alginate production not only under anaerobic but also under aerobic conditions. Likewise, two amino acid substitutions (C72A and C74A) within the Fe-S binding domain of PA4331 resulted in constitutive production of the exopolysaccharide. Using HPLC-MS/MS to measure cyclic-di-GMP produced by a purified soluble DGC fragment of SadC, we found that the addition of purified PA4331 significantly decreased the amount of cyclic di-GMP. Incubation of a synthetic peptide comprising the DGC domain of SadC with PA4331 resulted in hydroxylation of a prolyl residue. We propose that SadC, PA4330 and PA4331 form a signaling module that directly senses oxygen and modulates cyclic di-GMP levels produced by SadC to regulate alginate synthesis.

RSV03
RNase E and the small RNA SinZ affect the 5' UTR of the acyl-homoserine lactone synthase gene in *Sinorhizobium meliloti*

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In *Sinorhizobium meliloti*, a nitrogen fixing plant symbiont, quorum sensing relies on N-acyl homoserine lactones (AHLs), produced by the AHL synthase SinI. In this work we analyzed the roles of the endoribonuclease RNase E and a small non-coding RNA in the expression of *sinI*. Overexpression of *rne* (the gene coding for RNase E) prevented the accumulation of AHLs at detectable levels and resulted in lower levels and shorter half-life of *sinI* mRNA. Moreover, using translational *sinI-egfp* fusions, we found that *sinI* expression is specifically descreased upon induced overexpression of *rne*. The 5'-UTR of *sinI* was sufficient for this effect. 5'-RACE analyses revealed a potential RNase E cleavage site in the 5'-UTR of *sinI* [1]. Furthermore we identified an sRNA (tentatively called SinZ) that shows imperfect complementarity to the 5'-UTR of *sinI*. Overexpression of *sinZ* resulted in a decrease in *sinI* mRNA level and AHL amount in an RNase E-dependent and Hfq-independent manner. Using an *in*

vivo reporter system with a *sinI-egfp* translational fusion, we were able to show a direct interaction between SinZ and the 5'-UTR of *sinI*.
SinZ is conserved among the Rhizobiaceae but its interaction with *sinI* is specific for *S. meliloti*. We performed CopraRNA [2] analyses to search for conserved targets. Among the predicted targets, that show a significant change in expression in a *sinZ* overexpression strain, are an anti-sigma factor and a transcriptional regulator. We also found that the response of SinZ to cold shock and sodium chloride stress is conserved in *Sinorhizobium*, *Rhizobium* and *Agrobacterium*. This indicates that SinZ is a conserved ribolegulator with a species-specific function, which allows to link quorum sensing with stress response in *S. meliloti*.

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RSV04
Phosphatase activity of the histidine kinases ensures pathway specificity of heme-responsive two-component systems in *Corynebacterium glutamicum*

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Two component systems (TCS) represent a prevalent way which bacteria use to sense a variety of different stimuli and respond to environmental conditions. The Gram-positive soil bacterium *Corynebacterium glutamicum* contains two homologous TCS which are both involved in the regulation of heme homeostasis. Heme serves as an important cofactor for proteins of diverse functions and is used as an alternative iron source by many bacterial species. Whereas the HrrSA system is crucial for utilization of heme as an alternative iron source by activating the expression of the heme oxygenase (*hmuO)* under iron limiting conditions [1], the TCS ChrSA is required to cope with high toxic heme levels by activating the expression of a putative heme exporter (*hrtBA)* [2]. Activity profiling of the ChrSA and HrrSA target gene reporters and growth experiments with deletion mutants revealed both of them to inherit distinct roles in the control of heme homeostasis [3]. HrrSA and ChrSA share a high sequence identity and are likely the result of a recent gene duplication event. Growth experiments of deletion mutants and *in vitro* phosphorylation assays revealed a high level of cross-talk between the closely related systems. Thus, the question arises by which mechanisms these pathways are insulated in *C. glutamicum* to allow specific signal transduction. To counteract detrimental cross-talk many sensor kinases are bifunctional and act as kinase and phosphatase on their cognate response regulator. *In vivo* reporter studies as well as *in vitro* phosphorylation assays demonstrated that both, HrrS and ChrS, are capable of specifically dephosphorylating their cognate response regulator and confirmed the importance of a conserved glutamine residue within a putative phosphatase domain (DxxxQ) for phosphatase activity. Here, we present first evidence for a highly specific phosphatase activity as a key mechanism for ensuring pathway specificity of the TCS HrrSA and ChrSA [3].

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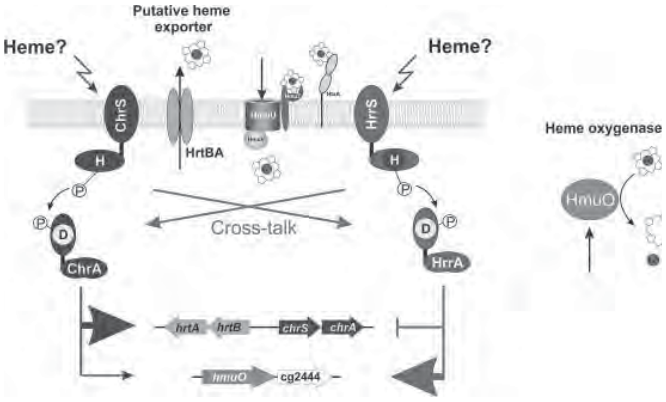
Figure 1



RSV05
Signaling Across the Membrane in *Escherichia coli* DcuS

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Aerobic and anaerobic C_4-dicarboxylate metabolism in *E. coli* is regulated by the two-component system DcuSR, comprising the membranous sensor histidine kinase DcuS and the associated response regulator DcuR [1]. Binding of C_4-dicarboxylates at the periplasmic portion of DcuS induces autophosphorylation of a cytoplasmic histidine residue as the starting point of a signaling cascade [2]. Hence, input and output of DcuS are physically separated and only connected through the second transmembrane helix (TM2). Repositioning of TM2 in the active signaling state of DcuS was studied using a scanning cysteine accessibility method (SCAM). Stimulation of DcuS by adding fumarate, removing the coregulating transporter DctA or introduction of PAS_C-ON-mutations [3] revealed considerable changes of TM2 localization. Compared to the non-induced state some previously hidden cysteine residues within the membrane became accessible while other ones lost their receptiveness to the cysteine label. Additionally, substitution of hypothetical inner membrane anchor tryptophans with highly charged arginines activated DcuS kinase and brought the TM2 position to the same signaling ON state. Thus, SCAM data and arginine mutagenesis suggest a piston type movement of TM2 towards the periplasm as the basis for transmembrane signaling in DcuS.

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RSV06
Understanding structural interactions and signal transduction within two component systems

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Two component systems (TCS) are the major systems used by bacteria to communicate with their environment. Although studied for more than three decades, it is still poorly understood how a signal is recognized and transduced by a TCS. A TCS is comprised of a sensor kinase and a response regulator. The model studied by our group is the Cpx-TCS which consists of three major proteins: the sensor kinase CpxA, the response regulator CpxR, and the accessory protein CpxP. To better understand the functionality and the dynamics of the Cpx-TCS, we investigated the affinities between all proteins.
First, we had to overcome the limitation of our established *in vitro* system [1]. CpxA incorporate into proteolipo-somes (CpxA-PLS), is inversely oriented. As a result, the periplasmic sensor domain of CpxA is not accessible for externally added CpxP. Consequently, we established the reconstitution of CpxA into nano-discs (CpxA-ND) and subjected to functionality analyses and spectroscopic interaction studies. Equal or improved kinase and phosphotransfer activities, respectively, could be shown using CpxA in nanodiscs compared to CpxA in proteoliposomes. Additionally, spectroscopic studies showed strong binding of CpxR to CpxA, which was controllable by adding Mg^{2+}-ATP. For immobilized CpxP

no interaction with CpxA-ND was detectable. This finding is in line with our observation that CpxP does not inhibit CpxA-ND.
From these cumulative results we hypothesize that the affinity between CpxA and CpxP is too low to be analysed in a freely diffusible system and that the weak transient interaction between both proteins is in living cells encouraged by the limited space between inner and outer membrane.

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RSV07

Dialkylresorcinols as novel bacterial cell-cell communication molecules in the human pathogen *Photorhabdus asymbiotica*

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Bacterial communication via small diffusible molecules to mediate group-coordinated behavior, referred to as quorum sensing, is well recognized. The prototypical quorum sensing system of Gram-negative bacteria consists of a LuxI-type autoinducer synthase that produces acyl-homoserine lactones (AHLs) as signals and a LuxR-type receptor that detects the AHLs to control expression of specific genes [1]. However, many bacteria possess LuxR homologs but lack a cognate LuxI-type AHL synthase. Those LuxR-type receptors are designated as LuxR orphans or solos. Recently we described, that the LuxR solo PluR of the insect pathogen *Photorhabdus luminescens* detects α-pyrones, named photopyrones (PPYs), instead of AHLs for cell-cell communication. PPYs are endogenously produced by the photopyrone synthase PpyS [2]. However the closely related insect and human pathogen *Photorhabdus asymbiotica* harbors the LuxR solo PauR that is homologous to PluR, but lacks any PpyS as well as any LuxI homolog. Therefore we were wondering about the specific signaling molecule that is sensed by PauR. We identified 2,5-dialkylresorinols (DARs), produced by the DarABC pathway [3], as signals that are specifically sensed by PauR. Upon induction with DARs, PauR activates the expression of the adjacent *pcf* operon, which results in cell clumping and contributes to pathogenicity. Generally LuxR-type proteins share six conserved amino acid residues in the signal-binding domain that are crucial for signal specificity. We identified specific amino acids in PauR that make up DAR sensing specificity and separates PauR from the pyrone sensor PluR and classical AHL-sensors. In summary, we showed that PauR and DarABC form a yet unknown endogenous cell-cell communication circuit in *P. asymbiotica*. Moreover, we identified DARs besides PPYs as novel bacterial cell-cell communication molecules, which demonstrated again that quorum sensing in Gram-negative bacteria goes far beyond AHL-signaling. As DarABC as well as LuxR solo homologs are also present in other, specifically human pathogens, cell-cell communication via DARs could be acquired specifically for human pathogenicity rather than communication via PPYs as alternative to AHL quorum sensing.

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RSV08

New insights in DNA binding modalities and gene regulation of the transcriptional regulator ComA in *Bacillus subtilis*

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Introduction: In *Bacillus subtilis*, the transcriptional regulator ComA is embedded in a complex cellular signaling network and is crucial for the development of genetic competence and sporulation. ComA is a response regulator belonging to the NarL-FixJ protein family and is phosphorylated by the membrane histidine kinase ComP. The activity of the ComPA two-component system is controlled by the small pheromone ComX which accumulates extracellular and acts as activation signal for ComP [1]. So far it is known that ComA binds to recognition elements (RE) within the target promoter region and thereby directly activates transcription [2,3]. The target gene promoters contain an inverted repeat (RE1+RE2) and a recently identified "half" repeat RE3 important for ComA-DNA interaction and transcriptional activation. Here we report experimental and computational evidences for a fourth RE that comprise a direct repeat together with RE3 and that is bound by ComA. This observation changes our view on the mechanism by which ComA was thought to work completely.
Materials and Methods: To examine the functionality of the newly found direct repeat on ComA-dependent gene regulation, we used fluorescence microscopy to determine the activity of truncated wild type and synthetic promoters *in vivo*. Recombinant protein overexpression and EMSA experiments were performed to investigate the physical interaction between ComA and direct repeats *in vitro*. Computational docking and molecular dynamic simulations were used to predict the structure of ComA bound to the direct repeat and to identify key molecular interactions.
Results: Sequence alignment of the known target promoters reveal the existence of a direct repeat consisting of RE3 and RE4 downstream of the inverted repeat. *In vivo* experiments show that the direct repeat alone is sufficient to activate ComA-dependent transcription. The relevance of key interactions between ComA and direct repeat were proved and evaluated by functionality experiments *in vivo* and ComA-DNA interaction studies *in vitro*.
Discussion: Our results suggest a new ComA-DNA binding pattern in which ComA dimers can bind to both, inverted and direct repeats to regulate transcription. Moreover we were able to investigate formerly unknown aspects of ComA-DNA interactions which remarkable contribute to understand the molecular mechanism of ComA-dependent gene regulation more deeply.

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RSV09

Multitasking by PII Proteins: Energy state sensing differentially affects NtcA activation and arginine synthesis in Cyanobacteria

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P_{II} signaling proteins comprise one of the most versatile signalling devices in nature operating on a highly conserved structure. In cyanobacteria, proteins PipX and *N*-acetyl-l-glutamate kinase (NAGK) are the paramount receptors of P_{II} signalling. These interactions control NtcA-dependent gene activation and the ornithine/arginine synthesis pathway, respectively. The PII interactions with PipX and NAGK are modulated by the PII effector molecules ADP, ATP, and 2-oxoglutarate. They bind interdependently to three anti-cooperative binding sites on the trimeric P_{II} protein thereby affecting its structure. Novel structures of Synechococcus PII revealed the sequential (asymmetric) occupation of its binding sites. Biochemical analysis were carried out to clarify the mutual influence of P_{II}-receptor interaction and sensing of the ATP/ADP ratio. In the presence of the P_{II}-receptor PipX, the affinity of ADP to the first binding site S1 strongly increases, whereas the affinity for ATP decreases owing to PipX favoring the S1 conformation of P_{II}-ADP. In consequence, P_{II}-PipX interaction is highly sensitive to subtle fluctuations in the ATP/ADP ratio. Thus, PipX is sequestered by PII when the ADP level raises and is no more able to bind the global transcriptional factor NtcA, although the 2-oxoglutarate concentration may remain high. Conversely, the DNA-binding activity of NtcA is strongly modulated by the binding of PipX in a DNA sequence-specific manner. Thus, different NtcA-dependent genes are differentially subjected to energy control via the network of interactions controlled by the PII signaling protein. The second path of PII signalling concerns ornithine/arginine synthesis control via the NAGK enzyme. Although the interaction between PII and NAGK is negatively affected by ADP, it is insensitive to subtle fluctuations of the ATP/ADP ratio. Recent experiments indicate even more targets of PII regulation. Modulation of the metabolite-sensing properties of P_{II} by its receptors allows P_{II} to differentially perceive signals in a target-specific manner and to perform multitasking signal

transduction.

RSV10
Regulation of the *Bacillus subtilis* ECF-sigma factor σ^X

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Bacillus subtilis encodes seven alternative sigma factors of the ECF family that control genes related to extracytoplasmic function. For the ECF sigma factor σ^W and its corresponding anti-sigma factor RsiW it has been shown that certain cell envelope stresses trigger degradation of the anti-sigma factor in a mechanism known as regulated intramembrane proteolysis (RIP), finally resulting in the release of σ^W and the transcription of σ^W-controlled genes. We are interested whether RIP of an ECF anti-sigma factor is the regulatory mechanism of other *B. subtilis* ECF sigma factors in general. The *B. subtilis* ECF sigma factor σ^X controls genes that are implicated in cell surface metabolism, regulation and cell envelope modification. RsiX was identified as the corresponding single-pass transmembrane anti-sigma factor. Distinct stress signals and the mechanism of σ^X release from RsiX are unknown so far. In order to analyze the involvement of RIP in σ^X regulation, a reporter system to monitor stability of RsiX was constructed using translational fusions to the GFP protein, and a transposon mutagenesis screen was performed. It became evident that the *sigX-rsiX* operon is silenced by the transcriptional terminator Rho by a mechanism of intracistronic polarity that seems to coordinate expression of *sigX* and *rsiX* that overlap by 62 basepairs. The Rho utilization site (*rut*) was mapped within the 5'-coding region of *rsiX* and could be destructed by five silent base pair exchanges. The mutation of *rut* enabled regulated expression of *rsiX* and the detection of a C-terminally truncated form of RsiX in membrane fractions of a *rasP* minus strain. Moreover, transcription of a σ^X promoter to *lacZ* fusion is clearly reduced in the absence of the RasP S2P-protease, suggesting that also σ^X activity is controlled by regulated intramembrane proteolysis of its anti-sigma factor.

RSV11
Interdependence between different layers of the cell envelope stress response in *Bacillus subtilis*

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The cell envelope is of vital importance for any bacterial cell and serves as a prime target for antimicrobial compounds. Consequently, in *B. subtilis* the response towards cell envelope stress involves several lines of defense: On the one hand, the expression of specific transport- and detoxification modules that directly remove or neutralize antimicrobials from their site of action. On the other hand, more unspecific mechanisms stabilize and protect the cell envelope as soon as damage is detected. Here we study the interdependence between these two lines of defense and scrutinize their correlations at the single cell level. To this end we focus on the effect of the ABC transporter BceAB and the UPP phosphatase BcrC, which are the major resistance determinants against bacitracin, on the expression of the *lia* operon of *B. subtilis*, which protects the cell envelope by a so far unknown mechanism. Besides an increased sensitivity of the Lia system towards bacitracin in *bceAB* and *bcrC* mutants, we found that noise levels in the expression of the Lia system differed markedly from that of a wild type population. Specifically, the *bceAB* mutant displayed an almost switch-like response with increasing inducer concentration and had lower noise levels than the wild type at intermediate inducer levels. This suggests that stochastic expression of primary resistance determinants might be the origin of the broadly heterogeneous induction of the secondary resistance conferred by the Lia system [1]. In contrast, a deletion of *bcrC* significantly increased the noise level in the Lia response and caused bimodal expression patterns. These observations suggest an intricate connection between primary and secondary resistance determinants and might give hints towards the underlying regulatory hierarchy within the cell envelope stress response of *B. subtilis*.

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RSV12
Pathogens talking to each other in dental biofilms: Crossfeeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*

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Polymicrobial biofilms are of large medical importance, but relatively little is known about the role of interspecies interactions for their physiology and virulence. Here we studied two human pathogens co-occuring in the oral cavity, the opportunistic fungus *Candida albicans* and the caries promoting bacterium *Streptococcus mutans*.
Methods used were co-cultivation experiments with green-fluorescent protein tagged whole-cell reporter strains and deletion mutants of *S. mutans*, fluorescent staining, scanning electron microscopy, metabolomics (GC/MS), microarray analysis, competence analysis on the single cell level using fluorescently labelled DNA, qPCR and qRT-PCR.
Dual-species biofilms reached higher biomass and cell numbers than mono-species biofilms and the production of extracellular polymeric substance (EPS) by *S. mutans* was strongly suppressed. To detect interkingdom communication, *C. albicans* was co-cultivated with a strain of *S. mutans* carrying a transcriptional fusion between a green fluorescent protein encoding gene and the promoter for *sigX*, the alternative sigma factor of *S. mutans* which is induced by quorum sensing signals. Strong induction of *sigX* was observed in dual-species biofilms, but not in single-species biofilms. Conditioned media from mixed biofilms but not from *C. albicans* or *S. mutans* cultivated alone activated *sigX* in the reporter strain. Deletion of *comS* encoding the synthesis of the XIP precursor abolished this activity, while deletion of *comC* encoding the CSP precursor had no effect. Transcriptome analysis of *S. mutans* confirmed induction of *comS*, *sigX*, bacteriocins and the downstream late competence genes, including fratricins, in dual-species biofilms.
We show here for the first time the stimulation of the complete quorum sensing system of *S. mutans* by a species from another kingdom, namely the fungus *C. albicans*, resulting in fundamentally changed virulence properties of the caries pathogen.

RSV13
Elucidating the function of the *mazEF* Toxin-Antitoxin System from *Staphylococcus aureus*

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Introduction: Toxin-Antitoxin (TA) systems are small genetic elements in prokaryotes that typically consist of a stable protein toxin that acts endogenously against vital cellular targets and an unstable protein or RNA antitoxin that counteracts toxicity. The human pathogen *Staphylococcus aureus* harbors at least four unrelated TA system families, one of which is of the *mazEF* type. The toxin MazF is an endoribonuclease that cleaves RNAs and as such could regulate gene expression. Although speculated to be involved in stress regulation or regulation, experimentally defined functions for this system are lacking to date.
Materials and Results: First, we validated the proposed cleavage of the *spa* transcript (encoding Protein A) by MazF *in vivo*. The *spa* transcript is cut at an UACAU site by MazF, six bases downstream of the transcriptional starting point. However, a second UACAU site located downstream of the first site was not cleaved and single base mutations of the upstream UACAU cleavage site also abolished cleavage, demonstrating strict sequence and likely structural specificity of MazF. Interestingly, mutation of the site to VUUV' (V and V'=[A, C or G]), an alternative proposed target sequence of *S. aureus* MazF, also abolished cleavage. Cuts of other VUUV' sites on *spa*

and other transcripts could not be observed. Inducible expression of *spa* verified that results from primer extension experiments were indeed due to cleavage and not an alternative transcription initiation site. Cleavage of transcripts with similar 5' untranslated regions and transcripts containing UACAU sites was not observed, suggesting that additional factors beyond a consensus sequence are required for cleavage. To identify further targets of MazF, we performed RNAseq with the *S. aureus* wild type and the *mazEF* mutant strain and quantified gene expression. We identified several differentially expressed genes. Amongst others, a regulator of hydrolase activity involved in penicillin sensitivity was strongly increased, as reflected by lower MIC values compared to the wild type. Indeed, survival upon penicillin challenge was strongly reduced. These results support the hypothesis that the *mazEF* TA system may function as a stress regulator.
Conclusion: *S. aureus* MazF is able to cleave mRNAs in a sequence specific manner and is therefore likely a regulator of gene expression. However, the extent to which MazF regulates genes is unknown and further research is being conducted to elucidate the MazF restrictome.

RSV14

Synechocystis sp. PCC 6803, a representative expressing multiple clock protein homologs

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Several Cyanobacteria are known to schedule their biological activities into a daily program. Use of an endogenous timing mechanism allows them to anticipate environmental changes accompanying the alternation of day and night. The model organism *Synechococcus elongatus* PCC 7942 harbors a true circadian clock consisting of only three proteins encoded by the *kaiABC* cluster. Interestingly, multiple *kai* gene copies are found in many cyanobacterial strains. Among them is *Synechocystis* sp. PCC 6803, which expresses one KaiA protein, three KaiB and three KaiC homologs, representing diverged subgroups of KaiC. *In vitro* and *in silico* analysis suggested that KaiA, KaiB1 and KaiC1 might constitute a functional oscillator (Wiegard *et al.* 2013) and deletion of the *kaiAB1C1* cluster resulted in impaired growth under light/dark cycles. Transcriptomic analyses revealed that *Synechocystis* coordinates its gene expression into a daily schedule, implying an endogenous temporal coordination but true circadian oscillations could not be detected (Beck *et al.* 2014).
Thus the question arises whether and how the presence of multiple Kai proteins leads to a modified timing mechanism in *Synechocystis*. We are analyzing the *in vitro* and *in vivo* behavior of the diverged Kai proteins to elucidate their function and putative interplay. Using specific antibodies for western blot analyses we could demonstrate that KaiA and all three KaiC proteins are expressed. In contrast to the cyclic protein abundance reported for *Synechococcus* KaiC, the amounts of all *Synechocystis* KaiC proteins seem to be rather constant under diurnal as well as circadian conditions. Less than one half of each KaiC protein was found in the cytosolic fraction and contrarily to *Synechococcus* even the KaiA protein was demonstrated to be partly membrane-associated. To learn more about the function of additional KaiC proteins and the diverged variants in general we are currently characterizing KaiC3, which represents a variant also present in *Microcystis* and *Cyanothece*. KaiC3 displays enzymatic activities that have been described for the well-studied *Synechococcus* homolog: it harbors a KaiA-independent kinase activity and seems to exhibit ATPase and ATP-Synthase activities as well.

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SECONDARY METABOLISM

SMV01

Proteomic analysis of *Scopulariopsis brevicaulis* LF580 and its mutant strain M26 for the production of the cyclodepsipeptides scopularide A and B

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Marine fungi are an excellent source for new natural products. However, their potential to produce bioactive compounds is insufficiently investigated. One good example for this is the *Scopulariopsis brevicaulis* strain LF580 isolated from the marine sponge *Tethya aurantium*. The strain is known as the producer of the scopularides A and B. These cyclodepsipeptides show distinct cytotoxic activity against tumor cell lines [Yu et al., 2008]. For this reason the strain was selected for genome analysis and optimization processes for the production of the two scopularides by random mutagenesis using UV radiation. For the screening of the mutant library for high producing scopularide A and B strains, we establish a novel fast and easy miniaturized screening approach. The method comprised a decreased cultivation volume, a fast extraction method and an optimized LC-MS analysis format [Kramer et al., 2014, accepted]. Using this approach, we could identify the mutant strain M26 demonstrating beside a changed morphological growth behavior also a faster growth combined with an enhanced production of the target metabolites. In order to gain a deeper understanding of the hitherto uncharacterized molecular mechanisms underlying the production of scopularide A and B by *S. brevicaulis* LF580, we applied quantitative proteomics to compare the proteomes extracted from the wild type strain and the mutant strain M26 harvested in the exponential growth phase. We have performed annotation and KEGG pathway analyses of the up- and down-regulated proteins by homology detection on the *S. brevicaulis* LF580 genomic sequences [Kumar et al., in preparation] using BLAST2GO tool. We hypothesize that our iTRAQ-based quantitative proteomic approach will provide novel biological information useful for the optimization of scopularide production by *S. brevicaulis* LF580.

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Kramer A, Paun L, Imhoff JF, Kempken F, Labes A (2014) Development and validation of a fast and optimized screening method for enhanced production of secondary metabolites using the marine *Scopulariopsis brevicaulis* strain LF580 producing anti-cancer active scopularide A and B. PLoS ONE. accepted
Kumar et al. (in preparation) Genome sequencing, assembly and annotation of a marine isolate of *Scopulariopsis brevicaulis* using three different next generation sequencing technologies.

SMV02

Engineering of *Aspergillus niger* for the production of secondary metabolites

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Filamentous fungi can each produce dozens of secondary metabolites which are attractive as therapeuticals, drugs, antimicrobials, flavour compounds and other high-value chemicals. Application of most fungal secondary metabolites is, however, hampered so far by the lack of suitable fermentation protocols for the producing strain and/or by low product titers. To overcome these limitations, we report here the engineering of the industrial fungus *Aspergillus niger* to produce high titers (up to 2,500 mg l^{-1}) of secondary metabolites belonging to the nonribosomal peptide class.
For a proof-of-concept study, we heterologously expressed the 351 kDa nonribosomal peptide synthetase ESYN from *Fusarium oxysporum* in *A. niger*. ESYN catalyzes the formation of cyclic depsipeptides of the enniatin familiy, which exhibit antimicrobial, antiviral and anticancer activities. The encoding gene *esyn1* was put under control of a tunable bacterial-fungal

hybrid promoter (Tet-on) which was switched on during early-exponential growth phase of *A. niger* cultures. The enniatins were isolated and purified by means of reverse phase chromatography and their identity and purity proven by tandem MS, NMR and X-ray crystallography. The initial yields of 1 mg l^{-1} of purified enniatins were increased about 1,000 fold by optimizing feeding conditions and the morphology of *A. niger* in liquid shake flask cultures. Further yield optimization (about 2.5 fold) was accomplished by cultivating *A. niger* in 5 l fed batch fermentations. Finally, an autonomous *A. niger* expression host was established, which was independent from feeding with the enniatin precursor D-2-hydroxyvaleric acid D-Hiv. This was achieved by constitutively expressing a fungal D-Hiv dehydrogenase in the *esyn1*-expressing *A. niger* strain, which used the intracellular α-ketovaleric acid pool to generate D-Hiv. This is the first report demonstrating that *A. niger* is a promising expression host for nonribosomal peptides with titers high enough to become industrially attractive. Application of the Tet-on system in *A. niger* allows precise control on the timing of product formation, thereby ensuring high yields and purity of the peptides produced.

SMV03

Elucidation of genes involved in the biosynthesis of the secondary metabolite sodorifen in *S. plymuthica* 4Rx13

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Microorganisms produce a variety of very complex natural compounds for diverse biological purposes, e.g. to increase their fitness, to communicate or as a repellent towards other microorganisms (1). Interestingly, the microbial potential of volatile metabolite synthesis has been overlooked and underestimated in the past. The rhizobacterium *S. plymuthica* 4Rx13 produces a rich volatile blend that includes a dominant compound with an unusual and unique structure. In a previous stud the nuclear magnetic resonance (NMR) was applied to elucidate the structure of the compound. This natural product was named sodorifen($C_{16}H_{26}$) and was shown to have a bicyclic ring structure entirely substituted with methyl groups (2). Due to the unusual structure of sodorifen, the biosynthetic pathway remains hitherto unknown. Thus, the strategy to unravel the biosynthesis of sodorifen was threefold: Firstly, the entire genome was sequenced and compared to other closely related *Serratia* species that are either sodorifen producers or non-producers (3). Hence, 138 unique and hypothetical genes were found. Secondly, a differential proteome approach was expected to show proteins that are up- and down-regulated under specific growth conditions. Thirdly, the transcriptome (RNA-Seq) established genes that are up regulated in comparison to non-producers. Subsequently, knockout mutants were generated. The phenotypic absence of sodorifen in the mutants was used as an indicator to search for target genes. One gene exhibited the respective phenotype. It belongs to a cluster of four co-transcribed genes that are possibly involved in the biosynthesis of the secondary metabolite sodorifen in *S. plymuthica* 4Rx13. The ko-mutant showed a volatile profile that was dramatically altered, not only was the sodorifen no longer present but also the belonging isomers and other compounds vanished from the profile. Instead a second compound that could be an interstage product or a precursor in the sodorifen biosynthesis was present. The structure elucidation with NMR depicted the similarity to sodorifen. The annotation of the gene as a cyclase appears to be the first reaction of the sodorifen biosynthesis. Additional mutants have to be studied in the future to find all genes that are involved in the biosynthesis of sodorifen.

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SMV04

Analysis of the biosynthesis of chlorinated pyrrole moieties from *Aster tataricus, Penicillium islandicum* and *Streptomyces albogriseolus*

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Astins are cyclic pentapeptides isolated from roots of the plant *Aster tataricus*. Astins show potent anti-tumour activity *in vivo* and *in vitro* (1). However, the amounts of astins that can be isolated from plants are very low and chemical synthesis is problematic. Therefore, the project aims at enhancing the production of astins using molecular genetics. Cyclochlorotine, a secondary metabolite with high similarity to astins, has been isolated from the fungus *Penicillium islandicum*. Cyclochlorotine is a hepatotoxic compound causing necrosis, vacuolation of liver cells and development of blood lakes (2). Because of the high similarity of the peptides (3), similar enzymes should be involved in the biosynthetic pathways. Both metabolites contain a dichlorinated pyrrole carboxylic acid derivative which is most likely derived from proline. It is assumed that chlorination occurs on the level of a peptidyl carrier protein (Pcp)-tethered pyrrole carboxylic acid moiety by a flavin-dependent halogenase. For genetic analysis the genome of *P. islandicum* was sequenced. A single gene for a potential halogenase was detected, cloned and heterologously expressed in *E. coli*. Other genes of the biosynthesis were not clustered together with the halogenase gene. With degenerated primers for pyrrole halogenases, a new halogenase in *Streptomyces albogriseolus* was found. Genome mining in the neighbourhood of the halogenase provided all necessary genes for the production of the Pcp-bound substrate for activity assays of pyrrole halogenases.

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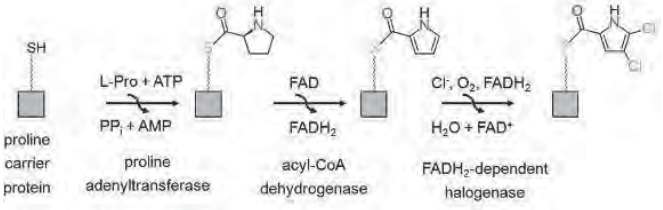
Figure 1



INTRACELLULAR TRANSPORT AND SECRETION

TSV01

Functional dissection of the tetracysteine motif of the competence ATPase PilF in *Thermus thermophilus* HB27

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Thermus thermophilus is the model bacterium for structural and functional analyses of DNA-transporters in Gram-negative thermophilic bacteria. *T. thermophilus* exhibits the highest known natural competence and is able to take up DNA from all three domains of life. The DNA-transport machinery is a highly complex system comprising of at least 16 proteins. Many of the DNA-transporter proteins show significant similarities to proteins of type IV pili and type II secretion systems and some proteins even play a dual role in DNA transport and in pili formation [1, 2]. Homopolymeric secretin complexes comprising of 12 secretin (PilQ) monomers guide pili through the outer membrane but are also suggested to guide the DNA through the outer membrane and the periplasm. To mediate pilus assembly and to power the DNA-translocator a AAA-ATPase, PilF, forming a hexameric complex, is required [3]. PilF has an unusual triplication of a general secretory pathway domain at its N-terminus and conserved Walker A and B motifs and a tetracysteine motif at its C-terminus. Functional analyses of the tetracysteine motif revealed that it mediates zinc binding [4]. Moreover, the

individual cysteine residues were found to be essential for heat-stability of the PilF complex and ATP and ADP were found to increase its thermostability. One of the four cysteine residues was essential for piliation and for pilus mediated functions such as twitching motility and adhesion. The finding that a pilus-defect mutant was unaffected in natural transformation led to the conclusion that pilus structures are not required for DNA transport and that the DNA-transporters and the type IV pili are distinct systems.

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TSV02

In vivo dynamics and regulation of the type III secretion injectisome

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Type III secretion systems are highly efficient inter-kingdom protein translocation devices used by Gram-negative bacteria to manipulate the behaviour of eukaryotic host cells. To analyse the type III secretion injectisome "in action" and study translocating injectisomes in live bacteria, we analysed functional fluorescently labelled injectisome components in *Yersinia enterocolitica*, using sensitive fluorescence microscopy at high temporal resolution. We found that at least one essential cytosolic component of the type III secretion system is dynamic, exchanging subunits between the injectisome and a cytosolic pool. Turnover correlates with the activation of the injectisome, linking exchange and function of the system.
Dynamic processes are targets for adaptation and regulation, which is currently poorly understood in type III secretion. As all our measurements are performed on live bacteria, we can analyse the regulation of the type III secretion system by subjecting the bacteria to various conditions that switch between different functional states of the injectisome. For the first time, this allows us to study the functional regulation of the type III secretion system in live cells, including the rearrangements of the injectisome upon activation and the enigmatic role of the proton-motive force in type III secretion. Our data reveals unexpected new aspects of the function and regulation of the type III secretion system *in vivo* and sheds light on the mechanism of protein translocation by the type III secretion injectisome.

TSV03

Structure and Function of a G+ Bacterial Type IV Secretion System (T4SS)

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Conjugative plasmid transfer is the most important means of spreading antibiotic resistance and virulence genes among bacteria and therefore presents a serious threat to human health. To date most well studied conjugative type IV secretion systems (T4SS) are of Gram-negative origin. Although many medically relevant pathogens are Gram-positive, their conjugation systems have received little attention so far.
We have studied the conjugation system of the multiple antibiotic resistance plasmid pIP501, which exhibits the broadest known host range for plasmid transfer in Gram-positive bacteria including enterococci, staphylococci and streptococci. Its transfer region is organized in an operon encoding fifteen putative transfer proteins. We have recently crystallized three of the conjugation proteins, TraK, TraM and TraN and solved their structures. The proteins are either putative members oft the T4SS core complex and/or fulfill a scaffolding function for building up the secretion machinery. In addition the TraN protein specifically recognizes double stranded DNA upstream of the *oriT* of pIP501. In this paper we present the structures of the conjugation proteins and their putative roles in the construction of the conjugation system.

TSV04

A Type Vi Secretion System Adaptor Protein Facilitates Translocation Of Diverse Effectors

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Vibrio cholerae is a Gram-negative bacterial pathogen that consists of over 200 serogroups with differing pathogenic potential. All *V. cholerae* strains sequenced to date harbour genes for the type VI secretion system (T6SS), a molecular puncturing device with homology to the tail-spike complex of the T4 bacteriophage that allows translocation of effectors into neighbouring eukaryotic and prokaryotic cells. Effector translocation is toxic unless the target cell produces an immunity protein against the cognate effector. We observed that the effectors encoded within otherwise conserved gene clusters differ widely among *V. cholerae* strains, allowing strains to co-exist or to outcompete each other. The mechanism that allows translocation of a diverse set of effectors by a conserved secretion system is currently unknown. Therefore, we set out to identify T6SS components required for the translocation of one effector with lipase activity, TseL. We investigated the genetic requirements for TseL-mediated prey killing and TseL-translocation into culture supernatants by western-blot analysis of pellet and supernatant samples of various T6SS mutants. We identified two mutants, V52Δ*vgrG-1* and V52Δ*VC1417,* which specifically lost their ability to translocate TseL and to kill in a TseL-mediated manner. VgrG-1 is believed to be part of a trimer complex at the tip of the T6SS, while VC1417 is a protein with hypothetical function that is encoded in a gene situated between *vgrG-1* and *tseL*. Bioinformatic analysis of VC1417 from two strains that encode a similar *vgrG-1* but different effectors reveal that the 3' end of *VC1417* co-segregates with the effector-encoding gene downstream. *VC1417* might serve as a recombination site for effector-encoding elements, giving rise to chimeric adaptor proteins that allow the attachment of diverse and newly acquired effectors to conserved components of the T6SS. Chimeric adaptor proteins like VC1417 may provide *V. cholerae* with a competitive advantage, because such a "mix-and-match" mechanism increases the combination of effectors a single T6SS can carry.

BIOLOGY OF CONVENTIONAL AND NON-CONVENTIONAL YEASTS

YEV01

Short-chain carboxylic acid transporters in *Yarrowia lipolytica*

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The yeast *Yarrowia lipolytica* is able to utilize various carbon sources, like glycolytic or hydrophobic substrates, but also carboxylic acids. However, only a few data exists on uptake of carboxylic acids. Undissociated forms of such weak acids can cross membranes by simple diffusion. In contrast the uptake of dissociated forms requires a mediating mechanism. In cases of *Saccharomyces cerevisiae* (*JEN1*) and *Kluyveromyces lactis* (*KlJEN1*, *KlJEN2*) a few of such mediated transport systems have already been reported. Sequence alignments in *Y. lipolytica* resulted in detection of a family of six putative carboxylate transporters (*YlJEN1-YlJEN6*) [1]. Here we report a first physiological and molecular characterization of these transporters, including examinations on gene expression during growth on different carboxylic acids as well as carboxylic acid production conditions. In doing so, we have observed an adapted gene expression due to changes in the available carbon source for selected *JEN* genes (mono-, di- and tricarboxylic acids). Further analysis revealed also growth defects on different carboxylic acids in strains deleted for certain *JEN* genes, which could be complemented by other JEN genes controlled by a constitutive promoter.

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YEV02

Engineering of glucose transporters into specific xylose transporters for simultaneous fermentation of glucose-xylose mixtures

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Recombinant xylose fermenting yeast strains prefer their natural substrate glucose over xylose and consume the two sugars sequentially in mixed sugar fermentations. We could previously show that this is due to the preference of the sugar uptake systems for glucose. It significantly increases fermentation times. Using an elaborate screening system we were able to engineer specific xylose transporters from *S. cerevisiae* glucose transporters. These mutant transporter versions do no longer transport glucose but only xylose, and xylose uptake is not inhibited by glucose. The transporters should prove valuable for fast glucose - xylose cofermentations.

YEV03

A novel yeast killer toxin attacking ribosomal RNA

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Some yeast species produce virus-like element (VLE) encoded killer toxins, which provide a selective advantage. The well studied killer toxins PaT from *Pichia acaciae* and zymocin from *Kluyveromyces lactis* act as tRNA anticodon nucleases cleaving the mcm^5-modified anticodon loops of $tRNA^{Gln}$ and $tRNA^{Glu}$, respectively [1,2]. The DrT toxin of *Debaryomyces robertsiae* is rather similar to PaT with respect to the amino acid sequence and also its molecular target, the $tRNA^{Gln}$ [3]. We identified the toxic subunit of another VLE-encoded toxin, PiT from *Pichia inositovora*, by conditional expression of *PiORF4* (ORF4 of the VLE pPin1-3). The gene shares regions of similarity with the toxic subunit of zymocin. In addition, catalytic residues of zymocin (E9 and H214) were found at conserved positions and were confirmed to be essential for toxicity. Hence, it was assumed that PiOrf4 represents another tRNA attacking toxin. However, in contrast to the known toxins, PiOrf4 did not require a modification of the wobble position of tRNAs for its toxicity, as mutants of the mcm^5-modification pathway (*elp3* and *trm9*) did not rescue the cells from toxin activity. Also, overexpression of mcm^5-modified tRNAs did not protect the cells from PiOrf4 action. Surprisingly, when the total RNA from cells expressing *PiORF4* was isolated, a decrease of the ribosomal RNA and the concomitant appearance of smaller fragments of the 25S and 18S rRNA was observed. A stable cleavage product of the 18S rRNA with a size of approximately 130 nt was purified and after linker ligation analysed by reverse transcription and sequencing. The 3`-termini of this fragment were mapped to nucleotide 131 and 132, a region showing some similarity to the anticodon loop of the zymocin target $tRNA^{Glu}$. Although the toxins have different targets and no clear sequence homology, their similar structure, the gene location on structurally related VLEs and conserved essential residues [4] possibly hint at a common evolutionary origin [5].

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YEV04

Ribosome Biogenesis in Yeast: Base Modifications in 18S rRNA.

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Ribosomes are highly complex macromolecular machines responsible for the synthesis of all cellular proteins. In eukaryotes, ribosome biogenesis needs the coordinated interaction of rRNAs and proteins. The 18S rRNA of the small 40S subunit, the 25S and the 5.8S rRNAs of the large subunit are processed from a 35S rRNA precursor. This precursor assembles with the 5S rRNA and the respective proteins to the 90S pre-ribosome at the nucleolus. During 35S rRNA processing a number of chemical modifications occur at the rRNAs including base modifications and 2'-O-methylations at specific nucleotides. The basic process of ribosome biogenesis is conserved within eukaryotes, but the most detailed results have been obtained with *Saccharomyces cerevisiae* as the model system.We identified the Nep1 (Emg1) protein family as an essential protein involved in ribosome biogenesis. Nep1 homologues are found in all eukaryotes and in some archaea. The yeast and the human Nep1 proteins are localized in the nucleolus and the human *Hs*Nep1 can complement the Nep1 function in a yeast *Δnep1* mutant. In yeast, S-adenosylmethionine (SAM), a cofactor for methyltransferases, restored growth of a temperature-sentisitve *Scnep1-1^{ts}* mutant indicating its function as a methyl transferase. The target site for Nep1 catalyzed base modification was identified as ψ1191. A mutation which abolished the yeast Nep1 RNA binding was also found as being responsible for the human Bown-Conradi-Syndrome (BCS). BCS causes growth retardation, psychomotor retardation, microcephaly and early child death. Analysis of yeast and human mutations showed that the mutated proteins lost their nucleolar location and their RNA-binding activity. Additionally, the human mutant protein hyperaggregated. Structure analysis of the Nep1 protein from the archaebacterium *M. jannaschii* unraveled two positively charged areas on the Nep1 dimer which interact with RNA. Our data suggest that Nep1 is a methyltransferase whose function is needed to remove sno RNA snR57 from the pre-rRNA before the Rps19 protein of the small subunit can associate to the pre-rRNA and that the essential phenotype of a *Δnep1* deletion could result from arrested ribosome biogenesis at the Rps19 assembly step. Further possible modifications within the 18S rRNA are discussed.

YEV05

Identification of novel base methyltransferases of the 25S rRNA in *Saccharomyces cerevisiae*

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RNA modifications are present in all three kingdoms of life and detected in all classes of cellular RNAs. RNA modifications are diverse, with more than 100 types of chemical modifications identified to date. These chemical modifications expand the topological repertoire of RNAs and are expected to fine-tune their functions. Ribosomal RNA (rRNA) contains two types of covalent modifications, either methylation on the sugar (Nm) or bases (mN), or base isomerization (conversion of uridine into pseudouridines,(Ψ). Pseudouridylations and ribose methylations are catalyzed by site-specific H/ACA and C/D box snoRNPs, respectively. The RNA component (snoRNA) of both types of snoRNPs is responsible for the site selection by base pairing with the rRNA substrate, whereas the protein component catalyzes the modification reaction. Contrastingly, base methylations are performed by snoRNA independent, 'protein-only', methyltransferases (MTases). rRNA modifications occur at highly conserved positions, all clustering around functional ribosomal sites. Mutations in factors involved in rRNA modification have been linked to severe human diseases (e.g. X-linked Dyskeratosis congenita). Emerging evidences indicate that heterogeneity in RNA modification prevails, i.e. not all positions are modified at all time, and the concept of 'specialized ribosomes' has been coined. 25S rRNA of the large subunit of ribosome in yeast was previously shown to contain seven base methylations: 2 m^1A (1-methy adenosine), 1 m^5C (5-methyl cytosine), 2 m^3U (3-methyl uridine) and 2 m^5U (5-methyl uridine), the precise location, physiological significance, and enzymes responsible for which remained unknown. Identification of these enzymes is absolutely instrumental to understand the significance of rRNA modifications in cell physiology. In the present study all base methylations of the 25S rRNA in yeast were subjected to comprehensive analysis, where each base methylation was accurately characterized and mapped using RP-HPLC (Reversed Phase High Performance Liquid Chromatography) and mass spectrometry. This analysis not only validated previously known base methylations but also demonstrated that the 25S rRNA of yeast contains two m^5C residues (only one m5C residue was known before), and lacks m^5U residues, as previously predicted. Furthermore using reverse genetics in combination with RP-HPLC and primer extension we identified all six base methyltransferases of the 25S rRNA. Taken together, the analyses of these modifications revealed that the base methylations play a significant role in 60S biogenesis and in antibiotic sensitivity. Surprisingly, most of rRNA modifications are dispensable for cell viability although the enzymes are essential for growth and ribosome biogenesis. This clearly highlights a dual

functionality of these enzymes in ribosome biogenesis. As ezymes and positions of most of these modifications are also highly conserved, the knowledge gained from yeast provides now an excellent opportunity to analyze the substrate specificities of their homologs in higher eukaryotes including humans.

YEV06

Biotechnological production of ω-hydroxy fatty acids with the help of metabolically engineered *Yarrowia lipolytica* strains

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It is known that the destruction of β-oxidation enables ω-oxidation of fatty acids in the non-conventional yeast *Yarrowia lipolytica*. Therefore strains lacking β-oxidation can be used for the biotechnological production of long chain α,ω-dicarboxylic acids [1]. Besides the production of dicarboxylic acids, the biotechnological production of ω-hydroxy fatty acids is of special economic interest since the chemical synthesis of both is not able without the formation of unwanted byproducts. ω-Hydroxy fatty acids can be used as monomers for the production of biobased plastics and as valuable components in lubricants, adhesives, cosmetic ingredients and anticancer therapeutics [2]. To enable the biotechnological production of ω-hydroxy fatty acids with the yeast *Y. lipolytica*, their degradation during ω-oxidation has to be stopped. Therefore eight relevant (fatty) alcohol dehydrogenase genes (*FADH*, *ADH1-7*) and one alcohol oxidase gene (*FAO1*) were identified in *Y. lipolytica* by comparative sequence analysis. All relevant genes were deleted in *Y. lipolytica* H222ΔP, a strain lacking β-oxidation by the deletion of the acyl-CoA oxidase genes (*POX1-6*). Hereby the deletion of the fatty alcohol oxidase gene, which has not been described yet in *Y. lipolytica*, exhibited the highest effect. Our results indicate that both (fatty) alcohol dehydrogenases and an alcohol oxidase are involved in ω-oxidation of long chain fatty acids whereby latter plays the major role. The *POX*, *ADH* and *FAO* deleted strain *Y. lipolytica* H222ΔPΔAΔF is able to convert *n*-alkanes to ω-hydroxy fatty acids in large quantities. The overexpression of *FAO1* can be further used to improve existing strains for the production of dicarboxylic acids.

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YEV07

Lipids from lignocellulose-grown oleaginous yeasts for food and biodiesel production

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Oleaginous yeasts such as *Lipomyces starkeyi* and *Rhodotorula glutinis* can convert carbohydrates into fatty acids at the highest known specific rates and the fatty acid content can exceed half of the total cell biomass. Carbohydrates, in the form of lignocellulose, are regarded as the main resource for a future fossil free renewable bio-based economy. Refining of side products such as pentoses to high value products must be improved to make lignocellulosic fuel production economical and resource efficient. We are developing a yeast-based system to convert the polysaccharides of pretreated lignocellulose to fatty acids and high value chemicals such as carotenoids. We have optimised analytical methods and cultivation conditions. Considerable differences in the fatty acid composition of different yeast species were found. Unraveling the physiological basis of e.g. forming differing amounts of unsaturated fatty acids such as linolenic acid will provide a basis for optimising the synthesis of essential fatty acids by yeasts, useful in food and feed formulations.

YEV08

The fraction of cells that resume growth after acetic acid addition is a strain-dependent parameter of acetic acid tolerance in *Saccharomyces cerevisiae*

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High acetic acid tolerance of *Saccharomyces cerevisiae* is a relevant phenotype in industrial biotechnology when using lignocellulosic hydrolysates as feedstock. A screening of 38 *S. cerevisiae* strains for tolerance to acetic acid revealed considerable differences, particularly with regard to the duration of the latency phase. In order to understand how this phenotype is quantitatively manifested, four strains exhibiting significant differences were studied in more detail. Our data show that the duration of the latency phase is primarily determined by the fraction of cells within the population that resume growth. Only this fraction contributed to the exponential growth observed after the latency phase, while all other cells persisted in a viable but non-proliferating state. A remarkable variation in the size of the fraction was observed among the tested strains differing by several orders of magnitude. In fact, only 11 out of 10^7 cells of the industrial bioethanol production strain Ethanol Red resumed growth after exposure to 157 mM acetic acid at pH 4.5, while this fraction was 3.6 x 10^6 (out of 10^7 cells) in the highly acetic acid tolerant isolate ATCC 96581. These strain-specific differences are genetically determined, and represented a valuable starting point to identify genetic targets for future strain improvement. In fact, we already identified genetic regions potentially carrying the crucial polymorphisms, and we are currently down-scaling these regions in order to identify the relevant genes and nucleotides.

ZOONOSES

ZOV01

Effect of Prophage Genes on the Growth of enterohemorrhagic *Escherichia coli* O157:H7 with 5-N-Acetyl-9-O-Acetylneuraminic Acid as a Carbon Source

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Enterohemorrhagic *Escherichia coli* (EHEC) are food-borne pathogens which can cause hemorrhagic colitis and the hemolytic-uremic syndrome (HUS). They are able to adhere to mucus-coated endothelium and proliferate competitively in the human large intestine. EHEC bacteria have therefore to compete with the autochthonous microbiota for carbon sources. All *E. coli* contain a chromosomal gene *nanS*, which encodes a 5-*N*-9-*O*-acetylneuraminic acid ($Neu5,9Ac_2$) esterase which cleaves the 9-*O*-acetyl group. The remaining 5-N-acetyl neuraminic acid can then be metabolized by proteins encoded in *nan* operon. Interestingly, Shiga toxin 2-encoding bacteriophage 933W of *E. coli* O157:H7 strain EDL933 encodes a homologue of NanS, which has been designated 933WP42. 933WP42 is also able to cleave $Neu5,9Ac_2$, which is an important compound of the human mucus. Analysis of the *E. coli* O157:H7 genome has shown that six further intact open reading frames with high sequence similarities to the 933WP42 encoding gene Z1466 were present. All these homologues are located in similar regions of different prophages. To clarify the question why 7 homologues genes putatively encoding $Neu5,9Ac_2$ esterases are present in EDL933 and other EHEC strains, deletions in these genes were constructed. Deletion of *nanS* and the corresponding EDL933 loci Z1466, Z3342, Z6054 and Z1793 were constructed in various combinations. Mutants were grown in M9 minimal medium using $Neu5,9Ac_2$ as a sole carbon source. Growth of single *nanS* or Z1466 deletion mutants did not show a decrease in growth compared to wild type EDL933. However, if both genes were deleted in the same strain, viable cell counts decreased by approximately 13 %. Further deletions of Z1466 homologues genes did not result in significant growth reduction as compared to the double deletion mutant, lacking Z1466 and *nanS*. The results of this study show that 933WP42 and NanS have an important role in the utilization of $Neu5,9Ac_2$. The other genes may probably have different enzymatic functions. Future analysis includes the characterization of the enzymatic activity of the six 933WP42 homologies genes and their function.

ZOV02

Multilocus Sequence Typing (MLST) for the identification of transmission routes of *Streptococcus gallolyticus* subsp. *gallolyticus*

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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is a commensal and facultative pathogen of the gastrointestinal tract in animals and humans. Traditionally, it is known as a member of *S. bovis* biotype I group. This opportunistic bacterium may cause endocarditis, septicemia and meningitis. The number of infections is increasing, but the transmission routes and the zoonotic potential remain unknown. Systematic analyses of an isolate of a blood culture from an infective endocarditis patient and fecal animal isolates of the associated laying hen farm should be pointed out to examine transmission routes and population structures of SGG.
Materials and Methods: SGG strains were isolated from pooled feces samples of laying hens from four groups of a multi-age system. Droppings from laying hens were collected at different time points (Apr., Dec. 2013; Feb. and Apr. 2014). Furthermore, stored manure and samples of dust were screened for SGG and information concerning their breeders and time of delivery of hens to the farm were included into the study. Multilocus sequence typing (MLST) serves as a basis for the characterization of the isolates of fecal laying hen.
Results: MLST analyses divided 34 isolates into seven sequence types (ST) that form two distinct clusters. In Apr. 2013, the same ST 13 was found in the human blood culture isolate as well as isolates from feces of two laying hen groups. Additionally, at this time ST 52 was detected. Since Dec. 2013 ST 52 is absent. Further analyses identified the STs ST 54/57/58/59/60 as well as ST 13 across the multi-age laying hen farm using systematic sampling. ST 57 was also isolated from samples of dust (from the surrounding of hen group one and two). eBURST proposes ST 58 as the predicted primary founder of these SGG isolates (ST 13, ST 57). Every time when pullets moved to the farm SGG was not identified, but was detectable after a few months. Isolates from different hen groups, breeders and times of delivery or sampling can be found in the same clusters. Determination of minimal inhibition concentrations show ciprofloxacin resistance (> 2 µg/ml ciprofloxacin) of all isolates with the exception of ST 52 isolates.
Discussion: The novel MLST scheme provides the basics to analyze the population structures and infection chains. This systematic approach resulted in a more representative view concerning the zoonotic potential of SGG. Our data demonstrate the independence of the transmission of SGG from the breeder of laying hens and time points of sampling and delivery of hens. The founding of ST 57 in dust particles as well as in feces of laying hens supports the hypotheses that contaminated environment can cause a time dependent colonization of the gut of animals. In addition, the spreading of different STs in hen groups of the multi-age system and the environmental occurrence supports our assumption of indirect and direct transmission routes of SGG within the laying hen farm.

ZOV03

Humoral immune response against different surface and virulence-associated *Chlamydia abortus* antigens in ovine and human abortion

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Introduction: The obligate intracellular bacterium *Chlamydia* (*C.*) *abortus* is the causative agent of enzootic abortion of ewes (EAE) and poses a significant zoonotic risk for pregnant women. Only very little is known about the epidemiology, transmission and clinical relevance, as there is currently no diagnostic tool to monitor these issues.
Materials and Methods: In previous work, we identified 48 immunoreactive *C. abortus* proteins using serological proteomic analysis and screening of a gene expression library. Here, we selected surface (MOMP, MIP and pmp13G) and virulence-associated (CPAF and TARP) as well as putative type-3-secreted proteins (CAB063, CAB408 and CAB821) for recombinant synthesis and development of a line assay to characterize the antibody response against these antigens.
Results: In a recently published model of experimental ovine abortion, sheep were intranasally infected with high versus low doses of infectious chlamydial elementary bodies. We investigated sera from these sheep and found that animals infected with high doses developed antibodies only against pmp13G two to five weeks post infection (sensitivity and specificity >90%). In the following observation period, anti-pmp13G-antibodies decreased below the detection level while none of these animals aborted. In contrast, in animals infected with low concentrations of chlamydial elementary bodies, antibodies were not detectable until the time of abortion. CPAF, MIP and pmp13G (sensitivity and specificity >90%) were the major antigens and antibodies persisted for months after abortion. In addition, we identified MIP and pmp13G as major antigens in naturally infected sheep as well as in women with septic abortion.
Discussion: We conclude that the selected proteins may be promising candidates for serodiagnosis of *C. abortus* infections both in animals and in humans.

ZOV04

Antigenic Mycobacterial Lipopeptides - Investigations in the Guinea Pig Model -

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Introduction: Tuberculosis, caused by *Mycobacterium tuberculosis* as well as *M. bovis*, causes an immense disease burden worldwide. Improved diagnostic tools and reliable, effective prevention strategies are urgently required. Two recent publications (Bastian et al., JI, 2008; Seshadri et al., JI, 2013) describe a highly immunogenic class of MHC-II restricted lipopeptide antigens of mycobacteria of the tuberculosis complex. Aim of this work is to further characterize the immunogenicity of this so far uncharacterized class of antigens *in vitro* and *ex vivo* using guinea pigs as small animal model for tuberculosis.
Materials and Methods: We use guinea pigs as animal model because they are sensitive to mycobacterial infections and develop a disease pattern similar to humans and cattle.
To test for the immunogenicity of lipopeptides *ex vivo* we sensitize guinea pigs with the mycobacterial vaccine strain, BCG. After four weeks we obtain blood, isolate the PBMC, stain with CFSE and stimulate them with different mycobacterial chloroform methanol extracts (CME) or a lipopeptide enriched subfraction (LF). For comparison we use Tuberculin (PPD), the most widely used diagnostic TB antigen. After 5 days, loss of CFSE staining is determined by flowcytometry as a measure for antigen-specific T cell activation.
To test for the antigen-specificity of responding T cells we sort the proliferated fraction by Magnetic-Activated-Cell-Sorting (MACS®). Subsequently, the sorted populations are re-stimulated with PPD, CME or LF.
Results: CME and the lipopeptide enriched subfraction induce a robust T cell proliferation, which is comparable to the PPD response. In accordance with the cited publications the majority of responding cells are $CD4^+$ T cells. Protease treatment or delipidation of CME or PPD significantly reduces the T cell response to the pretreated antigens. This indicates that the respective antigens contain both a peptide- and a lipid-moiety, which are both important for their stimulatory potential. In restimulation assays we observe that PPD-specific T cells respond comparably to PPD, CME and LF. Vice versa, CME expanded T cells recognize PPD, CME and LF.
Discussion: From our observations we conclude that mycobacterial lipopeptides are highly immunogenic and induce strong and antigen-specific T cell responses. The findings with antigen-specifically expanded, sorted T cells suggest that the highly immunogenic lipopetides are present in PPD and CME although both preparations differ substantially in their composition. Ongoing efforts aim to define this class of lipopeptide antigens and to determine whether they are specific for mycobacteria of the tuberculosis complex.

ZOV05

Interaction of the *Streptococcus canis* M-like Protein SCM with host proteins

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Introduction: *Streptococcus canis* is considered a neglected zoonotic species causing disease in domestic carnivores and human. Pathogenic streptococcal species have evolved a variety of mechanisms to escape the immune response of the host. For example, the decoration of the bacterial surface may serve as a protective shield or a proteolytic arm depending on the type of host protein. This investigation describes the molecular interaction of SCM, the M-like protein of *S. canis* with plasminogen and IgG and gives first insights into its role in pathogenesis.
Methods and Results: Binding studies with iodinated proteins as well as FACS analysis using SCM- positive and SCM-negative bacteria clearly demonstrate that SCM binds to IgG and plasminogen. In accordance to its zoonotic nature, *S. canis* binds to host proteins from different species including dogs, cats, horses, mice, rabbits, and humans. Furthermore, a simultaneous interaction of SCM with both, IgG and plasminogen, suggests that the specific binding sites are separated on the SCM protein. Indeed, by generating numerous C-terminal, N-terminal, and specific knock-out fragments of SCM we were able to narrow down the binding sites for plasminogen to amino acid 93 to 121 and for IgG to amino acid 172 to 202 of mature SCM. To further dissect the interactions of the proteins we used Dot Blot analysis. That clearly demonstrated that SCM specifically binds to the IgG subtypes I, 2, and 4 but not subtype 3. Furthermore, the SCM-IgG interaction occurs in a non-opsonic manner, since SCM bound specifically to Fc fragments but showed no affinity to the papain-treated IgG fragment Fab. Plasminogen is bound by its C-terminal part termed mini-Plg, defining SCM as the first proteinous bacterial receptor for the physiological cleavage product of the zymogen Plg. The recruitment of mini-Plg or its preoteoyltically active form mini-plasmin to the bacterial surface allows *S. canis* to degrade fibrinous structures, a hallmark for establishing invasive diseases. Although *S. canis* possessed no cysteine protease activity under *in vitro* conditions, Western Blot analysis revealed the presence of SCM in the culture supernatant. Soluble SCM is able to form protein complexes when incubated in human plasma. These aggregates are composed of a variety of host proteins including Plg but solely rely on the interaction between SCM and IgG.
Discussion: The genus streptococcus has evolved a variety of mechanisms to circumvent host immune defense. Our data describe two of them relying on the M-like protein SCM. Further investigations will elucidate detailed consequences for pathogenicity and its relevance for new therapeutical strategies.

ZOV06

Shiga toxin glycosphingolipid receptors of dog kidney epithelial MDCK II cells and their association with lipid rafts

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Introduction: Shiga toxins (Stxs) released by enterohemorrhagic *Escherichia coli* (EHEC), a greatly feared subgroup of Stx-producing *E. coli* (STEC), are the major and so far the best characterized EHEC-derived virulence factors. They are responsible for severe extraintestinal complications such as the life-threatening hemolytic-uremic syndrome [1]. The commonly used target cells for *in vitro* evaluation of Stx-mediated cellular cytotoxicity are monkey kidney-derived epithelial Vero cells, which are highly sensitive to Stxs and express the Stx-receptor glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer). Here we asked whether epithelial Madin-Darby Canine Kidney (MDCK) cells, which were originally isolated from the kidney of a female Cocker Spaniel, express Stx-receptors Gb3Cer and/or Gb4Cer and whether this cell type exhibits comparable toxin susceptibility like Vero cells. We employed MDCK II cells (a certain subtype of the MDCK cell line), which have become a widely used model for studying epithelial development and function [2].
Materials and Methods: MDCK II cells were cultivated in EMEM medium supplemented with 10% fetal calf serum. Lipids were extracted from confluent grown cells and neutral GSLs were isolated by anion-exchange chromatography. Stx-receptors Gb3Cer and Gb4Cer were identified by means of thin-layer chromatography (TLC) immunodetection using a collection of globo-series specific antibodies, combined with electrospray ionization (ESI) mass spectrometry [3]. The association of Stx-receptors with microdomains was determined by analyzing detergent-resistant membranes (DRMs), which were prepared by sucrose density gradient centrifugation [4].
Results: Various globo-series GSLs were TLC-immunodetected in the neutral GSL fraction of MDCK II cells such as the major Stx-receptor Gb3Cer and the less effective ligand Gb4Cer, which both appeared as variable species with varying fatty acid portions ranging from C16 to C24 acyl chain lengths in their ceramide moieties. Analysis of DRMs, which are suggested as lipid raft equivalent structures, revealed preferential distribution of the Stx-receptor GSLs, sphingomyelin and cholesterol to the upper DRM gradient fractions indicating their predominant localization in lipid rafts. Preliminary data suggest a lower sensitivity of MDCK II cells to Stx compared to Vero cells using Stx-containing STEC cell culture supernatants.
Conclusions: The data support the hypothesis that a supramolecular arrangement of Stx-receptors in the cell membrane might play an important role in the interaction of Stx with MDCK II cells and Stx-mediated cellular cytotoxicity.

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ANAEROBIC METABOLISM

AMP01
Identification of a gene cluster involved in the down-stream pathway of anaerobic naphthalene degradation

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The *Deltaproteobacterium* N47 is able to use naphthalene as sole carbon source under strict anaerobic, sulphate-reducing conditions (Meckenstock *et al.*, 2000). It was shown recently that after carboxylation to 2-naphthoic acid (Mouttaki *et al.*, 2012) and formation of the corresponding CoA-ester, the latter is stepwise reduced to a hexahydro-2-naphthoyl-CoA with unknown conformation of the remaining double bonds (Eberlein *et al.*, 2013). Also the following metabolic steps are still unknown, but there is strong evidence that β-oxidation like steps are involved (Annweiler *et al.*, 2002). The genes that most likely encode for the reductase catalysing the formation of hexahydro-2-naphthoyl-CoA are surrounded by a cluster of genes coding for hydratases, dehydrogenases, hydrolases, and thiolases. Some of these genes were shown to be up-regulated during growth on naphthalene in N47 (Bergmann *et al.*, 2011) or the marine naphthalene-degrading strain NaphS2 (DiDonato *et al.*, 2010). Using a PCR-based operon mapping approach of mRNA from an N47 culture grown on naphthalene, we could show that each of the 22 genes within this cluster is co-transcribed with at least one of the genes that are up-regulated during growth on naphthalene. This indicates that the whole gene cluster is involved in the down-stream pathway of anaerobic naphthalene degradation. When heterologously expressed in *Escherichia coli* under aerobic growth conditions, 15 of these genes yielded soluble His_6-tagged proteins which could be purified by affinity chromatography. Activity assays were conducted with linear (crotonyl-CoA, 3-hydroxybutyryl-CoA, and acetoacetyl-CoA) and with cyclic (cyclohex-1-enecarboxyl-CoA, 2-hydroxycyclohexanecarboxyl-CoA, and 2-oxocyclohexanecarboxyl-CoA) substrate analogues and the activity of hydratases, NAD-dependent dehydrogenases, and hydrolases could be demonstrated. The fact that some of these enzymes were exclusively acting on cyclic substrates, whereas other ones were only converting linear substrates, implies their respective position in the pathway (before or after ring-opening).

AMP02
Analyzing the metatranscriptome of an anaerobic digestion plant

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Introduction: Understanding anaerobic digestion of organic material is essential to optimize the efficiency of substrate use and to maximize gas yield. The multi-staged microbial conversion has to be investigated further because the whole process is prone to failures due to the difficult thermodynamics of the involved microorganisms (1). Monitoring via chemical parameters can be problematic because changes are mainly a consequence of disturbances between the microbes reacting directly to modifications of exogenous conditions (2). With advances of next-generation sequencing technologies, analyzing the metatranscriptome becomes feasible (4).
Materials and Methods: The fermenter, fed with renewable materials, was running stable at sampling time. Total nucleic acids were isolated and DNA and rRNA enzymatically digested. The remained mRNA was sequenced by GATC Biotech AG Konstanz/Germany. The resulting data were analyzed with the automated online server MG-RAST (5).
Results and discussion: Over 100.000 protein features were found after the automated quality control of the MG-RAST server. The hits are summed into diverse categories to get a general overview of the performance of the community. One third of all hits belong to the basic metabolism of organic macromolecules indicating a healthy population. RNA metabolism, respiration and regulation are covering about one quarter of the sequences. In context with the low number for sequences related to DNA metabolism and cell division suggest that an active community is more important than the absolute number of microorganisms and species.

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AMP03
Characterization of the heme d_1 biosynthesis enzyme NirDL/NirDLGH

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Pseudomonas aeruginosa generates energy under anaerobic growth conditions through the stepwise reduction of nitrate to molecular nitrogen during denitrification. In the second step of denitrification nitrite is converted into nitric oxide by the cytochrome cd_1 nitrite reductase (NirS). NirS contains two essential cofactors, heme *c* and heme d_1. The enzymes which are involved in the synthesis of the isobacteriochlorin heme d_1 are encoded by the *nirSMCFDLGHJEN* gene cluster.[1] In the course of heme d_1 biosynthesis siroheme is converted to 12,18-didecarboxy-siroheme by the heterotetrameric enzyme NirDLGH. In order to characterize the NirDLGH from *P. aeruginosa* we established an *in vitro* enzyme activity assay showing that 12,18-didecarboxy-siroheme is formed only in the presence of all four proteins. The heterodimeric complexes NirDL and NirGH were shown to catalyze only one of the two decarboxylation reactions, respectively. In contrast, the homologous NirDL protein from *Hydrogenobacter thermophilus* converts siroheme into 12,18-didecarboxy-siroheme *via* the intermediate monodecarboxy-siroheme.
Furthermore, we solved the crystal structure of NirDL from *H. thermophilus* in complex with the substrate analogue Fe-Uroporphyrin III. The putative active site of NirDL was identified as well as a potential DNA-binding domain of NirDL.
Interestingly, NirDLGH from *P. aeruginosa* also possesses a regulatory function in addition to its enzymatic activity. *In vivo* and *in vitro* experiments suggested that NirL and NirH bind to a DNA region upstream of the *nirJ* transcription start site thus regulating the expression of the *nirJEN* genes. In this context, we are particularly interested in the characterization of the DNA-binding sequence, the DNA-binding domain of the proteins and the conditions that affect the regulation of the *nirJEN* genes.

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AMP04
Action and abundance of propionate degrading bacteria in biogas reactors treating organic wastes

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Abstract will not be presented.

AMP05
Characterization of phenols biodegradation by compound specific stable isotope analysis

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Biodegradation of phenol and alkylphenols occurs under both aerobic and anoxic conditions. In the absence of molecular oxygen, the degradation of phenolic compounds is initiated by microorganisms through carboxylation, fumarate addition to the methyl moiety or anoxic hydroxylation of the methyl moiety (Rudolphi, Tschech et al. 1991,Schink, Philipp et al. 2000,Mechichi, Stackebrandt et al. 2002,Peters, Heintz et al. 2007,Schleinitz, Schmeling et al. 2009). Comparatively, under aerobic condition, the initiation mechanisms are revealed to be monoxygenation or dihydroxylation for phenol and ring hydroxylation or methyl group oxidation for cresols (Tao, Fishman et al. 2004,Corvini, Schäffer et al. 2006,Boyd, Sharma et al. 2011) . While several studies biochemically characterized the enzymes and reaction mechanisms in the relevant

degradation pathways, isotope fractionation patterns were rarely reported possibly due to constraints in current analytical methods. In the present study, compound specific carbon isotope ratio analysis method using isotope ratio mass spectrometry coupled with liquid chromatography (LC-IRMS) for phenol and cresols are successfully constructed. The enrichment factors for carbon (ƐC) upon the degradation of phenol and cresols by selected strains have been obtained. Cresols degradation by various strains showed generally moderate carbon isotope fractionation patterns with observable differences. For *p*-cresol degradation, three strains were examined. The aerobic strain *Acinetobacter calcoaceticus* NCIMB8250 exploits ring hydroxylation as initial reaction (Ehrt, Schirmer et al. 1995) and a ƐC value of -1.4±0.2‰ was obtained. *Pseudomonas pseudoalcaligenes* NCIMB 9867, an aerobic strain with side chain hydroxylation as activation mechanism upon cresols degradation (Hopper and Chapman 1971), yielded a ƐC value of -2.3±0.2‰. A similar ƐC value of -1.9±0.2‰ was observed for the anaerobe *Desulfosarcina cetonica* which utilizes fumarate addition as the first step of degradation (Muller, Galushko et al. 1999, Muller, Galushko et al. 2001). Moreover, for *m*-cresol degradation by *D.cetonica*, a ƐC value of -2.2±0.3‰ was gained. In respect to phenol degradation, the carbon isotope fractionation patterns differ more profoundly. For the aerobic strain *A. calcoaceticus* NCIMB8250 with monooxygenation as the initial reaction upon phenol degradation (Ehrt, Schirmer et al. 1995), a ƐC value of -1.5±0‰ was obtained. While the anaerobic *D.cetonica* showed a slight inverse carbon isotope fractionation with a ƐC value of 0.4±0.1‰.
In conclusion, carbon isotope fractionation analysis is a potentially powerful tool in the detection and monitor of phenolic compounds degradation in the environment. However, due to the methodology bottleneck in analyzing the corresponding hydrogen isotope fractionation at the moment, the one dimensional carbon isotope fractionation characterization shows limitation in differentiating certain degradation pathways.

AMP06
Effect of electron acceptor fluctuations on anaerobic toluene degrader communities in contaminated aquifer sediments

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Groundwater is an important resource but often polluted by aromatic hydrocarbons, such as BTEX compounds. To conceive efficient bioremediation technologies, it is crucial to better understand the ecology of degraders and ongoing biodegradation processes *in situ*. Hydrogeochemical fluctuations in groundwater alter the availability of substrates and electron acceptors, but it is not yet understood how degrader communities with different levels of diversity maintain specific functions, such as contaminant degradation under dynamic habitat conditions. We hypothesize that more diverse communities can withstand such disturbances better, since they harbour a greater level of functional redundancy.
To address this problem, we investigate how sedimentary anaerobic toluene degrader communities react to a change in electron acceptor availability. Sediment samples from a BTEX contaminated site are incubated with artificial groundwater medium and amended with toluene in batch microcosms. Alternating electron acceptor availability is applied to mimic *in situ* fluctuations. Changes in the anaerobic toluene degrader community diversity are assessed via the functional marker gene benzylsuccinate synthase (bssA) using T-RFLP and pyrosequencing approaches. We link changes in degrader diversity to degradation rates by monitoring toluene and electron acceptor concentrations. This ongoing project will help to better understand the interplay of abiotic and biotic drivers in anaerobic hydrocarbon degradation in groundwater.

AMP07
New reactions in anaerobic alkane and alkene metabolism

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Hydrocarbons like alkenes and alkanes are degraded under aerobic and anaerobic conditions. Numerous facts are known about the aerobic metabolisms but the anaerobic mechanisms are a research topic with many open questions especially regarding the enzymes involved. In our work we are interested in identifying and characterizing the enzymes of the anaerobic alkane and alkene metabolism in sulfate-reducing bacteria.
Desulfococcus oleovorans and *Desulfatibacillum alkenivorans* are well-known sulfate reducing bacteria which degrade alkenes and alkanes anaerobically. It was proposed that the initial step of the alkane metabolism in *D. alkenivorans* is initiated by fumarate addition leading to even chain fatty acids when grown on even chain alkanes (1). In contrast *D. oleovorans* is of special interest for lacking any genes involved in a fumarate-addition pathway and therefore uses a different, yet unknown alkane degrading pathway. This strain produces odd chain fatty acids when grown on even chain alkanes and vice versa. It was postulated that alkane degradation in *D. oleovorans* is initiated by a carboxylation reaction at C3, leading to a hypothetical intermediate which is further degraded by beta-oxidation to a fatty acid one carbon atom shorter than the primary alkane (2). We found an induced protein with high sequence similarity to ethylbenzene dehydrogenase (EBDH) from *Aromatoleum aromaticum* in alkane-grown cells of *D. oleovorans*. Since EBDH catalyzes the oxygen-independent hydroxylation of ethylbenzene to 1-phenylethanol (3), we propose that the initial step of the alkane metabolism in *D. oleovorans* is a hydroxylation reaction to form the corresponding 2-alcohols, as catalyzed by a large membrane-associated enzyme complex. We will present further evidence for this proposal and show that the same organism prefers alkenes over alkanes, which are degraded via a completely different route.

AMP08
Integration and folding of the β-barrel protein MtrB in *S. oneidensis* MR-1

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The facultative anaerobic bacterium *Shewanella oneidensis* Mr-1 can use a wide variety of respiratory electron acceptors. Some of these are insoluble like Fe(III)-oxides. Hence special adaptations of the organism are necessary to support electron transfer across the outer membrane. This step is catalyzed by the trimeric MtrABC protein complex that spans through the outer membrane. This complex consists of a periplasmic c-type cytochrome MtrA, the β-barrel protein MtrB and the cell surface localized decaheme cytochrome MtrC. For the integration of a β-barrel protein into the outer membrane the cell needs chaperons like SurA or Skp and the protease DegP to degrade missfolded proteins (1). In previous work, we could show that MtrA is not only an electron carrier protein in the periplasm, but also necessary to protect MtrB against degradation by DegP (2).

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AMP09
Genetic analysis of carbon monoxide utilisation in *Methanosarcina acetivorans*

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Methanogenesis is an important part of the global carbon cycle and exclusively carried out by methanogenic members of the *Archaea*. Most methanogenic archaea can only use one substrate, which makes it impossible to delete genes involved in order to study the function of the corresponding enzymes. However, *Methanosarcina* species are more metabolically versatile allowing disruption of individual methanogenic pathways. Unlike most methanogens, our model organism *Methanosarcina acetivorans* cannot use hydrogen and carbon dioxide as growth substrates, but is able to grow on methyl compounds (e.g. methanol), acetate or carbon monoxide (CO). During carboxidotrophic growth the organism produces, beside methane, substantial amounts of acetate and formate. While the pathway of acetate formation from CO has been established, it is unknown how *M. acetivorans*, which lacks formate dehydrogenase, generates formate from CO. In this study, attempts were made to delete the genes encoding formyl-methanofuran dehydrogenase isoform Fwd1 (*fwdDBAC*), formyl-methanofuran:tetrahydrosarcinapterin formyltransferase FTR (*ftr*), and energy converting methyl-tetrahydrosarcinapterin:coenzyme M methyltransferase MTR (*mtrE-H*). While deleting *fwdDBAC* and *ftr* aimed at elucidating the path of formate formation from CO, *mtrE-H* was

mutagenized to verify a proposed MTR bypass and to address the requirement of methane formation for CO-dependent growth.
We choose a combination of CO and methanol as substrates to make deletion of the otherwise essential *ftr* and *mtrE-H* genes possible. The results of our efforts will be presented and their implications on the physiology of *M. acetivorans* be discussed.

AMP10
Anaerobic degradation of aromatic compounds in Archaea

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Next to carbohydrates, aromatic compounds are the most abundant class of organic compounds in nature that can be fully degraded by aerobic and anaerobic bacteria and aerobic fungi. In anaerobic bacteria most mono- and homocyclic aromatic growth substrates are channeled to the central intermediate benzoyl-CoA that serves as substrate for dearomatizing benzoyl-CoA reductases (BCRs). There are two classes of BCRs: the class I BCRs are ATP-dependent, contain active site [4Fe-4S] clusters and are abundant in facultative anaerobes such as denitrifying and phototrophic bacteria; the ATP-independent class II BCRs contain an active site W-pterin and are present in obligately anaerobic bacteria (e.g. sulfate-, Fe(III)-reducing or fermenting bacteria). The distribution of BCRs has been explained by the differing energy yield in facultative and obligate anaerobes (3). To date, the hyperthermophilic *Ferroglobus placidus* is the only Archaeon that is known to degrade aromatic compounds under strictly anaerobic conditions. *F. placidus* couples the oxidation of a variety of monocyclic aromatics to the reduction of Fe(III) at 85°C (1). Recent genomic and transcriptomic analyses indicated that in *F. placidus* benzene, phenol or benzoate are converted to the central intermediate benzoyl-CoA (2). Surprisingly, the genome contained genes encoding an ATP-dependent class I but not a class II BCR. In these studies, BCR of *F. placidus* was suggested to reduce its substrate by four electrons to cyclohex-1-enoyl-CoA, resulting in a modified benzoyl-CoA degradation pathway that has so far only been described for the photoheterotrophic *Rhodopseudomonas palustris*. We present the results of first *in vitro* investigations of the degradation of aromatic compounds under hyperthermophilic conditions. In contrast to the genomic predictions we demonstrate that BCR reduced its substrate by two electrons to cyclohexa-1,5-dienoyl-CoA, and that enzymes of both, the common and modified benzoyl-CoA degradation pathway are involved in the further degradation. Results from heterologous expression and characterization of ATP-dependent BCR from *F. placidus* are presented.

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AMP11
Conversion of Pyruvate and Glucose to Methane by *Methanosarcina acetivorans*

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Degradation of organic matter in anoxic ecosystems is achieved by syntrophic communities of organisms from all three domains of life. The process involves four principle steps, hydrolysis, primary and secondary fermentation, as well as methane formation. Methanogenesis is only carried out by methanogenic archaea. Although methanogenesis is the only way how methanogens can conserve energy for their growth, the number of substrates utilized for methanogenesis is quite limited. Most methanogens are only able to grow with H_2+CO_2, some can utilize methylated compounds, and some can grow with acetate.
The model organism *Methanosarcina acetivorans* is capable to use various methylated compounds, CO, acetate and also pyruvate as the sole carbon and energy source for growth. Furthermore, *M. acetivorans* can also grow on pyruvate when methanogenesis is inhibited by 2-bromoethanesulfonate (BES). Suspensions of pyruvate-grown cells produce mainly CH_4 and CO_2 from pyruvate but in the presence of BES the major products are acetate and CO_2. Until now no methanogen is known, which is capable of converting exogenous carbohydrates to methane. However, *M. acetivorans* encodes most functions required for glucose oxidation, i. e. formation of methane from glucose, except for those of carbohydrate uptake and activation. By complementing *M. acetivorans* with the gene encoding the *Zymomonas mobilis* glucose facilitator (*glf*) a strain was generated which is capable of converting glucose to methane. However, this strain is not able to use glucose as the sole carbon and energy source for growth. To address this phenomenon, glucose utilization, both in growing cultures and in resting cells, was quantified. The results of this study indicate that exogenous glucose is used only to a minor degree as carbon and energy source, but instead mainly as cellular storage material.

AMP12
Transposon mutants in the anaerobic degradation of the monoterpene linalool in *Thauera linaloolentis* 47Lol

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Linalool (3,7-Dimethyl-1,6-octadien-3-ol) is a monoterpene used by the betaproteobacterium *Thauera linaloolentis* 47Lol^T as sole carbon and energy source under denitrifying conditions [1, 2, 3]. Transposon mutagenesis was performed with *Escherichia coli* BW20767 as donor of the plasmid pRL27 [4] to create random insertion mutants in ofloxacin and rifampicin-resistant strains of *T. linaloolentis* 47Lol. Mutants with impaired linalool metabolism were selected and the insertion sites were determined using genomic DNA or plasmids after rescue-cloning as templates for Sanger sequencing. Among the identified genes are an ABC transporter permease, an ADP-heptose synthase, a glycosyl transferase, a penicillin binding protein and a porin. All these proteins are involved in the synthesis of membrane and cell wall components. We correlated this finding to the toxicity of linalool. As monoterpene, linalool is expected to integrate into the membranes and to denature proteins by hydrophobic interactions.
Independently, a recent study published a draft genome of *T. linaloolentis* 47Lol^T (accession number AMXE00000000) [5]. A bioinformatic analysis of the genome suggested a degradation pathway involving genes from 4 genomic regions for the catalysis of linalool to acetyl-CoA molecules. Future research will test whether these genes are indeed involved in the linalool degradation.

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AMP13
Bacillus stamsii - a syntrophic glucose-oxidizing bacterium

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A *Bacillus* strain utilizing glucose as sole source of carbon and energy only in the presence of a methanogenic archaeon when grown under anaerobic, reducing conditions was described several years ago. Strain BoGlc83 was isolated from profundal sediments of Lake Constance and was the predominant glucose-fermenting bacterium according to MPN counts. In coculture, glucose was oxidized mainly to acetate and methane and minor amounts of lactate. This pattern of fermentation products suggests obligate syntrophic cooperation, as the free reaction enthalpy of glucose oxidation to acetate and hydrogen by strain BoGlc83 is insufficient for the anticipated ATP yield. However, the ability to produce lactate as alternative electron sink indicates that axenic growth of strain BoGlc83 should theoretically be possible. At first sight the strain did not grow aerobically nor under any

other anaerobic condition, suggesting that the strain is highly specialized on syntrophic cooperation. In the meantime it was found that aerobic growth is possible with a variety of different organic substrates but only in the presence of reduced sulfur compounds, especially thiosulfate. In contrast to previous findings, nitrate serves as electron acceptor, however only under auxotrophic conditions. Additionally, pyruvate stimulates axenic fermentative growth on glucose, yielding lactate as the major product and indicating a lack of certain biosynthetic pathways. Indeed, the genome sequence revealed that the pathways for the synthesis of certain amino acids are not encoded. Strain BoGlc83 represents the first facultatively anaerobic *Bacillus* strain capable of syntrophic coupling.

AMP14

Evidence for a Hexaheteromeric Methylenetetrahydrofolate Reductase in *Moorella thermoacetica*

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Moorella thermoacetica can grow on H_2 and CO_2, forming acetic acid from 2 CO_2 via the Wood-Ljungdahl pathway. All enzymes involved in this pathway have been characterized to date except for methylenetetrahydrofolate reductase (MetF). We report here that the *M. thermoacetica* gene that putatively encodes this enzyme, *metF,* is part of a transcription unit also coding for genes *hdrCBA, mvhD,* and *metV*. MetF co-purified with the other five proteins encoded in the unit in a hexaheteromeric complex with an apparent molecular mass of 300 kDa. The 40-fold enriched preparation contained per mg protein 3.1 nmol FAD, 3.4 nmol FMN and 110 nmol iron, almost as predicted from the primary structure of the six subunits. It catalyzed the reduction of methylene-tetrahydrofolate with reduced benzyl viologen but not with NAD(P)H neither in the absence or presence of oxidized ferredoxin. It also catalyzed the reversible reduction of benzyl viologen with NADH (diaphorase activity). Heterologous expression of the *metF* gene in *Escherichia coli* revealed that the subunit MetF contains one FMN rather than FAD. MetF exhibited high methylenetetrahydrofolate reductase activity with benzyl viologen only when produced together with MetV, which in part shows sequence similarity to MetF. Heterologously produced HdrA contained 2 FAD and had NAD-specific diaphorase activity. Our results suggested that the physiological electron donor for methylenetetrahydrofolate reduction in *M. thermoacetica* is NADH and that the exergonic reduction of methylenetetrahydrofolate with NADH is coupled via flavin-based electron bifurcation with the endergonic reduction of an electron acceptor, whose identity remains unknown.

AMP15

Diversity of carbon fixation pathways in thermophilic bacteria

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The six known autotrophic pathways do not seem to be evenly distributed across the three domains of life. Some pathways have only been found in Bacteria while others were found exclusively in Archaea, and to our current knowledge, no autotrophic pathway is shared by organisms from all three domains of life. But our picture is likely very incomplete. New genomic data for organisms from diverse habitats suggest not only that unknown autotrophic pathways may exist but also that some autotrophic pathways are more widespread than previously anticipated. We have discovered new examples for the convergent evolution of autotrophic cycles in Bacteria: An extremely thermophilic, strictly autotrophic, denitrifying member of the low-GC, gram positive bacteria, *Ammonifex degensii*, uses two autotrophic pathways in parallel. Apart from the Wood-Ljungdahl pathway, which is present in many relatives of *A. degensii*, it contains a highly unusual version of the Calvin-Benson cycle that utilizes enzymes with an archaeal origin. The moderately thermophilic, facultatively autotrophic, sulfur-reducing δ-proteobacterium *Desulfurella acetivorans* uses the 3-hydroxypropionate/4-hydroxybutyrate cycle which was previously thought to be restricted to *Archaea* of the phyla *Cren-* and *Thaumarchaeota*. Besides the Wood-Ljungdahl pathway, this pathway is a second example of an autotrophic pathway that occurs in both Bacteria and Archaea.

AMP16

Structure and function of the class II benzoyl-coenzyme A reductase from strictly anaerobic bacteria

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In anaerobic bacteria most aromatic growth substrates are converted into the central intermediate benzoyl-coenzyme A (benzoyl-CoA). Benzoyl-CoA reductases (BCRs) dearomatize benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). There are two classes of BCRs (1): the class I BCRs employed by facultatively anaerobic bacteria are ATP-dependent and contain active site-[4Fe-4S] clusters. Obligately anaerobic bacteria such as metal ion-reducing, sulfate-reducing and fermenting bacteria employ the class II benzoyl-CoA reductase, which is composed of eight different subunits (BamB-I). This ~1 MDa complex was purified and characterized from the Fe(III)-respiring *Geobacter metallireducens* and *Desulfosarcina cetonica*. The complex from *G. metallireducens* contained two tungstopterins, selenocysteine, three FADs and 23 FeS-clusters per $(BamBC)_2DEFGHI$. The proposed electron bifurcation mechanism is being studied, in which the endergonic reduction of benzoyl-CoA by reduced ferredoxin is coupled to the exergonic one of NAD^+ by reduced ferredoxin. A heterologous expression platform for the active-site subunit BamB has been developed. Using this platform, the structure and function of class II BCRs from numerous organisms are currently being studied.

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AMP17

Characterization of a putative pyruvate decarboxylase from *Clostridium acetobutylicum*

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Clostridium acetobutylicum represents the model organism of solventogenic clostridia, a group of strict anaerobic Gram-positive bacteria capable to produce *n*-butanol as a next-generation biofuel. Suitable genetic tools for clostridia became available only recently, and therefore, the complexity of the fermentative metabolism and its regulation is still not completely understood [1]. Interestingly, a couple of recently generated knock-out mutants exhibited a phenotype with predominant formation of ethanol, which is usually a minor end-product of *C. acetobutylicum* [2-4]. Hence, the functionality of a putative pyruvate decarboxylase (PDC), encoded by the gene *cap0025*, was investigated in this study to elucidate the occurrence of an alternative ethanol biosynthetic pathway. According to the available genome sequences, *C. acetobutylicum* and *C. pasteurianum* are the only clostridial species harboring a putative *pdc* gene, but experimental evidence for catalytic activity of the PDC was not reported so far [5]. Therefore, the *pdc* gene was cloned and heterologously as well as homologously overexpressed in *Escherichia coli* and *C. acetobutylicum* ATCC 824, respectively. Protein purification was controlled by SDS-PAGE and Western Blot analyses, and enzyme activities of the purified PDC were determined spectrophotometrically.

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AMP18

Benzimidazole and methylated analogues differentially affect the tetrachloroethene respiratory metabolism of *Sulfurospirillum multivorans*

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Sulfurospirillum multivorans is a microaerophilic ε-proteobacterium, which uses tetrachloroethene (PCE) as terminal electron acceptor for growth (organohalide respiration). This environmentally relevant pollutant is converted to *cis*-1,2-dichloroethene by the reductive dehalogenase (PceA).

The PceA enzyme harbors two [4Fe-4S] clusters and an unusual cobamide cofactor, termed norpseudo-B_{12}. The latter is synthesized *de novo* in *S. multivorans* and differs in its nucleotide loop structure from other natural cobamides. It contains an adenine moiety as lower ligand base of the cobalt ion, whereas the ubiquitous coenzyme B_{12} bears a 5,6-dimethylbenzimidazole (DMB) molecule at this position. It was recently shown that the uptake and incorporation of DMB as cobamide's lower ligand led to severe effects on the PCE-dependent growth and the PceA activity of *S. multivorans* [1].
In this study, the impact of benzimidazole (Bza) and 5-methylbenzimidazole (MeBza) on both, PCE-dependent growth and PceA enzyme activity was investigated. When Bza (25 µM) was added to the cultures neither a negative effect on the PCE-dependent growth nor on the PceA activity has been observed, although the adenine moiety in norpseudo-B_{12} was quantitatively replaced by Bza. In the presence of MeBza (up to 50 µM) only a small portion (30%) of norpseudo-B_{12} was replaced by the MeBza-containing cobamide. However, a negative effect on the PceA enzyme activity was detected under these conditions. Homologues of the nicotinate-mononucleotide phosphoribosyltransferase (CobT) from phylogenetically diverse bacteria were shown to activate different purines as well as benzimidazoles as precursors of lower ligand bases in cobamides [2]. In order to elucidate the role of CobT in *S. multivorans* for the activation and incorporation of DMB, MeBza, and Bza the enzyme was heterologously produced and characterized for its substrate spectrum.

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AMP19
The Influence of the *N*-and *C*-termini on Formate Translocation by the Formate Channel FocA

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During mixed acid fermentation enterobacteria such as *Escherichia coli* are able to convert up to one third of the carbon derived from glucose to formate, which results from the cleavage of pyruvate by PflB (pyruvate formate-lyase). Formate accumulation in the cytoplasm would reduce the internal pH, therefore if it cannot be metabolized it is translocated across the cytoplasmic membrane into the periplasm. In the stationary phase formate is quantitatively re-imported for internal consumption by the formate hydrogenlyase complex. One means by which formate is translocated across the membrane is via the channel FocA, which belongs to the formate-nitrite transporter superfamily of homopentameric membrane proteins. FocA translocates formate bi-directionally and it is encoded by *focA*, which is located in an operon with the gene encoding PflB. Tight coordination of PflB and FocA synthesis ensues. Recently, we have shown that PflB regulates formate passage through FocA by direct protein-protein interaction via the flexible *N*-terminal helix of FocA (1). Furthermore, during a recent amino acid exchange programme we noted exchange of K26 in the *N*-terminus of FocA also influenced formate transport (2). Therefore, here we analysed the effects on formate translocation of both *N*- and *C*-terminal amino acid truncations of FocA. These variants were analysed by monitoring changes in intracellular formate levels using a chromosomal *fdhF'-'lacZ* fusion, which is formate-inducible. We also analysed the influence of these exchanges on the growth inhibitory effects caused by the toxic formate analogue hypophosphite, which is translocated into *E. coli* by FocA. All variants of FocA with *N*- and *C*- terminal truncations were membrane-associated, but some were synthesised at a low level. Truncations that severely shortened the *N*-terminus, along with a 6 amino acid *C*-terminal truncated variant, showed no FocA-dependent transport of formate or hypophosphite. These results indicate that both the *N*- and *C*-termini of the FocA-protein are involved in the regulation of formate translocation through the FocA pore. These findings also suggest that formate translocation by FocA is not only dependent on constriction sites within the pore of each protomer (3, 4), but that the flexible cytoplasmic *N*- and *C*-termini regulate passage through the channel. The potential mechanisms underlying this regulation will be discussed.

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AMP20
Understanding the ecology of anaerobic polycyclic aromatic hydrocarbon degradation in natural oil seeps

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Polycyclic aromatic hydrocarbons (PAHs) are among the most recalcitrant substances occurring in nature. Nevertheless, the anaerobic degradation pathways for polycyclic aromatic hydrocarbons are not yet fully understood. For bioremediation of contaminated groundwater aquifers it would be useful to further expand the knowledge of the degradation pathways. The world's biggest natural tar lake in La Brea, Trinidad and Tobago has been discovered as a source for a range of microorganisms able to degrade PAHs. Samples of liquid asphalt emerging from the depths of the lake have been taken to gain access to fresh liquid oil. A metagenomic approach from directly extracted DNA from the oil as well as community analysis via Next-Generation-Amplicon-Sequencing and T-RFLP of organisms living in small water droplets of a size of 1 to 3 µl found within the oil are performed. Assessing the microbial community of the different water Figures revealed a high diversity of the communities of each individual droplet and of differences between different droplets. Amplicon sequencing has revealed that members of Burkholderiales and Enterobacteriales are most prevalent in the droplets. Besides those we found that members of Bacteriodales, Rhodospirillales, Sphingomonadales are highly abundant, and to a lesser extent, Thermotogales, and Nitrosomonadales. Most of these are very common in oil samples and previously taken solid samples from the tar lake (1). Further on we were able to identify methanogenic archaea, which indicate that the water inclusions were completely anoxic. To further extent our knowledge of the ecology in these water inclusions in both phylogenetic and functional diversity will broaden our understanding of degradation processes in these special habitats and might lead to the identification of novel marker genes in PAH degradation for bioremediation in contaminated groundwater aquifers.

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AMP21
Dissecting Hydrogen-Driven Organohalide Respiration by *Dehalococcoides* Species

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The genus *Dehalococcoides* belongs to the phylum *Choroflexi* and comprises strictly anaerobic bacteria that conserve energy exclusively by organohalide respiration (OHR). *D. mccartyi* species use H_2 as the sole electron donor and genome sequencing has revealed the presence of genes encoding 5 types of hydrogenases, termed Hup, Hyc, Vhu, Ech and Hym. Hym is predicted to be a [FeFe]-hydrogenase, while the others belong to the group of [NiFe]-hydrogenases. Hup (hydrogen-uptake) represents the only hydrogenase for which the active site is predicted to be located on the extracellular side of the cytoplasmic membrane, suggesting a role in energy conservation. Furthermore, the genomes of *D. mccartyi* strains also encode a membrane-bound formate dehydrogenase (Fdh-like), which proteomic studies have revealed to be one of the most abundant proteins during OHR along with the Hup hydrogenase. *D. mccartyi* is unable to use formate either as an electron donor or as a carbon source. The *fdhA-fdhB-fdhE* operon encodes the catalytic subunit (FdhA), a membrane anchor (FdhB) similar to that of some O_2-sensitive [NiFe]-hydrogenases and an accessory protein (FdhE) required for enzyme maturation. A lack of either a selenocysteine or a complete molybdenum cofactor biosynthetic pathway raises the question as to the function of the Fdh-like protein in *Dehalococcoides* species. Therefore, the aim of this project was to clarify the role of the Hup hydrogenase and the function of the Fdh-like protein during H_2-driven OHR. Hydrogenase activity was examined using native gel electrophoresis of crude extracts of strains CBDB1 and DCMB5 followed by a hydrogenase-specific in-gel activity assay.Three similar H_2-oxidizing enzyme complexes

were visualized in both strains. Mass spectrometric analysis of the protein complexes responsible for the hydrogenase-dependent activity identified FdhAB and HupSL polypeptides as the most abundant proteins along with different reductive dehalogenases and polypeptides corresponding of the hydrogenases Vhu and Hym. Furthermore, enrichment of the Fdh-like enzyme showed H_2-dependent activity and co-purification of Hup hydrogenase. Both, the Fdh-like and the Hup enzyme were heterologously synthesized in *E. coli* to clarify whether the Fdh-like and Hup enzymes form a complex. A slowly migrating and weakly active Fdh-like-Hup complex could indeed be identified but it was unstable. In contrast, no activity could be detected when the operon encoding only the Hup hydrogenase was expressed. These data provide the first indication that the Fdh-like enzyme has H_2-oxidizing activity and suggest the existence of a membrane-associated Fdh-like/ Hup supercomplex in *Dehalococcoides mccartyi*.

AMP22
Hydrogen metabolism in *Sulfurimonas denitrificans*

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Sulfurimonas denitrificans was originally isolated from tidal flats of the Dutch Wadden Sea as *Thiomicrospira denitrificans* and was later reclassified as *Sulfurimonas denitrificans*. Although *S. denitrificans* was initially grown on reduced sulfur compounds and characterized as a thiosulfate oxidizer, recent sequencing of its genome revealed that it encodes periplasmic and cytoplasmic [NiFe]-hydrogenases, but hydrogen uptake activities are still not available and the role that hydrogen plays for its growth remains unknown. We show the first experimental evidence that *S. denitrificans* can indeed express a functional hydrogen uptake active hydrogenase and hydrogen is consumed during the growth. In fact, reduced sulfur compounds are not necessary for the growth of *S. denitrificans* when hydrogen is present. *S. denitrificans* grew faster and denser on hydrogen than on thiosulfate alone. Under the provided conditions it can grow more efficiently with hydrogen than with thiosulfate. In our experiments, the hydrogen uptake activity was detected only in the membrane protein fraction but not in the soluble protein fraction, it indicates that the hydrogen uptake activity is related to the periplasmic hydrogenase but not the cytoplasmic hydrogenase. In summary, this data suggests that hydrogen may be more important for the energy metabolism of *S. denitrificans* than currently thought.

AMP23
Activation of acetone by the sulfate-reducing bacterium *Desulfococcus biacutus*

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Pathways for acetone degradation have been studied well for aerobic and nitrate-reducing bacteria. They all activate acetone through carboxylation to acetoacetate, consuming at least 2 ATP equivalents for the activation reaction. This reaction is not possible for sulfate-reducing bacteria due to their extreme energy limitation, and acetoacetate could not be detected as a reaction intermediate in sulfate reducers (1). Rather, acetoacetaldehyde was identified as a reaction intermediate, indicating that acetone is activated by a carbonylation reaction (2). The reaction requires ATP which is cleaved to AMP and PPi, and thiamine pyrophosphate as a further co-substrate (3). Furthermore, the genome of *Desulfococcus biacutus* has been sequenced and annotated, allowing to identify acetone-specific enzymes through a proteomic approach. In the present work, candidate enzymes are studied that are possibly involved in the activation of acetone. Anaerobic activity staining showed a specific band only in cell-free extract of acetone-grown cells, leading to the same thiamine pyrophosphate requiring enzyme that was identified previously. Furthermore, a protein annotated as isopropylmalate/homocitrate/citramalate synthase was found only in acetone-grown cells in 2D-PAGE. These two candidate enzymes were cloned into *E. coli* for overexpression and purification. These enzymes are to be tested in activity assays to allow a better understanding of the reaction mechanism of this novel carbonylation reaction.

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AMP24
Modification of the anaerobic metabolism of *Escherichia coli*: Accumulation of Mixed Acid Fermentation End Products

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Escherichia coli is one of the best investigated microorganisms so far. It is capable to thrive on glucose fermentatively or with different electron acceptors. Due to its good genetical tractability multiple modifications of the central metabolism were made to produce chemical compounds in a bioreactor. In this study the impact of deletions in genes coding for enzymes of the fermentative metabolism of *E. coli* (*ldhA* (lactated-dehydrogenase), *adhE* (alcohol-dehydrogenase), *frd* (fumarate reductase) and *pta-ack* (phosphotransacetylase and acetate kinase)) were investigated to compare different mutants under identical growth conditions. The deletion of *frd* causes an increase of the growth rate aerobically and anaerobically. The additional deletion of *adhE* nearly abolished fermentative growth and decreased the growth rate during anaerobic respiration. The triple mutant *frd adhE ldhA* was no longer able to consume glucose under fermentative conditions and the growth rate during anaerobic respiration was significantly lower with DMSO (17 % compared to the wildtype) or nitrate (70 %) as electron acceptor. However, aerobic growth was accelerated 1.25 fold. The following deletion of *pta-ack* nearly abolished anaerobic growth and decreased the aerobic growth rate (70% compared to the triple mutant). Interestingly, the triple mutant and the quadruple mutant accumulated acetic acid (*frd adhE ldhA*) or pyruvic acid (*frd adhE ldhA pta-ack*) in the media broth, respectively. Acetic acid was produced with a yield of 75% of the theoretical maximum. Industrial production of acetic acid depends on high pressure and temperature. Microbiological synthesis could decrease energy consumption and would broaden the spectrum of usable substrates. Pyruvic acid was excreted to the media under anaerobic conditions with a yield of 60%. It was also present in the aerobic culture broth. Hence, this strain seems to be an interesting host for pyruvic acid dependent processes like butandiol production, since the synthesis of butandiol starts with the consumption of two pyruvate molecules.

AMP25
Adaptation of *Listeria weihenstephanensis* to anaerobiosis

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L. weihenstephanensis is a recently new described *Listeria* species (1). It was isolated from the water plant *Lemna trisulca* from a fresh water pond in Bavaria, Germany. The isolate is non-haemolytic and has not been associated with animal or human diseases. It has an optimal growth at pH 7-8 and temperature at 28-34 °C. It requires a relative low demand of nutrients and is able to grow under both aerobic and anaerobic conditions. In the present study the adaptations of *L. weihenstephanensis* to oxygen availability were further investigated.

Growth analyses for *L. weihenstephanensis* were performed aerobically and anaerobically in BHI medium at 18 °C and 34 °C. At both temperatures *L. weihenstephanensis* was able to grow both aerobically and anaerobically, although anaerobic growth was clearly decreased.

To better understand the adaptations of *L. weihenstephanensis* to oxygen availability global transcriptional analyses, via next generation RNA sequencing, were performed for cells grown aerobically or anaerobically to an OD_{600} = 0.85-0.90. At 18 °C, 49 genes were found to be stronger transcribed aerobically while 40 genes were found to be stronger transcribed anaerobically. At 34 °C, 52 genes exhibited aerobically a stronger transcription while transcription of 27 genes was induced anaerobically. The oxygen dependent gene expression of selected genes was validated via qPCR. Among the differently regulated genes, many encode for metabolic enzymes indicating broad metabolic adaptations with respect to oxygen availability. Moreover, the transcriptional results clarified that temperature doesn´t play a key role in this oxygen-dependent regulation for *L. weihenstephanensis*.

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AMP26

Two propanediol utilisation like proteins (PduL) of *Moorella thermoacetica* act as phosphotransacetylases (Pta)

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Moorella thermoacetica, formerly known as *Clostridium thermoaceticum*, is reputed as the primary acetogenic bacterium for the resolution of the Wood-Ljungdahl pathway, also known as reductive acetyl-CoA pathway. This basic metabolic property enables homoacetogenic bacteria to grow autotrophically using CO_2 (or CO) as an energy and carbon source while forming acetate as main product. However, some homologues of enzymes of the Wood-Ljungdahl pathway could not be found in the genome of *M. thermoacetica* [1]. An example is a phosphotransacetylase (Pta) which is a key enzyme for the formation of acetate. This is remarkable because Drake *et al.* were able to enrich a protein fraction in 1981 revealing Pta activity [2]. An explanation was that the gene products of two open reading frames annotated as propanediol utilisation like protein (*pdul*) genes might act as phosphotransacetylases. In this work we were able to verify this hypothesis biochemically, expressing both PduL homologues (Moth1181 and Moth0864) of *M. thermoacetica* in *Escherichia coli*. Indeed, after their purification using *Strep*-TagII technology (*www.iba-lifesciences.com/strep_tag*) both enzymes revealed Pta activity *in vitro*. They were able to use acetyl-CoA, propionyl-CoA and one of them even butyryl-CoA as substrates. A detailed characterisation including temperature and pH optimum curves showed maxima at 65 °C and pH 7.6, respectively. Thus, we were able to prove that in *M. thermoacetica* PduL homologues function as Pta.

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AMP27

GASCHEM: Synthetic biology of industrial waste gas consuming and biofuel producing bacteria

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Large amounts of waste gases containing carbon monoxide and carbon dioxide are produced by steel mills every day. Their uncontrolled release into the atmosphere is problematic due to their role as greenhouse gases. However, acetogenic bacteria such as *Clostridium ljungdahlii* and *Clostridium autoethanogenum* can grow on these gases and produce industrially relevant compounds, albeit in relatively small quantities.
The GASCHEM project, located at the University of Nottingham and supported by substantial funding from the UK Biotechnology, Biological Research Council and the company LanzaTech, aims to address these issues by engineering *C. autoethanogenum* strains capable of producing ethanol and other, more desirable chemical commodities from waste gases at an industrial scale. The project is organised into four phases. First, a systems biology approach will provide comprehensive understanding of the organism's native fermentation metabolism. Based on this, rational metabolic engineering will be employed in phase 2 to redirect metabolism towards increased production of desirable native products, specifically ethanol and butanediol. In phase 3, a synthetic biology approach will we be employed to design and integrate heterologous metabolic pathways leading to the formation of non-native and of higher value fermentation products. Finally, selected promising strains will be evaluated by LanzaTech at pilot plant and, if appropriate, demonstrating plant level.

AMP28

Key Factors Governing [NiFe]-Hydrogenase Small Subunit Maturation

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Under anaerobic conditions *Escherichia coli* synthesizes three different [NiFe]-hydrogenases (Hyd). Each Hyd comprises a large subunit that carries a $NiFe(CN)_2CO$-cofactor and a small subunit, which harbors (iron-sulphur) FeS clusters. While the maturation of the large subunit has been well studied, in comparison the maturation of the small subunit is still poorly understood[1]. Each small subunit accommodates a dedicated set of electron-transferring FeS clusters but it is unclear what governs when and how they are inserted[2]. Only the small subunit of the hydrogen-oxidizing enzymes (Hyd-1 and Hyd-2) of *Escherichia coli* carries a signal-peptide for the twin-arginine translocon (Tat). Tat-dependent transport is only observable when cofactor synthesis and assembly of the heterodimeric enzymes are complete[3]. This fact suggests a key function in maturation control and transport mediation by the Tat-signal-peptide-binding chaperones HyaE and HybE, which are specific for the small subunit of Hyd-1 and Hyd-2, respectively[3]. Here we investigated the role of three different key maturation factors of the small subunits of *E. coli* Hyd-1 and Hyd-2. First, the role of the FeS protein ferredoxin in cofactor synthesis and delivery was studied. *E. coli* strains lacking the gene encoding ferredoxin failed to mature the small subunit of Hyd-1 and Hyd-2. Moreover, the deletion compromised stability of both the small and, to some extent, the large subunit. Second, we demonstrated the role of the *C*-terminal 'trigger'-peptide, which is cleaved off the large subunit after the $NiFe(CN)_2CO$-cofactor has been inserted, in controlling the maturation and assembly of the hydrogenase heterodimer. Third, putative roles for the hydrogenase-specific chaperones HyaE, HyaF and HybE in control of cofactor synthesis, subunit assembly, Tat-transport, and protein recycling were investigated.

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AMP29

NirJ and AhbC - two different Radical SAM enzymes involved in heme d_1 and heme biosynthesis

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Among enzymatic cofactors, there is a big group of those bearing a tetrapyrrole structure. They play important roles in various processes like in photosynthesis (chlorophyll *a*), oxygen transport (heme), electron transport (cytochrome *c*) or methanogenesis (cofactor F_{430}). For the biosynthesis of heme two different pathways exist, the classical heme biosynthesis route and the alternative heme biosynthesis pathway. The latter is used by sulfate reducing bacteria and the archaea. Both ways lead to the same product, heme *b*, but *via* different intermediates. Another pathway leads to heme d_1, a cofactor of the nitrite reductase NirS, which is part of the denitrification process. [1, 2] The heme d_1 biosynthesis route and the alternative heme biosynthesis pathway share the same intermediates up to the point of 12,18-didecarboxysiroheme (DDSH). Accordingly, the enzymes of both pathways show high similarities. In the route for heme d_1, DDSH is transformed by the enzyme NirJ into 3,8-dioxo-12,18-didecarboxysiroheme, the precursor of heme d_1. In contrast, in the before last step of the alternative heme biosynthesis, AhbC converts DDSH into iron-coproporphyrin III. Both, NirJ and AhbC are putative Radical SAM enzymes. Those enzymes carry iron-sulfur-clusters, which enable uncommon radical reaction mechanisms. Our aim is to characterize both enzymes experimentally and to further proof their proposed activity. Therefore, NirJ from *Dinoroseobacter shibae* and AhbC from *Methanosarcina barkeri* were recombinantly produced and characterized as Radical SAM enzymes, by UV/Vis-spectroscopy, iron- and sulfide-determination and cleavage of *S*-adenosylmethionine (SAM). [3]

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ARCHAEA

ARP01
Biodiversity in the Freiberger mine *Reiche Zeche*: What´s about Anaerobes and Archaea?

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The region *Erzgebirge* has a long tradition in mining and geology. Considered as economically not viable, mining activities have been terminated during the last century. With the increasing demand on high tech products attention is drawn to the winning of "stategic elements". In the Freiberger Biohydrometallurgical Center (BHMZ) interdisciplinary work is performed in this research area. On future approach is to install an in-situ leaching plant in mine *Reiche Zeche* in Freiberg. Up to now, efforts were made on the characterisation of acidic iron-oxidising bacteria. The study presented here is the first providing deeper insight into microbial diversity of the *Reiche Zeche*. 7 sites in the mine *Reiche Zeche* have been sampled for mining waters, with sites located in an area selected appropriate for in-situ bioleaching. Directly after sampling the waters were prepared for futher geochemical analytics. Additionally, from each site subsurface waters and sediments were taken and reduced immediately. Two of these were freshly inocultated in different anaerobic growth media which were incubated at 10° C, resembling the temperature in the habitat, and 30° C. Growth was monitored microscopically at regular intervals. Additionally, waters were membrane filtered directly after sampling and DNA-Isolation was performed. In two parallel approaches the resulting DNA was tested for the presence of bacteria and archaea. For the latter, different DNA-Isolation, -purification and PCR methods were tested. Our study revealed, that bacteria are present in all sampling sites with one exception. Moreover, in 4 of the 7 samples the presence of archaea could be detected by PCR-based methods. Furthermore, the enrichment of anaerobic microorganisms was successful not only in heterotrophic but also in autotrophic liquid media. Growth of the micoorganisms correlated with the incubation temperature and the inoculum itself. Studies for further characterisation of the archaeal and anaerobic diversity (FISH, clone library) are in progress.

ARP02
Examination of the interaction of a halophilic Archaea with uranium regarding the long term storage of radioactive waste in rock salt

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For the long term storage of radioactive waste in a deep geological repository rock salt is one of the possible host rocks, next to clay and crystalline formations. To date only little is known about the microbial diversity in German rock salt and the interactions of halophilic microorganisms with radionuclides. Microorganisms indigenous to potential host rocks are able to influence the oxidation state and speciation and hence the mobility of radionuclides. Therefore, for the safety assessment of a radioactive waste disposal it is important to know what microorganisms are present in the potential host rocks (e.g. salt) and how these microorganisms can affect the performance of a repository. The reference organism Halobacterium noricense DSM 15987 was used to investigate the interactions with uranium at high ionic strength. For instance this halophilic Archaea was found in an Austrian salt mine1 and in the halite of the Waste Isolation Pilot Plant (WIPP, Carlsbad, USA)2. The cells were incubated for 48 h with uranium concentrations between 5 - 60 µM in 3.0 M NaCl (pH 6.0, room temperature, shaking) for sorption studies. After 48 h the cells were still living when incubated with uranium concentrations up to 60 µM, which demonstrates that Halobacterium noricense can tolerate uranium concentrations up to this level. The formed uranium sorption species were examined with IR-spectroscopy and time-resolved laser-induced fluorescence spectroscopy (TRLFS). Furthermore the time-depending and pH-depending biosorption of Halobacterium noricense with uranium will be presented.

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ARP03
Transcription regulation in *Sulfolobus acidocaldarius*

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Beside unique features, archaea combine typical characteristics of bacteria and eukaryotes. Promoter structure and genetic information processing such as replication, transcription and translation, closely resemble that of their eukaryotic counterparts. The archaeal transcription machinery consists of a multi-subunit RNA-Polymerase (RNAP) and the general transcription factors (GTF) representing homologues of the eukaryotic TATA-binding protein (TBP) and the transcription factor TFIIB (TFB). The actual understanding of the transcription initiation implies that TBP binds to the TATA-Box. Subsequently, TFB binds to the TBP-DNA-complex and forms sequence specific interactions with a purine-rich TFB-responsive element (BRE). The N-terminal region of TFB recruits the RNAP to build the ternary pre-initiation complex. Generally, the archaeal transcription machinery is considered as a simplified model of the more complex processes which are known from eukaryotes. The thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* possesses three TFBs (TFB1, TFB2 and TFB3) and one TBP. TFB1 seems to be the most commonly expressed TFIIB homologue under standard growth conditions and supports transcription initiation *in vitro* (Bell and Jackson, 1998), whereas TFB3 is upregulated following UV-exposure (Götz *et al*., 2007) and acts as a co-activator in the presence of TFB1 (Paytubi and White, 2009). However, the role of multiple GTFs like TFB1-3 in crenarchaeota is still unclear and functions similar to bacterial sigma factors have been suggested. The aim of this project is to study the functions of the TFB homologues, especially TFB2, and to investigate their roles in stress response. Overexpression and purification of recombinant GTFs was performed successfully for TBP, TFB1, TFEα and RNA Polymerase (in genome tagging in *S. acidocaldarius*). For TFB2 and TFB3 insoluble protein was obtained and purification via denaturation/renaturation is currently established and optimized. The purified proteins will be used for *in vitro* transcription and protein-DNA binding assays (EMSA). Furthermore, reporter gene constructs (LacS) were established to determine promoter activities of the different GTFs *in vivo* in response to different stress conditions.

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ARP04
Regulation of Selenoprotein Gene Expression in *Archaea*

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Proteins containing the 21st amino acid, selenocysteine (sec), occur in the three domains of life, *Archaea*, *Bacteria*, and *Eukarya*. While the mechanism of sec biosynthesis and incorporation was characterized for representatives of each domain, regulation of selenoprotein gene expression is still poorly understood. The archaeal model organism *Methanococcus maripaludis* contains eight selenoproteins known to be involved in its primary metabolism, hydrogenotrophic methanogenesis. Interestingly, the organism also forms cysteine-containing isoforms of all selenoproteins with the exception of formate dehydrogenase. These isoforms seem to represent a backup system for the organism's ability to conserve energy during selenium starvation. Upon disruption of the selenoprotein biosynthesis pathway, expression of selenoprotein genes is dramatically downregulated, while levels of mRNA for those of the cysteine isoforms increase. Disrupting *hrsM*, which encodes a LysR-type transcription regulator, results in the same phenotype, suggesting its involvement in regulation of both, the sec- and cys-encoding, isogenes. Here, we present our efforts to elucidate regulation of selenoprotein gene expression in *M. maripaludis* in more detail. *hrsM*-dependent gene regulation was characterized by means of

quantitative reverse transcription PCR allowing the quantification of transcripts encoding HrsM, and sec- and cys-containing isoenzymes. Furthermore, Western blot analysis was conducted to assess HrsM abundance under different physiological conditions and in various genetic backgrounds.

ARP05
Sugar degradation in *Sulfolobus solfataricus* — Metabolic pathway reconstruction and growth on mixed carbon sources

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Sulfolobus solfataricus is a thermoacidophilic crenarchaeum with optimal growth at 80°C and pH 2-3. The organism possesses a great physiological versatility and is capable to utilize different organic compounds as carbon and energy source such as a variety of different C5 and C6 sugars (e.g. D-glucose, L-arabinose, D-arabinose, D-xylose), sugar acids, alcohols and peptides. However, the used metabolic pathways as well as their regulation in response to different carbon sources are still unknown. In the genome of *S. solfataricus* 14 different dehydrogenase paralogs were identified. Three of them were already characterized as sugar dehydrogenases [1,2,3] and two as alcohol dehydrogenases [4]. Here we report the characterization of a new glucose-1-dehydrogenase paralog that possesses broad substrate specificity and shows activity with a range of different sugars (e.g. D-glucose, L-arabinose, D-xylose, etc.). In addition the enzyme possesses dual co-substrate specificity for NAD^+ and $NADP^+$, however, with $NADP^+$ being the preferred co-substrate. In contrast to most sugar dehydrogenases, which rely on manganese and magnesium as metal ions, the enzyme exhibits unusual high activity with cobalt and cadmium. To get first insights into the regulation of carbon metabolism in response to carbon source we studied the growth of *S. solfataricus* in the presence of mixed carbon source (diauxic effects) and after carbon switch. First studies following the growth of *S. solfataricus* as well as the utilization of the different carbon sources will be presented.

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ARP06
Role of archaeal DnaG protein in tailing and degradation of stable RNA

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The archaeal exosome is a phosphorolytic 3'-5' exoribonuclease. In a reverse reaction it synthesizes A-rich RNA-tails. Its RNA-binding cap comprises the eukaryotic orthologs Rrp4 and Csl4, and an archaea-specific subunit annotated as DnaG. The subunit DnaG needs Csl4 for the interaction with the exosome. In *S. solfataricus* DnaG and Rrp4 but not Csl4 show poly(A)-preference. Archaeal DnaG contains N- and C-terminal domains (NTD and CTD) of unknown function flanking a TOPRIM domain. We found that the NTD and TOPRIM domains have comparable, high conservation in all archaea, while the CTD conservation is higher in exosome-containing archaea. We show *in vitro* that the NTD is a novel RNA-binding domain with poly(A)-preference cooperating with the TOPRIM domain in binding of RNA. Consistently, a fusion protein containing full-length Csl4 and NTD of DnaG led to enhanced degradation of A-rich RNA by the exosome. We also found that DnaG strongly binds native and *in vitro* transcribed rRNA and enables its tailing by the exosome. An rRNA-derived transcript with a heteropolymeric tail was faster degraded by the exosome than its non-tailed variant. Based on our data, we propose that archaeal DnaG is an RNA-binding protein which, in the context of the exosome, is involved in targeting of stable RNA for degradation by addition of heteropolymeric tails.
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BIOENERGETICS

BEP01
Energetic state of *Dinoroseobacter shibae* during short-term anoxia

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The Roseobacter clade is one of the most abundant groups of marine bacteria world-wide. This study focuses on Dinoroseobacter shibae DFL 12T, a model organism of this clade, capable of anoxygenic photosynthesis under oxic conditions. D. shibae uses light as additional energy source, which supports survival during long-term starvation (1). It has been shown that this organism is capable of fast ATP regeneration after being energetically depleted due to short-term anoxia (2). Our hypothesis was that the membrane potential ($\Delta\Psi$), as main component of the proton-motive force, undergoes drastic variations during this process. The intracellular pH (pHi) was determined in non-buffered cell suspensions after permeabilisation of the cell membranes with butanol (3). Live-dead staining with SybrGreen I and propidium iodide was performed to determine cell viability. This was evaluated with epifluorescence microscopy and flow cytometry. The latter method was also refined with a valinomycin as $\Delta\Psi$-disturbing ionophore. pHi was found to be around 7.3 and thus lower than that of the medium (7.5), for both oxic and anoxic conditions. Live-dead staining, however, revealed remarkable differences between these conditions. De-energized cells stained red (indicating cell death), while after air-flushing under illumination for 10 min cells stained green (alive). Valinomycin treatment resulted in a similar effect as anoxia, without being reversible. These results suggest that $\Delta\Psi$ influences the outcome of live-dead staining experiments by showing low $\Delta\Psi$ cells falsely as dead. For fast ATP regeneration $\Delta\Psi$ has to be restored since ΔpH remains close to zero. Quantification of $\Delta\Psi$ under the different conditions is going on.

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BEP02
How to obtain energy from H_2 under aerobic conditions - Role of novel cofactors and supermolecular states of a membrane-bound [NiFe] hydrogenase

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H_2 is utilized by many microbes as energy source and for disposing excess reducing equivalents. Both reactions are catalyzed by hydrogenases which are in most cases sensitive towards O_2. Certain [NiFe] hydrogenases, including the membrane-bound [NiFe] hydrogenase (MBH) from *Ralstonia eutropha* [1], perform H_2 conversion in the presence of O_2. This remarkable O_2 tolerance bases crucially on a unique [4Fe3S] cluster coordinated by six cysteines [2,3]. X-ray crystallography, pulsed electron paramagnetic resonance, resonance Raman spectroscopy as well as density functional theory calculations were employed to analyze the redox-dependent chemical, structural and electronic properties of the [4Fe3S] cofactor and its ligands. The [4Fe3S] cluster undergoes redox-dependent reversible structural

rearrangements, namely an iron swapping between a sulfide and a peptide amide N [4-6]. Moreover, our investigations unraveled the redox-dependent and reversible occurence of an oxygen ligand located at a different iron [6]. This ligand is hydrogen-bonded to a conserved histidine that is essential for H_2 oxidation at high O_2 levels. We propose that these transformations, reminiscent of those of the P-cluster of nitrogenase, enable the consecutive transfer of two electrons within a physiological potential range. The necessity for all modifications is the price for H_2 conversion in the presence of O_2, namely a minor oxidase activity [7] enabling the active site to recover rapidly from oxidative inactivation.
This fast recovery from oxidative inactivation enables utilization of oxygen-tolerant hydrogenases in a biological fuel cell setup. However, hydrogenases are usually inactivated anaerobically at high electrode potential [8]. The full heterotrimeric MBH, including the membrane-integral cytochrome *b* subunit, was investigated electrochemically using electrodes modified with planar tethered bilayer lipid membranes resembling native conditions. Cyclic voltammetry and chronoamperometry experiments show that MBH, in equilibrium with the quinone pool, does not anaerobically inactivate under oxidative redox conditions [9]. In aerobic environments, the MBH is reversibly inactivated by O_2, but reactivation was found to be fast even under oxidative redox conditions. This enhanced resistance to inactivation is ascribed to the oligomeric state of MBH in the lipid membrane [10].

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BEP03
Protonation Dynamics in Protein Function: Identification of key protonation sites and paths in photosynthetic water oxidation via site-directed mutagenesis of cyanobacterial photosystem II

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Sequential absorption of minimally four photons by the chlorophylls of the photosystem II (PSII) protein complex is required to drive one turnover of the water-oxidation cycle. In this process, the spatio-temporal orchestration of proton removal from the protein-bound Mn_4Ca complex is functionally crucial. However, these protonation dynamics as well as the structural basis for proton release are only insufficiently understood.
The tight coupling of local protonation dynamics and long-distance proton relocations is most likely mediated by the hydrogen-bonded clusters formed by charged amino acids of the PSII proteins and water molecules (~1300 water molecules per PSII monomer). To understand the protonation dynamics at the atomic level, various tools for monitoring proton relocations, crystallization as well as site-directed mutagenesis of crucial amino acids involved in the hydrogen-bonded network will be employed. Major focuses of the present study are both:

(1) The different mutagenesis approaches for genetic modification and/or knockout of different photosystem-II core proteins and variants (isoforms) in (i) strict photoautotrophic and (ii) facultatively photoheterotrophic cyanobacteria such as *Thermosynechococcus elongatus* and *Synechococcus* sp. PCC 7002, respectively.
(2) The different isolation approaches to obtain highly-qualified PSII core particles and crystals resulting in high-resolution crystal structures of "protonation mutants".

BEP04
How does *Staphylococcus aureus* generate membrane potential?

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In many aerobic and facultative aerobic microorganisms the first complex of the respiratory chain is represented by the NADH:quinone oxidoreductase (complex I). The protein complex oxidizes NADH generated during glucose degradation, thus inducing the respiratory electron transfer and generation of membrane potential. The resulting proton motive force enables the synthesis of ATP. In *Escherichia coli* complex I is well studied and is composed of 13 subunits; in contrast to *Staphylococcus*, where a corresponding complex has not been detected so far. We identified a hypothetical protein in *S. aureus* with conserved regions of *E. coli*'s H^+-translocating subunit of NADH:quinone oxidoreductase. In *S. aureus* the orthologue is organized in an operon comprising three genes. Deletion mutants of the corresponding genes were physiologically characterized. They show an SCV-like phenotype and are strongly impaired in growth, membrane potential generation and oxygen consumption rates. The results indicate a crucial role in aerobic respiratory chain, energy metabolism and cellular fitness.

BEP05
New functional insights into the Sox proteins from *Allochromatium vinosum*

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In the purple sulfur bacterium *Allochromatium vinosum* thiosulfate oxidation to sulfate is initiated in the periplasm by the Sox enzyme system [1]. Heme *c*-containing SoxXAK oxidatively links thiosulfate to cysteine-152 of SoxYZ forming a thiocysteine-S-sulfonate. Sulfate is then released by the thiohydrolase SoxB while SoxYZ needs to be regenerated for the next reaction cycle by a yet unknown mechanism. Here, we provide new functional and structural insights into SoxXAK, SoxYZ and SoxL, a putative sulfurtransferase that is encoded in the *soxXAKL* cluster.
SoxK was proven to be important for SoxXA activity and stability and thus crucial for the oxidation of thiosulfate *in vivo.* Under photolithoautotrophic growth conditions, the mutant strain *A. vinosum* Δ*soxK* was not able to oxidize thiosulfate completely. The intermediary formation of sulfur globules as well as the formation of the final product sulfate was strongly impaired. Heterologous production and purification of stable, fully heme-loaded SoxXAK enabled reconstitution of the complete *A. vinosum* Sox system *in vitro*. Mixtures of recombinant SoxXAK, SoxYZ and SoxB catalyzed thiosulfate-dependent reduction of horse heart cytochrome *c*, however, an unusual "lag"-phase was observed before a linear "activity"-phase was reached. The "lag"-phase was not present when SoxYZ was pre-treated with sulfide. MALDI-TOF measurements did not indicate persulfuration of SoxYZ, strongly suggesting that activation of SoxYZ rather involves a sulfide induced conformational change. Upon analytical gel filtration SoxYZ appeared in two different fractions corresponding to the heterodimeric (SoxYZ) and the heterotetrameric $(SoxYZ)_2$ forms. An activity lag phase was not observed for the heterotetramer even without pretreatment with sulfide. These findings prompted us to investigate enzymes that might switch the conformation of SoxYZ. Addition of SoxL significantly extended the "lag"-phase, while sulfide-treated SoxL had just the opposite effect and a "lag"-phase was no longer observed. Hence, SoxL appears to be able to induce conformational changes of SoxYZ. The SoxL protein consists of a carboxy-terminal rhodanese domain with two conserved cysteines (Cys_{179} and Cys_{184}), and an amino-terminal domain characterized by a thioredoxin-like motif [Cys_{68}ProProPheCys_{72}]. Fifteen SoxL mutant proteins were produced, thus covering all possible combinations of cysteine to serine exchanges. First series of experiments clearly showed that SoxL without any cysteines does not retain any effect on SoxYZ. While SoxL lacking the N-terminal cysteine pair influenced Sox reactions in almost the same way as the wild type protein, removal of the carboxy-terminal cysteine pair significantly decreased SoxL activity.

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BEP06
Structural Basis of Biological NO Generation by Octaheme Oxidoreductases

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Nitric oxide (NO) is an important molecule with significant biological functions in both pro- and eukaryotes. Anaerobic ammonium-oxidizing (anammox) bacteria, which contribute substantially to the release of molecular nitrogen into the atmosphere, use the oxidizing power of NO to activate ammonium into the highly unusual metabolite hydrazine (N_2H_4). Here we describe the enzyme kustC1061 from the anammox bacterium *Kuenenia stuttgartiensis,* that uses a novel pathway to synthesize NO from hydroxylamine. This enzyme is related to octaheme hydroxylamine oxidoreductase (HAO), a key protein in aerobic ammonium-oxidizing bacteria. Based on combined structural and biochemical investigations, we propose a mechanism for NO formation by kustC1061. The molecular structure of the *K. stuttgartiensis* enzyme was determined by X-ray crystallography at 1.8 Å resolution and a structural model of *Nitrosomonas europaea* HAO was reassessed and refined. Both protein structures were determined in the presence and absence of their substrates. Moreover, biochemical and biophysical studies, including UV-Vis spectroscopy, enzyme kinetic measurements, protein film electrochemistry and analytical ultracentrifugation, were carried out. In contrast to *Nitrosomonas* HAO, which oxidizes hydroxylamine to nitrite, it was found that kustC1061 specifically oxidizes hydroxylamine to NO. Both enzymes show a high structural similarity and contain the unique P460 heme cofactor in their catalytic centre, but show subtle amino acid differences near the active site that account for their reaction specificity. The presence of kustc1061 homologues in anammox and other bacteria might enable the detoxification of hydroxylamine, generating NO for respiratory purposes.

Reference: J. Biol. Chem. 289, 1228-42 (2014)

BEP07
Structural and biochemical analysis of the unusual acidophilic *c*-type cytochrome thiosulfate dehydrogenase from *Allochromatium vinosum*

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Thiosulfate dehydrogenases (TsdA) catalyze the reaction of two molecules thiosulfate to tetrathionate, thereby releasing two electrons. TsdA homologues are widespread among bacteria, which agrees with reports of tetrathionate formation not only by specialized sulfur oxidiziers but also by many chemoorganoheterothrophic bacteria [1]. In the purple sulfur baterium *A. vinosum*, thiosulfate dehydrogenase is a periplasmic, monomeric 27.2 kDa diheme *c*-type cytochrome. Electronic paramagnetic resonance spectroscopy indicated cysteine and methionine as sixth distal axial ligands of the two heme irons [1]. Here, we present structural data for the dithionite-reduced cytochrome, which clearly proves the proposed axial ligation of heme 1 by His_{53} and Cys_{96} and heme 2 by His_{164} and Met_{209}. This prompted us to elucidate the function of the heme-ligating potential active site Cys_{96} in more detail. TsdA variants containing exchanges of Cys_{96} to glycine, methionine or histidine were effectively produced in *E. coli*. The very high specific activity of 28.000 U/mg of recombinant wildtype AvTsdA with thiosulfate as the substrate [1] is completely abolished in all of the TsdA mutants, clearly indicating Cys_{96} as an essential active site residue. In accordance, none of the Cys_{96} variants exhibited any activity in the reverse direction, i.e. tetrathionate reduction. The AvTsdA wildtype protein effectively catalyzes tetrathionate reduction with reduced methylviologen as an electron donor, albeit with low specific activity (22 U/mg) [2]. For the AvTsdA wt protein, Vmax is about six times higher at pH 4.0 than at pH 5.0 in the thiosulfate oxidizing direction [1]. Still, the catalytic efficiency of the enzyme is signficantly higher at pH 5.0 (~5-fold higher k_{cat}) due to a considerable increase of substrate affinity. We assign this effect to increasing protonation of the catalytically active cysteine at lower pH. Significant differences in absorption intensities of α-, β- and γ-peaks observed by UV-vis spectroscopy at different pH values are in full agreement with this interpretation. Redox cycling of the wildtype protein between the fully reduced and fully oxidized forms revealed an intermediate state characterized by a weak absorption band at 634 nm, pointing at the transient presence of high-spin heme, possibly caused by movement of Cys_{96} out of the iron coordination sphere. This conclusion is supported by the absence of the intermediate 634 nm absorbance band in all of the TsdA Cys_{96} variants.

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BIOTECHNOLOGY (INCL. MICROBIAL BIOCATALYSIS)

BTP01
A modified Hydantoinase Process for the synthesis of enantiopure β-amino acids in an enzymatic reaction cascade

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β-amino acids are becoming increasingly important for the synthesis of chiral fine chemicals and pharmaceuticals. Though, the efficient production of chiral β-amino acids remains a challenge. A modified Hydantoinase Process for the synthesis of chiral D- or L-β-amino acids applying a racemase, a dihydropyrimidinase and a *N*-carbamoyl-β-amino acid hydrolase was analyzed (see figure 1). The enantioselective conversion of several unnatural dihydropyrimidines to *N*-carbamoyl-β-amino acids using whole-cell biocatalysis was successful. [1, 2] However, challenges like low substrate solubilities as well as transport limitations of applied substrates and resulting products occur. To avoid these limitations, approaches employing artificial enzymatic reaction cascades in cell-free systems with organic solvents will be tested with regard to their application in microfluidic reaction systems. For this purpose, suitable enzymes have to be identified and different reaction conditions and rates of enzymes need to be adjusted.

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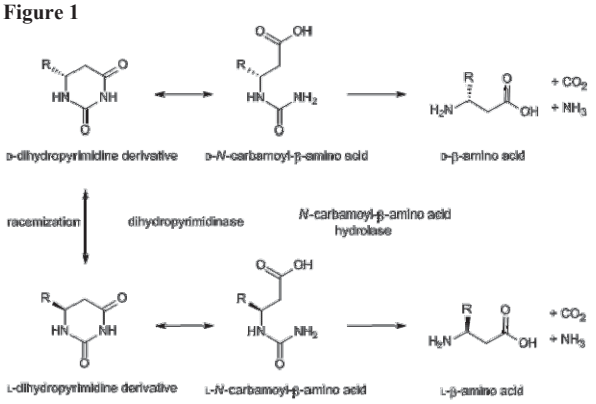
Figure 1



BTP02
Quantification of methanogenic heterodisulfide reductase activity in biogas sludge

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Biogas mainly consists of methane and carbon dioxide and is produced by anaerobic fermentation of organic matter in biogas plants. The microbial activity includes cleavage of complex organic polymers and the degradation of monomers to organic acids and to a lesser extent to alcohols. Subsequently, syntrophic bacteria convert these compounds to acetic acid, hydrogen and carbon dioxide, which are used to form methane via hydrogenotrophic or aceticlastic pathway of methanogenesis.
In general, the development of novel sensors in biogas plants is desirable to quantify the activities of key enzymes by simple but specific detection systems. A potential candidate is a key enzyme in all methanogenic pathways, the heterodisulfide reductase. Methanogenesis finally results in the formation of the heterodisulfide and the heterodisulfide reductase reduces this intermediate as a terminal electron acceptor of the methanogenic energy conserving systems [1]. In aceticlastic methanogens the heterodisulfide reductase is tightly bound to the cytoplasmic membrane. In contrast, the enzyme is located in the cytoplasm in hydrogenotrophic methanogens [2].
With our test system, we were able to detect heterodisulfide reductase activity in cell free extract prepared from 1 g biogas sludge. Reduced methylviologen was used for heterodisulfide reduction in the test system. The activity was detected photometrically and was 64 ± 17 mU x mg protein^{-1} (1 U = 1 µmol heterodisulfide reduced per min) [3].
Furthermore, cell lysate from 60 g of biogas sludge was separated by ultracentrifugation. The different localization of heterodisulfide reductase within the cells allows to separate the proteins from the sludge into the two cellular fractions, cytoplasmic membranes and cytoplasm. When reduced methylviologen was used as electron donor 26% of total heterodisulfide activity was found in the membrane preparation and the cytoplasmic fraction contained 74% of total activity [3].
Hence, the novel test system allows to quantify the performance of hydrogenotrophic and aceticlastic methanogens in biogas sludge and may be suitable to detect changes of metabolic activity of methanogens as a consequence of disorders of the biogas production process. Thus, there might be possibilities of application and the option to take early action before serious process failures of the entire system occur.

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BTP03
Expression of a functional recombinant human glycosyltransferase in *E. coli*

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Recombinant protein therapeutics have revolutionised the treatment of many diseases and are produced using well-established expression systems based on bacteria, yeast, insect and mammalian cells. However, the use of bacteria for expression of eukaryotic proteins is limited by the lack of post-translational modification pathways, including those for glycosylation [1].
The development of an *Escherichia coli*-based system for production of human glycoproteins could potentially lead to increased yields, as well as significant decreases in processing time and costs.
Here, we describe the expression of functional human-derived glycosyltransferase UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAcT2) [2] in a recombinant *E. coli* strain. A codon-optimised gene encoding amino acids 52-571 of GalNAcT2 [2] was inserted into a pET-23 derived expression vector encoding a polyhistidine-tag which was translationally fused to the N-terminus of the glycosyltransferase (HisDapGalNAcT2). HisDapGalNAcT2 was produced in *E. coli* using a recently published expression system including the pre-expression of the sulfhydryl oxidase Erv1p and protein disulfide isomerase PDI [3]. Soluble HisDapGalNAcT2 was purified using nickel affinity chromatography and was subsequently analysed by size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) and circular dichroism spectroscopy to determine molecular mass, folding state and thermal transitions of the protein. It has been shown that GalNAcT2 initiates O-glycosylation by the addition of the monosaccharide N-acetylgalactosamine to the hydroxyl group of the amino acids serine or threonine [4]. The activity of purified HisDapGalNAcT2 was monitored using a colorimetric assay based on the release of phosphate during transfer of glycosyl residues to a model acceptor peptide. Modifications were assessed by Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry (MALDI-TOF-MS) analysis, which indicated the presence of N-acetylgalactosamine residues at several putative glycosylation sites of the acceptor peptide, confirming that recombinant human HisDapGalNAcT2 had glycosyltransferase activity *in vitro*. Future investigations will assess the potential of *in vivo* glycosylation in *E. coli*.

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BTP04
Physiology and transcriptomics of the adaptation of the marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis* SK2 to its aliphatic substrates

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The marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis* is able to degrade mixtures of n-alkanes as they occur in marine oil spills. Growth behaviour and physiology of these bacteria cultivated with n-alkanes of different chain lengths (C6-C30) as substrates was investigated. Growth rates increased with increasing alkane chain length up to a maximum between C12 and C19, with no evident difference between even and odd numbered chain lengths, before decreasing with chain length greater than C19. Surface hydrophobicity of alkane-grown cells, measured as water contact angles, showed a similar pattern with maximum values associated with growth rates on alkanes with chain lengths between C11 and C19, and was significantly lower for cells grown on pyruvate. *A. borkumensis* was found to incorporate and modify the fatty acid intermediates generated by the corresponding n-alkane degradation pathway. Cell grown on distinct n-alkanes proved the capability to not only incorporate but also modify fatty intermediates derived from the alkane degradation pathway. Comparing cells grown on pyruvate with those cultivated on hexadecane showed similar tolerances towards toxic concentrations of chlorophenols, whereas tolerance to different n-alkanols was significantly increased when hexadecane was used as carbon source. These findings could be verified by a detailed transcriptomic comparison between cultures grown on hexadecane and pyruvate including solvent stress caused by addition of 1-octanol as the most toxic intermediate of n-alkane degradation.

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BTP05

New approaches of cell enumeration and activity quantification of *Acidithiobacillus ferrooxidans* in leaching samples

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Determination of microbial activity and biomass is an essential task in order to obtain reliable bioleaching rates.

Hence, we present a fluorometric approach as a new alternative to the established, but quite expansive and laborious q-PCR, with which reproducible *Acidithiobacillus ferrooxidans* cell enumerates were achievable. Total fluorescence of PicoPreen-stained cells was determined by a microplate reader after the detachment of cells from and removal of mineral precipitates. Reliability of this method was shown byepi-fluorescense microscopy following an automated cell counting. For further evaluation this method will be placed in correlation with extracted DNA and results compared to those obtained by commonly used q PCR method.
Besides the cell enumeration, we were able to apply two respiration methods for quantification of activity of iron-oxidizing bacteria. It was possible to show the applicability of an optode- as well as an OxiTopC™-based procedure with *At. ferrooxidans.* While the OxiTopC™-approach aimed at the monitoring over several days or even weeks, the optode-approach delivered results within minutes. Before the establishment of the approaches presented here, both methods were mainly used for the investigation of soil or marine communities.
By plotting the activities determines by the optode-based approach and the biomass as a function of time within the same diagram, it became obvious, that curve progressions were similar. With our OxiTopC™-approachwe could clearly differentiate between microbial ferrous iron oxidation and its chemical regeneration (i.e. leaching attack). Both methods are more suitable for activity determination than the previously used ferrous iron oxidation rate, since the detection principle depends, under thermostatic conditions, solely on the consumption of oxygen. Hence changes in the medium have only minor impacts.

BTP06

Itaconic acid pathway of *Ustilago maydis*

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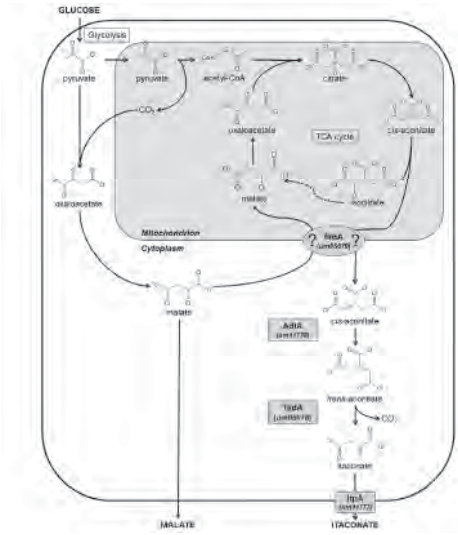
In recent years the chemical industry is making attempts to replace petrol-based with bio-based chemicals to become more sustainable and environmentally friendly. Among others, itaconate has the potential to become an adequate bio-based building block for the production of numerous valuable chemicals. Besides the currently industrial biosynthetic producer of itaconate, *Aspergillus terreus,* also other species are able to produce itaconate naturally, such as *U. maydis*. Although their itaconate yield is relatively low compared to an optimized *A. terreus*, they possess attributes, such as single cell growth, that can lead to considerable process advantages. One reason for the relatively low yield is that optimization of *U. maydis* via metabolic engineering was thus far hindered by a lack of fundamental knowledge about the itaconate biosynthesis pathway. Therefore, in this study the genes encoding the proteins responsible for the itaconate production of *U. maydis* were identified and a novel biosynthesis pathway for itaconate is proposed (Figure 1), which is different from the one established in *A. terreus*.
The pathway was confirmed by corresponding deletion and overexpression mutants as well as heterologous expression in *E. coli* and *S. cerevisiae* strains and resting cell assay experiments in *U. maydis* and *S. cerevisiae*.
The genes *cyp3* (putative cytochrome P_{450} monooxygenase), *tad1* (*trans*-aconitate decarboxylase), *itp1* (Major Facilitator Superfamily extracellular itaconate transporter), *adi1* (aconitate-Δ-isomerase), *mtt1* (mitochondrial tricarboxylate transporter), and *ria1* (transcriptional itaconate regulator) are arranged in a gene cluster involved in the itaconate biosynthesis and possibly its degradation. First metabolic engineering attempts were already successful enhancing *U. maydis'* itaconate production twofold by overexpressing the transcription factor regulating the gene expression of the itaconate cluster.
With the physiological and genetic characterization of *U. maydis'* itaconate biosynthesis pathway, this work lays the foundation for further optimization of *U. maydis*' itaconate biosynthesis and is therefore a further step towards industrial application, competitive with *A. terreus*. However, to compete with petrochemical-derived products several factors remain to be investigated and improved before *U. maydis* can meet its full potential as a biocatalyst for the production of valuable itaconate from renewable, non-food biomass feedstock at an industrial scale.

Figure 1



BTP07

Mirror image phage display for selection of Tau-binding D-enantiomeric peptides for therapeutic applications in neurodegenerative diseases

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A variety of neurodegenerative disorders, including Alzheimer's disease, are associated with amyloid fibrils and neurofibrillary tangles composed of the tau protein. Inhibitors of pathological amyloid fibril formation could be useful in the development of therapeutics, provided that the inhibitors were specific enough to avoid interference with physiological processes.
We have developed biotechnological strategies to identify specific peptides out of large phage displayed peptide libraries by specialized selection procedures. In order to avoid disadvantages of natural peptides (e.g. protease sensitivity, immunogenicity), we apply mirror image phage display procedures to yield D-enantiomeric peptides that are extremely protease stable and not or less immunogenic than L-peptides.
Employing mirror-image phage display with a large peptide library (> 1 billion different peptides), we have identified tau-fibril binding peptides consisting of D-enantiomeric amino acids. Selections were performed using fibrils of the D-enantiomeric hexapeptide VQIVYK, representing residues 306-311 of the tau protein, as a target. VQIVYK has been shown to be important for fibril formation of the full-length protein and itself forms fibrils with biophysical properties similar to full-length tau fibrils. Here, we report various on D-enantiomeric peptides which bind to VQIVYK as well as to full length tau fibrils and modulate the aggregation thereof. These peptide might be an interesting starting point for therapy development.

BTP08

Thermostable laminarinases as promising biocatalysts for the production of biofuels and chemicals

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Current environmental challenges and the limitation of fossil fuels are generating a high demand of feedstocks for production of renewable energy and chemicals. Different concepts have been recently developed for the utilization of plant biomass based on starch or lignocellulose. Macroalgae are a promising alternative due to their abundance and high content of carbohydrates (up to 60 %) [1]. Interestingly and in contrast to terrestrial biomass, macroalgae lack lignin and contain the polysaccharides laminarin,

carrageenan and alginate. Thus, efficient hydrolysis of macroalgae biomass is a challenging task and requires efficient enzymes for the bioconversion of this highly complex substrate. In this work we focused on hydrolysis of laminarin which is a main storage polysaccharide in brown algae and one of the most abundant carbohydrates in marine ecosystems. Laminarinases are capable enzymes to degrade laminarin into mono- and oligosaccharides. Depending on species, season and growing conditions laminarin represents up to 11 % of the dry weight in brown algae [2]. Laminarin consists of 20 to 30 linear β-1,3-linked glucose units with occasional β-1,6-linkages.
For the identification of novel laminarinases, a sequence-based screening of metagenomic data was performed and among others a gene encoding for a putative laminarinase from a thermophilic bacterium was identified. The putative laminarinase was heterologously produced in *E. coli* and successfully purified. This enzyme exhibits high activity on laminarin from *Laminaria digitata* at high temperatures representing a promising candidate for the hydrolysis of brown algae.

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BTP09
Carbon Dioxide Based Acetone Fermentation

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Acetone is an important bulk chemical for the industry. It is used as a solvent as well as an important value added chemical for the synthesis of polymers like poly(methyl methacrylate) (PMMA), also known as acrylic glass. Today, acetone is still mainly produced from fossil resources. However, future challenges require alternative strategies enabling the generation of chemicals and biofuels from renewable resources, most favourable based on the usage of the greenhouse gas carbon dioxide (CO_2) as a substrate. CO_2 is a less expensive feedstock and available in great quantities. Furthermore, CO_2 as growth substrate is not interfering with food production, as glucose and other sugars do. Thus, the aim of the project "CO_2-based acetone fermentation" (COOBAF) funded by the German Federal Ministry of Education and Research (BMBF) is the development of a fermentation process in which acetogenic bacteria produce acetone using CO_2 as starting material.
Although more than one hundred acetogenic bacterial species are described so far, in part there is little knowledge about their applicability as production strains. After a screening of 39 autotrophic acetogenic strains the actual work focuses on six organisms with potential for being a future biotechnological production strain. Here, we present a new vector system that can be adapted to a variety of acetogenic bacteria. Plasmids, containing a synthetic acetone synthesis operon, have been transformed either into thermophilic or mesophilic acetogenic bacteria and subsequently cultivated. Both, under heterotrophic conditions with an organic carbon source as well as under autotrophic conditions with CO_2 + H_2 as substrates, acetone production could be confirmed in flask batch cultures and the productivity is currently optimized in up-scaled reactor cultivations.

BTP10
Phenotypic heterogeneity during heterologous protein production in *Bacillus megaterium*

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Within the last years *Bacillus megaterium* was systemically developed for the gram per liter production of recombinant proteins using the strong xylose-inducible promoter system P_{xylA}. The expression system is based on a multicopy plasmid containing the functional elements of the system namely the gene encoding the repressor XylR and the promoter P_{xylA}. For a deeper understanding, P_{xylA} was fused to the coding sequence of the green fluorescent protein (GFP). If xylose is absent its expression is repressed by the xylose repressor XylR while in the presence of xylose the expression is derepressed. Although high yields of GFP were produced the culture showed a significant level of heterogeneity at single cell level during the recombinant protein production process with up to 30% of low-producing cells leading to three speculative working models - (1) an imbalanced repressor inducer equilibrium, (2) an unequal distribution of plasmids during cell division or (3) cell aging effects. Referring to the first model, XylR with and without bound xylose was recombinantly produced in *Escherichia coli* and purified. Native PAGE and XylR/DNA-binding studies showed *in vitro* the occurrence of the two different conformation and oligomerization states of XylR in the presence or absence of xylose while binding or omitting DNA. These studies indicated that the binding of xylose to the XylR is a transient phenomenon making these studies more complicated. On the other site *in vivo* studies of the XylR-free *B. megaterium* carrying the *xylR* free multicopy plasmid gave some hints that the phenomenon of phenotypic heterogeneity is more related to different plasmid copy numbers within the producing cells. In order to gain more information about the production dynamics in single cells XylR was translationally fused to mCherry and further analyzed using time-lapse fluorescent microscopy and flow cytometry. Further, the intracellular ratio of repressor molecules and DNA binding sites was modified leading to an altered equilibrium of promoter complexes and consequently affecting culture heterogeneity. Derived results led to the hypothesis that heterogeneous production behaviour might also be a consequence of cell aging effects. Finally, the observations were incorporated into an extended mathematical model in order to reproduce the bistabilty via computer simulations.

BTP11
The potential of acetate and ethanol to increase methane formation in biogas plants

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Biogas, which is formed during the degradation of organic material, is one of the most important renewable energy sources and is used for the generation of electric power and heat. Despite it´s great importance, biochemical processes in biogas plants are still not fully understood and improvements based on analysis of microbial activity might be possible. To shed light on the question which group of organism constitutes the limiting factor in the anaerobic breakdown of organic material, biogas sludge from different mesophilic biogas plants was examined under various conditions. Therefore, biogas sludge was incubated for 24 hours in anaerobic serum flasks under an atmosphere of N_2/CO_2. These batch reactors mirrored the conditions and the performance of the full-scale biogas plants. They were suitable test systems to observe short-term effects on biogas formation caused by the addition of different substrates for syntrophic bacteria (butyrate, propionate, or ethanol) and methanogenic archaea (acetate or H_2+CO_2). Methane production rates increased by 35 to 126 % when sludge from different biogas plants was supplemented with acetate or ethanol but other fermentation products such as propionate, butyrate or H_2 did not result in increased methane formation rates. The stability of important process parameters such as concentration of volatile fatty acids and pH indicated that ethanol and acetate increased biogas formation without affecting normally occurring fermentation processes [1].
With respect to possible future biotechnological applications, it was important to reveal if increased methane production rates due to ethanol addition can also be maintained over a longer time period. Therefore, small-scale and lab-scale continuous reactors filled with biogas sludge were developed. Those reactors were fed daily with the same substrates as the full-scale reactor the sludge derived from. Stable process conditions, which were comparable to those of a full-scale biogas plant, were observed during a period of 14 days. Methane formation was significantly increased when 10-20 mM ethanol was added to the biogas sludge daily in addition to normal feeding and also when higher ethanol concentrations of 50-100 mM were added every seven days. “Normal” methane production continued to take place but ethanol led to production of additional methane in the biogas plant. With these results, we provide evidence that aceticlastic methanogenesis and ethanol-oxidizing syntrophic bacteria are not the limiting factor in the process of biogas formation, respectively and that biogas plant optimization is possible with special focus on methanogenesis from acetate.

[1] Refai et al. (2014). Appl Microbiol Biotechnol, epub ahead of print

BTP12

Advances in Characterization of Dimethylphenol Degrading Bacteria from Pilot Scale Constructed Wetlands

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Constructed Wetlands (CWs) are technologies suitable for treating both municipal sewage and industrial wastewater. In these systems, the main role in the transformation and mineralization of organic pollutants is played by microorganisms. Dimethylphenols (DMP, xylenols) are toxic compounds with high environmental mobility in water and one of the main constituents of coal pyrolysis industry effluents. In order to understand the DMP degradative metabolic pathways activity and the role of these communities in CWs environment, it is necessary to isolate and characterize potential degraders from such environments. Therefore, samples from roots, gravel and water were collected from three different pilot scale CWs fed with artificial wastewater containing 40 mg/L of 2,6; 3,4 and 3,5-DMP in equimolar ratios. Sample from each CWs were inoculated in liquid minimum media with 70 mg/L of 2,6; 3,4 and 3,5-DMP as sole carbon and energy source. Isolated strains were physiologically characterized in liquid media containing 70 mg/ L of all DMP isomers. Bacteria were taxonomically identified by sequencing of 16s rDNA.
All samples from CWs in M9 liquid medium and DMP, showed degradation rates up to 92%, 100% and 35% for 3,4; 3,5 and 2,6-DMP respectively, during the first 6 days. A total of 20 isolates were obtained. Kinetic tests showed that *Delftia acidovorans* and *Burkholderia* sp. were both able to completely degrade o-xylenols (2,3 and 3,4- DMP). An absorbance increase at 375 nm during the oxidation processes of 3,4-DMP by *D. acidovorans* was detected, showing the accumulation of 2-hydroxymuconic semialdehyde, a compound formed by the meta-cleavage of catechol by the catechol 2,3-dioxygenase, one of the two most typical pathways for metabolizing phenols and other aromatics, after the first di-hydroxylation of the benzene ring to produce catechol.

BTP13

Effects of Ca^{2+} and Mg^{2+} on the degradation of polyethylene terephthalate by polyester hydrolases from *Thermobifida fusca*

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Polyethylene terephthalate (PET) is a synthetic polymer with a semi-crystalline structure. The efficient hydrolysis of PET by recently discovered polyester hydrolases from *Thermobifida fusca* requires reaction temperatures close to the glass transition temperature of PET at 71 °C where the polymer chains become more accessible for enzymatic degradation. The enzymes can hydrolyse PET at reaction temperatures up to 60 °C. It has been shown that their melting point can be increased by the addition of divalent cations. When PET was hydrolyzed by the polyester hydrolases TfH, BTA2, Tfu_0882, TfCut1 and TfCut2 from three strains of *T. fusca* in the presence of Ca^{2+} or Mg^{2+}, the reactions could be performed at temperatures up to 65 °C. The weight losses of PET films achieved at this reaction temperature were higher compared to those obtained at 55 °C and 60 °C. The binding of Ca^{2+} to the enzymes was studied using molecular dynamics simulations. Ca^{2+}-binding ligands could be identified in close vicinity to the active site of the polyester hydrolases.

BTP14

Application of *Streptococcus pyogenes* arginine deiminase in glioblastoma therapy

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Introduction: Arginine is a non-essential amino-acid , playing a critical role in human tumor cell proliferation. It is synthesized from citrulline and aspartate by two enzymes, argininosuccinate-synthetase (ASS) and argininosuccinatelyase (ASL). Previous studies presented evidence for arginine auxotrophy in several tumors due to ASS-deficiency, amongst them glioblastoma multiforme (GBM). Hence, ASS-deficient tumors are supposed to be sensitive for arginine-depleting substances, such as arginindeiminase (ADI). In this study, we aimed at elucidating the sensitivity of ultra-low passage GBM cell lines towards *Streprococcus pyogenes* ADI and drug combinations.
Materials and Methods: Sensitivity of 12 GBM cell lines towards ADI (25 mU/ml) and combinations with Palomid 529 (a TORC1/TORC2 inhibitor) or chloroquine were analyzed by means of proliferation, viability, apoptosis and necrosis. ADI originates from *Streptococcus pyogenes* (M49) and was produced in *E. coli* using the IBA System followed by an affinity-chromatographic purification step (Strep-Tag). For combination studies, drugs were applied in low doses ($<IC_{50}$ values). Treatments were performed for 72h and 144h, respectively.
Results: Half of the GBM cell lines responded well towards ADI monotherapy. In those cell lines, viability significantly decreased (up to 50% killing). Responding cell lines were subsequently applied in combination experiments to test if any additive or even synergistic effects may be anticipated. Such promising results were obtained 3/5 cases. In cell lines HROG02, HROG05 and HROG10, ADI and Palomid combinations were most effective yielding more than 80% killing after two rounds of treatment. Comparable boosted antitumoral effects were observed after adding chloroquine to ADI (up to 70 % killing). In initial studies on identifying possible underlying mechanisms, apoptosis was nearly completely excluded, as well as a significant influence on the cell cycle. In most, but not all cases, the expression of several enzymes of the arginine synthesis pathway (ASS and ASL) explained obtained Results:
Conclusion: Starting from these results, S. pyogenes ADI suggests itself as a very promising (since non-toxic) candidate especially for development of adjuvant combination treatments of GBM.

BTP15

Immobilized and Entrapped *E. coli* Cells are Metabolically Active

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Enzymatic catalysis in chemical industry is of particular interest. The enantio-, regio- and stereoselectivity of these biological catalysts facilitates complex conversions, increases product yields and decreases production costs. Furthermore, the specificity to selected reactions prevents byproduct formation and the reaction process can be easily controlled. Unfortunately, cofactor or cosubstrate requirement can prevent industrial application, if an appropriate regeneration system is not available. Also multistep reactions with several enzyme components are hardly applicable because of high costs when purified enzymes are used. Whole cell catalysis represents an alternative strategy which circumvents these disadvantages. Evolutionary optimized organisms are able to adapt to several circumstances and continuous metabolism keeps supplies coming. Furthermore, manipulation of the genetic code opens the possibility to equip microorganisms with additional tools. Thereby, also artificial components, that are necessary for a particular reaction, can be implemented into a single cell. Another advantage of cellular systems is the stabilization and protection of intracellular enzymes from the extracellular environment. Beside the utilization of free cells it is also possible to immobilize cells on solid supports or carriers. The simple removal and recyclability reduces operational costs and makes applications to flux continuous reactors possible. However, cell leaching reduces the metabolic capacity after repeating reaction cycles. Herein the immobilization of an engineered *E. coli* strain is presented. This exemplary strain converts 1-butanol to butyric acid. We immobilized these cells on activated and non-activated Accurel MP1001 carrier and screened their metabolic activity by analyzing the butyric acid content using HPLC. In comparison, activation of the carrier doubles the overall activity, probably due to a higher binding capacity. Immobilized cells were further successfully entrapped by *silCoat*-formation, a methodology, recently described from our group. [1] This prevents the leakage of cells from the carrier after immobilization. Although more constrained by mass transfer limitation, entrapped cells showed similar activity as non-treated cells. The metabolic activity of the immobilized cells illustrates that the chemical treatment does not influence cell viability. Additionally, the usage of this simple entrapment strategy in combination with cells illustrates its wide

applicability. Usage of other carrier- and cell-types should be conceivable without effecting cellular metabolism.

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BTP16

Secretion of bacterial aspartase and transaminase by the yeast *Yarrowia lipolytica*

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Heterologous gene expression and secretion of gene products by *Yarrowia lipolytica* mainly focuses on eukaryotic proteins. To evaluate the ability of *Y. lipolytica* to produce bacterial proteins, the secretion of a bacterial aspartase and a transaminase was investigated.
The release of functional proteins from the cell into the culture medium mainly depends on the interaction of the secretion signal with the heterologous proteins. Activity based investigations of secretion via different parts of the prevalently used secretion signal sequence of the extracellular alkaline protease Xpr2 revealed the Xpr2predp peptide as the best variant. These cultivation experiments additionally gave hints on a different structural influence of the N-terminal enzyme sequence of both enzymes on the LysArg restriction site of the Xpr2prepro precursor.
In order to increase the functional enzyme level in the culture supernatant, bioreactor fed batch cultivation experiments were performed using different nitrogen sources. The highest extracellular enzyme activity could be achieved with peptone, the most complex N-source, compared to casamino acids, sodium glutamate and ammonium.

BTP17

Enhanced periplasmic production of therapeutic proteins in *Escherichia coli*

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For manufacture of therapeutic proteins, that do not require translational modification (e.g. glycosylation) for their biological activity, the production in *Escherichia coli* is still the host system of choice. Its well-known biology, the ability to grow on inexpensive media, the availability of genetic tools for host cell modifications and last but not least its long history as pharmaceutical work host are only the most prominent benefits. However, pharmaceutical proteins became more and more complex and therefore the requirements on the expression systems grew. Many modern, biologically active proteins contain multiple disulphide bridges, what makes classical production routes (e.g. production as inclusions bodies and subsequent *in vitro* refolding) more and more challenging. Even though recent advances in folding technology (e.g. high pressure refolding) enable refolding in commercially relevant yields for many target proteins, Boehringer Ingelheim (BI) also significantly increased its efforts in production of properly folded target proteins in the *E. coli* periplasm.
We present several approaches to increase the amount of soluble therapeutic protein within the periplasmic space of the bacterial cell. Application of BI's expression system, which makes use of a genomically integrated product gene significantly increased the titre of properly-folded target protein when compared to one of the typically used plasmid-based expression systems. Furthermore, the secretion and refolding machinery was supported by co-overproduction of different helper proteins and chaperones to enhance cellular productivity. The fermentation process was optimized with regard to process variables to enhance yield of properly-folded target protein.

BTP18

Systematic mutational analysis of lipase secretion in *Bacillus subtilis*

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Bacillus subtilis is a Gram-positive bacterium classified as a generally regarded as safe (GRAS) organism and thus widely used in biotechnology for the production of homologous and heterologous proteins. Many of these proteins are secreted into the culture supernatant thus significantly reducing the costs for down-stream processing.
Secretion of heterologous proteins usually needs to be optimized, and the signal peptide has emerged as one of the major targets to increase the secretion yield. Current results obtained for bacterial proteins, but also for proteins of human origin indicate that 1) signal peptides seem to be specifically adjusted to the mature protein to allow optimal secretion, and 2) interactions of the mature protein with the translocation machinery influence secretion efficiency. We have chosen the well-characterized lipase A of *B. subtilis* as a model protein to systematically analyze the role of the mature protein for secretion efficiency. Benchmark constructs contain three different signal peptides fused to LipA, namely the native LipA signal peptide, a mutagenized LipA signal peptide and a homologous signal peptide from *B. subtilis*. The latter two signal peptides lead to a decrease in secretion of native LipA to 70 and 60%, respectively, of the secretion rate determined for native LipA. The *lipA* gene is mutagenized by *error prone*-PCR and site saturation mutagenesis. For screening of LipA variants we use an agar plate assay with tributyrin as the substrate, an activity assay in liquid medium with the substrate *p*-nitrophenyl-palmitate and an ELISA assay for enzyme quantification. While screening is still in progress, first results indicate a significant impact of hydrophobic patches in the N-terminal region of *B. subtilis* LipA. Amino acid substitutions reducing the hydrophobicity in the N-terminal part of LipA (positions 1-29) led to 20 - 50% improved LipA secretion. Our results will allow identifying amino acids in mature LipA which affect secretion by (1) interaction with the signal peptide and (2) independent from the signal peptide and thus presumably by interaction with the translocation machinery.

BTP19

SolB, a small noncoding RNA of *Clostridium acetobutylicum*

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Small noncoding regulatory RNAs (nc RNAs, sRNAs) are important for the regulation of eukaryotic and prokaryotic cells. Prokaryotic noncoding RNAs show a wide range of regulatory effects in different metabolic pathways. Mostly, they act as posttranscriptional regulators by binding to distinct mRNA targets. This interaction can regulate mRNA stability and translation initiation. In the last years, research concerning small noncoding RNAs of Gram-negative bacteria, such as *Escherichia coli* and *Pseudomanas aeruginosa*, provided a multitude of fascinating data. In contrast, only a few small noncoding RNAs of Gram-positive bacteria have been identified. Here, we report on a small noncoding regulatory RNA of the Gram-positive bacterium *Clostridium acetobutylicum*. This solvent-producing anaerobic bacterium is of importance as it naturally produces butanol, an important bulk chemical as well as a biofuel. Therefore, it is necessary to elucidate the regulatory mechanism involved in butanol production. This small noncoding regulatory RNA, called SolB, is involved in regulation of solvent production. It is located upstream of the *sol* operon, which plays a key role in the production of butanol. Via primer extension and Northern Blot analysis the length and the transcription start point of SolB were determined. A *solB* overexpression mutant showed a complete repression of butanol and acetone production. Further growth experiments using different *C. acetobutylicum* mutants, a reporter gene assay using *E. coli*, in silico binding analyses of SolB and qRT-PCR analyses using *C. acetobutylicum* mRNA highlight the mRNAs of the genes *spo0A* (CA_C2071) and *adhE2* (CA_P0035) as targets of SolB. As genes are involved in solvent production, the result indicates the importance of the regulatory RNA SolB for solvent

production.

BTP20

Enhancing biogas plant performance by implementation of the microbial fuel cells (MFC) technology.

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The technologies of applied methane production and electricity generation from biogas have been well known for a long time. Nowadays there are up to 8000 biogas power plants in operation in Germany [1]. In 2012 biogas alone generated around 25 bn kWh electricity [2]. In total, electricity from biomass accounted for more than 44 bn kWh [2]. This defines biomass besides wind power as the most important branch of the renewable energies. Nevertheless due to the amendment to the "Erneuerbare-Energien-Gesetz" (EEG 2012) the construction of new biogas plants has decreased drastically [1]. In order to increase the efficiency of the biogas plants, and thus to make it more attractive for the plant manufacturer, it is necessary to identify limiting factors in the fermentation process and to overcome them. These interfering factors are mainly process imbalances which can lead to rise of the hydrogen partial pressure and a pH shift resulting in the inhibition of acetogenesis and methanogenesis. In order to increase the stability and efficiency of the microbial processes in biogas plants new approaches are investigated in this study. First, process imbalances are monitored via a microbial electrochemical cell-based biosensor by detecting volatile fatty acids (VFA), which are indicators for process imbalances. Secondly we want to control the hydrogen partial pressure and the VFA concentrations using the principle of microbial fuel cells. For this purpose an anode will be installed directly into the biogas fermenter. Anode reducing organisms will consume VFA and hydrogen concentrations on demand. The rate of electrode based VFA and hydrogen oxidation will be regulated by a variation of the anode potential The concentrations of anode reducing organisms and methanogens are examined during these processes via genomic barcode assisted qPCR quantification.

[1]Fachverband Biogas: Branchenzahlen - Prognose 2013 / 2014
[2]BMUB: Entwicklung der erneuerbaren Energien in Germany im Jahr 2012

BTP21

Extension of the substrate spectrum of the NAD^+-reducing hydrogenase from *Ralstonia eutropha* for cofactor regeneration

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Many oxidoreductases used for industrial synthesis of chemical compounds and chiral precursors are dependent on redox cofactors such as NAD(P)H. Because these cofactors have low stability and are rather expensive, their addition in stoichiometric amounts is not feasible. One approach to circumvent this problem is to couple the product formation reaction directly with an enzyme-driven cofactor regeneration system[1][2]. The soluble, NAD^+-reducing hydrogenase (SH) from *Ralstonia eutropha* H16 represents a promising enzyme for cofactor regeneration, as it transfers a hydride from the cheap substrate molecular hydrogen to NAD^+. The regeneration reaction requires only H_2 and takes place even in the presence of O_2, without formation of interfering side products[3][4].Since there are many oxidoreductases that require the phosphorylated cofactor NADPH and only few $NADP^+$-reducing enzymes available for cofactor regeneration, we aim at changing the NAD^+ binding pocket in the SH into a site that also accepts and converts $NADP^+$. Specific amino acid exchanges designed by rational mutagenesis led to SH derivatives with synthetic $NADP^+$-reducing activity. To further improve the H_2-driven NADPH reduction capacity of the SH, we designed a directed evolution approach based on hydrogenase variants that are heterologously produced in the well-studied organism *Escherichia coli* [5]. Positive selection of transformed cells that harbor SH variants with H_2-driven $NADP^+$ reduction activity is accomplished with an *E. coli* mutant which cannot synthesize sufficient NADPH for growth in minimal medium[6]. Thus, successful synthesis of an $NADP^+$-reducing SH variant in this strain should allow growth in minimal medium with H_2 as the source of reductant. The kinetic parameters of the new SH derivatives will be tested systematically with purified enzyme. Eventually, their cofactor recycling capacity will be tested in in-vivo coupled enzyme reactions.

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BTP22

Photosynthetic bacteria as alternative platform organisms for the expression of human membrane proteins

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Heterologous expression of human membrane proteins is a major concern for medicinal and pharmaceutical research due to the fact that nearly 50% of available drugs are either directly or indirectly targeting human membrane proteins. However, the intricate nature of membrane proteins hampers their structural and functional studies because common expression hosts like *E. coli* are optimized for the production of soluble proteins. Therefore, we developed a new expression system based on the facultative phototrophic non-sulfur purple bacterium *Rhodobacter capsulatus*. During phototrophic growth, *R. capsulatus* forms a continuous system of intracytoplasmic membranes (ICM) harboring the integral membrane protein complexes of its photosystem. Thus, due to its phototrophic nature, *R. capsulatus* is perfectly adapted to synthesize heterologous membrane proteins by providing a highly enlarged membrane surface and an efficient membrane protein folding and translocation machinery. To allow a concerted induction of heterologous membrane protein expression and ICM formation, we first constructed a new set of *nif* promoter-based expression plasmids that enables the gradual expression of target genes in the bacterium under phototrophic growths conditions. To evaluate the applicability of the novel expression system, several human proteins that are either membrane-associated or integral membrane proteins exhibiting 1 to 7 transmembrane helices (TMHs), were comparatively expressed in *E. coli* and *R. capsulatus*. Protein accumulation and localization studies revealed that *E. coli* seems to be the preferable expression host for membrane proteins with a low number of TMHs, whereas membrane proteins with a higher number of transmembrane domains achieve higher protein yields with the newly developed *R. capsulatus* expression system. Therefore, the photosynthetic bacterium *R. capsulatus* is a promising alternative platform organism for the heterologous expression of more complex membrane proteins.

BTP23

The challenge of salt-free heterologous production of ectoine and hydroxyectoine

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The amino acid derivatives ectoine and hydroxyectoine belong to the best researched compatible solutes. These small organic compounds are accumulated by halotolerant and halophilic microorganisms to obtain osmotic equilibrium at high or fluctuating salinities. In addition to their function as osmoprotectants for whole living cells, compatible solutes have pronounced stabilizing effects on macromolecules exposed to physical stresses [1, 2]. Therefore, compatible solutes are of great interest for biotechnological applications and, especially ectoine, is already used as cell protectant in skin care products and as protein stabilizer in life science [3].
At present ectoine and hydroxyectoine are produced at industrial scale using the natural producer *Halomonas elongata* (a moderate halophile), which excretes the compatible solutes into the medium [4]. A time-consuming and costly issue of these production processes is the requirement to separate the desired products from salt. Therefore, many attempts have been undertaken to establish a salt-free heterologous production system for ectoine and hydroxyectoine in non-halophilic hosts such as *Escherichia coli*,

Corynebacterium glutamicum and *Hansenula polymorpha* [5-7]. Although the halophilic gene clusters (e.g. from *H. elongata*) were expressed under the control of strong promoters, the production rate for ectoines remained well behind expectations. A reasonable explanation would be that the ectoine-synthesizing enzymes need salt for optimal activity, as has been shown for the enzymes from *H. elongata* [8]. Hence, we conclude that efficient salt-free ectoine/hydroxyectoine production requires the use of non-halophilic variants of these enzymes. In a comprehensive search for potential donors we have recently investigated the non-halophilic *Acidiphilium cryptum* that exhibits an ectoine/hydroxyectoine biosynthetic gene cluster. *In vitro* assays revealed that one of the enzymes, ectoine synthase, has maximum activity in the absence of salt [9]. Furthermore, all enzymes of this biosynthetic pathway display an unusually low excess of acidic amino acids, and appear to be decisively non-halophilic. Thus, the *A. cryptum* gene cluster seems to be a promising candidate for salt-free heterologous production of ectoine/hydroxyectoine. Here we present our ongoing studies to meet this challenge.

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BTP24
Motive- and function- based search for novel alcohol dehydrogenases

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The production of chiral drug intermediates is getting increasingly important for pharmaceutical industry (reviewed by Patel, 2007). Thereby biocatalysis displays an environmentally friendly method with benefits such as high enantio-, regio-, and chemoselectivity in comparison to chemical approaches. Available systems are ranging from whole- cell biocatalysts to purified alcohol dehydrogenases. Whole- cell catalysts such as baker´s yeast (Katoh *et al*., 2006) and *Trichosporon cutaneum* (Conceição *et al*., 2003) were reported to convert diketones to diols or α-hydroxy ketones. Thus fungi provide for promising candidates for the delivery of novel alcohol dehydrogenases with the desired characteristics.
To name but a few of the well characterized alcohol dehydrogenases there are the *(R)*- ADH from *Lactobacillus kefir* (Kraußer *et al.* 2007), *(S)*- ADH from *Rhodococcus sp.* (Kraußer *et al*. 2007) and horse liver ADH (Adolph *et al*., 2000). Common procedures like the screening of microorganisms (e.g. Inoue *et al.*, 2005) or metagenome databases (e.g. Knietsch *et al*., 2003) predominate the search for novel alcohol dehydrogenases for asymmetric oxidation and reduction. In this study two different approaches were followed. First of all a motive- based search was performed yielding new enzymes with high similarities in NADH as well as Zn^{2+} binding sites to those of the carbonyl reductase from yeast *Candida parapsilosis* (CPCR2) (Jakoblinnert *et al*., 2012). The second approach is an innovative function-based search. Based on the presumable *in vivo* functions of the well-investigated CPCR2, new zinc- containing, medium- chain alcohol dehydrogenases were targeted, exhibiting a broad substrate range and thus increasing the scope of ADH catalyzed biocatalysis.

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BTP25
Development of a new anhydrotetracyline-inducible expression system based on *tetR* promoter of Tn*1721* for expression of heterologous genes in *Bacillus subtilis*

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Bacillus subtilis is a common workhorse for production of recombinant proteins. So far, several expression systems have been constructed for production of proteins in *B. subtilis*, such as IPTG-inducible or sugar-inducible systems [1]. In this study, a new gene expression system based on the tetracycline resistance genes (*tetA* and *tetR*) of Tn*1721* was constructed. The *tetA* and *tetR* genes are located in reverse orientation on Tn*1721* and their promoters, namely P_{tetA} and P_{tetR}, overlap each other. The *tetA* gene encodes an antiporter exporting the tetracycline molecules to the extracellular milieu. Transcription of both *tetA* and *tetR* is repressed by TetR due to the presence of TetR operators (*tetO*) insides the spacer of P_{tetA} and P_{tetR} as well as in the untranslated region of *tetA* [2]. To construct the expression system, both P_{tetA} and P_{tetR} were inserted upstream of *eGFP*, as a reporter gene, on a pUB110-derivative plasmid which was a high copy plasmid in *B. subtilis*. The results indicated that both P_{tetA} and P_{tetR} had a weak activity which was due to their 18 base pair spacer region. Optimization of the promoter core elements and shortening the spacer length to 17 base pair slightly increased the P_{tetA} activity, while P_{tetR} remarkably improved (P_{tetR2}). To regulate the activity of P_{tetR2}, P_{tetR}-*tetR* was inserted into the expression plasmid. Expression of *tetR* on the plasmid rendered P_{tetR2} inducible; however, the maximal activity of P_{tetR2} was drastically reduced. Therefore, other *B. subtilis* promoters, *i.e.* P_{mtlA}, P_{mtlR}, P_{ptsG}, were used for the expression of *tetR*. Interestingly, fusion of the P_{ptsG}-*tetR* resulted in a tight regulation of P_{tetR2}-*eGFP* with almost 44-fold induction. The final plasmid pKAM219 containing P_{tetR2}-*amo105*-ter-P_{ptsG}-*tetR* cassette was then used for production of a newly isolated neopullulanase (*amo105*) in *B. subtilis* [3]. This enzyme was able to degrade pullulan cyclodextrin as well as the synthetic substrate 2-chloro-4-nitrophenyl-α-d-maltotrioside (CNP-G3). By induction of P_{tetR2}-*amo105* with anhydrotetracycline, 15-fold P_{tetR2} induction and up to 45 U/mg neopullulanase activity was observed. This result verified that the newly designed expression system can be used for expression of other heterologous proteins than eGFP in *B. subtilis*.

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BTP26
Expanding the product scope of *Pseudomonas putida*

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Monoterpenoids can be applied as aroma and flavor compounds and as antimicrobial, antifungal and anticarcinogenic agents, respectively. Chemical synthesis is often difficult and expensive due to complex chiral structures of monoterpenoids, whereas extraction from natural sources is often wasteful due to low natural availability. Hence, biotechnological production constitutes an attractive alternative route. Since antimicrobial properties of many monoterpenoids impede a wide application of *E. coli* or *S. cerevisiae* as conventional production hosts, we tested *P. putida* as a promising alternative microbe due to its remarkable natural tolerance to many solvents and aromatic hydrocarbons. In this work, we show *P. putida* DSM 12264 as a highly efficient microbial cell factory as well as whole-cell biocatalyst for production of monoterpenoids. This strain was previously shown to be an efficient perillic acid producer in the fed-batch bioreactor due to its natural ability to convert limonene to perillic acid. To test *P. putida* DSM 12264 as a microbial cell factory, geraniol synthase of *Ocimum basilicum* and the mevalonate pathway of *Myxococcus xanthus* were introduced, leading to 219 mg/L geranic acid in 2 days in the bioreactor. To the best of our knowledge, this is the first example of *de novo* monoterpenoic acid production with an engineered microbe so far. Furthermore, we could show that *P. putida* is also an efficient system for P450-driven hydroxylations. By expressing model monooxygenase $P450_{cin}$ (CYP176A1) and its reductase of *Citrobacter braakii*, up to 5 g/L 2b-hydroxy-1,8-cineole can be produced in 3 days in the fed-batch bioreactor without further optimization. Our results show that *P. putida* can be used as a highly efficient catalyst for the production and conversion of different

monoterpenoids.

BTP27
Characterization of a toluene-degrading strain of *Magnetospirillum* isolated from a Planted Fixed-Bed Reactor (PFR)

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Magnetospirillum is a genus characterized majorly by some magnetic strains and their ability to break down aromatic compounds under microaerophilic and anaerobic conditions. Numerous studies on this genus are focused on their magnetic properties, however almost no studies have analyzed and characterized the genes related to toluene degradation under denitrifying anaerobic conditions. Furthermore a few studies have assessed the ability of *Magnetospirillum* to degrade toluene under the mentioned conditions. In this work, microcosm samples were taken from a Planted Fixed Reactor (PFR), planted with *Juncus effusus* and fed with toluene through several years. An isolated strain was taxonomically and physiologically characterized, and defined genes related to general toluene degradation pathways for anaerobic conditions were detected and specific primers were designed. Finally, their expressions were assessed in the reactors using quantitative polymerase chain reaction (qPCR). Through *16s rDNA*, our results showed that the strain were closely related to *Magnetospirillum* TS-6, a strain able to degrade phenol, previously describe by Shinoda et al. (2000). Furthermore, *Magnetospirillum* was able to degrade toluene up to a concentration of 50 mg/L in liquid cultures with nitrate as the electron acceptor under anaerobic conditions. Genes were analyzed through BLAST and related to toluene degradation, specifically *bssA* (benzylsuccinate synthase) and *bcrC* (benzoyl-CoA reductase), being the last gene responsible of toluene ring dearomatization. With qPCR the abundance of *Magnetospirillum* sp. were possible to quantify in two different PFR 9,17E+01 and 2,96E+03 (copy numbers/µl) respectively.

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BTP28
A cold-active alginate lyase from deep-sea sediment metagenome for degradation of marine biomass

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Cold environments like the deep-sea, which are one of the most abundant environments on Earth, have been successfully colonized by cold-adapted microorganisms. The ability of these psychrophiles to proliferate in their habitat is owed to their production of cold-active enzymes. These enzymes are attractive candidates for applications in industrial processes that run at low temperatures thereby saving energy by avoiding additional heating steps. For this purpose a sequence-based screening of a deep-sea sediment metagenome was accomplished to identify new marine biomass-degrading enzymes. In this regard marine macroalgae, especially brown algae are of great interest since they represent a promising feedstock for the production of biofuels and chemicals. The main component of brown algae is alginate, which makes up to 40 % of the dry weight (1). Alginate is a linear heteropolysaccharide consisting of α-L-guluronate and β-D-mannuronate covalently (1-4)-linked in randomly arranged sequences. Those building blocks can be enzymatically degraded into unsaturated oligosaccharides through a β-elimination reaction using alginate lyases. In our approach 17 alginate lyase-enconding genes were identified within a metagenomic data set. A phylogenetic analysis showed that eight putative alginate lyases belong to polysaccharide lyase family 6, while nine open reading frames encode for members of the polysaccharide lyase family 7. To identify alginate-degrading enzymes, the catalytic activity was directly monitored by measuring the absorbance at 235 nm on the basis of generated unsaturated uronic acid products. A novel alginate lyase from *Glaciecola sp*. 4H-3-7+YE belonging to polysaccharide lyase family 7 was identified (2). The cold active enzyme with a molecular mass of 39 kDa was purified and characterized.

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BTP29
A new "thermophilic-like" ene reductase from *Rhodococcus opacus* 1CP

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The increasing importance of Old Yellow Enzymes (OYE) or ene reductases (ER) as biocatalysts relies on their high selectivity and specificity towards asymmetric reduction reactions of activated C=C bonds of α,β-unsaturated carbonyl compounds. So far, two classes of ER's are known, the classical and the thermophilic-like OYE [1].
By genome analysis we identified the actinomycete *Rhodococcus opacus* 1CP to possess fourteen different ene reductase encoding genes. Multiple sequence alignment and phylogenetic analysis with previously characterized ER's revealed that one of these enzymes belongs to thermophilic-like OYE showing highest sequence identity to a thermostable ER from *Thermus scotoductus* SA-01 [2]. The present study focuses on the characterization of this thermophilic-like ER named OYERo2 in order to determine its potential for biocatalysis. The *oyeRo2* gene was recombinantly expressed in *E. coli* as a functional soluble protein. OYERo2 was purified by IMAC as an N-terminal his-tagged protein with a monomer molecular weight of approx. 42 kDa. Cofactor identification showed one molecule FMN per monomer. Size-exclusion chromatography showed OYERo2 to possess a tetramer structure. Stored at -20 °C the enzyme was very stable over several weeks. OYERo2 was able to catalyze the reduction of 2-cyclohexen-1-one and 2-methyl-2-cyclohexen-1-one but similar to other members of this subclass, no conversion was detected with cyclic Cβ substituted enones. High specific activities were detected when catalyzing the reduction of maleimide and N-methyl-maleimide through the oxidation of NADPH and NADH, with a preference for NADPH as electron donor (both substrates about 45 $U \cdot mg^{-1}$ with NADPH). A standard enzyme assay with maleimide as a substrate showed an optimum temperature at 37 °C for the protein. However, the enzyme was only stable up to 30 °C.
To conclude, OYERo2 from *R. opacus* 1CP is a novel ER in the group of thermophilic-like OYE. It shows the characteristic sequence motifs, as well as the tetramer structure and high activities towards typical substrates. The missing thermostability and the similarity to the previously discovered ER's DmER and RmER [3] lead to the assumption that a third class of OYE is existing next to classical and thermophilic-like OYE.

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BTP30
Engineering the citric/isocitric acid overproduction by *Yarrowia lipolytica*

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Functionalized carboxylic acids are highly versatile chemical species with a wide range of applications (e.g. as co-polymers, building blocks, acidulants). Therefore, they are of special interest as biotechnologically available targets. The yeast *Yarrowia lipolytica* secretes high amounts of organic acids, like citric and isocitric acid (CA/ICA) under conditions of growth limitation from a carbon source excess. Depending on the carbon source, *Y. lipolytica* produces a characteristic CA/ICA ratio, on carbohydrates or glycerol of 90:10 and on sunflower oil or n-alkanes of 60:40. To examine, whether this CA/ICA product ratio can be influenced, isocitrate lyase (*ICL1*), aconitase (*ACO1* or *ACO2*), NADP- (*IDP1*) or NAD- (*IDH1, IDH2*) isocitrate dehydrogenases gene-dose-based overexpressing strains were constructed

(using integrative multicopy vectors) containing multiple copies of these genes alone or combinations of them. The *ACO1* [1] (but not in case of *ACO2*) or the *IDP1* overexpression and a combination of them resulted in a product pattern shift in direction of ICA, reducing the undesired CA for ICA production. On sunflower oil the ICA proportion increased from 35-55% to 65-72% of total acid produced in shaking flasks experiments. Strains with increased copy numbers of both *ACO1* and *IDP1* showed the highest ICA selectivity up to maximally 75-80% in bioreactor experiments. Otherwise, overexpression of only one NAD-isocitrate dehydrogenase subunit genes (*IDH1* or *IDH2* to decrease IDH enzyme activity) resulted only in a moderate ICA-increase.
By using wild-type or engineered *Y. lipolytica* strains the enantiomerically pure form of D-*threo*-isocitric acid, currently available as a speciality compound, can be produced now in large amounts and used as a building block for organic synthesis [2].

[1] Holz et al. (2009) Appl Microbiol Biotechnol 81: 1087
[2] Heretzsch et al. (2008) Angew Chem Int Ed 47: 1958
Supported by the SMUL of the Saxony, Germany (138811.61/89 and 2620000240)

BTP31
Immobilisation of laccase - a comparison of different carriers and methods

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Introduction: Enzymes for bioremediation and industrial bioprocesses need to show high stability and it must be possible to run the process at low costs. For this purpose, the multiple uses of the enzymes are necessary which can be achieved by employing immobilised enzymes.
Materials and Methods: The immobilisation of laccase from *Trametes versicolor* on various carrier particles with different conjugation methods was investigated. Carrier particles of different sizes, morphologies, and with surface layers modified with diverse functional groups were chosen. Commercially available Sepa-Beads® with epoxy and amino groups, temperature-sensitive or pH switchable poly-N-isopropylacrylamide particles, and the renewable biopolymer chitosan were used. The immobilisation of laccase by entrapment polymerization and covalent linkage on the different particles was investigated.
Results and Conclusion: Best immobilisation yields are obtained by entrapment polymerisation. Efficiency depends on enzyme and cross linker concentration. For immobilisation by covalent linkage, best results were reached with chitosan as the carrier. Interestingly, a remarkably prolonged stabilisation of the enzyme was observed, too. The immobilised laccase did not lose any activity over a period of 25 days when stored at room temperature, whereas the free enzyme lost activity under these conditions. The properties of immobilised and free laccase were compared and they were used for bio-bleaching of textile dye effluents. With chitosan fibres as carrier, we successfully performed dye decolourisation for over 25 times. In cooperation with two textile factories in Saxony, the bleaching of relevant textile dyes was investigated under industrial conditions

BTP32
Metabolite biosensors for single cell analysis and the development of production strains

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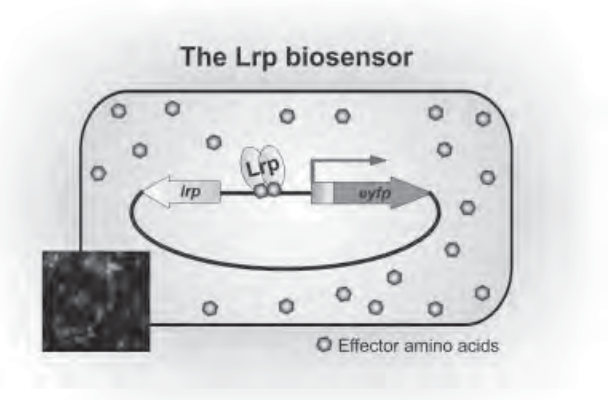
The analysis of microbial metabolite production is typically performed using bulk techniques, which obscure information with respect to single cell behavior. We have developed a genetically-encoded biosensor based on the transcriptional regulator Lrp (leucine-responsive protein) of *Corynebacterium glutamicum* (1). The sensor allows the intracellular detection of methionine and branched-chain amino acids at the single cell level by converting this information into a fluorescent signal. The suitability of the biosensor to mirror different intracellular concentrations of effector amino acids was applied to study population dynamics of the valine producer strain *C. glutamicum ΔaceE* lacking the pyruvate dehydrogenase complex. Flow cytometry-based monitoring of biosensor cells during lab-scale fed-batch cultivation revealed the appearance of subpopulations varying in productivity and viability. In addition, live cell imaging studies using microfluidic chip devices displayed different types of non-producing cells within isogenic microcolonies of *C. glutamicum ΔaceE* (2). The appearance of non-productive subpopulations might strongly impact the performance and stability of the production process; therefore, genetically-encoded biosensors have the great potential to reveal bottlenecks for improving fermentation processes. In further studies, the Lrp-biosensor was applied to improve growth and productivity of *C. glutamicum ΔaceE* by *in vivo* evolution. Using fluorescent-activated cell sorting (FACS), cells with the highest fluorescent output were iteratively isolated and (re-)cultivated. Isolated strains revealed a significantly increased growth rate, shortened lag-phase and an increased final optical density. The L-valine production of some strains was increased up to 100% compared to the parental strain while the formation of by-products (L-alanine) was reduced. These results emphasize biosensor-based strain evolution as a straightforward approach to improve growth and productivity of microbial production strains.

(1) Mustafi et al., 2012 *Met Eng* 14 (4), 449-457.
(2) Mustafi et al., 2014 PLoS One 9 (1): e85731.

Figure 1



BTP33
Grain size effects on microbial leaching of copper shale (black shale)

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Copper shale (black shale) is the most important natural deposit for base and noble metals found in Central Europe. However high contents of carbonate and organic matter makes the extraction of the ore by conventional techniques, like flotation, challenging. The aim of this study is to apply bioleaching of carbonate rich Polish copper shale and to further investigate if the grain size has an effect on copper extraction. Therefore, pure cultures of Acidithiobacillus ferrooxidans (DSM 14882) were used as leaching bacteria and three different grain sizes of 63-100 µm, 100-200 µm and 200-315 µm were examined. Because of the high carbonate content of the ore, microbiological leaching was only possible after neutralization of the carbonate or with simultaneous addition of sulfuric acid. The experiments were performed in shaken flasks as well as in stirred bioreactors for, 30 days in 9K media, 4% (wt) solid content, pH 2.0 and 30°C. The analysis of dissolved copper after 30 days of leaching showed that in the inoculated shaken flasks a copper recovery of 97% ± 3% and in the non-inoculated flasks of 93% ± 5% was reached. However, differences in the copper yield with regard to the three grain sizes did not become obvious. Tests with non-inoculated shaken flasks without the addition of ferrous iron a copper extraction of only 59%± 3% within the same time frame and, again, no differences between the three grain sizes could be detected. In this study it was shown that the grain size does not affect the process of copper bioleaching, but indicate that the acidic pH of the leaching media or the pretreatment of copper shale have an influence on copper dissolution. Additionally, bioleaching tests in bioreactors showed that copper extraction in presence of A. ferrooxidans was four times higher than in the abiotic approach. The comparison of data obtained from this study with previously ones that microbial leaching is more effective in bioreactors than in shaken flasks.

BTP34
Overproduction of intracellular cysteine as polythioester precursor

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Polythioesters (PTEs) are sulfur-containing biopolymers persistent to microbial degradation and could become valuable materials for industrial applications as replacement for synthetic petrochemical-based plastics. So far, toxic and/or expensive organic sulfur compounds are required as precursor substrates for PTE biosynthesis [1]. To ultimately allow cost-efficient bulk production of PTEs, it is required to establish biosynthetic pathways starting from inorganic sulfur sources, like sulfate. The first intermediate in sulfate assimilation, cysteine, already is a structural analogue of the PTE precursor 3-mercaptopropionic acid. So, by metabolically engineering amino acid biosynthesis to enhance availability of intracellular cysteine, we accomplished the first step towards this final goal. For that purpose, the two microorganisms *Escherichia coli* and *Advenella mimigardefordensis*, which already serve as PTE production strains [2, 3], were chosen as starting point to increase the intracellular cysteine level. For quantification of cysteine a new method based on the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and a determination via HPLC/MS was established and confirmed by a ninhydrin reaction. We detected ten-times higher concentrations of cysteine in *E. coli* compared to *A. mimigardefordensis*. Various combinations of the serine-acetyltransferases (SAT) of the two strains fused to different promoters were cloned into both strains. The fusion product of the promoter of SAT_{Am} to SAT_{Ec} led to a doubling of the intracellular cysteine concentration in both strains. The deletion of a cysteine degrading desulfhydrase and the introduction of several point mutations into SAT to avoid feedback inhibition by cysteine were additional approaches to achieve high intracellular cysteine levels and provide potential PTE precursors. The further conversion of cysteine to 3-mercaptopropionic acid is in the focus of ongoing research and will hopefully be successful in the near future to enable PTE biosynthesis from a simple carbon source and sulfate via cysteine.

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[3] **Xia Y, Wübbeler JH, Qi Q & Steinbüchel A (2012)** *Appl Environ Microbiol* **78:**3286-3297.

BTP35
Raman spectroscopy - casting (laser) light on microbe - mineral interactions

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Highly efficient and sustainable mining strategies gain importance due to the fact that available resources of base metals like copper but strategic elements such as gallium and molybdenum as well face a steadily decreasing grade. This issue is enhanced by the increased demand and production amounts of those metal compounds. Biohydrometallurgy - the use of microorganisms or related substances in metal extraction - provides the potential of processing low grade ores efficiently. Thus it is applied for instance in gold and uranium mining yet.
Also due to strategic reasons mining of regional resources such as the European Kupferschiefer come to the fore. Its complex composition including sulphide rich ores, carbonates and organic compounds challenges biotechnological strategies. Nevertheless promising approaches have been reported. We examine heterotrophic bioleaching of copper from Kupferschiefer ores. To investigate the interactions between mineral surface and microorganisms Raman spectroscopy offers a versatile applicability: Identification of minerals and differentiation of microorganisms is nicely provided and is accompanied by imaging opportunities in a two or even three dimensional manner. Thus biofilms, for example, can be analysed with respect to microbial diversity or preferences of minerals during the attaching process.

BTP36
Production of a periplasmic trehalase in Gluconobacter oxydans and growth on trehalose

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Gluconobacter (G.) oxydans is a Gram-negative aerobic alpha proteobacterium belonging to the *Acetobacteraceae*. The organism catalyses the regioselective and incomplete oxidation of many hydroxylated organic compounds. The reactions are catalyzed by PQQ or flavin-dependent dehydrogenases located in the cytoplasmic membrane with their active sites oriented towards the periplasm. These features give *Gluconobacter* strains great biotechnological potential and industrial importance. The organisms are specialized in the oxidation of monosaccharides, whereas growth and product formation from disaccharides is either very low (e.g. sucrose) or impossible (e.g. lactose). Therefore, we investigated the possibility of conferring the ability to use trehalose as substrate through metabolic engineering. Basic expression vectors with constitutive promotors for the overproduction of cytoplasmic or membrane-bound dehydrogenases have already been constructed [1]. To obtain a biotechnologically valuable expression system for the specific production of proteins in the periplasm of *G. oxydans*, the translocation efficiency of different signal peptides in this organism was investigated. The analysis of the translocation efficiency of the various signal peptides revealed that the signal peptide PelB in combination with the strong promoter p264 had highest PhoA activity and was used for the production of TreA, a periplasmic trehalase of *E. coli* [2]. When the growth behavior of *G. oxydans* with trehalose as the substrate was analyzed, it became evident that the periplasmic *treA* expression strain grew with a doubling time of 3.7 h and reached a final optical density of 1.7. The rapid decrease of pH from 7 to 3.5 as a consequence of conversion of glucose to organic acids, provided evidence, that the *treA* expression strain was able to cleave trehalose and to use the resulting glucose molecules for growth. Further analysis indicated that strep-tagged TreA was successfully transferred across the cytoplasmic membrane of *G. oxydans* where it was highly active. Moreover, analysis of substrate and product concentrations via HPLC showed a rapid decrease of trehalose within 30 h indicating the catalytic activity of TreA. Usually the consumption of glucose by *G. oyxdans* leads to the formation of gluconate and ketogluconates. However, the treA expression strain mainly formed acetate and 5-ketogluconate. In summary, the periplasmic expression of hydrolases is an important step for metabolic engineering of *G. oxydans* with respect to the extension of the substrate spectrum.

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2 Gutierrez, C., Ardourel, M., Bremer, E., Middendorf, A., Boos, W., Ehmann, U. (1989). Mol. Gen. Genet. 217, 347-354

BTP37
An L-glucitol oxidizing dehydrogenase from *Bradyrhizobium japonicum* USDA 110 for production of D-sorbose with enzymatic or electrochemical co-factor regeneration

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A gene in *Bradyrhizobium japonicum* USDA 110, annotated as a ribitol dehydrogenase (RDH), had 87% sequence identity (97% positives) to the N-terminal 31 amino acids of an L-glucitol dehydrogenase from *Stenotrophomonas maltophilia* DSMZ 14322 (Brechtel et al. 2002), which could not be reisolated from this strain. The 729 bp long RDH gene coded for a protein consisting of 242 amino acids with a molecular mass of 26.1 kDa and could be identified as a member of the short chain dehydrogenase reductase (SDR) family, because of its molecular weight, the NAD-binding site GAASGIG and the sequence YASSK as the active centre. The heterologously produced protein exhibited the main enantio selective activity with D-glucitol oxidation to D-fructose, but also converted L-glucitol to D-sorbose with enzymatic cofactor regeneration and a yield of 90%. The temperature stability and the apparent K_m value of 9.2 mM for L-glucitol oxidation let the enzyme appear as a promising subject for further improvement by enzyme evolution. We propose to rename the enzyme from

the annotated RDH gene (locus tag bll6662) from *B. japonicum* USDA as a D-sorbitol-dehydrogenase (DSDH, EC 1.1.1.14). The only other so far described DSDH with L-glucitol oxidizing activity was isolated from sheep liver (Lindstad et al. 1998). It is a medium chain zinc dehydrogenase with low affinity for L-glucitol oxidation (K_m: 167 mM) and the oxidation product is not identified.
The final goal is the construction of electrochemical reactors with electrode-immobilized enzymes, cofactors and mediators, which allow a production in continuous flow without waste generation (Bon Saint Come et al 2013). In principle the combination of several reactors with the appropriate enzymes could lead to all rare sugars and polyols of the so called Izumoring (Granstrom et al. 2004) and the enzyme of this study can be used in this system for oxidation of L-glucitol to D-sorbose.

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BTP38
A novel GDSL-lipase from *Burkholderia glumae* PG1

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Burkholderia glumae is a Gram-negative bacterium known for the production of biotechnological relevant compounds including e.g. rhamnolipids and lipases which can be used for the production of biofuels and chiral building blocks for pharmaceuticals. Here, we report on the isolation and characterization of LipG, a novel lipase produced by *B. glumae* PG1 which belongs to the GDSL-family of lipolytic enzymes.
Initially, LipG was identified by proteomics in the *B. glumae* PG1 secretome. The corresponding open reading frame (ORF) encodes a putative GDSL-lipase with a molecular mass of about 44 kDa. GDSL lipolytic enzymes represent a distinct family with a conserved GDSX motif around the catalytic serine which is positioned near the N-terminus of the enzyme [1]. A characteristic feature of GDSL-hydrolases is their broad substrate specificity; they hydrolyze glycoesters, arylesters, wax-like lipids, phospholipids, lysophospholipids, acyl-CoA thioesters, peptides and triacylglycerols. Interestingly, ORF *lipG* codes for an N-terminal secretion signal and a domain of unknown function (DUF) located between the secretion signal and the GDSL-domain. This domain composition is also found in other hypothetical GDSL-lipases encoded by bacteria of the family *Burkholderiaceae*. We have cloned and expressed in *B. glumae* PG1 both native and C-terminally histidine tagged LipG and have localized the enzyme in the culture supernatant. A *B. glumae* mutant with a deficient type II secretion system (T2SS) secreted almost no LipG to the culture supernatant suggesting that LipG is transported *via* the T2SS. Currently, LipG is biochemically characterized and putative posttranslational modifications are analyzed as well as the function of the DUF for LipG transport and/or activation.

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BTP39
Improving the performance of a biofuel cell cathode with laccase from Pycnoporus sanguineus

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Laccases are multicopper enzymes that are widely distributed in bacteria, fungi and plants. They catalyze oxidation of aromatic and nonaromatic compounds and use molecular oxygen as final electron acceptor. Fungal laccases are exoenzymes that are secreted into the culture medium and can be harvested from the culture supernatant. In biotechnology - among other applications – purified laccases can be used in enzymatic biofuel cells for the cathodic oxygen reduction to improve cathode performance. Essential in this case is the ability of laccase to mediate direct electron transfer between a copper atom in its catalytic center and the cathode, which eliminates the need for mediators.
Recent studies even show that crude laccase-containing culture supernatant of Trametes versicolor is similarly efficient as purified enzyme in improving cathode performance, which eliminates the need for expensive enzyme purification. However, various other fungi possess laccases that can potentially be used for the same purposes and can perform cathodic oxygen reduction under various conditions. Many fungal species need inducers, such as copper or aromatic compounds, to trigger increased laccase production. Here we present a laccase from ligninolytic white-rot fungus Pycnoporus sanguineus, which is expressed in high amounts during primary metabolism and is capable of mediatorless electron transfer on the cathode. The laccase-containing culture supernatant of P. sanguineus has significantly improved cathode performance in biofuel cell under different conditions.

BTP40
Biocatalytic synthesis of medium-chained ω-hydroxylated fatty acids

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Medium-chained fatty acids (MCFA, C_6 - C_{12}) and their oxidized derivatives are precursor molecules for a wide variety of products including plasticizers, lubricants, flavor compounds, biofuels, polymers, and pharmaceuticals. The aim of this proof-of-principle study was to develop a bacterial single-cell biocatalyst for the production of ω-hydroxyoctanoic acid. In order to achieve this, we hijacked mid-chain fatty acids from the *de novo* biosynthesis cycle of *Escherichia coli* by the heterologous expression of a specific plant thioesterase in a β-oxidation negative strain. In a second step, we used a recently engineered self-sufficient monooxygenase fusion protein, allowing the specific hydroxylation of MCFA's at the terminal position. With this approach, we achieved the production of 430 mg/L octanoic acid in a *fadD* deletion strain expressing a thioredoxin fusion variant of the thioesterase FatB2, representing a 360-fold increase when compared to the parental strain. With the additional expression of the monooxygenase $CYP153A_{Maq}$., we could further demonstrate the consecutive terminal hydroxylation of the produced octanoic acid, finally yielding 13 mg/L ω-hydroxyoctanoic acid.

BTP41
A Web-Based Knowledge, Modelling & Visualization Base

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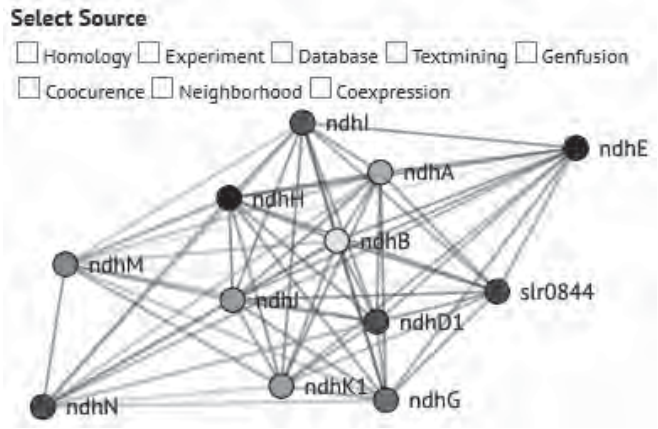
Introduction: CyanoFactory is both, a synonym for a visionary technology and a collaborate research project funded by the European Union. The primary goal of the research, by ten selected leading and highly complementary European partners, is aiming at applying synthetic biology principles towards a cell factory notion in microbial biotechnology. The vision is to build on recent progress in synthetic biology and develop novel photosynthetic cyanobacteria as chassis to be used as self-sustained cell factories in generating solar fuel. Our contribution in advancing the project is the creation of a web-based knowledge base. Its primary function is to collect and represent all experimental data as well as metadata. **Materials and Methods**: Our knowledge base CyanoFactory KB has been developed and further extended on the basis of WholeCell KB (Stanford University). Experimental omics data is obtained from the CyanoFactory

research partners. Furthermore data provided from other biological databases is obtained and integrated with the experimental Results: The interaction of proteins and enzymes is visualized using pathway maps and data from STRING and STITCH (EMBL). Furthermore CyanoDesign provides metabolic modelling and analysis of the efficiency of enzymatic reactions via Flux Balance Analysis.
Results: We created a productive knowledge base, which handles all the information from our partners. The advantage of our knowledge base is that, besides holding information, it provides cross-links to other sources, too. For example the "Chromosome Viewer" (fig.1) or the "Protein-Interaction-Map" (fig. 2) are compact forms of visualization which hold a lot of different information in a user friendly and simple way.
Conclusion: With the help of the web-based knowledge base, it is possible to concentrate information for a single organism and possible mutants. More information and more cross-links between these information enables a better understanding and working with the selected organism.

Figure 1

Figure 2



BTP42
Recombinant expression and characterization of a novel heme-peroxidase from the lichen *Leptogium saturninum*

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A lichen heme-peroxidase (*Lsa*POX) was purified and described for *Leptogium saturninum*, an ascomycete fungus that lives together with cyanobacteria of the genus *Nostoc* [1]. By oxidizing methoxylated aromatics, recalcitrant dyes and phenols, the *Lsa*POX shares catalytic properties with fungal dye-decolorizing peroxidases (DyPs, EC 1.11.1.19).
In this work we identified and characterized the full gene encoding this novel peroxidase, using common molecular biological methods as well as a semiconductor sequencing approach (Ion Torrent PGM). The *Lsa*POX gene shows no similarity with any other peroxidase on the sequence level but rather with NO-binding proteins (nitrobindins). This new nitrobindin-related peroxidase family so far contains the *Lsa*POX as well as about 100 distantly related sequences from ascomycetes and eubacteria. To prove functionality of the *Lsa*POX, we heterologously expressed it in *Saccharomyces cerevisiae* using a galactose inducible promotor.
We will give a summarizing overview on the catalytic and molecular properties of *Lsa*POX.

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BTP43
Mathematical modeling of Acetone-Butanol-Ethanol (ABE)-Fermentation by *Clostridium acetobutylicum* in continuous bioreactors

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Acetone-Butanol-Ethanol (ABE)-Fermentation by *Clostridium acetobutylicum* is a biphasic process. Production and secretion of growth related fermentation products, *i.e.* acetic and butyric acids, lead to a decrease of the pH the first phase of the process. At an external pH of about 5.1 the metabolism of the cells is switched to solventogenesis [1], meaning that butyric and acetic acid are taken up from the broth and neutral solvents, *i.e.* butanol, acetone and ethanol, are produced. While in batch fermentation processes acidogenesis and solventogenesis follow each other in time, a simultaneous occurring spatial separation of the two metabolic phases is possible with a continuous, multi-stage process design.
Here we present first steps towards a structured segregated model of Clostridial fermentation metabolism in a continuous bioreactor. It represents cell populations in differentiated metabolic states, which are characterized by specific production rates of acids and solvents. The respective production rates are functions of the external pH and/or acid concentrations. Furthermore the model compares the continuous fermentation process in a fixed-bed reactor, where cells are immobilized on a matrix, with those in a plug-flow reactor, where cells move through a tubular reactor without mixing in the axial direction. While in the first configuration individual cells are exposed to a relative constant chemical environment, cells in the second configuration experience changes in their surrounding pH and acid concentrations and thus need to adapt their metabolism.
Evaluation and refinement of this mathematical model with experimental data are work in progress. Once approved, the model will enable predictions of optimal substrate and biomass flow rates in a continuous butanol production process.

[1] Millat, T. *et al.* (2012) *Microbial Biotechnology* 6(5):526-39.

BTP44
Enhancing the detection rate of metagenomic-based screenings using a modified *E. coli* host strain

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In the search for novel biotechnological relevant enzymes, metagenomic libraries for functional screenings are used. The functional detection of enzymes from metagenomes is in general limited since only few reliable screening procedures are available and detection frequencies are often poor. Employing RNA-seq for the analysis of the expression profile of 19 fosmids suggested that transcription of metagenome-derived genes is a major limiting factor of functional metagenome searches. Working on the enhancement of the detection rate of metagenome-based screening, we are focusing on the improvement of the commonly used host *E. coli*. To overcome the limitation by using *E. coli* as a host strain, we modified the strain EPI300 by integration of an additional sigma-factor *rpoD* from *Clostridium cellolyticum*. The housekeeping sigma-factor was chromosomally integrated into the *bioF* gene and the resulted strain was designated UHH01. UHH01 was not impaired neither in growth nor in transduction frequencies and thus offered itself for construction of large insert libraries. Function-based screenings using sequenced fosmids suggested that the strain UHH01 was superior to the parent strain EPI300 and resulted in improved detection rate. Furthermore, we were able to demonstrate the advantage of the modified strain UHH01 compared to the parental strain EPI300 under laboratory conditions, by means of a screening

of a metagenomic library. Consequently, the integration of community-specific σ-factors into the *E. coli* host might be a reliable technique to improve the success of function-based metagenome searches.

BTP45
To be or not to be a PHB Depolymerase: PhaZd1 (PhaZ6) and PhaZd2 (PhaZ7) of *Ralstonia eutropha* are highly active PHB depolymerases but have no detectable role in mobilization of accumulated PHB

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R. eutropha is the model organism for investigation of the biosynthesis PHB granule formation and for intracellular reutilization (mobilization) of PHB (1). The putative physiological functions of two related intracellular PHB depolymerases, PhaZd1 and PhaZd2, of *R. eutropha* H16 were investigated. Purified PhaZd1 and PhaZd2 were active with native PHB granules *in vitro*. Partial removal of the proteinaceous surface layer of native PHB granules by trypsin treatment or the use of PHB granules isolated from Δ*phaP1* or Δ*phaP1-phaP5* strains resulted in increased specific PHB depolymerase activity especially for PhaZd2. Constitutive expression of PhaZd1 or PhaZd2 reduced or even prevented the accumulation of PHB under PHB permissive conditions in vivo. Expression of translational fusions of eYfp with PhaZd1/PhaZd2, in which the active site serines (S190/Ser193) were replaced by alanine, resulted only for PhaZd1 fusions in co-localization with PHB granules. C-terminal fusions of inactive PhaZd2 (S193A) with eYfp revealed the presence of spindle-like structures and no co-localization with PHB granules was observed. Chromosomal deletion of *phaZd1, phaZd2* or deletion of both depolymerase genes had no significant effect on PHB accumulation and mobilization during growth in NB or NB-gluconate medium (2). Moreover, neither proteome analysis of purified nPHB granules nor *lacZ*-fusion studies gave any indication that PhaZd1 or PhaZd2 were detectably present in the PHB granule fraction or were expressed at all during growth on NB-gluconate medium. In conclusion, PhaZd1 and PhaZd2 represent two PHB depolymerases with principally high capacity to degrade PHB when artificially expressed but they are apparently not involved in PHB mobilization in the wild type.

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2. Sznajder A., and Jendrossek, D. 2014. Appl. Environm. Microbiol. accepted for publication

BTP46
Biocatalysts for Waste Water Treatment - Trametes versicolor Laccase Immobilization on Porous Supports

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Introduction: Laccases are versatile multicopper oxidases with potential applicability in waste water treatment. Commercial laccase from the white-rot fungus *Trametes versicolor* was immobilized on porous supports such as polymer membranes and cryogels, both of which offer the advantage of a large surface area for biocatalyst binding and the possibility for simultaneous filtration applications in waste water treatment.
Experiment: Laccase immobilization was supported by electron irradiation. The laccase redox mediators2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and syringaldehyde were additionally immobilized in polymer membranes and cryogels instead of the enzyme, in order to assess possibilities to circumvent known drawbacks of freely dissolved redox mediators such as their loss from the reaction system when operated in continuous mode, and the formation of toxic by-products. Membranes as well as cryogels were characterized regarding their morphology and chemical composition.
Results and conclusions: Functional laccase was successfully immobilized onto membranes as well as cryogels. The apparent specific activity in removing the model pollutant bisphenol A (based on the activity towards the laccase substrate ABTS) followed the rank order cryogel-immobilized laccase (supported by electron irradiation) > laccase unspecifically adsorbed to cryogels > free laccase > membrane-immobilized laccase (electron irradiation-supported). The functionality of the cryogel-immobilized (supported by electron irradiation) redox mediators ABTS and syringaldehyde could be inferred from observed enhancements of the initial bisphenol A removal rates caused by the presence of redox mediator-containing cryogels during laccase catalysis. It remains to be elucidated inasmuch binding of redox mediators remains stable, and to which extent they may be released from the supports.

BTP47
Co-habitation in synthetic biofilm communities protects bacteria against chemical stress

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Bacterial cells within aggregates or biofilms are more resistant against adverse conditions than freely suspended cells. It is not known whether bacteria that do not form biofilms themselves but co-habitate biofilms formed by other bacteria do also acquire increased resistance properties. To investigate this, we designed proof-of-principle experiments with relevance to hygienic aspects and biotechnological applications.
Formation of cell aggregates by *P. aeruginosa* is induced in the presence of the toxic detergent Na-dodecylsulfate (SDS). Cells within such aggregates have a significantly higher resistance against severe chemical stress exerted by SDS in the presence of the uncoupling agent carbonylcyanide-m-chlorophenylhydrazone (CCCP) [1]. We investigated whether other bacteria can be integrated into these aggregates of *P. aeruginosa* and, if so, whether they are also protected against stress imposed by SDS and CCCP. For this, we constructed synthetic bacterial communities consisting of *P. aeruginosa* and either *Escherichia coli* or *Corynebacterium glutamicum*.
In the first bacterial community bioluminescent *E. coli* were used. In single culture of *E. coli* bioluminescence as a measure for viability was strongly affected by CCCP and SDS. In co-culture with *P. aeruginosa* bioluminescence was significantly sustained, when *E. coli* cells were integrated into the cell aggregates. In the second bacterial community a lysine-producing *gfp*-tagged *C. glutamicum* was used. In single culture growth of *C. glutamicum* was strongly inhibited by SDS. Co-cultivation experiments indicated that *C. glutamicum* could be integrated and, thus, protected in SDS-induced cell aggregates of *P. aeruginosa*. When a lysine-auxotrophic *P. aeruginosa* mutant was used, co-cultivation experiments without SDS showed that lysine production of *C. glutamicum* could fully restore growth of the auxotrophic mutant in minimal medium, and current experiments aim at investigating whether these strains can grow in a SDS-containing medium in a symbiotic manner. These results show that co-habitation in biofilms can protect bacteria against severe stress. This could be exploited in biotechnology for fermentations in toxic milieus or as an alternative immobilization method. Regarding hygiene, it might contribute to the understanding of pathogen survival in adverse environments.

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BTP48
Characterization of the MIIA-domain of the VIC_001052 protein of *Vibrio coralliilyticus* ATCC BAA450 and the development of an autocatalytic cleavable protein linker

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The metal ion-inducible autocleavage (MIIA)-domain (formerly DUF1521) is present in the protein VIC_001052 of the coral pathogen *Vibrio coralliilyticus* ATCC BAA450. Several divalent cations can induce an autocatalytic peptide bond cleavage in this domain at the sequence motif GD'PH. The cleavage reaction is fast and stable over a large pH and temperature range [1]. The conformational changes of the MIIA-domain in the presence of cations are analyzed by circular dichroism (CD) and fluorescence spectroscopy. For that purpose, protein variants carrying single amino acid exchanges or small deletions were generated. The inducible autocleavage activity of the MIIA-domain could be of use in a linker for the purification of recombinant proteins. For fast and easy purification recombinant proteins are often produced as fusion proteins with an affinity tag. In conventional systems the affinity tag has to be removed by protease treatment after the purification [2,3]. The MIIA domain was included in recombinant fusion proteins in order to separate the target protein from the

affinity tag by an autocatalytic reaction. In contrast to protease treatment, the affinity tag can be removed by addition of cations. No additional purification step to remove a protease is necessary. The autocatalytic cleavage can be performed with immobilized protein on an affinity column. After cleavage, about 44 amino acids of the linker sequence remain at the C-terminus of the target protein. Our current efforts are directed towards reducing this number of amino acids to a minimum without compromising the autocatalytic cleavage activity.

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BTP49

Expression of oxygenase encoding *cadA* is associated with the degradation of 4-chloro-2-methylphenoxyacetic acid in *Sphingomonas* sp. tfd26

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Phenoxyacetic acid herbicides like 4-chloro-2-methylphenoxyacetic acid (MCPA) are applied worldwide and potential groundwater contaminants. Microbial degraders are categorized into three groups, i.e., Beta- and Gammaproteobacteria (group 1), *Bradyrhizobium*- (group 2), and *Sphingomonas*- (group 3) related *Alphaproteobacteria*. It is well establishe that group 1 degraders are associated with MCPA degradation in soils. However, MCPA degradation was recently attributed to group 3 rather than group 1 degraders in agricultural soil. Group 3 degraders host *cadA* encoding a 2,4-dichlorophenoxyacetic acid oxygenase. However, it is unclear whether this gene is associated with MCPA degradation in the group 3 model organism *Sphingomonas* sp. tfd26. 40 µM MCPA was spiked to a *Sphingomonas* sp. tfd26 culture in the late exponential phase. MCPA was completely consumed within 10 hours of incubation as determined by HPLC-MS. Consumption was linear, and growth rates of cultures with MCPA were marginally higher than those of cultures without MCPA. A 10-fold induction of *cadA* expression was observed by qPCR and occurred without appreciable delay after spiking of MCPA. *cadA* transcripts were detected at low levels prior to spiking with MCPA and in controls without MCPA. Thus, the data indicate that (i) *Sphingomonas* sp. tfd26 is prone to react quickly to MCPA via induction or de-repression of *cadA*, (ii) *cadA* encoded oxygenases facilitate MCPA degradation in group 3 degraders, and (iii) support *cadA* as a suitable marker gene for analyzing group 3 MCPA degraders in the environment.

BTP50

Harnessing light to control the production of secondary metabolites in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a Gram-negative, water and soil bacterium as well as an opportunistic human pathogen causing serious nosocomial infections. This bacterium is well-known for its metabolic diversity and the production of a number of different secondary metabolites including rhamnolipids, which exhibit promising biotechnological potential. The synthesis of these metabolites is tightly regulated in a highly cell-density-dependent manner through the interplay of the two quorum sensing systems Las and Rhl. In this study, we investigated the use of monochromatic light as a key molecular switch to control both Las and Rhl regulatory networks and subsequently influence metabolite production in *P. aeruginosa*. For this purpose we constructed synthetic regulatory networks by combining three different, well characterized light-dependent switches from *Synecocystis sp.*, *Bacillus subtilis, Avena sativa* and *Bradyrhizobium japonicum* (Tabor *et al.* 2011; Ohlendorf *et al.* 2012) with the genes *lasI*, *lasR*, *rhlI* and *rhlR*, which are coding for the respective quorum sensing molecule synthase and corresponding transcriptional regulator, respectively. Using a subset of engineered PA01 strains in combination with a blue light switch, we obtained a light controlled increase of 30 - 60% of LasI dependent quorum sensing activity determined by metabolite analyses and reporter gene assays. In addition, a second red light based switch exhibited a controlled increase of approximately 40% in LasI dependent quorum sensing activity. Further studies are currently investigating various combinations of light dependent switches and quorum sensing regulatory genes for a controlled and increased metabolite production in *P. aeruginosa*.

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BTP51

Cellulolytic and hemicellulolytic symbionts from selected termites for the degradation of renewable plant material

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The intestinal tract of termites is an effective symbiotic system for lignocellulose degradation. The digestion of cellulose and hemicellulose is conducted in one day by symbionts and the termite's own enzymes. Hence, the termite gut is a promising source of cellulolytic and hemicellulolytic microorganisms (König *et al.*, 2013). We enriched the symbiotic cellulolytic and hemicellulolytic microbiota of the lower termite *Mastotermes darwiniensis* and isolated novel strains of yeasts and bacteria. The novel strains were screened for cellulase or xylanase activity. We used three screening methods: the congo red plate diffusion test, the "Miller-assay" and the cellulose azure or xylane azure dye release assay (McDonald *et al.*, 2012). Positive yeast isolates were identified *via* RFLP and sequence analysis of the ITS-regions (Divol and Lonvaud-Funel, 2005; White *et al.*, 1990). We identified three cellulase positive yeast strains, 23 cellulase positive bacterial strains and 29 xylanase positive yeast strains from six selected termites (*Mastotermes darwiniensis*, *Neotermes castaneus*, *Neotermes jouteli*, *Odontotermes obesus*, *Schedorhinotermes intermedius* and *Zootermopsis angusticollis*).
Novel or genetechnically modified cellulases and xylanases could provide improved tools for biofuel production. The application of an organism with both the abilities of cellulose digestion and bioethanol production could result in an immense commercial potential. Lignocellulose containing renewable crops, such as wheat straw, are available in abundant amounts (Chandel and Singh, 2011).

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BTP52

Generation of CO_2-neutral isoprene production using the cyanobacterium *Synechocystis* sp. PCC 6803

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Cyanobacteria are the only prokaryotes able to perform oxygenic photosynthesis. Thus, these bacteria can convert inorganic carbon as CO_2 into organic carbon compounds on the expense of light energy. Moreover, they need only a few additional inorganic nutrients and can be cultivated in high densities using non-arable land and seawater. This features qualified cyanobacteria as attractive organisms for the production of third generation biofuels as part of the development of a CO_2-neutral future energy production. *Synechocystis* sp. PCC 6803 represents one of the best-investigated cyanobacterial model strains. On the basis of its available genome sequence and genetic tools, many strains of *Synechocystis* have been generated producing different potential biofuels. Among those

attempts, isoprene is an attractive goal since this compound is not only an energy-rich biofuel but also used as chemical feedstock. Here, we are going to report our attempts to generate isoprene-producing strains of *Synechocystis*. To generate the isoprene production, cDNA of a plan isoprene synthase was cloned under the control of different *Synechocystis* promoters. For this purpose we used strong constitutive and regulated promoters. The expression of the isoprene synthase was quantified by semi-quantitative RT-PCR, whereas the amount of isoprene was quantified using GC-MS. Incubation of our strains at different light and salt conditions had marked impact on the isoprene production rates. The results of our experiments using a laboratory model strain can be used to guide future attempts establishing the large-scale isoprene production with cyanobacterial hosts.

BTP53

Recombinant Production of Rhamnolipid Derivatives

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Introduction: Rhamnolipids are glycolipidic biosurfactants mainly produced from often pathogenic *Pseudomonas* and *Burkholderia* species. They consist of one or two rhamnose residues linked to one or two ß-hydroxy fatty acids. This amphiphilic structure bears a great potential for a broad range of industrial applications. As rhamnolipids are biodegradable and can be produced from renewable resources they are environmentally friendly.
Materials and Methods: The genes *rhlA* and *rhlB* were cloned into an expression vector and expressed in a non-pathogenic bacterial host. The first enzyme RhlA links two ß-hydroxy fatty acids yielding the rhamnolipid precursor 3-(3-hydroxyalkanoyloxy)alkanoate (HAA). RhlB is a rhamnosyltransferase linking an HAA molecule to an activated dTDP-rhamnose resulting in a mono-rhamnolipid. Host strains carrying the expression vector were cultivated in LB medium containing glucose as additional carbon source. Rhamnolipids or their derivatives were produced at 30°C for up to 48 hours and finally analyzed by HPLC.
Results: Rhamnolipids and their precursor HAA could be produced by recombinant gene expression with non-pathogenic hosts. Expressing the genes from *P. aeruginosa* resulted in rhamnolipids and HAAs of *P. aeruginosa*-type, i.e., rhamnolipids with mainly C10-C10 chain lengths.
Discussion: The successful recombinant production of rhamnolipids or their derivatives is an alternative to the production with bacterial wildtype strains. The ease of genetic manipulation and process intensification opens plenty of opportunities for optimization of rhamnolipid synthesis. The avoidance of a pathogenic host simplifies official approval of such a microbial-based detergent production considerably. In addition, the production of rhamnolipid derivatives allows alternative applications. The challenges ahead are still considerable, as current detergent prices, depending on the application, are very low.

Literature
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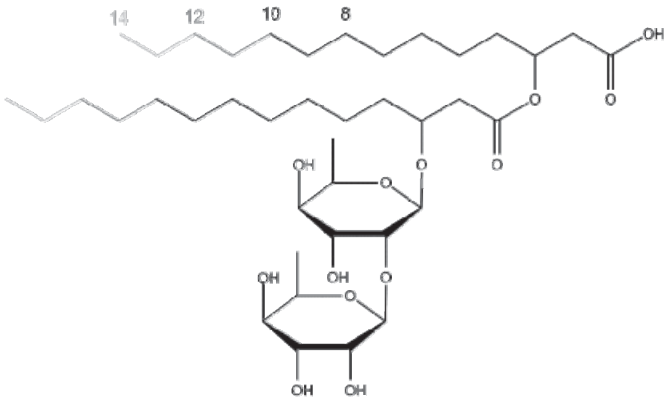
Figure: Molecular structure of a di-rhamnolipid

Figure 1



BTP54

Halomonas elongata as a novel host for the heterologous production of proteins in a unique stabilizing environment

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Increasing demand for recombinant proteins has attracted significant interest in the exploration of novel expression systems for recombinant proteins. Although it has long been established that stabilizing solutes (compatible solutes) can improve the functional expression of therapeutic proteins, as for example immunotoxins [1], the impact of a modified environment on expression, functionality and stability of the product has not yet been explored in depth. The use of compatible-solute producing and/or accumulating halophilic *Bacteria* as an alternative to established bacterial systems such as *E. coli* offers the following advantages:
1) by modifying the external salinity, the cytoplasmic concentration of stabilizing solutes can be adjusted according to needs
2) the type of stabilizing solute can be chosen by external addition to the medium (accumulation of solutes has precedence over synthesis)
3) periplasmic expression will expose the desired product to a saline environment. Recently we focused on the construction of suitable vectors for protein over-expression in the gamma proteobacterium *Halomonas elongata*. By fusing the promoter of the ectoine biosynthetic gene cluster to an optimized ribosomal binding site we obtained a suitable vector for salinity-controlled protein-expression [2]. Until now, our protein expression experiments in *H. elongata* were limited to the cytoplasm. However, the export of recombinant proteins into the periplasm might be equally interesting, especially when growing the organism in high-salt media. These conditions should favour folding and activity of halophilic proteins which tend to aggregate in non-halophilic hosts like *E. coli* due to a low salt concentration. Basically, two different pathways are known for protein translocation into the periplasm: The Sec translocon, exporting proteins in a largely unfolded state, and the Tat system, known to transport proteins in their folded form. Both systems depend on N-terminal signal peptides, which are processed after the transport. In this study we searched for sec- and tat-leader sequences in the recently sequenced [3] *H. elongata*. Suitable candidates were fused to fluorescent reporter proteins, namely Gfp_{uv} (tat) and mCherry (sec), and the resulting fusion proteins were expressed in *H. elongata*. The successful localization of the fluorescent proteins could be proven by fluorescence microscopy. To our knowledge we report here for the first time the directed periplasmic transport of recombinant proteins in a halophilic solute-accumulating *Bacterium*.

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BTP55

Bacterial production of high-value compounds by heterologous expression of clustered genes using TREX

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Microbes produce numberless metabolites with high-value activities such as antibiosis, cytotoxicity and immunosuppression. Their biosynthesis is often genetically encoded by clustered genes which are difficult to express using standard expression systems. Limitations are associated with cloning, transfer, stable maintenance and especially with the functional expression of all pathway genes. In addition, the microbial host provides a critical background for successful gene expression and drug production: (i) Functional enzymes must be synthesized requiring appropriate codon usage and protein folding, (ii) suitable precursor molecules need to be provided, and (iii) tolerance towards toxic intermediates and/or end products is required. Hence, completely different outcomes result from different pathway/host combinations. We have developed a novel genetic tool named TREX (pathway transfer and expression) which facilitates the expression of large gene clusters in bacteria. TREX consists of two gene cassettes which comprise genetic elements allowing the conjugational transfer into the expression host of large DNA fragments encompassing all genes of interest,

the stable integration of the TREX-labeled gene cluster into the host chromosome *via* transposition as well as the concerted expression of all pathway genes irrespective of their orientation and natural regulatory elements by convergent T7 RNA polymerase-mediated expression. TREX was applied to functionally express the entire prodigiosin biosynthesis pathway genes from *Serratia marcescens*, and we succeeded to construct different *Escherichia coli* and *Pseudomonas putida* production strains accumulating the respective antibiotic. A multi-step extraction and purification method allowed isolating highly pure prodigiosin as confirmed by HPLC, high-resolution mass spectrometry,NMR and chemical synthesis. TREX is the first toolkit that enables the transfer, randomized genome integration and bidirectional expression of complex gene clusters in a broad range of bacterial hosts. Consequently, TREX allows the reconstitution of pathways within a number of different and metabolically versatile screening hosts in a plug-and-play fashion which offers new perspectives in the fields of genome mining, synthetic biology and drug development.

BTP56

Recombinant ethylbenzene dehydrogenase for functional characterization of its reaction mechanism and potential biotechnological applications

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Ethylbenzene dehydrogenase (Ebdh) from *Aromatoleum aromaticum* EbN1 is an anaerobic enzyme which contains complex cofactors such as a molybdenum-bis-molybdopterin guanine dinucleotide, five Fe-S clusters and a heme b cofactor and catalyzes the stereospecific hydroxylation of ethylbenzene to (*S*)-1-phenylethanol. This study shows for the first time that active Ebdh was heterologously produced in different bacterial strains. The *ebd* operon (*ebdABCD*) was cloned into a broad-host range expression vector under an inducible *tet*-promotor. Overproduction of Ebdh was achieved aerobically or anaerobically under nitrate-reducing conditions in *Escherichia coli, Shimwellia blattae* and *Pseudomonas stutzeri*, but only anaerobic cultures yielded active enzyme. The highest activities were obtained from *P. stutzeri* and *E. coli* C41(DE3) containing the accessory plasmids pRKISC and pCodonPlus. We are currently involved in generating tagged versions of the enzyme and introducing site-directed mutations in the vicinity of the active site in EbdA, which will be assessed for their effects on activity and specificity of the enzyme. Upon further characterization and optimization of production conditions, recombinant Ebdh may be a good candidate for biotechnological production of chiral alcohols from their hydrocarbon precursors.

BTP57

Partial secretome and transcriptome analysis of *Caldariomyces fumago* reveals extracellular production of the CPO co-substrate hydrogen peroxide and the existence of two CPO isogenes

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The culture supernatant of the fungus *Caldariomyces fumago* grown in a fructose salt medium contains mainly the biotechnologically relevant enzyme chloroperoxidase (CPO) and only minor amounts of other proteins. Our approach to identify the nature of these proteins via peptide mass fingerprinting and transcriptome analysis demonstrated the presence of putative glycosyl hydrolase and glucose oxidase (GOx) enzymes. These activities could provide CPO with the co-substrate hydrogen peroxide from glucose or polysaccharides. The GOx activity has a pH optimum of 5, at which the glucose-driven CPO halogenation activity of the culture supernatant was up to 1.4 U/mL. Comparable GOx activity could be also detected in commercial CPO preparations. If further enhancement of GOx production can be achieved, the *C. fumago* culture supernatant could provide a simple and convenient CPO/GOx source for reactions with *in situ* hydrogen peroxide production. Inspection of the transcriptome data led to the discovery of two distinct CPO mRNA sequences. The *C. fumago* strain DSM1256 could be shown to contain the newly identified isogene as well as produce and secrete both isoenzymes. The CPO2 enzyme bears high sequence similarity to the well-characterized CPO (87% identity in case of the mature proteins). It shows two insertions in the signal peptide and in the C-terminal propeptide and one deletion in the mature polypeptide close to the C-terminus. Furthermore it lacks one of the serine residues known to be O-glycosylated in the CPO sequence. The demonstration of two distinct CPO polypeptides clarifies the nature of this isoenzyme previously presumed to represent a differently glycosylated CPO form.

BTP58

Production of the compatible solute α-D-glucosylglycerol by metabolically engineered *Corynebacterium glutamicum*

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Accumulation of compatible solutes is required for the acclimation of organisms to adverse environmental conditions, particularly to salt and drought stress. α-D-glucosylglycerol (αGG) serves as compatible solute in various halotolerant cyanobacteria such as *Synechocystis sp.* PCC 6803 (1). αGG is synthesized in *Synechocystis sp.* PCC 6803 in a two-step reaction in which the enzymatic condensation of ADP-glucose and glycerol 3-phosphate by GG-phosphate synthase (GGPS) is followed by the dephosphorylation of the intermediate by the GG-phosphate phosphatase (GGPP). Apart from its role in cyanobacteria as compatible solute αGG has been reported to have beneficial functions as a moisturizing agent in cosmetics and potential as a health food material, and therapeutic agent(2). For its production enzymatic synthesis is currently applied, development of strains and processes for microbial production of αGG has hitherto not been described.
We considered the Gram-positive soil bacterium *Corynebacterium glutamicum*, a well-established industrial workhorse for amino acid production, as a suitable candidate for the development of a synthetic production platform of αGG, because this organism did not utilize αGG as a substrate. Plasmid-bound expression of *ggpS* and *ggPP* from *Synechocystis sp.* PCC 6803 enabled αGG synthesis in osmotically stressed cells of *C. glutamicum* (pEKEX3-ggpSP). Astonishingly αGG was also present in the culture supernatants of *C. glutamicum* (pEKEX3-ggpSP), showing efficient secretion of αGG by this strain. The precursor for αGG synthesis ADP-glucose is used in *C. glutamicum* by the *glgA* encoded glycogen synthase as well as the *otsA* encoded trehalose-6-phosphate synthase. Upon deletion of both genes the final αGG concentration in culture supernatants after 24 h cultivation stress was increased from 0.5 mM in *C. glutamicum* (pEKEX3-ggpS-ggpP) to 2.9 mM in *C. glutamicum* ΔotsAΔglgA (pEKEX3-ggpSP) indicating an improved precursor supply in the latter strain. *C. glutamicum* usually synthesizes the amino acids proline, glutamate, and glutamine as compatible solutes in response to hyperosmotic stress. However in N-limited cultivations trehalose is preferentially synthesized upon hyperosmotic stress (4), which is not possible in *otsA* and *glgA* deficient *C. glutamicum* strains (3). Indeed, after further optimization of cultivation conditions finally more than 10 mM αGG (about 2g l^{-1}) were present in supernatants of N-limited cultures of *C. glutamicum* ΔotsAΔglgA (pEKEX3-ggpSP). This result shows, that *C. glutamicum* can be used as an efficient platform for the production of the compatible solute αGG.

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BTP59

Development of a new vector for metabolic engineering in *Sulfolobus acidocaldarius*

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The depletion of fossil resources propels the search for alternate ways of energy generation. Especially the production of 2nd generation biofuels from lignocellulosic biomass that does not compete with our feedstocks (e.g. grasses, nutshells, banana waste or the daily newspaper) by metabolic engineered microorganism is very promising [1]. The aerobic crenarchaeum *Sulfolobus acidocaldarius* is one of the best studied archaeal model organisms [2]. The thermoacidophilic life style of *S. acidocaldarius* with growth at 78°C and pH 2-3 as well as the available comprehensive genetic

tool box [3] makes it a very suitable chassis for metabolic engineering and thus opens up new horizons for industrial applications. To further increase the flexibility of the organism for metabolic engineering approaches and to optimize productivity, we established a new vector that allows for induction either by cold shock (65°C) or tryptone (80°C). This vector allows for new process control: (i) by low temperature induction e.g. for effective expression of less thermostable enzymes or production of less stable compounds at reduced temperature going along with low metabolic activity of the cell and thus less biomass formation, or (ii) by tryptone induction for expression at high temperature which is more suitable for applications like lignocellulosic biomass degradation or direct product distillation (e.g. of alcohols). The properties of the new vector as well as a detailed characterization of the promoter features by reporter gene studies (lacZ) [3] will be presented.

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BTP60
System-wide analysis of stress-adaptation in *Bacillus megaterium* and its applications

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For many years now, we have been successfully developing *Bacillus megaterium* as a host for production, secretion and purification of recombinant proteins. The g/L-scale for intra- as well as for extracellular recombinant products has already been reached. Generally, once a producer has been genetically designed, optimal process parameters are established to maximize its potential for industrial production. Despite this upstream work, bacterial cells are constantly exposed to various kinds of stress during the whole production process, including e.g. shearing, high nutrient or product concentrations and variations of temperature, medium composition or oxygen availability. To date, the impact of these conditions on the cellular activity is only slightly understood but the recent development of systems biology now provides precious tools for characterizing the cellular behavior of stressed cells. In this context, we carried out a holistic study including transcriptome, proteome, metabolome and fluxome analyses to apprehend the impact of osmotic stress (simulated with up to 1.8 M of NaCl) and harsh cultivation temperatures (15° and 45°C) on the metabolism of the wild-type *B. megaterium*. After analyzing the data from each omics-technique separately, they were combined together to offer an integrated picture of cellular adaptation and to find underlying genetic targets for the development of more robust production hosts. Interestingly, while biomass yields and substrate uptake rates were decreased under both stress conditions, the flux distribution within the central carbon and energy metabolism as well as the levels of the corresponding mRNAs and proteins were only locally affected. However, in both cases specific responses occurring at every biological level involving membrane modification and intracellular accumulation of certain amino acids were detected. More surprisingly - although *B. megaterium* has long been known for its capacity to produce polyhydroxybutyrate (PHB) - we could demonstrate for the first time that under osmotic stress the intracellular PHB concentration positively correlates with salt concentration. As neither the concentration of the enzymes involved in the classical PHB-pathway nor that of their related mRNAs significantly increased, we systematically overproduced these proteins, resulting in an even higher PHB concentration. To summarize, this work on stress-adaptation underlines the potential of systems biology comprehending the dynamics of living organisms in detail followed by the use of this knowledge for strain improvement.

BTP61
Inhibition of the enzymatic hydrolysis of polyethylene terephthalate by bis(2-hydroxyethyl) terephthalate and methyl-(2-hydroxyethyl) terephthalate

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The inhibition of the polyester hydrolase TfCut2 from *Thermobifida fusca* by intermediate degradation products of polyethylene terephthalate (PET) was investigated. Nanoparticles prepared from PET films were used as substrate and were hydrolyzed by TfCut2 in the presence of bis(2-hydroxyethyl) terephthalate (BHET) and methyl-(2-hydroxyethyl) terephthalate (MHET). The formation of the reaction products was followed by reversed-phase HPLC. Kinetic analysis of the initial reaction rates using a model for PET degradation revealed BHET and MHET as competitive inhibitors with similar binding constants. The hydrolysis of MHET and BHET by TfCut2 showed a considerably lower reaction efficiency (E_f) for MHET confirming this degradation product as a relevant inhibitor of the hydrolysis of PET by TfCut2.

BTP62
Sporobeads: the utilization of *Bacillus subtilis* endospores for protein display

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Bacillus subtilis is a soil bacterium that can form metabolically inactive endospores under nutrient limitations. The process of endospore formation is commenced through an asymmetrical cell division, resulting in the formation of a larger mother cell and the smaller forespore. The forespore provides proteins to protect the DNA against environmental factors like UV-light. Moreover, the spore is encased in three protective layers, the cortex, the coat and the crust, which are produced by the mother cell. The cortex is comprised of peptidoglycan, while the coat and crust are made up of at least 70 different proteins, which are also produced by the mother cell.
Here we provide evidence that the spore surface can be utilized to functionalize the spores, by genetically fusing a gene of interest to a crust protein gene. The resulting spore, a so-called **Sporo**bead, displays a protein with a desired function on the surface, similar to commercially available beads used in filters or in the laboratory. The possible applications of our biological beads are very widespread, from enzymatic functions to filter-function using proteins able to bind to wanted (toxic) compounds.
As a proof-of-principle and for easy evaluation, we chose GFP as the first protein to be displayed. It was also used to evaluate the consequences of deleting the native crust proteins. Moreover, we chose to display the laccase from *B. pumilus* on the surface of the spore. Laccases are enzymes that have the potential to degrade a wide range of polycyclic carbohydrates and xenohormones, which are found as pollutants in our drinking and fresh water supplies. The resulting Laccase-**Sporo**beads had a 20-fold higher activity in the ABTS-assay than the reference strain without a laccase. We propose that these Laccase-**Sporo**beads could be utilized in sewage treatment plants to remove chemical contaminations from water.
Sporobeads only remain functional as long as they are in there spore state. Upon germination, they lose their crust and therefore there functional fusion proteins. It is therefore essential to prevent the **Sporo**beads from germinating in order to maintain their function. Towards that end, the GerminationSTOP module was developed, which is based on knock-outs of genes required for germination. The resulting strains showed a reduction in germination of six to eight orders of magnitude. As an additional safety feature, we are currently developing a suicide switch, which would be useful to kill the remaining germinating spores.

BTP63

Recombineering and biosensor-guided FACS screening for strain development of *Corynebacterium glutamicum*

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Corynebacterium glutamicum is widely used in industry for amino acid production. We have recently established single strand DNA (ssDNA) recombineering for genome engineering [1] and a biosensor technology enabling strain development at single cell level [2], [3]. Whereas ssDNA recombineering enables the use of oligonucleotides, double strand DNA (dsDNA) recombineering would allow the use of large PCR fragments for the genetic modification of bacterial genomes. However, dsDNA recombineering requires both, RecE (exonuclease) and RecT (ssDNA binding protein) activity, as compared to ssDNA recombineering, which depends solely on RecT. Consequently, a variety of expression vectors were constructed, either encoding RecE and RecT from the prophage Rac, or from a prophage present in *Corynebacterium aurimucosum*. As a test strain to evaluate successful recombineering, a *C. glutamicum*-strain with an integrated KanR gene carrying a frame shift mutation was used. Various experiments indicate that the appropriate expression of recET from prophage Rac together with the introduction of a correct kanamycin fragment of 600 bp via electroporation can restore kanamycin resistance. About 1.1 x 10^5 recombinants per assay can be obtained. This "healing" requires only a short homology region of the DNA substrate used and the genomic target region. Therefore strains are currently constructed, which will enable to study dsDNA recombineering also with different experimental set-ups. In addition to dsDNA recombineering, experiments with ssDNA recombineering in combination with FACS (Fluorescence Activated Cell Sorting) were performed. This technology uses the transcriptional regulator LysG, which in response to elevated L-lysine concentrations drives the transcription of eYFP [1]. We call the combined technology RecFACS, which is a new biosensor-guided technology to create producers as well as genetic diversity in one single step. We successfully used this technique to mutate and screen a site-saturation library of murE, which is known to have an influence on L-lysine production in this organism. I will present an additional application of RecFACS for improving *C. glutamicum* strains for L-lysine production.

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MICROBIAL CELL BIOLOGY

CBP01

The role of ER-related BiP/GRP78 in IFN-γ induced Chlamydia pneumoniae infection

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Introduction: *Chlamydia pneumoniae* (*Cpn*) is an obligate intracellular bacterium and causes pharyngitis and bronchitis. Further, chronic obstructive pulmonary disease (COPD) and atherosclerosis are supposed to develop through chronic inflammatory processes of persistently infected tissues. It is well accepted that *Chlamydiae* directly interact with the endoplasmic reticulum (ER) during infection, but little is known about the precise role of the ER for intracellular chlamydial development and survival. The ER has multicellular functions and plays a key role in ER stress responses that are regulated by the unfolded protein response (UPR). The ER chaperone BiP/GRP78 is known to regulate UPR to maintain cellular homeostasis. In this study we investigated functions of BiP/GRP78 in *Cpn*productive and persistent infections.
Materials and Methods: Interferon (IFN)-γ was used to induce persistent infections of *Cpn*. *Cpn* inclusions and the ER interactions were investigated by confocal microscopy and electron microscopy. siRNA was used to silence BiP/GRP78. The UPR in *Cpn* infection was analyzed by western blot and RT-PCR.
Results: We could show that the ER co-localizes with productive and IFN-γ induced persistent *Cpn* inclusions. Knockdown of BiP/GRP78 significantly reduced infectious progeny in productive and persistent *Cpn* infection. BiP/GRP78 expression itself was temporally induced upon IFN-γ induced persistent but not productive *Cpn* infection. In addition, enhanced BiP/GRP78 expression was accompanied by activation of the eukaryotic initiation factor-2α (eIF2α) and down-regulation of the vesicle-associated membrane protein-associated protein B (VAPB). During persistent *Cpn* infection, BiP/GRP78 expression attenuated activation of eIF2α and host cell apoptosis by an exogenous ER stress inducer.
Conclusion: ER-related BiP/GRP78 protein plays a key role during the intracellular chlamydial developmental cycle and contributes to the maintenance of the host cell homeostasis in persistently *Cpn* infected cells.

CBP02

MreB paralogues of Bacillus subtilis associate with a lipid bilayer and co-polymerize into filaments *in vitro*

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Many non-spherical bacteria with more complex morphologies express one or more MreB proteins. Among other cellular tasks, these actin-like cytoskeletal elements are suggested to function in cell shape maintainance by spatially organizing cell wall synthesis. Super resolution microscopy revealed that in the model bacterium *B. subtilis*, MreB and its paralogues Mbl and MreBH assemble into discontinuous filaments of variable length underneath the lateral membrane, which move at various angles to the diametric cell axis.
In this study, an *in vitro* assay was performed that allowed inspection of the polymerization properties of purified *B. subtilis* MreB proteins by fluorescence microscopy as well as electron microscopy. Taking advantage of the intrinsic membrane affinity of MreB proteins, a lipid bilayer derived from *E. coli* lipid extract was created on glass slides, to which monomeric YFP-MreB, CFP-Mbl and mCherry-MreBH were added. Each MreB paralogue polymerized into helical filamentous structures at a characteristic concentration, after the addition of divalent cations,while monovalent cations inhibited polymerization.
Neither the formation of filaments nor their helical arrangement are artifacts of the fluorescence marker but an intrinsic property of MreB, as a mixture of Strep-YFP-MreB at a concentration not leading to the formation of filaments and Strep-MreB in a ratio of 1:30 formed filaments indistinguishable to that of Strep-YFP-MreB. This is consistent with the data obtained by electron microscopy, which resolved the Strep-MreB filaments to be curved sheets of protofilaments.
Strep-YFP-MreB, -CFP-Mbl and -mCherry-MreBH - each protein fusion below the minimal concentration - polymerized into single, helical filaments, indicating that the paralogues form mixed protofilaments rather than bundles of single protofilaments.

CBP03

Analyzing the regulatory mechanisms underlying the ‚sodorifen' emission in *Serratia plymuthica* 4Rx13

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The effects of bacterial volatiles on other organisms are widespread and gain increasing interest in science. A very prominent example are plant growth promoting rhizobacteria (PGPR) being able to positively modulate the plant development directly (e.g. by production of plant hormones) or indirectly (e.g. by inhibition of pathogens). An example for PGPR is *Serratia plymuthica* 4Rx13 which was originally isolated from the rhizosphere of *Brassica napus*. In dual culture assays the emitted volatiles showed significant inhibiting effects on the mycelial growth of different plant pathogenic fungi [1]. Analysis of the volatile organic compounds (VOC) of

this bacterium revealed a broad spectrum of about 100 different volatiles. Interestingly the main compound is making up to 45 % of the volatile bouquet. NMR was used for structural elucidation of this so far unknown compound [2]. It was identified as a polymethylated bicyclus with a structure new to science. Its molecular formula is $C_{16}H_{26}$ and it was named ‚sodorifen' [3]. It is still unknown why *Serratia plymuthica* 4Rx13 is emitting ‚sodorifen' in such high amounts and what its biological or ecological function might be. Therefore we are now trying i) to unravel the biosynthesis of this compound and ii) to understand its regulation. By using a transcriptomic approach we compared the transcripts of *S.p.* 4Rx13 with a sodorifen-non-producing *Serratia* strain (*S. plymuthica* AS9). Furthermore we studied the expression of target genes (RT-PCR and Northern Blot technique) under different growth conditions (media supplemented with different aminoacids and carbon sources).
The results show that the transcription level of target genes investigated increases proportionally to the amount of 'sodorifen' being emitted by *S.p.* 4Rx13 when exposed to different carbon sources. This indicates that the feature of 'sodorifen' emission is not a question of genes being present or absent in bacteria but rather due to specific regulation.

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CBP04
Germination proteases of *Clostridium acetobutylicum*

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During germination and outgrowth of endospores of Gram-positive bacteria usually small acid-soluble proteins (SASP) of the spore are degraded into free amino acids which are needed for protein biosynthesis of the development of a new vegetative cell. This degradation is initiated by specific enzymes, named germination proteases (Gpr). In general Gpr are synthesized as inactive zymogens and converted into its active forms later in sporulation by an autoprocessing reaction removing an N-terminal propeptide immediately prior to spore dormancy. Due to the low water content of the spore Gpr are inactive until hydration of the spore during germination. In the genome of strict anaerobe *Clostridium acetobutylicum* four putative *gpr* genes have been identified due to the amino acid residue similarities of the deduced proteins against a well characterized Gpr of *Bacillus megaterium* including two conserved aspartic acid residues, which are important for Gpr activity (Pei and Grishin, 2002).
Here we present a comparative investigation of these four Gpr of *C. acetobutylicum* after their heterologous expression in and purification out of *Escherichia coli*. Only for Gpr1 and Gpr2 of *C. acetobutylicum* autoprocessing could be verified, whereas Gpr3 and Gpr4 revealed no autoprocessing. Furthermore, Gpr3 and Gpr4 seemed to be immediately active without processing. Degradation assays (Carrol and Setlow, 2005) showed that the autoprocessed forms of Gpr1 and Gpr2 were able to hydrolyze SASP specifically and thus can be classified as 'regular' Gpr. Interestingly also Gpr3 and Gpr4 were able to degrade SASP. However, protein degradation was not restricted to SASP raising the question, if these proteases are 'regular' Gpr or if they are involved in other sporulation processes.

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CBP05
Ccrp proteins influence pathogenicity of *Helicobacter pylori*

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Pathogenicity of the human pathogen *Helicobacter pylori* relies on its capacity to adapt to a hostile environment and to escape the host response. Although there have been great advances in our understanding of the bacterial cytoskeleton, major gaps remain in our knowledge of its contribution to virulence. In this study we have explored the influence of coiled coil rich proteins (Ccrp) on pathogenicity factors of *H. pylori*. Deletion of any of the *ccrp* resulted in a strongly decreased activity of the main pathogenicity factor urease. We further investigated their role using *in vitro* co-culture experiments with the human gastric adenocarcinoma cell line AGS modeling *H. pylori*- host cell interactions. Intriguingly, infection with any *ccrp* deletion mutant was unable to induce comparable levels of the so called "scattering/hummingbird" phenotype, in which cells are transformed from a uniform polygonal shape into a severely elongated state characterized by the formation of needle-like projections. Furthermore, infection with the *ccrp59* mutant resulted in reduced type IV secretion system associated activities, e.g. IL-8 production and CagA translocation/phosphorylation. Thus, next to their role in maintaining the helical cell shape of *H. pylori* Ccrp proteins influence many cellular processes and are thereby crucial for the virulence of this human pathogen.

CBP06
Characterization of proteins of the Asp23 protein family in *Bacilus subtilis*

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The Pfam database is a useful protein classification system providing a broad overview of a wide range of protein families. Nevertheless, as over 20% of these proteins belong to domain of unknown function (DUF) families, a major part of the proteins represented in the Pfam database is in need of further investigation. This is especially the case for the Asp23 protein family. The name giving alkaline shock protein 23 (Asp23) is one of the most abundant proteins in *Staphylococcus aureus* and the members of this family are both highly conserved and highly expressed in Gram-positive bacteria. Despite these facts, neither the structure of any protein of the family has been solved nor is anything known about the functionality of Asp23 proteins. The aim of our work is the characterization of the so far unknown genes *yqhY* and *yloU*. Their encoded proteins, YqhY and YloU, are the only known representatives of the Asp23 protein family in *B. subtilis*. A first hint on their physiological task was provided by a minicell-like phenotype as an result of a deletion of *yqhY*. This result indicated a possible involvement of YqhY in cell division, which was further supported by an interaction between YqhY and DivIVA in a bacterial two hybrid screen. In addition to this, a co-localization of these proteins at the cell poles was observed. Another conceivable possibility is the involvement of YqhY in fatty acid synthesis, since the *yqhY* gene forms an operon with *accB* and *accC*. The latter genes encode two subunits of the acetyl-CoA carboxylase that is responsible for the formation of malonyl-CoA. In accordance to this, the deletion of *yqhY* led to the occurrence of suppressor mutants carrying a point mutation in *accA*, which encodes another subunit of the acetyl-CoA carboxylase. The results of ongoing investigations about this possible connection will be presented.

CBP07
How do plasmids spread in the recipient mycelium during conjugative DNA transfer in *Streptomyces*?

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Conjugative plasmid transfer in the Gram-positive soil bacterium *Streptomyces* involves a single plasmid-encoded protein (TraB), which directs the transfer of a double-stranded DNA molecule into the recipient cell [1, 2, 3]. Similarity of TraB to the septal DNA-translocator proteins FtsK/SpoIIIE in sequence, domain architecture, enzymatic activity and mode of DNA recognition indicated that *Streptomyces* adapted the chromosome segregation system to transfer DNA between two distinct *Streptomyces* cells[2]. Following the transfer, the recipient mycelium is colonized by plasmid-copies in a process called plasmid spreading. Plasmid spreading depends on five to seven "spread" (Spd) proteins with unknown molecular functions and is manifested on agar plates by the formation of "pock" structures. Pocks are temporary growth retardation zones surrounding the plasmid donor and indicate the area, where the recipient has acquired a plasmid. The speculative concept of intra-mycelial plasmid spreading postulates the transfer of DNA across the septal walls in the opposite direction to that of the growing tip.
To prove the concept of intramycelial plasmid spreading we established a reporter system allowing to visualize transfer of a conjugative pIJ101 derivative in living mycelium by fluorescence microscopy. We observed spreading of the plasmid in "old" compartments of the recipient mycelium, providing evidence that intra-mycelial DNA-translocation across septal cross walls had occurred.
In genetic crosses efficient spreading of a *traB* deletion mutant plasmid was only detected if *traB* was provided in *trans* in the donor and in the recipient,

demonstrating that TraB is not only required for the primary transfer, but also for intra-mycelial plasmid spreading.
To test if TraB and Spd-proteins physically interact with each other, bacterial two-hybrid studies were performed. These analyses revealed a complex interaction network of Spd-proteins and TraB suggesting that TraB and Spd-proteins work together to distribute the plasmid in the recipient mycelium. Since TraB and some of the Spd-proteins localize to the membrane/cell wall, we propose a multi-protein apparatus that pumps the DNA across pre-existing septal walls to allow efficient colonization of the recipient mycelium by the plasmid.

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CBP08
Spore wall biosynthesis in *Streptomyces coelicolor*

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The gram-positive soil bacterium *Streptomyces coelicolor* undergoes a complex life cycle with differentiation in substrate mycelium, aerial mycelium and spores. In contrast to the early developmental steps, the formation of proper spores is dependent on a protein complex similar to the lateral cell wall synthesizing complex of rod-shaped bacteria[1]. Several diverse proteins are involved in this so called "*Streptomyces* Spore Wall Synthesizing Complex", including penicillin binding proteins, cytoskeletal proteins and putative wall teichoic acid biosynthetic enzymes. Unlike in other bacteria the deletion of various of the encoding genes causes only an impaired spore wall but is not lethal[1,2]. Therefore *S. coelicolor* is an interesting model organism for studying the synthesis of the bacterial cell envelope. In order to increase the understanding of the function and interplay of the involved proteins we biochemically analyze and compare wildtype and mutant spore walls, focusing on the two major components - peptidoglycan and anionic cell wall polymers.

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CBP09
Analysis of the apical cell growth machinery and antibiotic stress response in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive soil bacterium with high industrial importance. Furthermore, it serves as non-pathogenic model organism for related pathogens causing tuberculosis (TB), diphtheria and leprosy. Like its pathogenic relatives, *C. glutamicum* lacks several conserved cell division and shape determining proteins such as the actin homologue MreB, the nucleoid occlusion system Noc and the division site selecting Min system, which all play important roles in the model organisms *E. coli* or *B. subtilis* respectively. Instead, morphology and polar elongation is ensured by a protein complex composed of the polar determinant DivIVA and several penicillin-binding proteins (PBPs). To date, only little is known about the spatial and temporal regulation of the apical cell growth machinery, which has been proposed as a new target for antibiotic (AB) intervention. We recently showed that DivIVA directly interacts with the Par system, thereby acting as polar tethering factor in chromosome segregation. Furthermore, we now provide evidence that DivIVA interacts with the lipid II flippase RodA for spatial regulation of apical cell growth. Depletion of *divIVA* as well as deletion of *parB* has a coccoidal morphology. In addition, a Δ*rodA* mutant strain had a strong growth defect and increased sensitivity to several ABs. A complementation strain allowed subcellular localization of RodA-GFP at the cell poles. Using our established synthetic *in vivo* system, where *E. coli* cells are used as expression vessels, we established a FRET assay to quantify protein-protein interaction. We demonstrate that DivIVA-RodA interaction recruits RodA to the cell poles and that this interaction is based on a single amino acid residue in DivIVA. Point mutations abolished the interaction. To verify the specificity of this interaction, we included the second lipid II flippase FtsW into our FRET system. However, interaction of DivIVA and FtsW could not be observed. To our surprise, time-lapse microscopy revealed sustained polar growth after *rodA* deletion, implicating a lateral lipid II movement from midcell to the polar PBPs. This hypothesis could be confirmed with Vancomycin-FL staining and addition of the lipid II capturing lantibiotic Nisin. Making use of our experiences in apical growth mechanisms, we analyse stress response mechanisms of Ethambutol (EMB) and the benzothiazinone BTZ043, two widespread drugs in TB treatment. We found that morphology plays an important role in EMB sensitivity. Furthermore we observed altered protein levels, cell wall composition and asymmetric recovery after EMB treatment. Thus we provide evidence that EMB has another target in actinobacteria.

CBP10
Changes in cell wall constituents in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a member of the *Corynebacterineae*, a branch of the High-GC Gram-positive bacteria. *Corynebacterineae* are characterized by a complex cell wall consisting of the thick peptidoglycan layer overlayed by an arabinogalactan polysaccharide and a second membrane, the mycomembrane. Another characteristic of corynebacteria is the morphology change from club shaped cells in the logarithmic to coccoid cells in the stationary phase of growth. Important pathogens like *Mycobacterium tuberculosis*, *M. leprae* or *C. diphtheriae* are members of this group. *C. glutamicum* as a nonpathogenic bacterium is used in our lab as a model organism for apical growth and processes involved in cell division. Antibiotics Ethambutol (EMB) and Benzothiazones (BTZ) inhibit different steps in cell wall synthesis (1, 2) leading to a diminished amount of arabinose and in turn also mycolic acids. Cells treated with these antibiotics show a change in cell shape. We are investigating changes in cell wall constituents of mutants impaired in elongation growth. Proteins of interest are DivIVA, which binds to concave membranes and recruits enzymes of cell wall synthesis to the cell poles and RodA, the lipid II flippase (3). Our results show the expected reduction in arabinose and mycolic acid content in antibiotic-treated cells. Untreated cells show a reduction of arabinogalactan and mycolic acids in the stationary phase. Arabinogalactan and mycolic acid content are also affected in *divIVA* and Δ*rodA* mutants. These investigations might help us understand how change of cell shape leads to resistance against EMB and BTZ.

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CBP11
Regulation and Heterogeneity in Dual Flagellar Systems

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In dual flagellar systems, lateral flagella are known to be primarily induced for swarming across surfaces or swimming in high viscous environments. A signi-cant subpopulation of planktonic *Shewanella putrefaciens* CN-32 cells also induces expression of the secondary system under nutrient rich conditions and forms, in addition to the primary polar fi-lament, one or two flagella located at lateral positions. These additional fi-laments enhance the cells' capability for eff-cient spreading in liquid environments. The environmental trigger for lateral flagella formation and the regulation of this heterogenous flagellation is still unknown. Transcriptional analysis of the regulatory cascade of the polar and the lateral system revealed that both systems are cross-regulated. Additionally, external regulators were identi-ed using a transposon mutagenesis approach. Our data suggests that, while some regulators seem to act primarily on the polar system, other regulatory elements might act on both systems on the transcriptional level, allowing strict regulation of these metabolically expensive multi-protein complexes.

CBP12

Penicillin binding proteins functions essential for growth and cell division of *Listeria monocytogenes*

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Listeria monocytogenes is a Gram-positive pathogen belonging to the firmicutes and found ubiquitously in the nature. The consumption of food contaminated with *L. monocytogenes* can lead to listeriosis, a gastrointestinal infection that can develop into a more severe systemic condition, especially in immunocompromised persons, pregnant women and the elderly. Standard therapy of listeriosis involves treatment with high doses of β-lactam antibiotics such as ampicillin, amoxicillin or meropenem. Thus, it is important to understand the physiological function of their targets, which are the penicillin binding proteins (PBP). However, their precise roles during cell growth and division are largely unknown. *L. monocytogenes* possesses five high molecular weight penicillin binding proteins (HMW-PBP). HMW-PBPs can be divided into two classes: Class A HMW-PBPs are bi-functional PBPs which have a transglycosylase and a transpeptidase domain. In contrast, class B HMW-PBPs only possess a transpeptidase function. GFP-PBPs fusions either localized at the septum, the lateral wall or both, suggesting distinct as well as overlapping functions during the cell cycle. For analysis of the physiological function, we generated genetically stable marker-less deletion mutants of all HMW-PBPs and characterized their contribution to growth, cell division, antibiotic resistance and virulence. For HMW-PBPs which turned out to be essential, IPTG-dependent conditional mutants were constructed. We were able to show essentiality of PBP B2 and synthetic lethality of the bi-functional penicillin binding proteins PBP A1 and A2. Microscopic analysis of the mutant strains allowed us to assign specific functions in cell growth and cell division to the individual HMW-PBPs. Infection experiments revealed that most HMW-PBPs contribute to invasion into and cell-to-cell spread inside eukaryotic host cells. PBP B1 depletion dramatically reduced β-lactam susceptibilities and stimulated spontaneous autolysis. This shows that PBP B1 is a major determinant for the intrinsic resistance of *L. monocytogenes* against β-lactams.

CBP13

A closer look on the polyhydroxybutyrate- (PHB-) negative phenotype of *Ralstonia eutropha* PHB$^-$4

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The undefined poly(3-hydroxybutyrate)- (PHB-) negative mutant *R. eutropha* PHB$^-$4 was generated in 1970 by 1-nitroso-3-nitro-1-methylguanidine (NMG) treatment. Although being scientific relevant, its genotype remained unknown since its isolation except a recent first investigation. In this study, the mutation causing the PHA-negative phenotype of *R. eutropha* PHB$^-$4 was confirmed independently: sequence analysis of the *phaCAB* operon identified a G320A mutation in *phaC* yielding a stop codon, leading to a massively truncated PhaC protein of 106 amino acids (AS) in *R. eutropha* PHB$^-$4 instead of 589 AS in the wild type. No other mutations were observed within the *phaCAB* operon. As further mutations probably occurred in the genome of mutant PHB$^-$4 potentially causing secondary effects on the cells' metabolism, the main focus of the study was to perform a 2D PAGE-based proteome analysis in order to identify differences in the proteomes of the wild type and mutant PHB$^-$4. A total of 20 differentially expressed proteins were identified which provide valuable insights in the metabolomic changes of mutant PHB$^-$4. Besides excretion of pyruvate, mutant PHB$^-$4 encounters the accumulation of intermediates such as pyruvate and acetyl-CoA by enhanced expression of the observed protein species: (i) ThiJ supports biosynthesis of cofactor TPP and thereby reinforces the 2-oxoacid dehydrogenase complexes as PDHC, ADHC and OGDHC in order to convert pyruvate at a higher rate and the (ii) 3-isopropylmalate dehydrogenase LeuB3 apparently directs pyruvate to synthesis of several amino acids. Different (iii) acylCoA-transferases enable transfer reactions between organic acid intermediates, and (iv) citrate lyase CitE4 regenerates oxaloacetate from citrate for conversion with acetyl-CoA in the TCC in an anaplerotic reaction. Substantial amounts of reduction equivalents generated in the TCC are countered by (v) synthesis of more ubiquinones due to enhanced synthesis of MenG2 and MenG3, thereby improving the respiratory chain which accepts electrons from NADH and succinate.

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CBP14

RegAB two-component system homolog BPSL0201/0202 in Burkholderia *pseudomallei* controls nitrate respiration and is required for full virulence in mice.

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Introduction: *Burkholderia pseudomallei* is the causative agent of melioidosis and adapts to permanently changing oxygen-concentrations in its environmental habitat or during infection. The pathogen is able to grow under anaerobic conditions by using nitrate as an alternative electron acceptor. It is known that the absence of oxygen and presence of nitrate can influence tolerance against antibiotics and virulence of *B. pseudomallei*. However, the regulation of genes involved in denitrification pathways has rarely been addressed so far. Here, we identified a *B. pseudomallei* two component sytem (TCS) BPSL0201-0202 with strong homology to known RegAB TCS´s of other bacteria which control genes for nitrate respiration.
Materials and Methods: A *B. pseudomallei* Tn5 mutant with a defect in the BPSL0201 sensor kinase was isolated during a screen for mutants with restricted anaerobic growth. Additionally, we constructed a deletion mutant of the response regulator BPSL0202. Both mutants were tested in growth experiments in different media and in cellular *in vitro* experiments for adhesion, cytotoxicity, invasion and intracellular survival. *In vivo* virulence of mutants was assessed in a BALB/c infection model by determining mortality and bacterial burden in different organs (lung, spleen and liver). Furthermore, we performed DNA microarrays to reveal genes directly regulated by this TCS.
Results: Under aerobic conditions, both mutants grew normally like the wild type strain, but were strongly growth restricted under anaerobic condition in the presence of nitrate. Mortality experiments in Balb/c mice showed a strong reduction in virulence, accompanied with a significantly lowered bacterial burden in different organs of mice. Interestingly, no changes in cellular adherence or invasion between mutants and wild type strain were observable, but the mutant strains exhibited a decrease in intracellular survival in RAW and Hela cell lines after prolonged incubation time. DNA microarray experiments revealed a number of *B. pseudomallei* RegAB TCS regulated genes involved in nitrate metabolism and respiration pathways.
Conclusion: In this study we firstly described a RegAB homolog in *B. pseudomallei* necessary for full virulence in mice. The system regulates the pathways for anaerobic nitrate metabolism. Further construction of mutants with defects in genes directly regulated by this TCS should reveal their impact during *B. pseudomallei* infections in humans and animals.

CBP15

Interactions of the lipid-biosynthetic protein MprF of *Staphylococcus aureus*

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The multiple peptide resistance factor (MprF) found in *Staphylococcus aureus* and many other bacterial pathogens consists of separable domains for synthesis and translocation of lysyl-phosphatidylglycerol (Lys-PG). Exposure of Lys-PG at the outer leaflet of the cytoplasmic membrane protects the cells against cationic antimicrobial peptides and antibiotics such as daptomycin. Additionally MprF has been shown to be a hot spot for amino acid exchanges that are implicated in daptomycin resistance and clinical treatment failure by an as yet unknown mechanism. [1, 2, 3]
By screening a genomic library of *S. aureus* MW2 using a bacterial two-hybrid (BTH) approach, we identified several potential interaction partners of MprF. These proteins are involved in a range of cellular processes, including transport, cell division, cell wall synthesis and cell wall modification. To establish a functional link between MprF and its binding partners, lipid composition and minimum inhibitory concentrations of daptomycin were analysed in the respective knock-out mutants. Furthermore the influence of *mprF* deletion on cell wall synthesis is currently under investigation.

BTH assays suggest that MprF interacts with major cellular processes. This idea seems to be corroborated by promising results from the ongoing functional analyses. Whether these results could be caused by indirect regulatory effects, still needs to be investigated. Eventually elucidation of the MprF interaction profile will lead to a deeper understanding of its role in pathogenicity and antibiotic resistance.

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CBP16

A pre-divisional function for DivIVA in *Listeria monocytogenes*

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DivIVA is a cell division protein that is well conserved in firmicutes as well as in actinobacteria (1), and is known to localize at strongly bent membrane areas such as the division site and the cell poles. DivIVA in *Bacillus subtilis* is responsible for the spatial control of the MinCDJ division site selection system, allowing for symmetrical cell division (2). Deletion of either of the Min system genes or *divIVA* in *B. subtilis* leads to defects in positioning and recycling of the divisome, resulting in filamentation and the occurrence of achromosomal mini-cells with a high frequency (3). In contrast, deletion of *divIVA* in the closely related and pathogenic firmicute bacterium *Listeria monocytogenes* led to the formation of long chains of cells resulting from an autolysin secretion defect which involves the accessory secretion ATPase SecA2. Moreover, defects in the cell division process itself were not apparent (4). This observation, coupled with a lack of discernable mini-celling in listerial Δ*divIVA* mutants, led us to believe that the Min system of *L. monocytogenes* functions in a manner that is DivIVA-independent. We here set out to test this hypothesis using genetic and cytological experiments. Bacterial two-hybrid analysis displayed direct interactions between DivIVA, MinD and MinJ, indicating a possible impact of DivIVA on the MinCDJ system. Localization patterns of fluorescently tagged MinC and MinD were evidently dependent on DivIVA, while that of MinJ was not. A cell division phenotype of the Δ*divIVA* mutant was confirmed via comparative cell size measurements of Δ*secA2* and Δ*divIVA* mutant strains. A DivIVA-independent role of MinJ in the division site selection process became apparent upon conducting gene deletion studies, which showed that deletion of *minJ* suppressed the cell division defect of the Δ*divIVA* strain. We hence propose a non-linear association of DivIVA with the MinJ and MinCD cell division proteins in *L. monocytogenes*, which is in contrast to the situation observed in *B. subtilis*.

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CBP17

Genetic competence in *Bacillus licheniformis* - a matter of deregulation?

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Natural genetic competence - enabling bacterial uptake and chromosomal integration of extracellularly supplied DNA - provides a powerful tool for genetic engineering. While its complex regulation has been studied thoroughly in the model organism *Bacillus subtilis*, little is known about the conditions triggering competence in the industrially relevant *Bacillus licheniformis*. Auxotrophic strains of *B. licheniformis* 9945A derived from repeated rounds of random mutagenesis were long known to develop natural competence (Thorne and Stull 1966. J Bacteriol 91: 1012-20). Our inspection of the colony morphology and extracellular enzyme secretion of two of them, M28 and M18, suggested that regulator genes are collaterally hit. Indeed, in M28, we identified a mutation affecting *degS*, which encodes the sensor histidine kinase that is part of the molecular switch directing the cells either to genetic competence, or the synthesis of extracellular enzymes, or biofilm formation. For M18, sequencing of the suspected gene revealed a deletion in *abrB*, which encodes the major transition state regulator. Both of the mutations, when newly generated on the wild type *B. licheniformis* 9945A genetic background, resulted in phenotypes resembling M28 and M18, respectively, thereby identifying *B. licheniformis* genetic competence as a matter of deregulation.

CBP18

The staphylococcal ser/thr kinase PknB and its influence on the cell wall biosynthesis.

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Prokaryotic signal transduction pathways regulate cellular functions in response to environmental cues and enable bacteria to react immediately to changing conditions like antibiotic stress. Besides two-component regulatory systems (TCS), one-component regulatory systems (OCS) represent one of the most abundant signaling systems in prokaryotes. These OCS include eukaryotic-like serine/threonine kinases (ESTKs) and phosphatases (ESTPs), which are increasingly recognised as important regulators of major processes such as cell wall metabolism and division, virulence/ bacterial pathogenesis and spore formation. One such ESTK/ESTP-couple has recently been identified in *Staphylococcus aureus* designated PknB/YloO [1]. The extracellular sensor part of the kinase contains three daisy-chained PASTA-domains assumed to be capable of binding peptidoglycan subunits, suggesting that PknB monitors the coordinated assembly of peptidoglycan biosynthesis and cell division.
Microscopic analysis revealed the co-localisation of GFP-PknB and GFP-YycG at the septum, the site of active cell wall biosynthesis in cocci. Deletion of the PASTA-domains resulted in delocalisation of GFP-PknBDPASTA, suggesting an interaction with soluble and lipid-bound cell wall precursors, which also modulates the activity of the kinase.
To further investigate the role of PknB we analysed the interplay with the essential YycFG TCS on the molecular level and show phosphorylation of the response regulators YycF implying fundamental crosstalk between the regulatory systems.

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CBP19

A cell wall amidase with novel function: AmiC2 is involved in forming a nanopore array for cell-cell communication in *Nostoc*

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To face environmental changes, the terrestrial filamentous cyanobacterium *Nostoc punctiforme* ATCC 29133 can differentiate into several cell types: Spore-like akinetes, motile hormogonia and N_2-fixing heterocysts. The ability for cell differentiation requires concerted action of the filament and therefore, cell-cell communication within the filament is indispensable. For cell-cell communication, signal molecules and metabolites have to overcome the septal cell-wall barrier. Isolated peptidoglycan (PG) sacculi of *Nostoc* maintain the shape of the filament. The filament sacculus is one huge macromolecule with single cells not separated. Our previous studies showed that the septal PG has a disc-like shape with a central array of approx. 160 nanopores (Ø20 nm), possibly involved in formation of cell-cell connections [1]. Mutation of the *amiC*2-gene encoding a cell wall amidase leads to filament dystrophy, as well as loss of cell differentiation and cell communication [2]. This pleiotropic phenotype was probably caused by the absence of the nanopore array. AmiC2 is therefore necessary to transform the septal PG into a nanopore array, which allows formation of cell joining elements. As expected, AmiC2 localizes to the septa between the cells,

shown by immune fluorescence labeling. Truncated versions of AmiC2 fused to GFP revealed the function of the AMIN domains of this amidase for septal targeting. These data show a novel function of AmiC in filamentous cyanobacteria compared to *E. coli*, in which AmiC is involved in daughter cell separation after cell division. If AmiC2 activity of *Nostoc* is restricted to drilling pores into the septal PG, it has to be spatiotemporally regulated in a different way. *In vitro* studies using labeled PG showed a constantly active enzymatic domain (EZD) of AmiC2. To elucidate the mechanism of AmiC2 activity regulation, the crystal structure of the EZD was solved with high resolution (1.3 Å). Interestingly, a regulatory α-helix that is present in several other amidases is missing. Hence, a different regulatory mechanism can be assumed and further studies to find regulatory interaction partners are under way.

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CBP20

Bactofilins play a dynamic role in the flagellar assembly of *Bacillus subtilis*

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Bactofilins are a widely conserved protein family implicated in bacterial motility and cell shape maintenance. Based on their ability to form stable filaments *in vitro*, they have been proposed to act as cytoskeletal elements, providing localization scaffolds for other proteins. BacE and BacF of *Bacillus subtilis* are essential for motility. We show that the proteins are required for the assembly of the flagellum, as bactofilin mutants lack the flagellar filament. BacE forms a stable stoichiometric complex with FliW, a direct interactor of flagellin and a crucial partner in the regulatory mechanism of its synthesis, and also interacts with BacF. Both bactofilins can form extended membrane-associated filamentous structures when co-expressed in a heterologous cell system (S2 Schneider cells), but were observed as discrete assemblies with a diameter of 60 to 70 nm at the *B. subtilis* cell membrane. BacF assemblies are relatively static, but only partially colocalize with flagellar basal bodies, whereas BacE assemblies are highly mobile, inferring a dynamic interaction of bactofilins with components of the flagellar apparatus.

CBP21

The role of the Cpx-system within the envelope stress systems in *Escherichia coli* analyzed by MRM and co-localization studies

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Bacteria rely on two-component systems (TCS) to acclimate in response to environmental changes and therefore to different stimuli [1].These systems use a phosphorylation cascade from a transmembrane sensor kinase (SK) to a cytoplasmic response regulator (RR) and are set back to the initial state via dephosphorylation of the RR [2]. The Cpx-envelope stress TCS consists of the membrane-bound SK CpxA, the cytosolic RR CpxR and the periplasmic accessory protein CpxP, which inhibits the autophosphorylation activity of CpxA [3, 4]. To better understand the functionality and the dynamics of the Cpx-TCS, it is important to know the absolute amounts and the co-localization of CpxA, CpxR and CpxP under different growth conditions. Therefore, absolute quantification of the Cpx-TCS by multiple reaction monitoring (MRM) was performed under different Cpx-system affecting conditions. Since a crosstalk between CpxA and the RR ArcA of the Arc-TCS is suggested [5], we additionally quantified the absolute amounts of ArcA and ArcB. By analyzing the relative abundance of other envelope stress systems as for example the Bae-TCS or the Rcs-TCS, we furthermore want to assess the role of the Cpx-TCS within the stress network in *E. coli*. These studies are currently accomplished by analyzing the co-localization of CpxA, CpxR and CpxP under various conditions. Together, our results give a deeper insight into regulation mechanisms within the Cpx-TCS and its role within the stress network of *E. coli*.

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CBP22

Upgrade of flagellar motility in *Shewanella oneidensis* MR-1

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Many bacteria are motile by rotating flagella. The rotation is energized by gradients of either protons or sodium ions conducted across the membrane by the stators. Our model organism *Shewanella oneidensis* MR-1 possesses a polar flagellum as well as the sodium ion-driven stator PomAB and the proton-dependent stator MotAB, which was likely acquired via horizontal gene transfer. Both stators are concurrently transcribed, localize to the flagellar motor and are able to drive rotation of the polar flagellum in liquid environments. *S. oneidensis* MR-1 may therefore employ a hybrid motor composed of both stators at the same time. MotAB, however, cannot promote motility on soft agar plates and is dependent on the availability of oxygen. Surprisingly, small mutations in the putative "plug" domain of MotB (MotB*) result in an up-motile phenotype which shows a more robust motility under all tested conditions. The "plug" domain, a predicted amphipathic alpha helix, may therefore be important for the stator activation or function. Our results show that despite the more efficient motility, MotAB* is found in equal numbers in the motor and is exchanged at higher rates. Apparently, MotAB of *S. oneidensis* MR-1 is be more stable at the motor but less efficient in promoting a robust motility.

CBP23

The yes and no of the unique traits in the phylum *Planctomycetes*

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Planctomycetes are bacteria that comprise a huge variety of peerless cell biological features, such as an unusual cell plan, a unique endocytosis-like uptake mechanism for macromolecules and the unique FtsZ independent cell division through polar asymmetrically budding. However, several of these traits are currently subject of debate. Two opposing theories exist for example in case of compartmentalization. One argues for two compartments within a proteinaceous cellwall, the cytosole, the pirellulosome and the parayphoplasm. In contrast a more Gram negative like planctomycetal cell plan was suggested by other studies. However, another unique characteristic is broadly accepted: the uptake of macromolecules as previously demonstrated for GFP. Besides that, *Planctomycetes* comprise a complex life cycle, with a planktonic swimmer and an adult sessile stalked stage. Since *Planctomyces limnophilus* is genetically accessible, it was possible to generate deletion mutants for key genes putatively involved in these unusual planctomycetal traits. Employing mutants and specific chemical dyes, we demonstrate that the cell organization of *P. limnophilus* reflects more that of a typical Gram negative bacterial cell, than that of the proposed compartmentalized organism. However, we confirmed the presence of several unique structures employing cryo-electron tomography and light microscopic techniques that were not described for other bacteria thus far. According to the literature, the uptake of GFP could only be reproduced for *Gemmata obscuriglobus*. Thus additional experiments, implying different polysaccharides were performed and demonstrated the uptake of macromolecules such as dextran in *P. limnophilus*. Regarding the asymmetric cell division of *P. limnophilus*, all technical requirements such as timelapse-microscopy, generation of defined mutants and labeling via chemical dyes were established. Furthermore, the number of putative cell division related proteins previously postulated by our group could be further reduced, using genome sequencing of novel cultivated *Planctomycetes* and bioinformatics analysis. Those proteins are currently subject for GFP labeling and localization studies. Taken together we present several novel aspects of the planctomycetal cell biology. Besides a more ordinary Gram negative-like cell plan, a unique uptake of macromolecules and new insights of the FtsZ independent cell division in *P. limnophilus* were revealed.

MICROBIAL DIVERSITY AND ECOLOGY (INCL. SOIL AND WATER MICROBIOLOGY)

DEP01
Micropredator Networks in Wastewater Treatment Plants: A Potential Way to Improve Pathogen Removal

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The permanent availability of clean water is taken for granted in most Western countries. However, water shortage is a growing concern worldwide and a main issue in Israel and Palestine. An approach to overcome this problem might be the exploitation of predator-prey interactions to reduce pathogens in waste water more effectively.
In this trilateral study we investigate the diversity of bacteria and their micropredators, i.e. protists, bacteriophages, and specific predatory bacteria (*Bdellovibrio*-and-like organisms, BALOs) in wastewater treatment plants (WWTP). We took monthly wastewater samples over a period of a year from three different treatment plants located in Germany, Israel, and Palestine to track seasonal community changes. We used the Illumina Miseq amplicon platform to analyse the microbial diversity (16S and 18S rRNA genes) of all bacteria, Protists and BALOs. In addition we quantified the target organisms using qPCR. We also sampled bacteriophages to unravel phage-host systems and their dynamics to get a better understanding of the fate of phages and their hosts during treatment processes. At the same time we recorded chemical and environmental factors which enable us to correlate our findings. The results show networks of co-occurring microbes and potentially unravel positive and negative interactions between the studied micropredators and their prey under different environmental conditions.
We believe that studying the microbial diversity in WWTP will answer central questions in microbial ecology and predator-prey-theory, and might lead to an improvement of the current technology. In the future, this might result in a better management of microbial resources and in a more efficient reduction of pathogenic bacteria during the wastewater treatment process.

DEP02
Microbial diversity and adaptations of marine microbes in the Red Sea

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The Red Sea is an understudied harsh marine environment with strong gradients in productivity, salinity, and temperature from the North to the South. The epipelagic microbial communities in this environment have to withstand temperature of over 35 °C, salinities of up to 40 psu, and very high UV irradiance year-around. In order to understand adaptations of marine prokaryotes to this environment, we used a combination of community analyses, metagenomics, single-cell genomics, and transcriptomics approaches. As a result, we could show that the epipelagic microbial communities in the Red Sea are not very different from other less extreme oligotrophic counterparts with SAR11 and *Prochlorococcus* dominating the communities. Metagenomic analyses indicated high relative abundances of genes involved in degradation of osmolytes in Red Sea SAR11 populations and genes involved in light stress and DNA repair in Red Sea *Prochlorococcus* populations. Single-cell genomics and genomes of pure cultures from the Red Sea identified more than 2000 novel genes in the pan-genome of these two groups; most of with encode for hypothetical proteins with so far unknown function. Comparisons with metagenomic datasets from other marine environments indicated that these genes are not exclusive to Red Sea populations. Current analyses of metatranscriptomes and bioinformatics approaches aim to analyse the importance and possible function of the novel genes.

DEP03
Gaining proteomic insights into the interaction of the marine bacterium *Marinobacter adhaerens* HP15 with the diatom *Thalassiosira weissflogii*

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The interaction of marine bacteria with microalgae in marine systems facilitates reallocation of carbon compounds and nutrients, therefore having a crucial role in biogeochemical cycling of elements in the water column. The relation between bacteria and microalgae may be of various types; parasitic, competitive, and synergistic interactions have been found between the two partners (review by Amin *et al*., 2012). In previous studies, our group has identified a synergistic interaction between the marine bacterium *Marinobacter adhaerens* HP15 and the diatom *Thalassiosira weissflogii*. Based on these findings, a model system for bacteria-diatom interaction has been established (Gärdes *et al*., 2011; Gärdes *et al*., 2012) that aims at a better understanding of such relations. In our present work we focus a detailed look at changes in the proteome of *M. adhaerens* HP15 during co-cultivation with *T. weissflogii*. Thereby bacterial proteins are identified that are quantitatively altered in the presence of the diatom; once the functions of those proteins are known, further information on the actual level of synergism between bacterium and diatom can be retrieved. To identify such differential protein expression, the aimed workflow includes the sampling of proteomic fractions of *M. adhaerens* HP15 after co-cultivation with the diatom. With reference to a control treatment differential protein patterns are identified via 2D acrylamide gel approaches. Proteins of interest are further analyzed applying matrix assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS). Currently, stated co-cultivation experiments and protein analyses are under progress. Preliminary results refer to the context of iron availability and specific degradation of carbon compounds; the analysis of further data is expected in the nearest future. The identification and interpretation of changes on the proteomic level will not only add to a better characterization of the synergism between the two partners *M. adhaerens* HP15 and *T. weissflogii*. Moreover our results will offer a more detailed understanding of turn-over processes in marine systems with reference to bacteria-diatom interactions.

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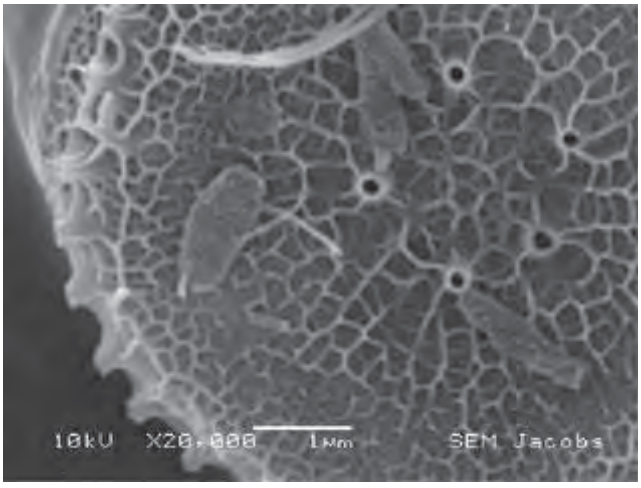
Figure 1



DEP04
Characterization of bacteriocines produced by spore former bacteria isolated from soil

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Out of all the sources, soil is the best microbial ecosystem in the nature. Of all the bacteria, which they are living in the soil sporeformer bacteria are important bacteria because of their potential for production of antimicrobial metabolites such as bacteriocin. The present study was conducted to investigate on production and characterization of bacteriocins produced by sporeformer bacteria isolated from soil . In this study thirteen bacteriocin producing sporeformer bacteria were isolated from 107 soil samples. Then the antimicrobial effect of them was evaluated against pathogenic bacteria using Agar Well Diffusion. Eventually these strains were identified by phenotypic and molecular methods. To continue the study optimization of bacteriocin production has been done based on their production at different pHs, temperatures and C- sources. Then activity of all bacteriocins was assessed against organic solvents and protease K. Finally the antimicrobial spectrum of the bacteriocins against pathogenic bacteria and fungi was investigated. In this study molecular weight and Aribitrary Units (before and after purification) of bacteriocins were determined. The results showed that molecular Weights of the bacteriocins were ranged from 42 - 72 kilo Dalton and the Arbitrary Units were increased after their purification. In addition, our results indicated that *Salomnella paratyphi, Escherchia coli* and *Bacillus cereus* were sensitive and *Pseudomonas aeruginosa, Kelbsiella peumoniae* and all fungi were resistant to the bacteriocins. Therefore, our results showed that the bacteriocins can effect on the bacteria far from their family. Hence we suggest that bacteriocins should be considered as an area of investigation for introducing the new remedy. Probably it might reduce the frequency of occurrence of antibiotic- resistant bacteria.

DEP05
Intestinal Escherichia coli colonization in a mallard duck population over four consecutive winter seasons

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Introduction: We report about one *E. coli* population of wild mallard ducks in their natural environment over four winter seasons, following the characterization of 100 isolates each consecutive season. The study should give comprehensive insights into the structure and dynamics of one *E. coli* population over years in a natural environment.
Materials and Methods: Macrorestriction analysis was used to analyze and define isolates variously as resident and transient PFGE types. Isolates were characterized genotypically based on virulence-associated genes (VAGs), and phenotypically based on hemolytic activity, antimicrobial resistance, adhesion to epithelial cells, microcin production, motility and carbohydrate metabolism.
Results: Only 12 out of 220 PFGE types were detectable over more than one winter season, and classified as resident PFGE types. There was a dramatic change of PFGE types within two winter seasons. Nevertheless, the genetic pool (VAGs) and antimicrobial resistance pattern remained remarkably stable. The high diversity and dynamics of this *E. coli* population were also demonstrated by the occurrence of PFGE subtypes and differences between isolates of one PFGE type (based on VAGs, antimicrobial resistance, and adhesion rates). Resident and transient PFGE types differed in antimicrobial resistance, VAGs and adhesion. Other parameters were not prominent colonization factors.
Discussion: In conclusion, the high diversity, dynamics and stable genetic pool of an E. coli population seems to enable their successful colonization of a host animal population over time.

DEP06
Calcite-accumulating large sulfur bacteria of the genus *Achromatium* in Sippewissett Salt Marsh

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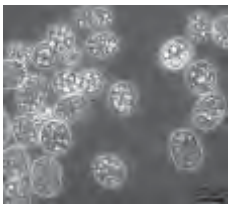
Large sulfur bacteria of the genus *Achromatium* are exceptional among *Bacteria* and *Archaea* as they can accumulate enormous amounts of internal calcite. Although known for more than one hundred years, they remain uncultured, the biological function of the internal calcite is still ambiguous, and only freshwater populations have been studied so far. Here we investigate a marine population of calcite-accumulating bacteria that is primarily found at the sediment surface of tide pools in a salt marsh, where high sulfide concentrations meet oversaturated oxygen concentrations during the day, as shown by microprofiling. Molecular analyses revealed their co-occurrence with highly abundant phototrophic sulfide-oxidizing and heterotrophic sulfate-reducing bacteria in the surface sediment of the marsh. The affiliation of the large calcite-accumulating cells with the *Achromatiaceae* family was confirmed by 16S rDNA sequencing and CARD-FISH hybridization. Their sequences form a monophyletic cluster suggesting the introduction of a new species for this population of marine *Achromatium*. Using confocal Raman spectroscopy and SEM-EDS, we confirmed the presence numerous internal calcite inclusions and elemental sulfur. The ratio of calcite:sulfur per cell seems to increase during the night and decrease during the day, while cell counts suggest a migration towards the sediment surface during the day. We also obtained genome data from a single marine *Achromatium* cell - the first data available for this family - which will complement our study on the interesting ecophysiology of calcifying *Achromatium* bacteria in general, but also their specific adaptation to a marsh habitat. Overall, our study extends the presently known diversity and habitat range of this extraordinary genus and carries on the open questions about the biological function of internal mass calcification in bacteria.

Figure 1



DEP07
Microbial ammonia oxidation in a sand filter used to remove arsenic from drinking water in Vietnam

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Withdrawn.

DEP08
Effects of Indoor Plants on and from their Surrounding Microbiome in Built Environments

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Human beings shape their surrounding microbiomes to a large extend by architecture, usage management and maintenance. These handlings might result in healthy or unhealthy environments e.g. in hospitals, clean rooms, and offices. Generally, sterile environments for humans are not only

impossible, but very often also unpreferable. Recent studies showed that controlled indoor environments are characterized by a reduction of their microbial diversity. Hence, our hypothesis is that a well-balanced microbiome with higher diversity will result in more healthy environments (Berg *et al*., 2014; overview see Fig. 1). Besides opening our windows to increase microbial diversity, we could also embellish a built environment by indoor plants. Whereas humans in the built environment are a main source for microbes we currently know very little of the contribution of indoor plants to the room microbiome and vice versa. To reduce this lack of knowledge this study examined the interplay of indoor plants with their surrounding environment in a two-sided approach: a) effects of the built environment on the phyllosphere microbiome of 14 plant species were investigated over different climatic zones in a greenhouse; b) effects of the common indoor plant *Chlorophytum comosum* on its surrounding environment were investigated over half a year in an isolation experiment. Microbial diversity was examined by cultivation-based methods including volatile organic compounds - VOC's tests for antagonistic potential; and BOX-PCR fingerprinting as well as DNA-based 16S/18S rRNA gene and ITS amplicon deep sequencing using Illumina's MiSeq. In addition, the application of PMA (propidium monoazide) as a selective DNA marker for compromised cells allowed conclusions towards the potential viable microbiome. Finally, qPCR examined the transfer of microbes in a quantitative way. This study revealed new microbial transfer routes into the built environment, and a selective enrichment of certain microbial genera dependent on time, space and surface texture. QPCR results showed significant grouping of samples for 16S rRNA gene and ITS copy numbers in an analysis of variances. Bacterial abundance on different media ranged between 10^3 - 10^6 CFU/cm^2 and could be correlated to plant state, location and climate. The indoor plant-associated microbial community harbored high antagonistic potential against pathogens, since 229 of 1284 bacterial isolates tested in VOC's assays showed promising activity. These isolates could be used as potential beneficials for indoor plants.
This two-sided approach broadened our perspective on beneficial room maintenance, future biotechnological potentials and healthy indoor environments in general.

Berg G, Mahnert A and Moissl-Eichinger C (2014) Beneficial effects of plant-associated microbes on indoor microbiomes and human health? *Front. Microbiol*. 5:15. doi: 10.3389/fmicb.2014.00015

Figure 1

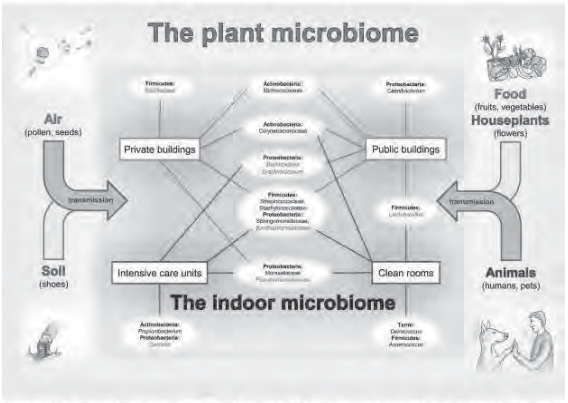

Figure 1. Relationships between the plant and indoor microbiome. The indoor microbiomes, influenced by transmissions via air, soil, food, houseplants and animals from plant microbiomes, presents an overview on typical and dominant bacterial groups occurring in the built environment. Schematic chart represents occurrence of the bacterial inhabitants indoors. Bacterial families and genera (white ellipses) are arranged according to their phylum affiliation (bold) and are connected to certain types of the built environments (red squares). Taxa highlighted in green are typical phyla detectable in plant microbiomes. This image has no demand of being complete. (Berg *et al*., 2014)

DEP09

In-situ Protein-SIP in a constructed wetland model system reveals *Ralstonia* as the key genus for aerobic toluene degradation

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Constructed wetlands are economical and efficient options for the treatment of wastewater. The removal of organic contaminants in constructed wetlands is performed by microbes which inhabit the rhizosphere. Their degradation activity is stimulated by oxygen and organic exudates released by the roots. However, little is known about the degradation pathways and the interplay within the microbial community and the plant.
In order to identify the bacterial key players involved in catabolic toluene degradation and the respective pathway(s) involved, we applied protein-based stable isotope probing (Protein-SIP)[1] with ^{13}C-labelled toluene to a 10 L lab scale planted fixed bed reactor (PFR)[2]. The degradation of toluene was followed for 50 h and samples were taken in order to examine the dynamics of toluene removal. In addition, abiotic parameters such as temperature, redox potential, oxygen concentration, and pH were monitored continuously. Metaproteome analysis revealed that the active rhizospheric bacterial community is complex, consisting of different members of the *Proteobacteria* and *Acidobacteria*. Notably, *Burkholderiales* was identified to be the most abundant order in the rhizosphere. Protein-SIP analysis could prove that *Ralstonia* is almost exclusively responsible for toluene degradation. We assume that other members of the rhizospheric bacterial community feed on the exudates released by the plants. Although there were only low concentrations of oxygen in the water, toluene was preferentially degraded by aerobic processes. The initial degradation step was apparently catalysed mostly by a ring-activating monooxygenase and cleavage of the (methyl)catecholic intermediate was carried out by a catechol 2,3 dioxygenase.
Thus, a detailed insight into the toluene transformation processes in the rhizosphere of a wetland system was gained, showing that *Ralstonia* perform well at hypoxic conditions.

1. Jehmlich, N., et al., *Protein-based stable isotope probing.* Nat. Protocols, 2010. **5**(12): p. 1957-1966.
2. Kappelmeyer, U., et al., *Operation of a Universal Test Unit for Planted Soil Filters - Planted Fixed Bed Reactor.* Engineering in Life Sciences, 2002. **2**(10): p. 311-315.

DEP10

Characterization of microbial community composition of vineyard and fruit-growing soil environments in contact to preserved wood

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Only a limited number of biocides are approved for EU to preserve wood in soil contact. Most wood preservatives are based on a combination of biocides and copper compounds. However, copper-based fungicides are also used for plant protection, which leads to an enrichment of copper tolerant soil microbial communities in respective environments. To test the efficacy of wood preservatives accelerated soil incubation studies at 28 °C and 65 % relative air humidity were carried out. Wood specimens were impregnated using water as reference or different formulations: salt A (containing copper, Triazoles and Benzalkonium Chloride), salt B (containing Triazoles and Benzalkonium Chloride; encapsulated), salt C (containing Triazoles and Benzalkonium Chloride; non-encapsulated) and salt D (containing copper). Specimens were partly buried in soil of five vineyard and fruit-growing environments (North and South of France, Portugal, North and Middle of Germany). Soil samples were collected at the interface of wood and associated soil after 8, 16, 24 and 32 weeks of incubation as well as soil samples without wood contact. To assess temporal and wood preservative effects on the bacterial and fungal soil community amplicon sequencing and quantitative PCR by 16S rRNA and ITS gene were performed, respectively. In addition, soil characterization, mass loss and bending elasticity of

specimen were analyzed. Each soil was characterized by specific soil properties and a specific succession of the phylogenetic composition and bacterial and fungal copy numbers. Bacterial copy numbers were significant higher in Middle German site (3×10^{10} to 1×10^{11}) compared to Portugal site (8×10^{9} to 4×10^{10}). Bacterial copy numbers were significantly reduced over time by salt A and salt D containing wood at three of five sites. Moreover, wood samples containing salt A and salt D had a lower mass loss and bending elasticity over time in contrast to other formulations. Amplicon sequencing results will elucidate further details in the phylogenetic composition of the respective soil environments and thus provide hints for functional groups and their pathways to tolerate preservatives in wood.

DEP11

Towards understanding the *Planctomycetes* - cyanobacteria interspecies interaction: A duck pond snapshot.

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Planctomycetes are bacteria that are ubiquitously distributed and environmentally important, playing major roles in global carbon- and nitrogen cycles. They possess a variety of unique features, such as a complex life cycle, where planktonic living swimmer cells develop into an attached-living adult state. In aquatic habitats, *Planctomycetes* represent major fractions of phytoplankton-associated bacteria and were found to dominate biofilms on micro- and macroalgae. In this study we analyzed the planctomycetal community associated with a cyanobacterial bloom in a German duck pond. Using differential filtration, we separated free-living from aggregate forming microorganisms, like cyanobacteria including their attached living bacteria. Based on genomic DNA isolated from the differential filters we generated *Planctomycetes*-specific 16S rRNA gene clone libraries and found first indications for planctomycetal species-specific interaction with cyanobacteria. In order to get deeper insights into the planctomycetal community associated with the cyanobacteria bloom, we developed a deep sequencing pipeline based on the Illumina technology, targeting the 16S rRNA gene variable region V3. The sequencing strategy is based on techniques described by Caporaso et al. (PNAS, 2011) and was combined with the web-based analysis service SILVAngs (Quast et al., Nucl. Acids Res., 2013) and is called **BactiSeq** (**Ba**cteria Bar**c**oded Mul**ti**plex **Seq**uencing). Employing our novel analysis approach, we compared classification results of SILVAngs with RDP classifier version 2.6 and found that RDP significantly underestimates the planctomycetal diversity and abundance. With the SILVAngs service we found that *Planctomycetes* form a major fraction (31%) of the cyanobacteria-associated community and we were able to verify the species-specific interaction of *Planctomycetes* with cyanobacteria that was deduced from the clone library analysis. Interestingly, cultivated planctomycetal type strains were rarely found in the deep sequencing data and thus belong to the so-called "rare biosphere" in this habitat.

DEP12

Diversity of Sulfur Oxidizing Bacteria involved in the Biogenic Sulfuric Acid Corrosion within Sludge Digesters

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Several billions of dollars are spent for the reparation and maintenance of sewage systems every year, whereupon the deterioration of concrete due to biogenic sulfuric acid corrosion (BSA) is one of the most serious and costly problems. The corrosion is caused by biogenious sulfuric acid which is produced by aerobic sulfur/sufide oxidizing bacteria (SOB) growing on the moist concrete surface. While the corrosion process is well studied in sewer pipes, a lack of understanding exists concerning BSA in sludge digesters. The predominant anaerobic condition in a digester would suggest no sulfuric acid production by SOB which need oxygen for growth. However, characteristic BSA damage patterns were also detected in different digesters. Thus, the aim of this study was to verify BSA in digesters by isolating and characterizing the relevant SOB. For SOB analysis, biofilm was scratched off from the concrete surface within the headspace of five different digesters. The focus of this study was on the isolation of active sulfuric acid producing microorganisms. Therefore, specific liquid media, differing in pH and energy source, were used for the enrichment and cultivation of SOB. Pure cultures were obtained by cultivating SOB on agar media and identified by polymerase-chain-reaction (PCR) and sequence analysis. The SOB diversity within the enriched SOB cultures was investigated by PCR combined with denaturing gradient gel electrophoresis (DGGE) and sequencing. The diversity studies by PCR-DGGE within the enriched mixed SOB cultures revealed five different SOB species participating in the BSA process: *Acidithiobacillus thiooxidans, Thiomonas intermedia*, *Thiomonas perometabolis, Thiobacillus thiooparus* and *Paracoccus solventivorans.* From the first three bacteria also pure cultures were obtained. *A. thiooxidans*, the key organism within the corrosion process, is known to be acidophilc (pH optimum 2-4) whereas the other four SOB species are neutrophilic (pH optimum 5.5-8). Since every SOB is characterized by a certain pH optimum, statements about the progression of microbial corrosion can be made. Especially the finding of the acidophilic *A. thiooxidans* within the headspace of three different digesters provides evidence of a progressed BSA attack. A lower extent of corrosion is assumed in the other two digesters examined, since only neutrophilic SOB were detected. Finally, different SOB communities could be identified in sludge digesters for the first time indicating that BSA is not only a problem occurring in sewer pipes, but also in digesters. Furthermore, the different SOB characterized by a certain pH-optimum could be correlated to the degree of damage.

DEP13

Large fractions of bacteria in pristine limestone aquifers are involved in CO_2-fixation coupled to the oxidation of sulfur and nitrogen compounds

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Despite growing evidence of an important role of lithoautotrophy in aquifer carbon flow, the microbial metabolisms linked to CO_2-fixation and their effects on aquifer biogeochemistry are still poorly understood. We investigated groundwater samples from two superimposed limestone aquifers in the Hainich-Dün region (Thuringia, Germany) with sampling depth ranging from 12 to 88 m. The aims of this study were (i) to assess the quantitative importance of autotrophic microbial groups using gene markers for the Calvin-Benson-Bassham cycle (*cbbM*, *cbbL*) and the reverse tricarboxylic acid (rTCA) cycle (*aclB*), and (ii) to identify the relevance of these groups within the total microbial community by combined analysis of bacterial 16S rRNA, *cbbL, cbbM,* and *aclB* genes and transcripts.
Microbial communities were dominated by members of the Proteobacteria and Nitrospirae with clear differences between the lower, oxygen-rich and the upper, oxygen-deficient aquifer. Quantitative PCR targeting RubisCO-encoding genes indicated that up to 17% of the microbial population had the genetic potential to fix CO_2 via the Calvin-Benson-Bassham cycle. A large fraction of *cbbM* and *cbbL* reads was closely related to thiosulfate and ammonia oxidizers such as *Sulfuricella denitrificans* and *Nitrosomonas sp.*. The presence of *aclB* genes encoding citrate lyase of the rTCA cycle related to *Nitrospira* sp. together with a large fraction of *Nitrospira*-related 16S rRNA reads pointed to an important role of CO_2 fixation by nitrite oxidizers. Incubation experiments confirmed the potential for nitrification and for denitrification coupled to thiosulfate oxidation. Our findings support an important role of carbon autotrophy linked to the oxidation of reduced sulfur and nitrogen compounds in pristine limestone aquifers.

DEP14

Diversity and seasonal variation of the Bacterioplankton community of a drinking water reservoir revealed by 16S rDNA based pyrosequencing

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Reservoirs are important sources for drinking water. For example, in Saxony 50% of the drinking water is derived from reservoirs. Microorganisms play an important role in aquatic ecosystems, due to their physiological activities with distinct influences on nutrient cycles and on different trophic levels. Therefore, information on the microbial community of reservoirs, especially its seasonal changes and reaction to environmental factors, are of importance with regard to the water quality. While several studies revealed the microbial community composition in lakes, there is much less information about reservoirs yet.
The Saidenbach drinking water reservoir is located in the Saxony and has a capacity of 22.4 million m³. Water samples were taken monthly from four sampling sites located along the flow of water from a predam (F) to the main basin (sites H, S and E). The microbial community was analyzed in two size fractions (>5µm, 0.2-5µm) by 16S rDNA based pyrosequencing, and compared to results from DGGE, cloning, and microscopical analyses. A correlation of the microbial community to environmental factors was calculated by statistical analyses. Pyrosequencing and cloning revealed *Proteobacteria* (35%), *Actinobacteria* (20%), *Bacteroidetes* (16%) and *Cyanobacteria* (20%) as dominating phyla in the water body. The Phylum *Proteobacteria* is dominated by *Beta-* and *Alphaproteobacteria* with the percentage of *Alphaproteobacteria* rising towards the end of the year. The freshwater cluster LD12 is the dominating group within *Alphaproteobacteria*. Among *Betaproteobacteria, Burkholderiales* is the most important order, dominated by *Comamonadaceae* (mainly Genus *Rhodoferax)* in all samples, indicating a stable composition of *Betaproteobacteria* throughout the year, which was also confirmed by CARD-FISH. The phylum *Bacteroidetes* is composed mainly of *Sphingobacteria* and a few *Flavobacteria*. Sequences assigned to the phylum A*ctinobacteria* belong to *Actinomycetales* (mainly genus *Frankinae*) and less abundant *Acidimicrobidae*. In the phylum *Cyanobacteria* chloroplast sequences make up a large part (80-90%) in the size fraction >5µm. The dominating phytoplankton in the Saidenbach reservoir in spring are diatoms. Pyrosequencing showed percentages of up to 23% *Bacillariophyceae* with maxima in April and May. The spring bloom of diatoms and other eukaryotic algae is followed by the establishment of the cyanobacterial population in early summer. Important genera among *Cyanobacteria* were *Cyanobium, Synechoccous, Planktothrix* and *Anabaena.* Cluster analysis showed a seasonal clustering especially in the large size faction (>5µm) indicating that the algae community showed a larger seasonal variation compared to the microbial. The two sites within the reservoir were more similar to each other than to the predam site. SRP, nitrate and Acetate had a significant effect on the microbial community.

DEP15

Red Fox and the City: About urban lifestyle and the risk of carrying resistant *E. coli* in wildlife

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Introduction: Antimicrobial resistant bacteria in wildlife animals can be seen as an indicator for a leakage of these microorganisms to the environment. *Escherichia coli* are the most frequently isolated bacterial pathogens from both humans and animals, and are able to carry one of todays' most frequent resistance mechanisms, extended spectrum beta-lactamases (ESBL). At the same time *E. coli* are part of the autochthonous gut microbiota, therefore are shed by almost every living being and may be easily distributed in the environment. Although it is known that wild animals also carry antimicrobial resistant *E. coli*, the way how the animals achieve these bacteria are still unknown.
In our study we used resistance to cefotaxim as a marker for possible human or companion animal origin of *E. coli* isolates in Red Foxes (*Vulpes vulpes*) from the Berlin city area, which was verified through phylogenetic analyses. Spatial analysis on possible factors fostering the spread of these bacteria to the urban environment and wildlife should demonstrate risk factors for urban wildlife to obtain these bacteria.
Methods: Samples from 518 foxes were taken from April 2010 to December 2012 and screened for cefotaxim (CTX) resistant *E. coli*. The bacteria were investigated for their clonal relatedness by macrorestriction analysis (PFGE). Via PCR the phylogenetic group was determined and isolates of EcoR groups B2 and D supposed to be of higher pathogenic potential were compared to known pathogens from humans and other animals by multi locus sequence typing (MLST). Spatial analysis was performed, including factors like hotspot analysis, proximity to hospitals, restaurants, or bigger roads. Also human population density at the location of the carcasses finding was correlated to the carriage of CTX resistant *E. coli*.
Results: A total of 52 CTX resistant *E. coli* isolates were obtained from 44 foxes (8.5%). Eight animals each carried two different resistant clones. Two isolates were assigned to EcoR group B2 and sequence type ST131, well known for its pandemic spread. Furthermore 17 isolates were assigned to EcoR group D and various STs, the most common (3/17) being ST69, which is also known from various clinical cases. One isolate belonged to the just recently described ST648.
Spatial analyses showed an even distribution of the fox samples all over Berlin. There was no significant difference between foxes carrying resistant bacteria and those who don't for any of the tested spatial factors.
Discussion: Our results show that foxes in urban areas carry resistant *E. coli* phylogenetically related to those from humans and animals. We showed independence on the proximity to hospitals, the human population density or any other of the spatial factors we tested for. It seems like there are various sources for resistant bacteria which urban wildlife gets in contact with, but further risk factors should be investigated to verify this.

DEP16

Myxobacteria as Biological Control Agents of Phytopathogens on Some Economic Crops in the Philippines

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The Philippines is mainly an agricultural country with food crops such as rice, corn and tomato as primary contributors to the country's agricultural gross domestic products. However, up to date, there is an unending struggle by our local farmers against phytopathogenic fungi and bacteria causing serious losses in yield. Among these pathogens are the fungi *Helminthosporium maydis* (*Hel*), *Curvularia lunata* (*Cur*) and *Rhizoctonia solani* (*Rhi*) on corn, *Pyricularia grisea* (*Pyr*) on rice, and the bacteria *Xanthomonas oryzae* (*Xan*) on rice and *Ralstonia solanacearum* (*Ral*) on tomato. Common method of disease control is by the use of commercial fungicides, many of which are harmful to humans and animals. Thus, there is a need for biocontrol measures which are safe and environment-friendly.
Local several isolates of myxobacteria such as *Cystobacter*, *Myxococcus*, *Pyxicoccus*, *Chondromyces*, *Stigmatella*, *Corallococcus* and *Nannocystic*, all gliding-fruiting myxobacteria, and one isolate of *Cytophaga*, a gliding but non-fruiting myxobacterium, were studied for their potentials as biocontrol agents against the aforementioned phytopathogenic fungi and bacteria. In dual culture challenge on agar, the myxobacteria exhibited selective and weak to strong suppression of mycelial growth. Hyphal filaments in the zone of contact with myxobacterial swarms became gnarled and coralloid, the branches shortened, and the distal segments lysed but with intact cell wall.
Pathogenicity of the bacteria was tested on 2-5- week-old rice and corn and 4-5-leaf-stage tomato plants. Myxobacteria were screened *in vitro* for their lytic activity against the plant pathogens via a modified lawn-spotting method. Isolates positive for lysis/inhibition were then subjected to the metabolite-based assay using the Kirby-Bauer susceptibility method. The *in vivo* potentials of myxobacterial methanolic extracts in the control of selected diseases of rice, corn, and tomato caused by the phytopathogens were also determined.

DEP17

Insights into the distribution, diversity and ecological role of *Dehalococcoidia*, phylum *Chloroflexi*, in the marine subsurface

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Bacteria of the class *Dehalococcoidia* (DEH), represent a diverse and major phylogenetic group within the phylum *Chloroflexi.* DEH have been detected by 16S rRNA gene sequencing in a wide range of shallow and deep marine sediments often with high relative abundances, yet little is known about their metabolic properties and ecological role in organic matter mineralisation. We first developed specific PCR primers to document the presence and abundance of DEH in transects though continental margins and through different sediment depths. By deep-sequencing we have defined sub-groups within the DEH and documented their presence depending on biogeochemical gradients, *i.e.* the sulphate-methane transition zone, indicating DEH fulfil diverse ecological roles in marine sediments. The characterization of a DEH model organism, *Dehalococcoides mccartyi* strain CBDB1, revealed growth with a wide range of halogenated organics including naturally occurring brominated aromatics as electron acceptor suggesting this reaction as one of the ecological niches occupied by DEH. Single cell genome sequencing after flow-cytometric isolation and Phi29 whole-genome amplification has revealed a third set of information of the physiological capacity of DEH. A total of five single genome sequences has shown that DEH are not necessarily bound to organohalide respiration but encode fermentative and respiratory pathways. Distinct genomic features appear to be present in members of different clades. Members of the GIF9 subgroup have the capacity to oxidise hydrocarbons and channel the carbon into an acetogenic pathway, while members of the 'Dehalococcoidia sister clades' may be specialised for oxidation of aromatic molecules, and harbour the potential to respire with multiple electron acceptors.

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DEP18

5-year tracking of soil bacterial communities and keystone bacteria involved in plant-derived carbon flow in a maize field

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Microorganisms control the turnover, fluxes, mineralisation and sequestration of organic carbon in soil. In plant-influenced soils, microbial decomposers channel carbon derived from rhizospheric inputs or plant litters into the soil food web. Despite the fundamental importance of soil bacteria as primary decomposers, they are poorly understood especially in terms of their identities, specific activities, spatial distribution and temporal dynamics. At an experimental maize field site in Göttingen, we sampled soil from 2009 to 2013 at 10, 50 and 70 cm of depth. We hypothesized that depth-dependant bacterial community distinctions as well as the development of a maize-adapted microbiota would become more and more apparent over the years. Keystone bacterial constituents of the rhizosphere and detritusphere food channels in this field have been previously identified using rRNA-based stable isotope probing (SIP) in combination with pyrotag sequencing.T-RFLP fingerprinting, quantitative PCR and pyrotag sequencing are now applied to trace overall soil bacterial community development and keystone bacterial food web members in the field.
Based on T-RFLP fingerprinting, bacterial community composition was strongly affected by soil depth. Some key bacterial populations (T-RFs) exhibited clear depth-dependant distribution pattern, playing important roles in explaining the variations among the overall bacterial communities. For example, *Humicoccus* spp., a taxon found to actively degrade glucose in the soil, and *Flavobacterium* spp., characteristic for the maize rhizosphere at the site, were found to be of clearly elevated abundance in top soil compared to deeper horizons. However, we did not observe a clear annual trend in either overall bacterial community composition or key food web constituents. Ongoing work combines quantitative PCR and pyrotag sequencing data for the field microbiome, to determine the abundance of keystone bacterial food web members and estimate the corresponding bacterial carbon fluxes. Our results provide novel insights the distinct roles of specific bacterial populations in soil carbon cycling.

DEP19

Interaction between marine diatoms and bacteria

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Interaction between diatoms and bacteria are of high importance in the carbon cycle of marine ecosystems. Diatoms are responsible for around 25 % of fixed carbon in the oceans. Organic matter produced by diatoms is an important carbon source for heterotrophic marine bacteria. In return, certain bacteria are speculated to provide diatoms with nutrients like iron, nitrogen compounds or vitamins. In order to detect and investigate mutualistic interactions between marine diatoms and bacteria, methods were developed for generation of axenic diatom cultures and for non-invasive chlorophyll quantification. Interactions between selected marine bacterial strains and the model diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* were analyzed in co-cultures compared to axenic diatom cultures. For generating axenic diatom cultures, diatoms were incubated in and repeatedly transferred to antibiotic-containing artificial sea-water medium without shaking. After six to eight weeks of incubation in antibiotic-containing medium, no bacterial growth in liquid and solid complex growth media was detectable. To enable a high throughput screening of diatom growth, a non-invasive method for chlorophyll quantification was established. For this, the chlorophyll fluorescence of diatom cultures was measured with the imager ChemiDoc™ MP (BioRad) and set in proportion to the amount of chlorophyll extracted with 90 % (v/v) acetone with following spectrophotometric measurement[1]. Thus, growth of diatoms in suspension and aggregates could be measured in microtiter plates over several days. Marine bacteria were isolated from the North Sea with artificial sea water medium with additional carbon sources. The isolated bacteria were investigated in co-culture experiments with *T. pseudonana* or *P. tricornutum* in 24-well plates under photoautotrophic growth conditions. Several specific bacterial isolates were identified that led to increases in chlorophyll content and aggregation of *P. tricornutum* in co-culture experiments. Also for *T. pseudonana*, bacterial isolates were identified leading to increased aggregation in co-cultures.
The results show that the development of the non-invasive chlorophyll quantification method allows a fast and reliable screening for marine bacteria influencing growth and aggregation of marine diatoms in comparison to axenic cultures. Further studies investigating the physiological and molecular basis of these interactions are on the way.

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DEP20

The hidden sulfur cycle in rice paddy soil: identification of key players by stable isotope probing and next-generation amplicon sequencing.

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Rice paddy fields are indispensable for human food supply but at the same time are one of the most important sources of the greenhouse gas methane. Recently, a hidden sulfur was proposed to occur in freshwater wetlands such as rice paddy fields that effectively cycles the various sulfur species between their oxidized and reduced states and at the same time counter balances methane production. Dissimilatory sulfate reduction as the driving force of the hidden sulfur cycle occurs in rice paddy soil at rates comparable to marine surface sediments, despite the significantly lower sulfate

concentrations. As a consequence, sulfate reduction as the thermodynamically favorable process over fermentations coupled to methanogenesis diverts organic matter degradation from methane towards more carbon dioxide production. To identify the responsible microorganisms, we set up greenhouse experiments where whole rice plants were grown in soil amended with gypsum ($CaSO_4$) in amounts relevant for rice agriculture (0.15% w/w). Rice plants grown in soil without gypsum served as control. In their late vegetative phase, rice plants were grown under an atmosphere enriched with $^{13}CO_2$ to induce production of ^{13}C-labeled root exudates and identify microorganisms involved in the turnover thereof under sulfate reducing and methanogenic conditions. Gypsum amendment significantly reduced methane emission from rice plant mesocosms by up to 98% showing that sulfate reducers were active and effectively competed with microorganisms involved in the methanogenic degradation pathways. 16S rRNA gene-targeted high throughput amplicon sequencing revealed a clear effect of gypsum amendment on the total microbial community in the rhizosphere and bulk soil. In particular, the abundance of members of the *Desulfobulbaceae*, *Desulfovibrionaceae*, and *Synthrophobacteraceae* increased under conditions that stimulated sulfate reduction. Parallel RNA stable isotope probing revealed label uptake by microorganisms in the presence as well as in the absence of externally supplied sulfate. Using comparative analysis, we currently investigate which of these actively labeled microorganisms were involved in sulfate reduction. Our results corroborate the importance of the hidden sulfur cycle in controlling production of the greenhouse gas methane and identified key players involved in sulfate reduction as the driving force of this biogeochemical phenomenon.

DEP21
Long-term stability of raw milk microbiota

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Introduction: Raw milk microbiota are both an important factor of raw milk quality and an indicator for the health status of cows. Furthermore, they may have a considerable impact on the quality of resulting dairy products. The composition of raw milk microbiota is complex and largely influenced by housing and feeding conditions of cows as well as the hygienic conditions of milking and the milking equipment.
Materials and Methods: Fresh milk of a single farm was analysed over several months for cell counts and species composition using a cultivation dependent approach and a comparably large random sample of 300 colonies per time point. Altogether, six samples were collected between April and August 2012 and isolates were identified by FTIR spectroscopy and 16S rDNA sequencing. Afterwards, isolates of frequent species were typed below the species level by RAPD and rep-PCR analyses in order to find out whether identical strains can be detected in different samples.
Results: Between 41 and 68 different species were detected in each sample and a considerable overlap in species composition was observed. 14 species were found in all samples analysed and these accounted for more than 60% of all isolates identified. Additionally, typing of isolates by RAPD and rep-PCR revealed, that for many species identical strains were isolated from different samples. Comparisons of rep patterns with isolates from earlier analyses in 2007, 2008 and 2009 even resulted in matches of new and older isolates.
Discussion: A large fraction of isolates belonged to species and even strains detected in all samples indicating a highly stable and well established microflora in the herd. The fact that identical strains can be found over a period of several years strongly indicates that there must be a transmission of the microbiota also to new cows joining the herd. As the cows are housed under very constant conditions and the herd is maintained by its own offspring alone, it is likely that this contributes to the establishment of a stable microbiota.

DEP22
Comparing the nitrifying communities of two different freshwater recirculating aquaculture systems connected by the water flow

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Fish farming in land based recirculating aquaculture systems (RAS) is gaining more and more importance to meet the increasing demand for fish products. These facilities depend highly on a well-designed nitrification process to clean the water efficiently and at low costs. Nitrification takes place in open moving bed reactors filled with biocarriers that contain the nitrifying biofilm, including ammonia and nitrite oxidizing bacteria (AOB and NOB). The investigated freshwater RAS for zander (*Sander lucioperca*) and rainbow trout (*Oncorhynchus mykiss*) are operated under differing conditions, according to the requirements of the fish. This includes the pH-value and temperature of the water, tank size, feeding regime, fish density etc. and could lead to the development of different nitrifying communities with diverse characteristics in both systems. However, at this particular plant, both systems are connected via the water flow. So the questions arise how these two communities differ from each other or if they are populated by the same nitrifying microorganisms.
To evaluate the differences, laboratory based activity and growth tests were performed with colonized biocarriers. The nitrifying biofilms were investigated with regard to their pH optimum at different temperatures. AOB and NOB were identified via specific primers in PCR reactions, especially main organisms like *Nitrospira* and *Nitrosomonas*. Since the rainbow trout RAS is operated at low temperatures, the cold adapted Candidatus *Nitrotoga*, originally found in permafrost-affected soil from Siberia (1), could be detected as well. The biofilm structure was visualized using FISH and Scanning Electron Microscopy.
By comparing distinct RAS operated under different conditions, it is possible to gain a deeper understanding of parameters that influence nitrification in technical systems. This helps to maintain more stable processes and to avoid disturbances.

DEP23
The importance of biopores for the microbial diversity and nutrient turnover in the subsoil

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Soils below the plough layer provide a stock of nutrients and trace elements and a habitat for microbes, which received little attention in the past despite their importance for nutrient cycling and plant nutrition [1]. Previous studies performed so far suggest that the microbial biomass, diversity and activity rapidly decrease with depth in soil [2]. However, the investigations have mostly focused on bulk soil neglecting the soil heterogeneity. In contrast to topsoils, which are regularly homogenized by the agricultural management, in subsoils spatial heterogeneity patterns are far more pronounced. Especially biopores, which are formed by tap roots and soil fauna and link the subsoil to the topsoil, might serve as important hotspots for microbes in the subsoil due to higher nutrient availability. To investigate the role of biopores for the microbial performance, microbial diversity and their functional potential for nutrient turnover in the subsoil of an arable field up to the depth of 1 m was assessed in different soil compartments including drilosphere, rhizosphere, and bulk soil, and compared to topsoil. Besides 16S rRNA gene fingerprint and 454-pyrosequencing, potential microbial activities related to carbon and nitrogen turnover were measured.
Interestingly, all measured parameters indicated that the differences between topsoil and subsoil in the drilosphere and rhizosphere were very low or absent: The microbial biomass, enzymatic activity and the absolute or relative abundance of functional gene markers for nitrification and denitrification did not decrease with increasing depth as it has been observed in bulk soil. Additionally, the bacterial community fingerprints of the drilosphere and rhizosphere were more restricted to the investigated compartment than to the depth, whereas for the bulk soil a significant difference between topsoil and subsoil community patterns was found [3]. These results indicate the importance of biopores as hotspots for microbial activities mainly in deeper soil layers. The restriction to bulk soil

measurements in subsoils leads to an underestimation of the subsoil diversity and its potential for nutrient turnover.

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DEP24
Morphology and Host Range of Bacteriophages Infectious to *Aeromonas* Spp. Bacteria. Potential Candidates for Phage Therapy

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Introduction: *Aeromonas* spp. are ubiquitous water-borne bacteria responsible for important economic losses in fish farming plants. These opportunistic microorganisms have also been associated with human diseases caused by contaminated water. The common carp and rainbow trout are popular food fish in Poland and should be considered as a source of *Aeromonas* infection; therefore, virulent phages may represent a viable alternative to antibiotics to inactivate pathogenic bacteria.
Aim: We asked whether bacteriophages isolated from local aquacultures have an impact on *Aeromonas* spp. strains and can be considered as potential candidates for phage therapy.
Materials and Methods: Bacteriophages infectious to *Aeromonas* spp. bacteria were isolated from samples of pond water, river water (Wieprz), and local lakes (Zemborzycki, Bilgorajski) using standard methods. TEM examination of negatively stained particles was employed for assessment of phage morphology. The lytic specificity and host range were assayed in app. 50 *Aeromonas* spp. strains using the double-layer technique. The one-step growth experiments were performed according to Adams (1959).
Results: All isolated phages had isometric, polyhedral heads and contractile tails, and were classified in the *Myoviridae* family. The bacteriophages varied in their lytic activities against *Aeromonas* spp. strains. Four out of ten, i.e. Φ207F, Φ401Ch, Φ401Cl, and Φ10, productively infected ~30% of *Aeromonas* isolates and almost 50% of those belonging to the species *A. salmonicida* and *A. bestiarum*, whereas the ΦJ4N, Φ15S, and Φ15m phages had their lytic activity restricted to only few strains. The one-step growth experiments revealed that the phages had similar cycles of multiplication. After a latent period ranging from 20 to 60 min, a quick rise in the phage titer was observed giving plaque yields from 130 to 250 particles per infected bacterial cell.
Conclusion: The results suggest a possibility of phage-typing analysis of the distribution and identification of *Aeromonas* spp. strains. The success of phage therapy depends on the impact of phages on the bacterial community, thus virulent phages with effective burst size can be utilized to reduce the population of potentially pathogenic bacteria in fish farming plants.
This work was supported by the Grant of the Vice-Rector of M. Curie-Sklodowska University, Lublin, Poland (BW-01-1000-14-09) and National Science Centre research grant (DEC-2011/03/B/NZ1/01203).

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DEP25
Investigation of the microbial community in a polluted river plume in the Gulf of Naples

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The Gulf of Naples (Italy), which connects coastal waters with the open Tyrrhenian Sea, offers optimal conditions for a highly diverse marine environment, which has been investigated intensively since <140 years. However, this environment is affected by anthropogenic pollution, which may cause serious damage to the pristine ecosystem. The Sarno river is, beside the extensive ports of Naples, the main source of anthropogenic pollutants introduced into the Gulf of Naples. The release of waste water from tanneries along the river causes high concentrations of arsenic, chromium and a broad variety of hydrocarbons, in the stream waters and subsequently in the Gulf of Naples. This intoxication of the coastal waters not only affects the city of Naples and the population of the gulf region, but could also cause serious damage to the diverse marine ecosystems.
Here, we present the first results of the investigations of the microbial community within the plume of the Sarno river released into the Gulf of Naples. We aimed to find genetic responses of the microbial community towards pollutants washed into the Gulf of Naples with the fresh water from the river, and to identify possible indicator genes for these pollutants. We analyzed four stations along a transect following the salinity gradient from the river estuary towards the open waters of the Gulf of Naples. The diversity and genomic potential of the microbial community at these stations was investigated using four metagenomes. The bacterial and archaeal community was quantified using catalyzed reporter deposition fluorescence in situ hybridization with probes covering the main marine and fresh water groups, combined with microautoradiographic measurements of the bacterial and archaeal activity. Furthermore, we used four metatranscriptomes to inform on the genetic activity and identify potential genes which might be used as indicator genes for pollutants in marine environments. We focused on genes involved in the degradation of xenophobic compounds and carbon cycling, both of which were found in in decreasing quantities along the transect from the river outlet towards the gulf. Hence showing an adaptation of the microbial community towards these compounds and suggesting a major role of marine bacteria and archaea in anthropogenic pollutant degradation.

DEP26
Drivers for ammonia oxidizers at local and regional scales along a land-use gradient in grassland soil

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We investigated drivers for ammonia-oxidation and the related microbial communities (ammonia-oxidizing bacteria and archaea) in grassland soils on the local as well as on the regional scale focusing on the role of land-use intensity (LUI). To this end, 150 sites from three distinct regions across Germany were selected (www.biodiversity-exploratories.de), covering the whole range of LUI levels (from natural grasslands up to intensive managed meadows). Furthermore, the role of contrasting soil types was analyzed in one of the regions (high vs low organic matter content) for ammonia-oxidation. We revealed a significant increase in potential nitrification rates and abundance of ammonia-oxidizing microbes at two sites on the local level from extensively to intensively managed sites, which indicates that the response pattern of ammonia-oxidizing microbes in grassland soils is likely triggered to a large extent by LUI. However at a third site, where two different soil types were investigated, no correlation between LUI and potential nitrification rates was observed, and only a site-specific effect was apparent. At this site, on the one hand the specific soil type (Histosol) and the related continuous nutrient mobilization from the former peat matrix, as well as the high groundwater level, which could induce a high abundance of methane- oxidizing microbes in the top soil, may be of greater importance as a driver for potential nitrification rates and abundance of ammonia-oxidizing microbes than LUI. On the other hand, the mineral soils of this site were characterized by extreme water shortage, which may also explain the lack of potential nitrification and the abundance of ammonia-oxidizing bacteria and archaea. Thus any extrapolation of local data to regional predictions must be made with care, as factors other than LUI may be of importance if the nitrification potential of a soil is to be described.

DEP27

Delineation of *S. maltophilia* isolates from cystic fibrosis patients by fatty acid methyl ester profiles and MALDI-TOF mass spectra using hierarchical cluster analysis and principal component analysis

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Objectives: *Stenotrophomonas maltophilia* is an opportunist multidrug resistant pathogen, which is associated with a high mortality rate. Various cystic fibrosis (CF) centres have reported an increasing prevalence of *S. maltophilia* colonisation/infection among patients with this disease. The purpose of this study was to assess specific fingerprints of *S. maltophilia* isolates from CF patients by investigating the fatty acid methyl esters (FAME) through gas chromatography (GC) and high abundant proteins by Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS), and finally, to compare them to isolates obtained from intensive care unit (ICU) patients and the environment.
Materials and Methods: A total of 100 *S. maltophilia* isolates (71 isolates from CF patients, 20 isolates from ICU patients and 11 isolates from the environment) were analysed. GC was performed as described previously (Müller *et al*., 1998). The peak area index (PAI), which indicates the amount of fatty acids produced by the bacterial culture in the broth culture during growth, was defined as follows: total peak area/solvent peak area × 10,000. Peak lists of MALDI-TOF mass spectra obtained with the Vitek® MS Plus identification were processed to perform a hierarchical cluster analysis (HCA) using Euclidean distance and Ward's method, in order to calculate the degree of similarity among the isolates. Principal component analysis (PCA) of the processed peak lists was performed as a type of multivariate analysis to identify the first three principal components and also to uncover differences of the isolates in respect of these components. DataLab™ software was used to perform PCA and HCA.
Results: Principal component analysis of GC-FAME patterns did not reveal a clustering corresponding to distinguished CF, ICU or environmental types. Based on the PAI, it was observed that *S. maltophilia* isolates from CF patients produced significantly higher amount of fatty acids in comparison to ICU patients and the environmental isolates. HCA based on the MALDI-TOF MS peak profiles of *S. maltophilia* revealed the presence of five big clusters, suggesting a high phenotypic diversity (Simpson´s index value of 0.794). Although HCA of MALDI-TOF mass spectra did not result in distinct clusters predominantly composed of CF isolates, principal component analysis (PCA) revealed the presence of a distinct cluster composed of *S. maltophilia* isolated from CF-patients.
Conclusions. Our data suggest that *S. maltophilia* colonising CF patients tend to modify not only their fatty acid patterns, but also their protein patterns as a response to fit in the CF-lung unfavourable environment.

DEP28

Cultivation of eDNA-forming bacteria in a continuous flow reactor and visualization of biofilms by Confocal Laser Scanning Microscopy

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The significance of extracellular DNA (eDNA) in biofilms was overlooked for a long time, but recent studies show that extracellular DNA is a major component of the EPS (extracellular polymeric substances) in biofilms and it was found to have widespread importance in biofilm formation. However, detailed knowledge about how it promotes biofilm formation and how it influences biofilm morphology is just starting to emerge (1). The γ-Proteobacterium F8 was isolated from the South Saskatchewan River in Canada. It was found to produce huge amounts of extracellular DNA forming a filamentous network when grown on agar plates containing FBM medium (2, 3). Till now, this phenomenon wasn´t examined for F8 biofilms grown under continuous flow conditions. The aim of this study was to grow F8 under controlled conditions in a continuous flow biofilm reactor and to monitor eDNA-production and biofilm morphology over time. Cells and EPS components were visualized by differential staining with fluorescent dyes and confocal laser scanning microscopy (CLSM). A continuous flow biofilm reactor was constructed and operated with FBM medium. Microscopic glass slides served as a surface for attachment of biofilm forming F8 cells. They where harvested on 5 time points between day 1 and day 16. Biofilms where stained with DAPI, Syto 9 and Propidium Iodidide for visualization of DNA. Sypro Ruby was used for protein staining and Nile Red for lipid staining. Total cell numbers as well as cfu of planktonic and biofilm cells in the reactor where determined by Flow Cytometry and the Drop Plate method, respectively.
F8 cells attached to glass slides and showed biofilm formation from day 1 on. Filamentous eDNA could be detected by staining with Syto 9 and Propidium Iodide, but not with DAPI.

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DEP29

VBNC Legionellae - Transition from culturable to the viable but non culturable state by starvation

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Introduction: Under adverse conditions legionellae switch to the viable-but-non-culturable (VBNC) state from which they may be resuscitated by intracellular growth in host organisms. Whether the VBNC-form of legionellae is of health relevance is not known.
Objectives: To obtain a better understanding of the health relevance of VBNC legionellae, we established a VBNC-induction and detection system and in parallel two host models.
Materials and Methods: Starvation was chosen as the model procedure to follow the transition process. We tested several assays to confirm viability of legionellae in the VBNC state (BacLight, modified esterase activity assay, incorporation of radiolabelled aminoacids, etc.). Additionally, the binding affinity of monoclonal antibodies against the LPS and against membrane bound proteins was monitored during the transition process.
Findings: The culturable *Legionella* population was more stable in tap water than in deionized water. It could be shown, that the transition process is temperature-, strain- and matrix-dependent. In a starving Legionella population the number of esterase active cells and the activity per cell drops down exponentially till it reaches a plateau after around one month. The membrane activity is retained for a longer period of time. Elisa results show that all tested LPS epitopes are still available in the VBNC state some of them with quite increased signals, nevertheless some LPS structures are shedded. The virulence associated membrane-bound proteins mip and momp are stable during the transition to the VBNC state.
Conclusions: Different methods to verify the viability of legionellae lead to different Results: Only the combination of more than one detection technique with different induction protocols seems to be adequate to study vbnc formation. VBNC legionellae may be health relevant regarding the stability of some virulence factors like momp, mip and certain LPS structures. Whether and to what extent VBNC legionellae after different treatments are resuscitable and virulent or not remains to be examined.

DEP30

Bacterial diversity in bioaerosol-emissions from the industrial livestock farming

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There is still a lack of information on the emissions of bioaerosols from animal processing plants. In Germany, therefore great efforts were made to standardize measurement methods and analytical procedures for the quantitative determination of bioaerosols during the last decade.
The resulting impinger based sampling procedure (VDI guideline 4257 part 2) was used in this study to investigate the bacteria in the exhaust air from different broiler houses. Microorganisms in bioaerosol samples were quantified via fluorescence microscopy after DAPI-staining. For qualitative analyses bacterial DNA was extracted from bioaerosol samples followed by bacterial specific 16S rRNA-Gen-PCR, and a subsequent analysis of 16S rRNA gene composition by a clone library and 454-Sequencing approach.

The total cell counts in the investigated exhaust air samples varied between 4.9- x 10^7 and 2.2 x 10^8 cells per m³ (mean: 1.2 x 10^8 cells per m^3) and exceeded the natural background concentrations by about 3 order of magnitudes. Molecular detection methods reveal that species of the genus *Staphylococcus* (most frequently *S. arlettae*, *S. lentus* or *S. cohnii)* were the predominant bacteria in emission samples. However, the molecular approach indicates as well species of genera *Jeotgalicoccus*, *Brevibacterium*, *Lactobacillus*, *Brachybacterium, Subdoligranulum, and Corynebacterium* as further dominant members of the emitted bacterial communities. In conclusion emissions from poultry houses represent considerable sources for distribution of a quite diverse composition of bacteria into the environment by airborne transport. However, the results from the clone libraries however, also indicate the emission of bacteria whose 16S rRNA gene sequences are next related to those of risk group II bacteria, among them *Aerococcus viridans*, *Enterococcus hirae* or *Staphylococcus saprophyticus*. Under consideration of the high concentration of airborne bacteria and the detected composition, from the preventive point of view it's advisable for worker inside poultry houses or inside the vent stack e.g. at service operation to wear an adequate breathing protection.

DEP31

Synthesis of novel palladium(0) nanocatalysts by microorganisms from heavy-metal-influenced high-alpine sites for dehalogenation of polychlorinated dioxins

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In a search for new aqueous-phase systems for catalyzing reactions of environmental and industrial importance, we prepared novel biogenerated palladium (Pd) nanocatalysts using a "green" approach based on microorganisms isolated from high-alpine sites naturally impacted by heavy metals. Bacteria and fungi were enriched and isolated from serpentinite-influenced ponds (Totalp region, Parsenn near Davos, Graubünden, Switzerland). Effects on growth dynamics were monitored using an automated assay in 96-well microtiter plates, which allowed for simultaneous cultivation and on-line analysis of Pd(II)- and Ni(II)-mediated growth inhibition. Microorganisms from Totalp ponds tolerated up to 3 mM Pd(II) and bacterial isolates were selected for cultivation and reductive synthesis of Pd(0) nanocatalysts at microbial interfaces. During reduction of Pd(II) with formate as the electron donor, Pd(0) nanoparticles were formed and deposited in the cell envelope. The Pd(0) catalysts produced in the presence of Pd(II)-tolerant Alpine *Pseudomonas* species were catalytically active in the reductive dehalogenation of model polychlorinated dioxin congeners. This is the first report which shows that Pd(0) synthesized in the presence of microorganisms catalyzes the reductive dechlorination of polychlorinated dibenzo-*p*-dioxins (PCDDs). Because the "bioPd(0)" catalyzed the dechlorination reactions preferably via non-lateral chlorinated intermediates, such a pathway could potentially detoxify PCDDs via a "safe route". It remains to be determined whether the microbial formation of catalytically active metal catalysts (*e.g.*, Zn, Ni, Fe) occurs *in situ* and whether processes involving such catalysts can alter the fate and transport of persistent organic pollutants in Alpine habitats.

DEP32

Antifungal and plant-growth promoting effects of diffusible and volatile sulfur-containing compounds produced by *Pseudomomonas fluorescens* strains

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Introduction: Rhizobacteria exhibit direct and indirect mechanisms to beneficiate plant growth promotion. Direct mechanisms include the synthesis of phytohormones, while indirect mechanisms comprise antagonism toward phytopatogens. Very few works in literature report strains containing both mechanisms. Here, we present four novel strains containing both, direct and indirect mechanisms of plant-growth promotion.

Materials and Methods: Four *Pseudomonas fluorescens* strains were characterized by sequencing their 16S ribosomal gene and phylogenetic analysis. The presence of potential phenazines (*phzCD*), cyanogens (*hcnAB*), DAPG (*phlD*) and ACC deaminase (*acdS*) were detected by PCR amplification. Siderophore production was determined by the chrome azurol S (CAS) assay, while Skim Milk agar (SM) plates were used to detect protease production (Kumar *et al*., 2005). Biofilm formation capacity in bacteria was analyzed by following the protocol by Wei and Zhang (1996). Determination of IAA production was analyzed in filtered-culture supernatants from strains by GC/MS. The volatile blends of all four pseudomonads strains were also analyzed by SPME-GC-MS. We evaluated the plant growth-promotion by diffusible and VOCs emission as previously reported (Orozco-Mosqueda *et al*. 2013). We used the *Medicago truncatula-Botrytis cinerea* pathosystem to evaluate bicontrol action.
Results: It was analyzed the antifungal and plant growth-promoting (PGP) effects of diffusible and volatile organic compounds (VOCs) produced by new rhizospheric isolates. The pseudomonads showed a high grade of antagonism against the grey mold phytopatogen *Botrytis cinerea* during confrontation assays. In addition, all strains increased significantly the *Medicago truncatula* biomass and chlorophyll content. Interestingly, the previous activities were exerted by the production of either diffusible or volatiles compounds emission. Importantly, during biocontrol experiments, the four *P. fluorescens* strains were able to protect *Medicago truncatula* plants from *B. cinerea* infection, by reducing stem disease symptoms and root browning. The presence of potential phenazines, cyanogens and ACC deaminase activity, as well as the production of biofilm, siderophore, proteases, DAPG and indole-3-acetic acid (IAA) were identified in most of the strains. Interestingly, *B. cinerea* induced *phlD* gene expression in all *Pseudomonas* strains, which suggest bacterial strains are sensing and defending from the pathogen trough synthesis of this antibiotic. The volatile blends of all four pseudomonads strains were similar, and sulfur-containing compounds were among the most abundant, including dimethyl disulfide (DMSD). The application of pure compound DMSD significantly reduced the mycelial growth of *B. cinerea*.
Conclusion: The *Pseudomonas* strains analyzed here exert multiple antagonistic and PGP mechanisms, and represent an excellent option to be used as either biocontrol or biopromoting agents in crops.

References

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DEP33

Process of biocontrol in industrial water cooling systems: Ecology of *Legionella pneumophila*

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In aquatic systems, particularly in multi-species biofilms, the bacterial genus *Legionella* spp. including the type *Legionella pneumophila* can survive for long periods. For this reason, the proliferative abilities of *Legionella pneumophila* and other microorganisms in these biofilms were examined. It was shown that *Legionella pneumophila* was stabilised and was able to reproduce in a biofilm matrix of *Klebsiella pneumoniae* together with *Flavobacterium* spp. as well as *Pseudomonas fluorescens*. After 12 days a dynamic biofilm, with a density of 4 x 10^4 CFU bacteria/cm² was detected on a steel surface. The data show that this specific biofilm matrix promotes the presence and the growth of *Legionella pneumophila*. It is interesting that *Legionella* also colonize a two-species biofilm of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* - where it could be demonstrated that a species such as *K. pneumoniae*, is permissive for *Legionella* whereas one such as *P. aeruginosa* may show inhibitory effects. Indeed it was also found that a biofilm consisting only of *P. aeruginosa* showed no growth of *L. pneumophila*. This confirms that some bacteria are permissive for the colonization by *Legionella* while others are antagonistic, such as *Aeromonas* spp. or *Stenotrophomonas* spp. against *Legionella pneumophila* serogroup 1. Thus data in the literature show that *L. pneumophila* has the ability to persist at a high level in a biofilm matrix of *K. pneumoniae*, *Flavobacterium* spp., *Xantomonas* spp. or *Pseudomonas flourescens*, but not in a biofilm in the presence of *P. aeruginosa* and *Aeromonas* spp. [Cotuk et al., 2005; Stewart et al. 2012].
There is need for further work in this area. There are only a few objective, original methods of research on the mechanisms and activity of microbial growth (especially *Legionella* spp.) within biofilms of the industrial ecosystem. Moreover, there are no reports for self-monitoring of cooling water systems, or for the relationship (i.e. *Quorum-Sensing*) between the

microorganisms present. The so-called *quorum-sensing* (QS) is a mechanism that allows microorganisms, by chemical communication, to measure the cell density in the population. Through *quorum-sensing,* the synthesis of secondary metabolites, enzymes and virulence factors is controlled, whereby bacteria can colonize various ecological niches [Winstanley & Fothergill, 2009].

DEP34
Highly diverse growth characteristics among acid-tolerant sulfate reducers

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Dissimilatory sulfate reduction is one of the target processes to biologically remediate metal contaminated waters. Metal rich waste waters are often acidic, especially those emanating from acid mine drainage. Hence, acidophilic sulfate reducers are in the focus for treatment. Sulfate reducing prokaryotes are generally considered neutrophilic, although both alkaliphilic and acidotolerant, or, mildly acidophilic strains have been described. In order to obtain aciodophilic/acidotolerant sulfate reducing bacteria, media mimicking pore water concentrations of inorganic compounds were inoculated with anoxic sediment samples from an acidic pit mining lake. The initial pH was posed to pH 3, 4, 5, and 6. Autotrophic (H_2/CO_2) culture conditions were chosen to circumvent toxic effects of organic acids exhibited in low pH environments. In a previous enrichment, results from T-RFLP fingerprinting and 16S sequence analysis had shown that at initial pH 5 and 6, *Desulfosporosinus* spp. predominated, whereas at pH 3 and 4, *Thermodesulfobium* spp. were responsible for sulfate reduction [1]. A second, independent enrichment gave similar results with an increased proportion of *Thermodesulfobium*-related t-RFs at higher temperatures (25 vs. 15°C). For subsequent isolation a modified roll-tube procedure was applied. With the resulting pure strains batch growth experiments were performed in a pH range of 2.5 - 7 and specific growth rates (μ) and molar growth yield coefficients (Y) were determined. Strains isolated at different initial pH-values showed pronounced differences with respect to pH growth range and growth parameters. Differences in growth behavior will be discussed against the background of direct and indirect inhibitory effects of low pH conditions and corresponding adaptive responses of sulfate reducing bacteria.

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DEP35
German and African soils as potential reservoirs for the facultative human and animal pathogen *Clostridium difficile*

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Clostridium difficile is a facultative nosocomial pathogen responsible for antibiotic-associated diarrhea or pseudomembraneous colitis, which is a major cause of hospital acquired diarrhea in Europe and North America. Many studies focused on the epidemiology of *Clostridium difficile*-associated diarrhea (CDAD) in hospitals but until now only little is known about the presence of *C. difficile* in the environment and about the factors influencing its environmental distribution. Here we present the results of a systematic screen of 150 different grassland and 150 forest soil sample sites in Germany as well as 67 savannah soil sites in subsaharan Africa for the presence of *C. difficile*. Extracted RNA from soil was reverse-transcribed into cDNA, the 16S rRNA V3 region amplified and sequenced using a HighSeq system (Illumina), resulting in over 500 million reads. Whereas African samples revealed only very few sites positive for *C. difficile*, both the proportion of positive sites (up to 80%) and the relative abundance of *C. difficile* reads was found to be substantially higher in German soil environments, especially in grassland soils. Interestingly, the proportion of positive sites decreased from North to South and irrespective of vegetation type. The identification of *C. difficile* sequences in German soils was confirmed by the respective isolation of *C. difficile* strains.

DEP36
Phenotype switch of *Legionella pneumophila* strains - new insights into the LPS structure

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Objectives: The lipopolysaccharide (LPS) molecule represents the immunodominant surface component of *Legionella pneumophila* strains. A panel of monoclonal antibodies (mAb) against various LPS structures is able to differentiate the species *L. pneumophila* into 15 serogroups (Sg). Epidemiological studies report for years that more than 70 % of legionellosis cases in Europe are caused by *L. pneumophila* Sg 1 strains. The 'Dresden mAb-Panel' of eleven mAb's was set up to further separate Sg1 strains into nine mAb-subgroups.
The genotypic basis of the LPS variety is mainly unknown. Only two genes of a large LPS-biosynthesis cluster were identified to have a direct impact on the LPS structures detected by mAb's: i) the *O*-acetyltransferase encoded by *lag-1* acetylates legionaminic acid derivate, the main components of the LPS O-chain and ii) the open reading frame 8 (ORF 8) which encodes for a methyltransferase, recognized by mAb 20/1.
Recent analysis of the LPS-biosynthesis locus identified several open reading frames (ORF) as putative genetic basis for the mAb-based differentiation of Sg1-strains. It is thought that these ORFs contribute to modifications of the Leg-homopolymer and are therefore responsible for altered LPS structures. This could result in differences in the mAb-reactivity, but more interestingly, in altered chemo-physical characteristics of the outer membrane. Additionally, it could have impact on the infectivity or the environmental stability in e.g. aerosols.
Materials and Methods: Using a modified restriction-free cloning approach we generated PCR-constructs harbouring a kanamycin resistance cassette flanked by homologies to ORF 7 of *L. pneumophila* Sg1 strains Heysham-1 (mAb-subtype Heysham), Corby (mAb-subtype Knoxville) and a Corby derived *lag-1* mutant TF3/1. By natural transformation the PCR-constructs were introduced into the wild type genomes. Screening of the transformants confirmed the successful integration into the ORF 7. The mutants were analyzed with regard to growth behaviour, the ELISA based mAb-reactivity of an extended Dresden Panel and other phenotypic assays.
Results: The mAb-reactivity of the transformed strains was altered compared to the wt-strains Heysham-1 and Corby. In more detail, the transformants gained or lost reactivity with two specific mAb's of the Dresden mAb-selection. This indicates that ORF 7 is involved in the modification of LPS structures in both, Corby and Heysham-1. ORF 7-deletion has no influence on the replication kinetic.
Conclusion: Our results show that the ORF 7 of the LPS-biosynthesis locus is involved in the biosynthesis of the LPS structures of *L. pneumophila* Sg1 strains Heysham-1 and Corby. The interruption of ORF 7 results in an altered mAb-profile of the tested strains. Further analysis is necessary to identify the exact function of ORF 7 with respect to structural properties of the LPS molecule, surface properties and infectivity.

DEP37
The versatile metabolism of *Desulfitobacterium* spp. - *O*-demethylation as a growth-selective process for enrichment

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Desulfitobacterium spp. are strictly anaerobic bacteria that belong to the phylum Firmicutes. They are mainly known for and studied because of their ability to reductively dehalogenate man-made organohalide compounds. Nevertheless, not much is known about their occurrence in uncontaminated niches such as forest soil. Recently, focus has been placed on the methylotrophic metabolism of Desulfitobacteria, namely on the *O*-demethylation of phenyl methyl ethers [1, 2], the main decomposition products of lignin. The utilization of these compounds as electron donors by Desulfitobacteria points to their contribution in the process of lignin degradation in soils and to an alternative lifestyle not linked to reductive dehalogenation. In order to study their involvement in lignin degradation processes and their geographical distribution in forests, samples were extracted out of five different topsoils collected in the vicinity of Jena, Germany, with the aim of enriching Desulfitobacteria from the corresponding soils. *O*-demethylation of the phenyl methyl ether syringate as electron donor served as the growth-selective process during five sub-cultivation steps, with thiosulfate being supplied as terminal electron acceptor. *O*-demethylation could be observed in all enrichment cultures. *Desulfitobacterium* spp. were detected via qPCR and FISH in every

enrichment. An increase in the 16S rDNA amount of Desulfitobacteria was observed until the second or third sub-cultivation step. During the later sub-cultivation steps, a gradual loss of Desulfitobacteria was observed in the enrichments, probably due to out-competition by e.g. acetogenic bacteria. The amount of *Desulfitobacterium* species enriched in every culture was dependent on the soil type used for inoculation. We conclude that *Desulfitobacterium* is a ubiquitous genus in forest soils and that it is involved in the *O*-demethylation of phenyl methyl ethers in the environment. Further experiments will aim at characterizing the microbial community in sampled soils and the enriched microbial communities to shed light on the *O*-demethylating *Desulfitobacterium* species and the associated microorganisms.

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DEP38

Does iron sulfide precipitation impede growth of sulfate reducers in semisolid media?

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Microbial sulfate reduction leads to the generation of hydrogen sulfide which, in turn, reacts readily with ferrous or ferric iron to form iron sulfides. The precipitation of minerals as a consequence of metabolic activity is often regarded to impede growth unless the microorganisms find a way to escape mineral encasement. Precipitation preferentially occurs on cell surfaces and extracellular biogenic structures. Planktonic cells may avoid this fate as motility or mixing of the solution prevents the buildup of high concentrations of metabolites in the vicinity of the cell. In contrast, in diffusion controlled microenvironments - as they occur in biofilms and/or sediments - mineral precipitation happens at the site of highest metabolic activity, i.e. in the vicinity of the cells. In order to learn more about iron sulfide formation in sessile sulfate reducing communities and to elucidate possible mineral-cell interactions, isolates obtained from sediments of an acidic pit mining lake were grown in semisolid, artificial pore water medium posed to pH 5. Hydrogen and carbon dioxide served as electron donor and carbon source, respectively. To visualize the distribution of minerals and cells within the bacterial colonies, thin sectioning was performed followed by staining with nucleic acid specific fluorescent stains and confocal laser scanning microscopy (CLSM). Mineral precipitates were detected in the CLSM-reflection mode. The typical colony shape was lenticular or derivatives thereof, often with a brightly fluorescent outer rim indicating a high cell density and probably high activity. Occasionally colonies exhibited a pronounced layered structure. An inner core of cells with minerals randomly distributed among them was enclosed by a mineral rich layer with only few cells present, followed by a densely populated outer layer devoid of minerals. Iron sulfides did not appear to precipitate on cell surfaces but rather in the bulk phase. Here, they formed larger mineral particles apparently 'pushing' the cells aside. Cells 'pushed' to the outside allowed continuation of colony growth. As a consequence, instead of inhibiting growth, the formation of iron sulfide seems to rather support growth as it diminishes hydrogen sulfide concentrations and thereby preventing the accumulation of toxic concentrations.

DEP39

Regrowth of *P. aeruginosa* in drinking water systems is promoted in absence of an autochthonous biofilm population

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Introduction: Household installations may play a substantial role in waterborne diseases since they can provide favorable conditions for persistence and growth of pathogens. In contaminated drinking water systems an extreme regrowth of *P. aeruginosa* was observed shortly after disinfection measures were performed. This unexpected phenomenon deteriorates the hygienic state in the drinking water system and may lead to a risk to the consumer. Following important questions arise: What is the cause of this extreme regrowth, which impact factors play a key role and by which measures can this be prevented?

Materials and Methods: Close-to-practice experiments: Semi-industrial test rigs with commonly used pipe materials were used in long term experiments simulating household drinking water systems. After establishing an autochthonous drinking water biofilm in the pipes, contaminations with *P. aeruginosa AdS* were conducted. After 7 weeks a cleaning and subsequent disinfection were performed. Concentrations of *P.aeruginosa* in biofilm and water phase were monitored by using standard cultivation methods and qPCR. Laboratory experiments: To simulate the situation in a pipe with upstream contamination source, shortly after disinfection, a defined number of *P. aeruginosa* was introduced into the pipes containing an intact biofilm and pipes with a biofilm damaged by disinfection. After 16 h stagnation at 37°C, biofilm and water phase were analyzed.
Results: Close-to-practice test rigs: compared to the initial state a strong regrowth of *P. aeruginosa* was observed under specific conditions (37°C, elevated nutrient concentration) shortly after disinfection procedures were completed. The lab experiments showed that in presence of an autochthonous biofilm *P. aeruginosa* didn't grow significantly in the water phase while about 10 % of the pathogens remained in the biofilm. In disinfected biofilms, *P. aeruginosa* multiplied fast in the water phase and reached 10 to 100fold concentrations compared to results from pipes with intact biofilms. The numbers of *P. aeruginosa* on the pipe surfaces increased by a factor of 10 to 1000 compared to the numbers in intact biofilms.
Conclusions: The presence of an autochthonous biofilm can limit the growth of *P. aeruginosa* in drinking water systems while the pathogen is able to multiply rapidly to high numbers in absence of such a biofilm. The biofilm bacteria seem to be competitors for nutrients and space on the pipe surface. If the biofilm is removed or damaged by disinfection, *P. aeruginosa* is the r-selected species and exhibits strong regrowth in the whole drinking water system. These findings emphasize the importance of removal of the source for contamination before disinfection measures are performed. Otherwise the hygienic situation in a drinking water system may worsen.

This work was financially supported by BMBF grant number 02WT1154.

DEP40

SchussenAktiv*plus*: elimination of fecal indicator bacteria in advanced wastewater treatment - an application-oriented field study at the Lake Constance tributary Schussen

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With respect to the European Water Framework Directive, the increasing release of micropollutants and pathogens into surface waters via wastewater treatment plants (WWTPs) should be reduced by additional purification processes. Within the joint research project SchussenAktiv*plus* [1] the efficiency and practical suitability of technologies like ozonation or active charcoal filtration were assessed in a field survey at the river Schussen, an important tributary to Lake Constance (drinking water reservoir, recreational and natural protection area). The microbiological exposure analyses presented here attempts to determine the efficiency of different forth sewage treatment with regard to the release of fecal indicator bacteria (FIB) into the environment. In total, five existing test systems (three WWTPs of different size and two storm water overflow basins (SOB)) and environmental samples from the rivers Schussen and Argen were monitored within a triennial timespan (2012-2014) before and after application of different sewage and rainwater treatment technologies (combinations of ozonation with different sand and charcoal filters, powdered activated charcoal filter, a lamella separator connected to a SOB, a retention soil filter). *E. coli*, intestinal enterococci and total viable count were quantified using standard plate techniques in raw wastewater, effluents of the different sewage cleaning stages as well as river water and sediments.
Sampling will be completed in summer 2014 and we will present the final data set. Up to now evidently is that WWTP after implementation of advanced technologies were more efficient to different degrees in reducing FIB than before. The SOB equipped with a lamella separator was inefficient in reducing FIB, while the retention soil filter possess a good natural elimination efficiency comparable with a WWTP. Finally, the results of microbiological analytic will contribute to the holistic approach of SchussenAktiv*plus* providing a scientifically proven concept for advanced waste - and rainwater treatment that reduces the risks for human and environmental health.

1. Triebskorn, R. et al., Environmental Sciences Europe 2013, 25:2

DEP41
Isolation of previously unknown marine bacteria from the Channel Sea

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Among microorganisms, bacteria show the highest diversity. So far this diversity is not sufficiently accessible due to the low cultivation success for most bacteria. This is particularly true for marine samples with low culturability values often below 0.1%. At the same time, the high fraction of physiologically active bacteria in natural marine samples indicate that these bacteria are capable of dividing and that previous cultivation methods are not optimally suited to meet the growth requirements of these microorganisms. The focuses of the current study conducted within the framework of the European MaCuMBA project was to assess cultivation protocols targeting members of different bacterial groups, combining cultivation-independent and -dependent methods. A suite of Channel sea water samples were investigated. Two new oligotrophic media based on artificial sea water with trace elements were developed, one with trace of complex carbon substrates (glucose, peptone and yeast extract), and another one amended with a mix of 5 different polymers (chitin, curdlan, pectin, cellulose and xylan). So far 110 strains were isolated, 103 from the medium with trace of carbon substrates and 7 from the polymer mix medium. The strains were identified on their 16S rDNA V3 region. Our method permits to isolate bacteria belonging to the *Bacteroidetes, Alpha-, Epsilon-* and *Gamma-proteobacteria* phyla. Among other isolates, 4 new strains belonging to the *Epsilonproteobacteria* were isolated. These bacteria are related to *Arcobacter nitrofigilis* DSM 7299 but according to DNA-DNA hybridization results belong to two different *Arcobacter* new species. The physiological, genomic and proteomic characteristics were determined and compared to their specific ecological niche (salinity, temperature, carbon sources).

DEP42
`*Candidatus* Rickettsiella isopodorum´, a new intracellular bacterium associated with woodlice in Europe and North America

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Introduction: The taxonomic genus *Rickettsiella* (*Gammaproteobacteria; Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. While *Rickettsiella* are traditionally described as arthropod pathogens, mutualistic relationships to the host have been described recently. The genus *Rickettsiella* currently comprises four recognized species. However, numerous *Rickettsiella* isolates or pathotypes await conclusive species assignment.
Materials and Methods: Isopod associated *Rickettsiella*-like bacteria have been investigated by light and electron microscopy, and phylogenetic reconstruction using a previously developed multilocus sequence analysis (MLSA) scheme has been combined with likelihood-based significance testing in the molecular systematic description of these isolates.
Results: Ultrastructural together with genetic evidence is provided for a *Rickettsiella* bacterium occurring in Germany in the common rough woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae). The new bacterium is found very closely related to a *Rickettsiella* strain from California that infects the pill bug, *Armadillidium vulgare* (Isopoda, Armadillidiidae). Both bacterial isolates display the ultrastructural features described previously for crustacean-associated bacteria of the genus *Rickettsiella*, including the absence of well-defined associated protein crystals; occurrence of the latter is a typical characteristic of infection by this type of bacteria in insects, but has not been reported in crustaceans. Both bacteria - despite their distant geographic origins - form a tight sub-clade within the genus *Rickettsiella* for all MLSA markers used. In the 16S rRNA gene tree, this sub-clade includes other bacterial sequences from woodlice. These clades are both well bootstrap supported and found highly significant when confidence limits are explored by a one-sided Kishino-Hasegawa test.
Discussion: As the combination of ultrastructural and genetic evidence clearly demonstrates that both woodlice associated bacteria i) belong to the genus *Rickettsiella* and to the same infrageneric taxon therein and ii) are different from each of the four recognized *Rickettsiella* species, a new candidate species has been introduced for their assignment. The designation `*Candidatus* Rickettsiella isopodorum´ has been proposed to refer to this new lineage of *Rickettsiella* bacteria.

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DEP43
Ammonia-oxidizing bacteria as indicator organisms to assess the environmental status of river Elbe sediments

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Lithotrophic ammonia-oxidizing bacteria (AOB) form a functional group which is characterized by the ability to gain energy via oxidizing ammonia to nitrite under aerobic conditions. Together with thaumarchaeal ammonia-oxidizing archaea (AOA) ammonia-oxidizing bacteria are the only guarantors of the first and most limiting step in the nitrification process, making them to key organisms in global nitrogen cycle. With the exception of the marine genus *Nitrosococcus* all AOB belong to a monophyletic group within the Betaproteobacteria. Comparisons between the phylogenetic substructure of these betaproteobacterial AOB, their ecophysiological properties and distribution patterns revealed that parameters like NH_3-affinity, tolerance against ammonia, capability to utilize urea as ammonia source and salt requirement are particularly well suited to reflect phylogenetic processes (Koops & Pommerening-Röser, 2001). Because of their ecological importance, their physiological uniqueness and monophyletic nature betaproteobacterial AOB have been considered as “a model for microbial microbiology” (Kowalchuk & Stephen, 2001). The study presented here can be taken as a proof of principal. The diversity, structure and dynamic of AOB-populations in the longitudinal course of the river Elbe was examined via illumina-based sequence analyses using ammonia monooxygenase gene sequences as marker. Our results revealed significant distribution patterns of AOB populations and thus allow conclusions on specific characteristics of the habitats.

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DEP44
Lighting up the dark matter of snottite biofilms

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Acid Mine Drainage (AMD) is an environmental threat, which endangers large zones around mining areas. Microorganisms play the major role in these processes. They catalyze the rate limiting step in pyrite oxidation, the reoxydation of ferrous to ferric iron. In the end, the oxidation of pyrite leads to acidification of the mine water and thus to the dissolution of heavy metals. Understanding the microbial consortia in AMD areas is important for prevention of this problem. In this work acidic snottite biofilms of an abandoned pyrite mine in the Harz Mountains (Germany) were analyzed. They are growing in stalactite-like structures and ferrous-sulfate-rich water with a pH of 2.3 drops constantly through these snottites. The biofilm matrix contains carbohydrates and bio-/geochemical products of pyrite oxidation. Sulfate and iron concentrations are up to 200 mM and 60 mM, respectively. XANES measurements showed jarosite as the major mineral element. So oxidative dissolution of pyrite is the primary energy source of the consortium. $^{14}CO_2$-fixation measurements indicate carbon fixation mainly in the outer parts of the snottites. *In situ* oxygen-measurements showed a

rapidly declining oxygen-content. A 700 µm width rim surrounds the large anoxic core of the biofilm. 16S rDNA analyses showed a composition of bacteria predominantly consisting of *Acidithiobacillus* and *Leptospirillum*. Uncultured *Thermoplasmatales* and ARMAN represent the majority of the archaea. XANES spectra as well as CARD-FISH-pictures in comparison with the oxygen measurements indicate that the most important factor for the distribution of bacteria and archaea is oxygen. *Acidithiobacillus* occurs all over the biofilm, whereas *Leptospirillum* species where only found in the oxic outer parts. Surprisingly so far uncultured *Thermoplamatales* and ARMAN could only be detected in the anoxic inner parts. With these results the following hypothesis can be proposed: Lithotrophs like *Leptospirillum* and *Acidithiobacillus* oxidize ferrous to ferric iron. Reduced sulfur species could be oxidized by *Acidithiobacillus* species to sulfate. Together with ferric iron sulfate could be reduced by *Ferroplasma* and some of the uncultured *Thermoplasmatales*. Therefore, the biofilm contains a full iron and sulfur cycle within a micrometer scale habitat. Based on these analyses we designed an anoxic medium for the enrichment of the uncultured archaea. It contains $Fe(III)SO_4$ as well as an organic carbon source. The head space has an H_2/CO_2 atmosphere. CARD-FISH pictures and 16S rDNA analyses show the enrichment of so far uncultured *Thermoplasmatales*. After a prolonged incubation time, to our surprise, even a good development of ARMAN could be detected. Therefore, against the so far existing results a facultatively anaerobic metabolism for the ARMANs can be postulated.

DEP45
VBNC state of pathogens associated with biofilm bacteria typical for potable water

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Pathogens like *Legionella pneumophila* and *Pseudomonas aeruginosa* which are known for their ability to persist in house installations pose a risk of infections for humans. The standard approach for detection is still cultivation on selective media. However, these bacteria may be in a physiological state called "viable-but-not-culturable" (VBNC) and thus remain undetected by this method. The aim of this work is to gain insight in which manner the viability of *P. aeruginosa* / *L. pneumophila* is influenced by several *Aquabacterium* strains with respect to VBNC. These strains were isolated in our group as primary biofilm formers from water distribution biofilms and are known to enter the VBNC state due to nutrient limitations. A continuous flow through biofilm reactor was used to simulate a low nutrient environment and to cultivate mono-species / multi-species biofilms containing above-mentioned pathogens. Culturing (e.g. Heterotrophic Plate Count) and culture-independent methods for identification of bacteria and for differentiation between live and dead cells like "Live/Dead"- essay, PAC (a direct-viable-count method), (PMA)-qPCR and FISH were compared. The conversion of *Aquabacterium* into VBNC was observed (around 60 % not culturable) and the consequence for co-cultured *P.aeruginosa* / *L. pneumophila* was estimated and examined. It was found, that three *Aquabacterium* strains converted into a VBNC-state under oligotrophic conditions. *P. aeruginosa* did not enter a VBNC state as response to nutrient limitation while *L. pneumophila* did when grown alone or in mixed biofilms.

DEP46
Microbial and chemical monitoring of basin water in different leech breeding systems

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Medicinal leeches are blood sucking Annelida of the order *Hirudinis* which are applied as natural medical treatments in human therapy. Leeches occur naturally in freshwater systems and are breeded in water tank systems. Several blood feedings are normally required before the leeches have the medical treatment size. Leeches cultured in water basins with relative high numbers of individuals are often susceptible for diseases, especially after blood meals. Water associated microbes and chemical water parameters play an important role for the health status of leeches. Controlled water quality is therefore a prerequisite for efficient leech breeding systems.
The aim of our study was to compare three different water systems for leech water basins over the time of blood feeding to establish an optimized system for leech breeding. Water systems with an additional sand filter beside the biological filter, with unaffected water and water in which the pH was decreased either chemically or biologically were compared over a 17 week period by sampling every three weeks. After 6 weeks leeches were fed with horse blood. Lactose peptone medium (DEV) was used to determine the concentration of culturable bacteria in the water tanks. Most abundant colonies were purified and identified by 16S rRNA gene sequencing. Total cell numbers in tank water were analyzed by SybrGreen I staining. Chemical water parameters were daily monitored.
Water parameter after blood feeding showed a rapid increase of ammonia followed by an increase of nitrite and nitrate. The concentration of culturable bacteria were for all water systems in the range of 10^3 to 10^4 CFUs mL^{-1} with only a slight increase after blood feeding in the chemically decreased water pH system. No significant differences were obtained for total cell numbers. Clear differences were obtained with respect to most abundant taxa. In the neutral pH system, *Sphingopyxis*, *Flavobacterium*, *Nocardia*, and *Rhodococcus* spp. were most abundant before, and *Flavobacterium*, *Sphingopyxis* and *Ochrobactrum* spp. after blood feeding. In the water with chemically decreased pH values, the compositions were not changed after feeding and *Flavobacteriu*m, *Ochrobactrum*, and *Chryseobacterium* remained most abundant. In contrast in the biologically decreased water pH system, most abundant colonies represented *Rhodococcus*, *Norcardia*, *Chryseobacterium* and *Ochrobactrum* spp. before feeding, but only *Chryseobacterium* spp. were detected after blood feeding. The fractions of deceased leaches were highest in the biologically decreased water pH system. First results of our study indicated, that changes in the compositions of water associated bacteria after blood feeding are affecting the leech health.

DEP47
Controls of soil microbial functions and community composition in Subsaharan savannah soils

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Savannah ecosystems cover a significant fraction of Earth´s terrestrial surface and are widely used for agriculture. The savannah soils of the African Okavango basin typically show a low fertility but are exploited heavily for subsistence farming. In the present study, exoenzymatic activities, ammonification and nitrification rates, and the effects of nutrient addition on resident soil microorganisms were examined to identify key microbial processes of nutrient cycling in the different soils and under different land use. The first step in the degradation of soil organic matter (SOM) as an important absorber and nutrient source is effected by the activity of exoenzymes (i. e. aminopeptidase, β-glucosidase, β-xylosidase, and phosphatase). The exoenzyme activity was determined with fluorescently labeled substrate analogues in several sampling sites differing in land use type (woodland, bushveld, fallow, slash and burn, drought agriculture, irrigation agriculture, horticulture) and soil type (peatland, old flood plains, Kalahari Sands) with highest activity values in the nutrient rich peatland soils of the Angolan highlands and the riparian woodland and bushveld soils of the old flood plains in Namibia and lowest in the nutrient poor sandy soils and the agriculturally used soils of Angola and Namibia. As nitrogen is a major important nutrient in Subsaharan savannah soils, nitrogen liberating processes as ammonification and nitrification were quantified in ten soils differing in land use type by the Pool Dilution Technique. Highest nitrogen turnover rates were observed in the pristine woodland soils and decreased with increasing anthropogenic impact on the soils, reaching lowest values in agriculturally used soils. First results of metatranscriptomic analysis of Namibian soil samples supplemented with different nutrients containing carbon, nitrogen and phosphorus sources also showed a negative effect of drought and land use on the community composition and activity of the soil microbial community. These results have implications for future sustainable land use management in the Okavango basin.

DEP48
Employing the carrying capacity to describe the microbial community properties of pristine groundwater

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Groundwater ecosystems are an essential resource for our drinking water and at the same time constitute fascinating habitats hardly explored and faced by a lot of (anthropogenic) disturbances. We look for ways to qualitatively and quantitatively access, and predict the resistance and resilience (potential) in consequence of such disturbances. As a central goal

we hope to identify and quantify the underlying biological and ecological key drivers of the microbial Carrying Capacity (MCC) and/or microbial Standing Stock (MSS) directly connected to the resistance and resilience of groundwater ecosystems of different productivity. We hypothesize, that the ecosystems' MCC and/or MSS, which is assumed a result of available energy and system productivity, constitutes a promising proxy for the potential of groundwater ecosystems to withstand impacts and recover from it. The MSS of the groundwater ecosystems can be determined at any time by counting the total number of microbes. In consequence, the MCC can be estimated based on available energy (e.g. assimilable organic carbon) and growth rates. Now, the link to the ecosystem's resistance and resilience needs to be established. In a first approach the growth parameters of a single species model will be investigated, expanding to the whole community of a pristine groundwater aquifer, in 2-D sediment flow-through systems. Varying parameters will be different concentrations of natural DOC and flow velocities. Later we will expend this approach by using bacteriophages as a proxy for 'top-down' effects to assess the influence of these effects on the prokaryotic community. In any case, it has to be considered, that groundwater ecosystems harbor both 'mobile' (free floating) and 'sessile' (sediment attached) microbial communities.
Adopted from 'chemical engineering', the 'Damköhler number', a dimensionless number that relates the reaction timescale (here: reaction rate with respect to the substrate) to the residence time (here: substrate transport rate) in a system, might be a promising tool to classify groundwater systems according to its productivity and following from this its resistance and resilience power.

DEP49
Diversity of the microbial community in volcanic soils from South of Chile

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Soil microbes play important roles in maintaining ecosystem functions. Volcanism in Chile is a continuous process that has a strong influence on landscape and geology. Llaima volcano is one of the largest and most active volcanoes in Chile. During its history, Llaima has had three detailed lava eruptions at different sites of the volcano. The aim was to evaluate the structure of total microbial community composition at these sites with different defined age and soil characteristics, to obtain information regarding recolonization by Bacteria and Archaea after a lava flow. Soil was collected from three vegetated sites recolonized after lava eruptions in 1640, 1751 and 1957. Bacterial and archaeal 16S rRNA genes were analyzed by 454 high-throughput pyrosequencing. These data indicated major differences in microbial diversity and community composition in the different soils. Diversity increased with soil age, particularly between the 1957 and 1640 soils. Thaumarchaeota were detected in all soils but Euryarchaeota only in the oldest ones. Dominant bacterial phyla included Proteobacteria, Actinobacteria, Acidobacteria and Chloroflexi. Chloroflexi were lowest in the oldest soils. In conclusion, we show that there is a slow reestablishment of the microbial community in volcanic soils following an eruption and that specific microbial groups play a role in the early stages of recovery.

DEP50
Molecular characterization of the ammonia oxidizing community in a pre-alpine freshwater lake (Lake Constance)

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Microbially driven ammonium oxidation to nitrite is the rate limiting step in nitrification and as such an important part of the global nitrogen cycle. This process has been extensively studied in marine and soil environments but is not yet well understood in freshwater ecosystems. Although nitrification does not directly change the inventory of fixed N in freshwater ecosystems, it constitutes the only known biological source of nitrate and as such a critical link between organic N and its eventual loss as N_2 by denitrification or anaerobic ammonia oxidation to the atmosphere. In addition, it changes the quality of N available for assimilation by plants, phyto- and bacterioplankton since the energy necessary to assimilate nitrogen is lowest for NH_4^+-N and increases for NO_3^--N. Thus, nitrification has a direct impact on primary producers in ecosystems such as freshwater lakes. Our primary goal was to characterize the ammonia oxidizing microbiota, which typically consists of both ammonia oxidizing archaea (AOA) and bacteria (AOB), in Lake Constance as a model for large oligotrophic freshwater lakes. The water column of Lake Constance was sampled at four distinct depths which span from the epilimnion through the metalimnion to the hypolimnion. Initially, two different DNA extraction procedures were tested that either used beat beating or enzymatic treatment for cell lysis. Thereafter, clone libraries based on the *amoA* gene (coding for the alpha-subunit of the ammonia monooxygenase) were constructed to capture both AOA and AOB species richness. *amoA*-based T-RFLP analysis extended this approach by capturing the (semi-) quantitative composition of the AOA and AOB community. In future, we plan to extend these analyses by following the ammonia oxidizing microbiota throughout the annual cycle of plankton succession and by correlating ammonia oxidizing activity to the captured AOA and AOB diversity.

DEP51
Quantification and activity of the methane oxidizing bacterial community in the river Elbe estuary

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Rivers represent a transition zone between terrestrial and aquatic environments, as well as a transition zone between methane rich and methane poor environments. Methane concentrations are generally higher in freshwater systems than in marine systems. The Elbe River is one of the crucial drainages into the North Sea and by this high amounts of methane are imported into the marine water column. Oxidation of methane by aerobic methanotrophic bacteria is the major biological sink. Six cruises from November 2013 until June 2014 were conducted along the salinity gradient from Hamburg towards Helgoland. Methane oxidation rate was measured with radiotracers and the abundance of methanotrophic bacteria was assessed via real-time PCR. A newly designed primer targeting the genomic sequence encoding the α-subunit of the functional pMMO enzyme in water column organisms was amplified and tested against the conventional primer set.
At the marine stations the cell number was relatively stable with 3×10^4 cells per L, while in the Elbe cell numbers ranged between 10^3 - 10^6 cells per L. Environmental parameters (temperature, salinity, SPM) seemed to have no influence on the abundance. However the interaction between activity and abundance seemed to be more complex.

DEP52
Interactions of Ag NPs with near-natural lotic biofilms

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The application of engineered silver nanoparticles (Ag NPs) in a wide range of consumer products finally results in their release into sewage systems and subsequently into the aquatic environment. Therefore, biofilms as the prominent life form of microorganisms in natural running waters may be impacted by Ag NPs leading to a potential impairment or loss of essential ecosystem functions ensured by microbial biofilm communities. In order to obtain ecotoxicological insights, mesocosm experiments were performed. Biofilms were grown in stream mesocosms containing water and sediment from the river *Rhine* and were subsequently exposed to two different sizes of citrate-stabilized Ag NPs (30 and 70 nm in size) in a concentration of 600 µg/l for 14 days. The physico-chemical parameters such as temperature, flow velocity, pH, O_2, electric conductivity, total phosphor, nitrate, nitrite and ammonium were closely monitored throughout the entire duration of the experiment. In respect of physico-chemical parameters, conditions were alike in treated and untreated systems. The biomass parameters (chlorophyll *a* and protein as well as gravimetric measurements) indicated no difference between the control biofilms and the biofilms exposed to Ag NPs. In contrast, T-RFLP fingerprints based on 16S rRNA gene amplicons indicated that Ag NPs lead to an altered bacterial community structure in the biofilm. Sequence analysis of cloned 16SrRNA genes revealed that changes in community structure were coming along with considerable displacements of bacterial taxa, such as the emergence of *Pseudomonas putida* in treated biofilms. Overall, a shift in bacterial community structure may indicate a change in metabolic and ecophysiological capabilities and hence ecosystem functioning.

DEP53
Characterization of a thermophilic electrolithoautotrophic growing consortium

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Microbial electrosynthesis is a form of metabolism that was recently discovered. The term describes the capability of some organisms to use electrical energy provided from a cathode as a source for metabolic electrons. This electron uptake can be coupled to the fixation of carbon dioxide, which results in electrolithoautotrophic growth. The biochemistry behind this process is so far unknown, but some methanogens as well as homoacetogens were shown to thrive using this strategy. It is the aim of this work to isolate novel electrolithoautotrophs from environmental samples with a particular focus on thermophiles. Hence, the inoculum for our enrichments was a mixed sample from hydrothermal systems. The enrichment was performed in a 2 L self-designed three electrode setup reactor that was incubated at 60°C and continuously purged with a N_2/CO_2 gas mixture. A carbon cloth cathode was inserted into the reactor and was poised to a constant potential of -350 mV vs. SHE. The system runs for nine months and meanwhile shows a constant current of -1.1 mA. Products of microbial metabolism could be monitored. The community in the electrosynthesis reactor comprises a mixture of archaea and bacteria. To gain further insight into the processes that take place inside the reactor system we now focus on elucidating the composition and capabilities of the microbial consortium on the cathode with the use of metagenomics. Metatranscriptomic analysis will be applied to detect contributing biochemical pathways. With the results of the meta-analysis we aim for a reverse engineering of an electroautotrophic growing reactor by imitating the community with distinct isolated organisms. The ultimate goal would be to biotechnologically engineer both process and organisms in a way that allows a targeted production of chemicals of industrial value.

DEP54
Exploring functional contexts of symbiotic sustain within lichen-associated bacteria supported by the *Prophane* bioinformatics pipeline

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Symbioses represent a frequent and successful lifestyle on earth and lichens are one of their classic examples. Recently, bacterial communities were identified as stable, specific, and structurally integrated partners of the lichen symbiosis, but their role has remained largely elusive in comparison to the well-known functions of the fungal and algal partners. We have explored the bacterial contributions to the symbiosis using the lung lichen *Lobaria pulmonaria* as the model. Metaproteomic data analysis were supported by *Prophane* (http://www.prophane.de) exploring the functional and taxonomic contexts of all identified proteins. Metagenomic data were comparatively assessed. We have found that more than 800 bacterial species have the ability to contribute multiple aspects to the symbiotic system, including essential functions such as i) nutrient supply, especially nitrogen, phosphorous and sulfur, ii) resistance against biotic stress factors (*i.e.* pathogen defense), iii) resistance against abiotic factors, iv) support of photosynthesis by provision of vitamin B_{12}, v) fungal and algal growth support by provision of hormones, vi) detoxification of metabolites, and vii) degradation of older parts of the lichen thallus. Our findings suggest that the bacterial microbiome of lichens contributes to the health, growth, and fitness of their hosts. We argue that the strategy of functional diversification in lichens by multi-player networks supports the longevity and persistence of lichens under extreme and changing ecological conditions.

DEP55
Isolation of acetic acid, propionic acid and butyric acid producing bacteria from biogas plants

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In recent years, the production of biogas from renewable resources and agricultural wastes aiming a sustainable and CO_2-neutral energy generation increased vigorously [1]. Biogas plants contain a complex and dynamic microbial community that causes the anaerobic degradation of organic biomass to biogas. In order to improve the process of the biogas production, the knowledge of the concomitant microorganisms in biogas plants is important. Up to now, the majority of these microorganisms is unexplored. In the past, molecularbiological methods have been applied to get an insight into the diversity of microbiota. Metagenome analyses revealed that many microorganisms have been not classified so far [2]. Because of the small amount of isolates from biogas plants there is a lack of microbial reference data. Especially bacteria, which form acetic acid, propionic acid and butyric acid have not been characterized in detail. Therefore, we have isolated fermenting bacteria of the acidogenic step in biogas plants. For the isolation of acid forming bacteria, we took samples from mesophilic (39 °C) and thermophilic (54 °C) biogas plants, which have been fed with renewable substrates (e.g. maize) and swine or cattle manure. The above-mentioned acids can be produced from various intermediate products such as glucose, alcohols (glycerol, ethanol), acids (e.g. lactate, succinate) and amino acids (e.g. glutamate, lysine). Using the deep agar shake method we have been able to isolate a great variety of bacteria from enrichment cultures growing on different carbon sources, which will be presented in the present poster.

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DEP56
The importance of lactic acid for the formation of methanogenic substrates in biogas plants

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The microbial process of biogas formation from renewable substrates has not been enlightened in detail yet. The production of methanogenic substrates, mainly H_2, CO_2 and acetic acid, can be grouped in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Up to now, especially the first two steps are poorly investigated. The concentration and composition of organic acids may influence the process stability, degradation rate and methane production. Under certain conditions, lactic acid is an important precursor of other acids, e.g. propionic acid. In higher concentrations propionic acid leads to a disturbance of the whole biogas process and it is therefore suitable as an indicator for digester failure (Nielsen et al., 2007). In this project, five mesophilic biogas plants (39°C) with different substrate feeding strategies were examined. Substrates were mainly maize and grass silages as well as liquid manure from cattle or pigs. In order to investigate the role of lactic acid in the process of biogas formation, various microorganisms were isolated and tested for their capability to produce lactic acid. Those bacteria which were able to form lactic acid were identified. Up to now six species of the genus *Lactobacillus* and *Pediococcus parvulus* were found. These isolates belong to the group of lactic acid bacteria, which may occur in a number of up to 10^7 cells per ml of digester sample. In this poster the biochemical and physiological features of the isolates will be presented.

Literature

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DEP57
Distribution and diversity of functional marker genes targeting different prokaryotic carbon dioxide fixation pathways in stratified lakes

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While the biochemistry and molecular biology of different CO_2 fixation pathways in prokaryotes are well understood for many isolates under laboratory conditions, the driving forces and biogeochemical processes determining the occurrence of diverse phototrophic and especially chemolithotrophic CO_2 fixation strategies in the environment are mostly unexplored. We selected stratified freshwater lakes as study sites, because these ecosystems are characterized by distinct concentration gradients of oxygen and different redox states of elements such as nitrogen and sulfur, thus allowing the investigation of CO_2 fixation strategies in the ecological framework of measurable habitat heterogeneity. Three autotrophic pathways were selected for investigation: the Calvin-Benson-Bassham cycle, the reductive tricarboxylic acid cycle, and the recently discovered archaeal 3-hydroxypropionate/4-hydroxybutyrate pathway. DNA extracts from 52 samples, obtained from 6 lakes, served as template for the PCR-amplification with 15 different primer pairs targeting a variety of genes coding for selected key enzymes of the three pathways. PCR products from selected samples were used to construct clone libraries and over thousand clone-inserts were subjected to sequencing and phylogenetic analysis. Different forms of RubisCO (IA, IC and II) were found in almost all samples investigated, the phylogenetic affiliation of the genes indicating that stratified lakes are likely to create functional niches occupied by bacteria with different metabolic properties. The reductive tricarboxylic acid cycle was exclusively detected in anoxic and especially in sulfidic zones of the lakes, confirming the environmental requirements of this pathway. Autotrophic mesophilic *Thaumarchaeota* using the 3-hydroxypropionate/4-hydroxybutyrate cycle for CO_2 fixation were common residents in the aphotic and (micro)oxic zone of deep lakes, with putative thaumarchaeal ammonia oxidizers playing a central role. Our results indicate that different chemolithotrophic CO_2 fixation strategies are widespread in lakes, showing distinct distribution and diversity patterns within and across our study sites. In this context, different energy requirements of the pathways, the characteristics of the key enzymes and the distribution of electron donors and acceptors are likely to create unique niches that favor individual CO_2 fixation strategies.

DEP58
Impact of AOP on antibiotic resistant bacteria as part of the wastewater effluent microbiom

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Wastewater treatment plants are one source of microbial contamination. In the light of recent studies there are reports of increased levels of multi-resistant bacteria and antibiotic resistance genes (ARGs) in wastewater treatment plants (WWTPs) and downstream habitats.
Conventional wastewater treatment represents a reservoir for the dissemination of ARGs in the bacterial community. Horizontal gene transfer (HGT) of antibiotic resistance genes contributes to increase abundances of hygienically relevant, antibiotic resistant bacteria in aquatic systems. AOPs as additional wastewater treatment techniques are investigated to decrease the bacterial load and the dissemination of antibiotic resistances in adjacent downstream aquatic habitats. To investigate the efficiency of ozonation, the project involves molecular biology detection and quantification of facultative pathogens and medically relevant antibiotic resistance genes in microbial communities by qPCR analysis in the effluent of an ozonation system. We investigate the impact of different ozon concentrations, reflux systems and filters by molecular quantification of the methicillin resistance gene *mecA* from staphylococci, the ampicillin resistance gene *ampC* from *Enterobacteria*ceae, the transposon mediated vancomycin resistance gene *vanA* from enterococci, the imipenem resistance gene bla_{VIM} from *Pseudomonas aeruginosa*, and the erythromycin resistance gene *ermB* from streptococci. Further, we quantified taxon-specific DNA-marker for the carrier of the mentioned bacteria. Aiming to reduce facultative pathogenic bacteria and antibiotic resistance genes investigations are performed at the WWTP of Darmstadt, where ozonation in combination with different filter units, like MBR, were additionally applied to the routine technology. As expected, most of the hygienically relevant bacteria were reduced during oozonation, but it became obvious that bacteria carrying antibiotic resistance genes showed a considerable robustness and fitness against ozonation. This is indicating a selection-like process favoring already present antibiotic resistant bacteria during oxidative wastewater treatment despite the overall bacterial reduction in the effluent. Further investigations regarding drug-dependent predispositions and different bacterial stress response mechanisms are currently analyzed to characterize the bacterial adaptation.

DEP59
Multi-Carbon Compounds as Substrates for Methanol-Utilizing Microorganisms of a Forest Soil Community

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Aerobic methylotrophs in temperate soils are sinks in the global methanol cycle. Parameters determining their diversity and activity have scarcely been understood. Previous studies revealed a correlation between the vegetation type and diversity of soil methylotrophs in aerated soils. Previously detected genotypes reflected a large diversity even when only gene markers for gram negative methylotrophs were analyzed, i.e., genes of the PQQ-dependent methanol dehydrogenase (MxaFI/XoxF), methylene-tetrahydromethanopterine-dependent cyclohydrolase (MCH), and formaldehyde-activating enzyme (Fae1). Most of the cultivated and soil derived methanol utilizers are facultatively methylotrophs meaning they are polycarbotrophic and utilize multi-carbon compounds, such as mono-, di- and polysaccharides, alcohols, carbonic acids, and aromatic compounds. We hypothesize that the multi-carbon substrate spectrum of methylotrophs is a determinative ecological niche parameter in soil. In the current study, we tested if soil methylotrophs have divergent preferences to soluble substrates that are products of lignin breakdown (vanillic acid), anaerobic organic matter degradation (acetate), or monomers of abundant plant polymers or root exudated sugars (xylose, glucose). In a multi-substrate DNA stable isotope probing (SIP) experiment on forest floor soil samples, methanol-utilizing taxa that responded to acetate, vanillic acid, glucose or, xylose by assimilation of that multi-carbon substrates were identified. The abundance of *mxaF/xoxF*-harboring methylotrophs was evaluated by qPCR to determine growth on tested substrates. The degradation rate of the supplemented substrates acetate, glucose and xylose were quicker than the initial rate of the lignin derivative vanillic acid. Genotypes of bacterial methylotrophs were assessed by pyrosequencing of bacterial 16S rRNA genes and *mxaF/xoxF*. Diversity and abundance of fungal taxa were analysed by ribosomal genes and ITS region to resolve the relevance of yeast and ascomycota species

DIAGNOSTIC MICROBIOLOGY

DVP01
Successful application of Hemoccult® test cards for the detection of gastrointestinal pathogens by multiplex PCR

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Introduction: Up to 60% of international travelers experience episodes of travelers` diarrhea (TD). Reliable data on the etiology of TD is lacking, as comprehensive laboratory testing is mostly not feasible in the destination countries. Important pathogens in the genesis of TD include for example Campylobacter spp., especially in South and Southeast Asia. Norovirus is responsible for 10 to 15% of TD cases. Especially in countries with a low hygienic standard, infections with Entamoeba (E.) histolytica can be acquired. By the application of Hemoccult® test cards (Beckman Coulter®) travelers can easily acquire stool samples for diagnostic testing in their home countries. By using multiplex PCRs, for example the Gastrointestinal Pathogen Panel® (GPP, Luminex), a wide range of viral, bacterial and protozoal agents responsible for TD can be simultaneously detected.

Aims of the study: In this study the application of Hemoccult® test cards for stool sample acquisition was evaluated in combination with testing for model gastrointestinal pathogens by GPP. A travel duration of up to 6 weeks was simulated by storing the samples on Hemoccult® test cards.
Materials and Methods: Three negative stool samples were spiked with gastrointestinal pathogens to achieve a defined concentration of Campylobacter spp. and E. histolytica in the samples. Three norovirus G I- and three G II-stool samples were acquired from a reference laboratory. These samples had already been tested by real time PCR (CT values ranging from 11 to 23). Each sample was brought onto both detection fields of a Hemoccult® test card. Altogether seven Hemoccult® test cards were created for duplicate testing at the start of the study and then in weekly intervals for up to six weeks. The test cards were stored at room temperature. DNA/RNA extraction was done by using the NucliSENS miniMag®. The detection of gastrointestinal pathogens was achieved by GPP in combination with the Luminex MAGPIX® system.
Results: Following the extraction of the stool samples on Hemoccult® test cards the detection rate for Campylobacter spp. was 97.6%, for E. histolytica 100%, and for norovirus G I and GII 97.6% respectively 100%. Gastrointestinal pathogens could be reliably detected at weekly intervals up to the end of the study at day 42. Even samples on Hemoccult® test cards with pathogen concentration at the limits of detection of the GPP could be successfully tested.
Conclusion: Hemoccult® test cards could be used as a reliable tool for stool sample acquisition and testing for gastrointestinal pathogens in combination with a commercially available multiplex PCR. DNA and RNA were stable on the test cards and the samples could be successfully tested for up to 42 days. The examination of stool samples on Hemoccult® test cards seems therefore to be an effective and promising tool to acquire reliable data on the etiology of TD. Possible uses also include the evaluation of vaccines against TD pathogens or the study of the effectiveness of antibiotic prophylaxis for travelers.

DVP02
Simplified Detection of *cfiA*-positive *Bacteroides fragilis* by MALDI-TOF MS and Prevalence of these Carbapenemase-producing strains in a German Medical Laboratory

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Objectives: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is widely used for identification of microorganisms in clinical microbiology. Recent publications indicate that MALDI-TOF MS can be used to differentiate *cfiA*-negative and *cfiA*-positive *Bacteroides fragilis* strains. As the latter produce a metallo-ß-lactamase causing decreased susceptibility against carbapenemes this may have a clinical impact.
Here, we evaluated a new algorithm for detection of *cfiA*-positive *B. fragilis* isolates during standard identification process. Furthermore, we screened past data for the occurrence of such strains in an independent German laboratory.
Materials and Methods: Based on characteristic peaks a mathematical algorithm was developed. A "log(IQ)" score calculated by this algorithm indicates the respective *B. fragilis division*, with or without the *cfiA* gene, based on the specific peak pattern.
Mass spectra were acquired with a microflex LT instrument and MALDI Biotyper Software version 3.1 (Bruker Daltonik, Germany). Spectra of strains identified as *B. fragilis* in routine diagnostics of our laboratory were additionally evaluated with the new log(IQ) score. For susceptibility testing meropenem Etest® strips (bioMérieux, Marcy l'Étoile, France) were used with EUCAST breakpoints. Prevalence of *cfiA*-positive strains was determined by applying the log(IQ) algorithm to all recorded spectra since year 2009. Evaluation of the spectra and statistical analysis was conducted using custom Python scripts, the R statistical package and Microsoft Excel.
Results: Between August and December 2013 a total of 116 *B. fragilis* strains were analyzed. 8 spectra (6.9%) yielded to positive log(IQ) values > 0.5 indicating the presence of *cfiA*. These strains also showed elevated MICs in susceptibility testing against meropenem (14.5 g/L mean, range 1-32 g/L) compared to the strains flagged *cfiA*-negative by log(IQ) (0.15 g/L mean, range 0.064-1.5 g/L). All *cfiA*-negative isolates were susceptible according to EUCAST breakpoints. 10 additional log(IQ) positive isolates were tested in 2014, again showing decreased susceptibility against meropenem with a mean MIC of 1.7 g/L (range 0.5-4 g/L).
Retrospective loq(IQ) analysis of spectra acquired in routine diagnostics in the years 2009 to 2014 from 3716 *B. fragilis* strains resulted in 202 presumably *cfiA*-positive cases alluding to a prevalence of about 6.1%.
Discussion and Conclusion: In this study we evaluated a new algorithm for detection of *cfiA*-positive *B. fragilis* isolates by MALDI-TOF MS. The log(IQ) algorithm can easily be applied automatically during identification process. As *cfiA*-positive strains show higher MICs against carbapenems this can be important for early therapeutical decisions and should be further validated. Our data indicate that about 6% of *B. fragilis* strains may be *cfiA*-positive.

DVP03
Optimization of Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) database for rapid identification of staphylococci composing the *Staphylococcus intermedius*-group (SIG)

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Introduction: Among coagulase-positive staphylococci (CPS) of companion animal origin, staphylococci composing the *Staphylococcus intermedius*-group (SIG) are common opportunistic pathogens, capable of causing a wide range of different purulent and toxin-mediated diseases in dogs, cats and horses. While there is a rising rate of methicillin-resistant *S. pseudintermedius* (MRSP) among microbiological specimens noticeable, phenotypic species identification techniques for the distinct members of the SIG might be more or less imprecise and time-consuming. In recent years, first severe infections with multi-drug resistant MRSP were also reported for humans. A fast and reliable identification of SIG, for instance by use of matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS), is necessary to disclose sound identification rates in both veterinary and human medicine.
Materials and Methods: As a first step, a reliable reference database for spectra associated with the different members of the SIG was created using isolates unambiguously identified by gene-based methods. A total of 27 MALDI-TOF MS spectra were acquired for each isolate of the following species: *S. pseudintermedius* (n = 43, including 20 MRSP), *S. intermedius* (n = 5) and *S. delphini* (n = 12) and a reference library was set up with BIOTYPER 3.0 software (Bruker Daltonics, Bremen, Germany).Subsequently, a broad convenience sample consisting of 200 CPS strains was evaluated using a) the original database content and b) the database after extension with distinct hierarchical clustered reference spectra for 60 SIG.
Results: A significant improvement (average rise of log score value: 0.24) of the SIG identification score values was obtained for 200 SIG. Moreover, for 17 isolates the initial identification as "*S. intermedius*" changed to "*S. pseudintermedius"* as best match with improved score values by applying the in-house reference spectra extended database version.
Conclusion: Data presented here highlight the opportunities of sequence-based refinement of the Bruker database content with respect to improvement of MALDI-TOF MS-based bacterial species identification.

DVP04
MALDI-TOF Mass Spectrometry Following Short-Term Incubation on Solid Medium is a Valuable Tool for Rapid Pathogen Identification from Positive Blood Cultures

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Introduction: In bloodstream infection, early onset of effective antimicrobial therapy is critical to a favorable patient outcome. Thus, rapid identification of the causative microorganism is essential. Although MALDI-TOF Mass Spectrometry (MALDI-TOF MS) has already proven useful for accelerated pathogen identification, traditional analysis of positive

blood cultures requires subculturing over at least 16-24 h, while subculture-independent sample preparation procedures enabling direct MALDI-TOF MS (e.g. Sepsityper Kit, Bruker Daltonics) are associated with additional effort and costs. Hence, we integrated an alternative MALDI-TOF MS approach in diagnostic routine using a short-term incubation on solid medium.
Materials and Methods: Positive blood cultures (in total n=553, BacT/ALERT, bioMérieux, collected from 319 patients including 377 different isolates) were routinely plated on chocolate agar plates (bioMérieux) and incubated for 4.0-4.5 h (37°C, 5 % CO_2). Subsequently, MALDI-TOF MS using a Microflex LT instrument (Bruker Daltonics) was performed once per sample according to the manufacturer's instructions. For successful identification of bacteria at species level, score cut-off values were used as proposed by the manufacturer (≥2.0) and in an adapted form (≥1.5 for gram-positive cocci, ≥1.7 for all remaining bacteria). For further data analysis, copy isolates were excluded (i.e. same pathogens detected in repeated blood cultures were considered only once per patient).
Results: When compared to traditional analysis, MALDI-TOF MS subsequent to a short-term pre-culture, applying the modified score cut-off values, led to an overall correct species identification in 69.5 % with misidentification in 3.4 % (original cut-offs: 49.2 % and 1.9 %, respectively); for gram-positive cocci, correct identification in 68.4 % (100 % for *S. aureus* and enterococci, 80 % for beta-hemolyzing streptococci*)*, for gram-negative bacteria including *P. aeruginosa, S. maltophilia*, anaerobic rods and Enterobacteriaceae, correct identification in 97.6 %. In polymicrobial blood cultures, in 72.7 % one of the pathogens was correctly identified. Results were not reliable for gram-positive rods and yeasts. Furthermore, difficulties typically arose from coagulase-negative staphylococci and viridans group streptococci. The approach was easy to implement in diagnostic routine without additional expenditure of consumables, costs or hand-on processing time. In cases with available clinical data, in 49.0 % our approach enabled us to give a differing treatment recommendation one day earlier due to quicker pathogen identification.
Conclusion: MALDI-TOF MS following short-term pre-culture is a valuable tool for rapid pathogen identification from positive blood cultures, allowing easy integration in diagnostic routine and the opportunity of considerably earlier treatment adaptation.

DVP05
Evaluation of a rapid test for confirmation of ESBL activity

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Introduction: According to EUCAST guidelines susceptibility to betalactam antibiotics is reported as measured. It is, however, a current matter of debate whether *in vitro* susceptibility to cephalosporines predicts their in vivo efficacy. Since inefficacy of the initial antibiotic regime due to ESBL expression prolongs the clinical course and worsens the prognosis rapid detection of ESBL-producing organisms is a diagnostic and therapeutic priority.
Objective: The bLACTA test (BioRad) offers a rapid screen for ESBL, AmpC and carbapenemase-mediated resistance by assessing third generation cephalosporine resistance. Here, we evaluated the test for its usefulness as a predictory and/or confirmatory test for ESBL-expression in enterobacteriaceae (ESBL-E).
Materials and Methods: A total of 200 preselected clinical and veterinary enterobacteriaceae isolates were studied. 125 of these were *E. coli*, 58 *Klebsiella spp*. and 17 isolates belonged to other species. The results of ESBL-testing by VITEK2 (Biomerieux), Phoenix 100 (Becton Dickinson), chromID ESBL (MAST) and ESBL genotyping (GenID) were compared to bLACTA testing and controlled by disc diffusion and E-test where critical.
Results: The data obtained reveal that the bLACTA test is easy to handle and highly reliable in the detection of cefotaxime and cefpodoxim resistance. On the contrary, there was no correlation with susceptibility or resistance to ceftazidime and piperacillin/tazobactam. Furthermore, all but one genetically proven ESBL positive isolates were detected but AmpC detection was only ≤ 25%. Moreover, ESBL positivity in VITEK2 was less predictive of ESBL genotype than those Phoenix analysis and bLACTA testing. Notably, K1 *Klebsiella* isolates could not be distinguished from ESBL-bearing *Klebsiella* spp. using this test.
Conclusions: Overall, our data highlight the high reliability of the bLACTA™ test in the prediction of ESBL positivity in *E. coli* strains and piperacillin/tazobactam susceptible *Klebsiella* spp. isolates. It can further be used to confirm ESBL positivity in isolates typed as ESBL positive by VITEK2 or Phoenix analysis, thus omitting the requirement for further and more time consuming ESBL confirmative testing.

DVP06
Does exposure to duck hatchery bioaerosols cause adverse healtheffects in workers?

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Introduction: In today's large-scale industrial poultry production with high stocking density of a single species, employees are exposed to complex bioaerosols which are related to symptoms like decline in lung function or increased airway responsiveness and adverse health effects like chronic obstuctive pulmonary disease or hypersensitivity pneumonitis. However, knowledge about causalities between work related adverse health effects and inhalation of complex bioaerosols is limited. The aim of this study was to evaluate workers' immune response to prolonged bioaerosol exposure.
Materials and Methods: In this study we determined the microbial exposure of duck hatchery workers during workshifts (sorting of ducklings at eclosion days) and their immune response. In a new approach utilizing bacterial isolates cultured from duck hatchery working place air we determined workers' IgG titers against 8 gram-negative and 3 gram-positive bacterial strains with an inhouse immunofluorescence assay. In order to overcome low precision, sensitivity and reproducibility of visual evaluation we used a tailor-made image software to measure fluorescence intensity of single cells. In addition, workers' sera were tested for immunological parameter levels (total IgE, C-reactive protein (CRP), clara cell protein 16 (CC16), soluble CD14 (sCD14), lipopolysaccharid binding protein (LBP)). During workshifts personal and stationary bioaerosol sampling was performed. Furthermore, we compared the across shift changes in lung function of duck hatchery workers at days with and without duckling sorting. Statistical analysis included correlation of exposure data and comparison of parameter levels of duck hatchery bioaerosol-exposed workers (n=7) and non-exposed persons (n=10).
Results and Discussion: Personal bioaerosol sampling yielded a mean total cell count of $6.9x10^6$ microbial cells per m^3 air and cultivation dependent quantification of airborne bacteria resulted in mean $4.2x10^5$ CFU per m^3 air. Exposure data showed positive correlation between inhalable dust, endotoxins and total cell count (r= 0.97 and 0.81 respectively). Analysis of sera displayed slightly but not significantly increased workers' IgG titers to most duck hatchery bioaerosol isolates. Soluble CD14 was significantly increased in hatchery workers ($p<0.05$). Furthermore, the results of lung function tests revealed a decline of workers' FEV_1 (forced expiratory pressure in 1 second) during workshifts at days with duckling sorting. In conclusion, these results seem to indicate an immune response of duck hatchery workers to inhalation of working place bioaerosols and we recommend adequate ventilation engineering.

DVP07
Fast, direct and quantitative protein microarray-based characterisation of *Staphylococcus aureus* isolates from culture material.

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Besides genes associated with drug resistance and adhesion to host tissues *etc*., *Staphylococcus aureus* harbours a complex array of virulence factors that includes superantigens (toxic shock syndrome toxin, *tst1*, and some 30 enterotoxin genes), exfoliative toxins, proteins that interfere with various functions of the host immune system (complement and chemotaxis inhibitors *etc.*), leukocidins (*i.e.*, toxins that specifically destroy white blood cells by formation of polymeric pores in cell membranes) and different haemolysins including alpha toxin. Therefore, a fast and economic antibody assay for the direct parallel measurement of different toxins and proteins directly from colony material and culture supernatants was developed based on a microarray spotted with specific antibodies. Most experiments for protocol development and optimisation were performed using the alpha toxin as model target. The alpha toxin of *S. aureus* is a pore forming toxin. It penetrates host cell membranes causing osmotic swelling, rupture, lysis and, subsequently, cell death. Haemolysin alpha is toxic to a wide range of different mammalian cells; i.e., neurotoxic, dermonecrotic, haemolytic, and it can cause lethality in a wide variety of animals. Staphylococcal protein A,

PbP2a, enterotoxins A and B, TSST1, haemolysins alpha and beta, staphylokinase and PVL (F-component) were used to generate monoclonal antibodies via phage display that were spotted on microarrays. These protein microarrays together with specific software allowed after calibration measuring concentrations of staphylococcal toxins in culture supernatants.
The alpha toxin production of 648 previously genotyped isolates of *S. aureus* was measured. Isolates originated from medical and veterinary settings and were selected in order to represent diverse clonal complexes and defined clinical conditions. Generally, the *in vitro* production of alpha toxin is related to clonal complex affiliation. For clonal complexes CC22, CC30, CC45, CC479, CC705, and others, no alpha toxin production was found under the given *in vitro* conditions, while others, such as CC1, CC5, CC8, CC15 or CC96 secreted variable to high levels. There was no correlation between alpha toxin yield and the clinical course of the disease, or between alpha toxin yield and host species. Similarly, the *in vitro* yield of PVL also was found to be related to clonal complex and strain affiliation. Furthermore, a new labelling approach using a newly designed fluorescence reader and fluorescent beads allowed more rapid and simultaneous testing of all targets covered directly from colony material within a very short time (~30 min). A very good concordance of pheno- and genotype was observed using a set of ~100 isolates. This assay can, besides the hla and PVL measurements, be used as culture conformation test for MRSA, for diagnostic purposes especially with regard to PVL-related conditions, Toxic Shock and food intoxications. Strains yielded characteristic expression patterns so that a future, expanded assay could also be used for presumptive strain identification.

DVP08

Automation of RIDA®GENE Bordetella by the use of the BD MAX™ Open Mode platform - a feasibility study

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Introduction: *Bordetella pertussis, B. parapertussis* and, on occasion, *B. holmesii* cause whooping cough or pertussis. Infections by *B. parapertussis* tend to take a milder clinical course. Usually, clinical diagnosis is confirmed by PCR testing of nasopharyngeal aspirates or swabs. The RIDA®GENE Bordetella multiplex real-time PCR assay (R-Biopharm AG, Darmstadt, Germany) simultaneously detects and differentiates *B. pertussis, B. parapertussis* and *B. holmesii.* Its targets are the IS*481* and IS*1001* elements. In this study we tested the feasibility of automating this multiplex real-time PCR assay by the use of the BD MAX™ Open Mode platform (Becton Dickinson, Heidelberg, Germany).
Materials and Methods: Nasopharyngeal swabs and washes were tested for *B. pertussis*, *B. parapertussis* and *B. holmesii*, respectively, by using a modified RIDA®GENE Bordetella real-time detection kit. The assay was tested for analytical sensitivity, reactivity and analytical specificity on the BD MAX™ system. The adaption steps included extraction and amplification based on the DNA-3 extraction kit from Becton Dickinson and master mix provided by R-Biopharm.
Results: The modified RIDA®GENE Bordetella assay showed good overall performance on the BD MAX™ system. The analytical sensitivity hit ≤ 10 cp/µL for all 3 targets. In 30 different bacteria tested for specificity no target signal was detected except for *Campylobacter jejunii* which showed a potential cross reaction in the channel of *B. holmesii*. Further investigation on this issue is currently under way. Time to result was around 100 min for 24 specimens.
Conclusions: In its current format, RIDA®GENE Bordetella allows for the sensitive and specific detection of *B. pertussis*, *B. parapertussis* and *B. holmesii*, respectively. Adaption to the BD MAX™ Open Mode platform was feasible and resulted in an easy-to-use, automated test requiring minimal hands-on time, infrastructure and training. IS*481* is shared by *B. pertussis* and *B. holmesii*, and - unlike the RIDA®GENE Bordetella assay - most current assay formats do not discriminate among the two species. As a consequence the relative contribution of *B. holmesii* to pertussis in vaccinated persons might be underestimated and erroneously attributed to vaccination failure.

DVP09

Direct and Fast Detection of Carbapenemase Genes with an Oligonucleotide Microarray using different exponential Multiplex Amplification Methods

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Rapid molecular identification of carbapenemase genes in native patient samples is crucial for infection control and prevention, surveillance and for epidemiological purposes. Furthermore, it may have a significant impact on the selection of an appropriate initial treatment and great benefit for ICU patients. For this purpose, an assay was developed to analyze low copy number samples of different reference strains containing the carbapenemase genes *bla*KPC, *bla*IMP, *bla*VIM, *bla*GIM, *bla*NDM, *bla*OXA-23, *bla*OXA-40, *bla*OXA-48, *bla*OXA-51 or blaOXA-58. In a first step the bacterial DNA was labelled and exponentially amplified using two different multiplex amplification methods, RPA (Recombinase Polymerase Amplification) and PCR. Subsequently, the labelled amplicons of the selected carbapenemase genes were hybridized and specifically detected with a novel oligonucleotide microarray. The sensitivity of entire assay was determined using dilution series of reference DNA samples from different strains (*i.e. Klebsiella pneumoniae*, *Acinetobacter baumannii*) in a range of 10^1 to 10^7 genomic equivalents. First analyses of all target genes with PCR and RPA showed a good sensitivity and specific signals were detected on the microarray with templates comprising as few as 10^1 genomic equivalents. As reference system for target quantification in spiking experiments, a real time PCR system was established targeting the *bla*VIM gene. The sensitivity and speed, especially of of the isothermal amplification strategy allows a future development of a point-of-care device for the detection of clinically important carbapenemase genes in native patient samples.

DVP10

Automated tcdB PCR assay for routine diagnostics of Clostridium difficile infections in hospitalized patients.

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Introduction: *Clostridium difficile* infection (CDI) is the most frequent cause of diarrhea in hospitals. Early diagnosis of CDI is required for early treatment and implementation of specific hygiene precautions. Automated NAAT systems have been developed for fast and sensitive detection of CDI. In the present study tcdB PCR using BD MAX Cdiff assay (Beckton Dickinson) was directly compared to combined antigen testing and toxigenic culture as the gold standard.
Materials and Methods: 529 routine stools samples were analyzed in parallel by GDH and toxin A and B EIA (Quik Check complete, Alere) and BD Max Cdiff assay. All samples with positive results in any assay were independently confirmed by anaerobic culture (CLO-agar and GENbox anaer, Biomerieux). Isolates were characterized by PCR ribotyping, multiplex-PCR for toxin genes and by antibiotic resistance testing.
Results: *C. difficile* infections with toxigenic strains were detected by automated tcdB PCR with high sensitivity (97.7%) and specificity (96.9%). Sensitivity of *C. difficile* common antigen screening (GDH) was 98.8% with lower specificity (95.2%) due to the detection of non-toxigenic strains. Sensitivity of toxin A and B testing was lowest (70.0%) but specificity was high (99.5%). The knowledge of sensitivities and specificities may have direct implication for the selection of appropriate assays for routine C. difficile testing. GDH proved to be a fast and easily applicable screening test requiring subsequent confirmation of toxigenic infection which is now easily available using automated tcdB PCR. Toxin A and B antigen testing is specific for CDI; however, 31% of toxigenic isolates remained undetected. Therefore, a molecular confirmation assay is required at least for toxin negative but GDH positive samples. Screening by automated tcdB PCR cannot discriminate between toxin gene carriers (13%) and patients with culture proven CDI. Therefore we propose that PCR-based screening will require an independent conformational assay. *C. difficile* antigen- and PCR-based algorithms will be discussed according to clinical and cost aspects.
Conclusion: Fast and easily applicable *C. difficile* detection is available by antigen testing and also by automated point of care PCR systems. Toxigenic culture remains the diagnostic goldstandard, however with limitations for routine diagnostics due to prolonged turn-around-time (TAT).

DVP11

Latex agglutination test compared with mannan antigen in combination with antibodies as serum biomarkers for candidemia

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Introduction: Candidemia often occurs in intensive care unit patients and in patients with haemato-/oncological disease. For early diagnosis, serological assays are available to determine *Candida* related antigen or antibodies. One of the commercial tests is a latex agglutination (Cand-Tec®-Test, Ramco Laboratories), but it detects an insufficiently defined cytoplasmic antigen. A modern test uses ELISA to measure mannan antigen (Mn-Ag) (PLATELIA™ *Candida* Ag Plus, BIO-RAD).
Antibodies are detected by complement-fixation test (KBR) and indirect haemagglutination test (HAT). Mannan antibodies (Mn-Ak) are detected by ELISA.
Standard blood cultures are essential for the identification of *Candida* species (spp) and for susceptibility testing. But varying time-to-positivity (TTP) restricts their use for early detection.
Materials and Methods: 24 serum samples of patients with candidemia and 21 serum samples of patients without candidemia were used to compare positivity, sensitivity and specifity of latex agglutination and Mn-Ag alone and in combination with KBR and HAT respectively with Mn-Ak.
Data were collected concerning the distribution of *Candida* spp, for the involvement of colonized central venous catheters (ZVK) and for TTP.
Results: Serology:In serum samples of patients with positive blood cultures, latex agglutination was positive in 8% of the cases, Mn-Ag in 33%, HAT in 35%, KBR in 26%, and Mn-Ak in 58%. In serum samples of patients without candidemia, latex agglutination showed high levels in 100% of the cases, Mn-Ag in 13%, HAT in 15%, KBR in 23%, and Mn-Ak in 47%. The combination of antigen and antibody was positive in 48% of the cases for latex agglutination and HAT/KBR and positive in 75% for the combination of Mn-Ag and Mn-Ak.
Sensitivity of Mn-Ag was low with 0.29, but higher than latex agglutination with 0.083. Specifity of Mn-Ag was nearly 1.0, latex agglutination 0%. In comparison, the measurement of Mn-Ak showed a higher sensitivity in comparison with HAT and KBR, but a lower specifity.
The combination of Mn-Ag and Mn-Ak had high specifity, but very low sensitivity. *Candida* spp: 67% of *Candida spp*. were due to *C.albicans,* followed by *C. tropicalis* 17%, *C. glabrata* 13% and *C. parapsilosis* 4%. In 42% of patients with candidemia a colonized ZVK could be found. TTP:Blood cultures with *C.albicans* were mainly positive after 24-48h, with *C. glabrata* 48-120h, with *C. tropicalis* 24h and with *C. parapsilosis* 72h.
Conclusion: None of the serological parameters showed high sensitivity combined with high specifity whereby the measurement of Mn-Ag seemed to be superior to latex agglutination. The combination of Mn-Ag and Mn-Ak could not distinguish between candidemia and colonization.
C. albicans still seems to be the major yeast causing candidemia, associated with 74% of the colonized ZVK. Blood cultures were detected positive mostly within 24-48h, the time needed for serological Results:

DVP12

Comparison of VITEK2, MALDI-TOF MS, and 16S rDNA sequencing for identification of *Myroides odoratus* and *Myroides odoratimimus*

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Introduction: The genus Myroides comprises medically relevant species that (as opportunistic pathogens) may cause diseases in immunocompromised patients. In this study we attempt to compare three diagnostic approaches (biochemical, molecular and mass spectrometry) for identification of the two most frequently occurring species, *Myroides odoratus* and *Myroides odoratimimus*, and evaluate the accuracy for identification for each method.
Materials and Methods: The bacteria were collected by both laboratories involved during routine diagnostics over a period of two years. The isolates (16 *M. odoratimimus* and 8 *M. odoratus* strains) were subcultured on Columbia blood agar prior to testing. ATCC type strains *M. odoratimimus* (ATCC® BAA-634™) and *M. odoratus* (ATCC® 4651™) were used as controls. The strains were analysed by the two automated systems Vitek 2 (bioMérieux, Nürtingen, Germany) and MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). The procedures were carried out and the results were evaluated according to the instructions provided by the manufacturers. As a third method 16S rDNA sequencing was conducted using the primers TPU-1 (5′-AGA GTT TGA TCM TGG CTC AG-3) and RTU-4 (5′-TAC CAG GGT ATC TAA TCC TGT T-3′). Subsequently, a homology search against the NCBI nucleotide collection (nr/nt) was performed with the obtained DNA sequences, using BLAST algorithm (http://blast.ncbi.nlm.nih.gov).
Results: Vitek 2 reliably identified *Myroides* at genus but not on species level. However, the identification score was excellent with a mean score of 98%. Both, MALDI-TOF MS and 16S rDNA sequencing distinguished between *M. odoratimimus* and *M. odoratus*. 17 strains were reliably identified by MALDI TOF-MS on both genus and species level (score ≥ 2.0), 2 strains on genus level (score between 1.7 and 2.0) and 3 strains could not be reliably identified (score ≤1.7). Homologies ≥ 97% are regarded to be reliable on species level. In our study 19 of 22 strains showed homologies ≥ 99% only in three isolates the homology was lower (96% two times and 95% once).
Discussion: Vitek 2 is a reliable method to identify *Myroides* on genus level. Both, MALDI-TOF MS and 16S rDNA sequencing are able to distinguish between the two species *Myroides odoratimimus* and *Myroides odoratus*. Additional main spectra enlarging the biotyper database will lead to more reliable results in the future.

DVP13

Towards rapid identification of *Clostridium difficile* ribotype 027 using MALDI-TOF MS

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Objective: The ribotype 027 (RT 027) is associated with severe cases of Clostridium difficile infection (CDI) and its detection is being surveyed by public health authorities, however, the complexity of the currently available ribotyping methods prevents routine identification of RT 027. We investigated the performance of MALDI-TOF MS for detection of RT 027.
Materials and Methods: Mass spectra of 3 RT 027 strains, obtained on a Bruker Autoflex III platform were analyzed for unique features to enable separation from spectra of *C. difficile* strains with ribotype 001, 072, 014, 020, 078, 126 using the MaldiQuant software-package. The sub-species-classification main spectrum (MSP) generated based on these unique features using the MALDI Biotyper 2 software (Bruker Daltonics) was evaluated for diagnostic performance using RT 027 strains, that were selected for genetic diversity.
Results: Bayesian classification revealed that spectra of the tested RT 027 strains could be separated with an accuracy of 100% from spectra of all other investigated ribotypes of the training set. Features unique for RT 027 were used to construct a MSP linked to MSPs specific for RTs 126, 078, 001/072, 014/020 or 027, respectively. 10 of 11 RT 027 matched the RT 027 MSP with a score greater than two (sensitivity = 90.9 %) and none of the 10 strains with another RT than 027 was falsely classified as RT 027 (specificity = 100 %).
Conclusion: Our data indicates that MALDI-TOF MS may represent a useful avenue to enable routine screening for RT 027 in CDI.

DVP14

Performance of ChromID-ESBL Agar for detection of multiresistant enterobacteriaceae as compared to VITEK 2 in clinical material of very low birth-weight infants in the neonatal intensive care unit (NICU).

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Introduction: Gram negative bacilli cause many episodes of late onset-sepsis among very low birth weight infants and the RKI recommends the screening for Enterobacteriaceae resistant to aminopenicillins and 3.-/4. generation cephalosporins independent of the resistance mechanism

(2MRGN NeoPäd); however, little is known about the performance of ESBL screening media for this purpose.
Methods: A prospective study over 6 month was performed during which surveillance samples (rectal- and nasopharyngeal swabs) from a NICU were collected and recovery of 2MRGN NeoPäd from a ChromID ESBL agar read after 24 h was compared to recovery on non-selective media followed by identification of the antibiotic resistance pattern on a VITEK 2 device.
Results: During the study period 620 specimens (305 anal- and 315 naso/pharyngeal swabs) of 169 individuals were analyzed from which 954 isolates were recovered. 42 infants were identified to be colonized with *E. cloacae*, 10 with *K. pneumoniae*, 50 with *S. aureus*, one with *S. marcescens*, one with Acinetobacter spp. and one with *P. aeruginosa*. No VREs or MRSA were detected. Eight individuals were colonized by 2MRGN NeoPäd Enterobacteriaceae (two with ESBL E. coli, five with *E. cloacae* and one with *C. freundii*), all of which were also detected on selective agar. However, using the selective media, eight additional newborns colonized with 2MRGN NeoPäd were identified (one *C. freundii* and seven *E. cloacae*).
Conclusion: The presented data suggests, that colonization with 2MRGN NeoPäd can be detected with superior sensitivity and less effort by selective ESBL media as compared to conventional cultural detection methods.

DVP15
Digital-Read-Out (DRO) of inhibition zone diameters for antibiotic susceptibility testing using the agar disk diffusion method.

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Introduction: Agar disk diffusion is one of the commonest methods for testing of antibiotic susceptibility in clinical microbiology; however, the growing number of species related zone diameter break-points of the current guidelines render manual reading and interpretation of disc diffusion antibiogramms increasingly difficult. We here introduce a convenient way for Digital-Read-Out of inhibition zone diameters using a caliper, thereby enabling semi-automated, low- to medium scale throughput documentation and interpretation of inhibition zones.
Materials and Methods: The system consists of an electronic caliper featuring a serial data-port which interfaces with the USB-Port of a Windows-PC via an IC capable of reading the serial protocol of the caliper and send it to an USB port. Using a keyboard emulator software the readings from the USB-port can be transferred to any Laboratory-Information-System (LIS), database- or spreadsheet software for storage and statistical analysis. The device is equipped with a foot switch to enable hands free triggering the storage of the caliper readings.
Results: The presented device allowed the quantitative reading and documentation of about 70 distinct parameters by a single technician in roughly 7 minutes. The data were automatically analyzed and interpreted according to programmed break-points and additional criteria (i.e. target value and range).
Conclusion: The presented caliper with DRO enables fast and easy quantitative documentation of inhibition zone diameters that can be further interpreted by break-points to predict antibiotic sensitivity or by expert-systems to assess the resistance phenotype for enhanced interpretative reading.

DVP16
Qualitative and quantitative high-throughput screening of biofilms by automated fluorescence microscopy

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Biofilms are communities of microorganisms which live in a self-produced matrix of extracellular polymeric substances. This alternative lifestyle enables survival in different environmental niches which is constituted by their resistance to immune defense and antibiotics. Once bacteria form a biofilm in the host, infections are hard to treat and can change into chronic infections. Therefore it is important to develop tools to investigate biofilms for therapy improvement. The aim of this work is to establish a method for the fully automated large-scale screening of biofilms which allows the comparison of different strains, the impact of different conditions during biofilm formation and most importantly the determination of the effects from different antibiotics. We enhanced our previous published VideoScan technology to perform high-throughput screening of biofilms. This technology is based on fully automated fluorescence microscopy. The camera focuses automatically and captures images which are processed via sophisticated digital image processing methods. VideoScan enables the analysis of multiplex assays such as microbead or cell-based assays [Rödiger et al. 2013; Frömmel et al. 2013].
We use a 96 well plate format for the formation of biofilms. In a study we screened 20 strains for biofilm formation where one strain was found as a strong biofilm former. Our reference strain for biofilm is *E. coli* MG1655 F'tet *tra*D (kindly provided by Dr. J.-M. Ghigo, Pasteur Institute Paris). After 16h of incubation biofilms were stained with SYTO-9 and propidium iodide for the Live/Dead staining followed by a VideoScan analysis. This analysis represents a two-step evaluation realized by our software and image processing. In the first step which indicates the global fluorescence Live (coumarin)/Dead (cyanine 3) ratio, single images are taken and assembled to an overview picture of Live/Dead stained bacteria. In addition we tested different antibiotics (Amp, Cam, Kan, Gen) in different concentrations to look for the changes in the Live/Dead ratio. The second step is a fine-grained analysis of the biofilm. We developed a tool to count bacteria in different z-stacks of the biofilm, where images are captured in the coumarin/cyanine 3 channel at an variable interval (e.g. 5 µm).
With our VideoScan technology it is possible to study biofilms in a fully automated large-scale screening. The use of the 96 well format constitutes a cheap, reproducible and simple method. With this high-throughput platform we can check bacteria for biofilm formation under various conditions. This time-saving technology could facilitate the evaluation of biofilms regarding to applied antibiotics.

DVP17
Evaluation of chromogenic media for detection of *Streptococcus agalactiae*

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Introduction: *Streptococcus agalactiae* belong to the beta-haemolytic streptococci of the Lancefield group B (GBS). GBS is part of the normal bacterial flora colonizing the gastrointestinal and genital tract. If the pregnant woman colonized with GBS transmission to the newborn child can take place during the birth and cause heavy neonatal infections.
According to guideline (1) a screening is recommended between the 35th and 37th week of gestation. In case of GBS colonization an antibiotic treatment shortly before birth (penicillin or alternatively clindamycin) is recommended. The aim of this study is to compare different chromogenic selective culture media and blood-containing culture media depending on the cultivation time and sensitivity, as well as the evaluation of the differences in the resistance development after changing the testing guidelines from CLSI to EUCAST.
Materials and Methods: Vaginal swabs of patients (n=140) were inoculated on different culture media (CNA-agar (bioMerieux), CHROMagar StrepB (Mast Group), Thermo Scientific^{TM} Brilliance^{TM} GBS Agar (Oxoid), Granada agar (bioMerieux) and StrepB Select (Bio-Rad)) and incubated overnight (35 +/- 1°C; 5% CO2). After the identification by MaldiBiotyper (Bruker) and detection of the B-Lancefield-group (DiaMondial), the antibiotic testing was carried out with VITEK (bioMerieux).
Results: 140 samples were examined for GBS. 11 of these were tested positively for *Streptococcus agalactiae* on all used culture media. However, the culture media differ in sensitivity, cultivation time and inhibition of the vaginal flora. A susceptibility testing was preformed in each case. All tested *S. agalactiae* showed a penicillin-sensitivity. However, a decreased clindamycin- sensitivity as well as an inducible clindamycin resistance was detected at two samples respectively, shown by VITEK system.
A comparison of the resistance statistics for a period of three months shows an increasing clindamycin and erythromycin resistance after conversion to EUCAST.
Discussion: The prevalence rate of GBS with pregnant women is 10-30% in Germany (1), the ascertained results are slightly lower (8%). By the use of chromogenic selective culture mediaand modern identification methods like Maldi-TOF a reliable proof of *Streptococcus agalactiae* is possible in 18 h. Further 12-16 h are required for susceptibility testing, indicated by penicillin intolerance. However, changing from CLSI to EUCAST leads to an increased resistanceinparticular ofmacrolides and lincosamides.

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DVP18

Rabbit Monoclonal Antibodies Directed at the T3SS Effector Protein YopM Identify Human Pathogenic *Yersinia* Isolates

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The Yersinia outer protein M (YopM) is a type 3 secretion system (T3SS)-dependent effector protein of pathogenic *Yersinia enterocolitica, Yersinia pseudotuberculosis* and *Yersinia pestis.* Although translocation of YopM into host cells is indispensable for full virulence, its molecular functions still remain largely elusive. It has been shown that YopM forms complexes with members of the PRK and RSK kinase families and recent studies indicate that YopM of *Y. pestis* and *Y. pseudotuberculosis* YPIII strain inhibit caspase-1. In previous studies, we could identify the recombinant YopM (rYopM) protein derived from the *Y. enterocolitica* strain JB580 (pYV 8081) as a cell-penetrating protein that, without the need for a T3SS or the presence of other bacterial factors, is able to enter target cells autonomously. Intracellular rYopM is functional and reduces the expression of pro-inflammatory cytokines including TNF-α. In the present study, we have generated rabbit monoclonal anti-YopM antibodies (RabMabs). With regard to their superior properties and for studies in murine disease models, such YopM-specific monoclonal antibodies produced in rabbits would provide major advantages. Epitopes of these RabMabs were mapped by Western blotting using various truncated versions of rYopM. RabMabs recognizing either the N- or C-terminus of YopM were characterized further and validated using a collection of 61 pathogenic and non-pathogenic *Yersinia* strains as well as exemplary strains of further *Enterobacteriaceae* such as *Salmonella enterica* ssp. *enterica*, *Shigella flexneri* and intestinal pathogenic *Escherichia coli*. RabMab 41.3 directed at the N-terminus of YopM of *Y. enterocolitica* strain JB580 reliably discriminated between pathogenic and non-pathogenic *Yersinia* strains evaluated in this study. Thus, RabMab 41.3 might be applicable for the detection of pathogenic *Yersinia* strains.

DVP19

The Diagnosis of implant associated infections: a comparison of different methods

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In the past decades several new techniques for the diagnosis of prosthetic joint infections have been developed, but the detection of the causative bacterial species is still challenging for microbiologists. The main reason therefore is (i) the ability of bacteria to form biofilms on the implant surface. Thus, it is necessary to break up the biofilm structure and to dissolve the embedded bacteria before cultivation. (ii) Some bacterial species associated with implant infections possess an anaerobic metabolism and thus, their identification is more difficult due to the more ambitious cultivation. To find an accurate diagnostic methodology for implant associated infections, between 2012 and 2014 we investigated hip and knee implants of 14 patients with suspicion of septic loosening. We combined and compared new techniques and established methods, including the sonication of whole prosthetic joints, tissue samples (up to 6 per patient) and swaps from the implant surface (up to 4 per implant). For the cultivation we applied three different solid and two liquid media including two optimized for anaerobic growth conditions. Additionally a sample of the sonication fluid was cultivated in blood culture flasks. All cultures were incubated at 37°C for up to 14 days under anaerobic or anaerobic conditions. Furthermore the tissue samples and the sonication fluid were used for molecular analysis. For the confirmation of the cultural and molecular biological results, two tissue samples per patient of the periprosthetic membrane were histopatological analysed. Combining the tested techniques, 11 of the 14 patients were tested positive for an implant-associated bacterial infection, which could be confirmed by the histopatological analysis in most cases. Analysis of the performance of single techniques (sonication, tissue samples and implant swaps) allowed the overall identification of the 11 infected patients, application of only one technique would have drastically underdiagnosed infected patients. Additionally, identification of false positive results due to contaminations would have been impossible without the additional informations from the alternative technical approaches.
All together, we conclude that all the tested techniques are suitable to identify an implant associated infection but only the combination of several techniques lead to accurate diagnostic Results: Furthermore, we urgently suggest the additional histopatological analysis in parallel to confirm the results from culture techniques.

DVP20

Evaluation of a Multiplex PCR Cartridge System for Simultaneous Detection of Pneumonia Causing Pathogens in Respiratory Samples

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Introduction: The early initiation of an appropriate treatment in infections can be favorable for the outcome. Regarding pneumonia, studies show that polymicrobial episodes vary between 11% and 60%. As this could be important for the choice of antimicrobial agents we evaluated a multiplex PCR cartridge system for respiratory samples being able to simultaneously detect major pathogens causing pneumonia.
Materials and Methods: In addition to conventional culture, 31 respiratory samples (BAL, sputa, aspirates) suspected to be from severe infections or having multiple pathogens in culture were also tested with the Unyvero™ cartridge system (Curetis AG, Holzgerlingen, Germany). This molecular assay can detect 17 microorganisms relevant in pneumonia (*S. mitis* group, *S. aureus, Ps. aeruginosa, A. baumannii, Legionella pneumophila, M. catarrhalis, S. maltophilia, Enterobacter spp., E. coli, K. pneumoniae, K. oxytoca, M. morganii, Proteus spp., S. marcescens, H. influenzae, Chlamydophila pneumoniae and Pneumocystis jiroveci*) as well as resistance markers. Culture was performed according to standard procedures. For identification the MALDI Biotyper™ (Bruker Daltonik, Bremen, Germany) was used as well as VITEK® (BioMérieux, Marcy l'Étoile, France). Results of the conventional methods were compared to those of the multiplex PCR cartridge.
Results: From the 31 samples selected for the molecular assay 24 had valid results for a comparison and were further investigated. Over all these samples the Unyvero™ system found a total of 50 bacteria. With regard to the species covered by the PCR assay 47 bacteria were isolated in culture. 11 cultures (45 %) were polymicrobial while the molecular assay was positive for more than one pathogen in 14 cases (58%). In 9 cases the PCR found organisms that did not grow in culture (e.g. like *A. baumannii)* whereas the conventional methods found species that were not marked as positive in the assay in 6 samples. In addition, 15 further species that were not included in the molecular panel were recovered by culture. Time to result for the multiplex panel was about 4.5 hours.
Discussion: A rapid detection of disease-causing microorganisms can be of clinical relevance since empirical treatment in some cases might not cover all pathogens appropriately. This is especially true for polymicrobial infections.The tested multiplex assay for pneumonia causing pathogens found a few more polymicrobial samples than culture (14 vs. 11) and with both methods pathogens were found that were not detected with the other method, respectively. The current design of the assay can not distinguish *S. pneumoniae* from members of the *S. mitis group*. The data shown here suggest that rapid multiplex PCR assays might be a good addition to conventional methods as they can support early adjustment of initial treatment. Further analysis has to be done with respect to the resistance markers in the assay.

DVP21

Novel bead-based enrichment methods of bacterial pathogens from liquid matrices and microarray-based detection

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The rapid detection of pathogenic microorganisms in liquid matrices is a great challenge in environmental monitoring as well as human healthcare.
We report on the development of simple and fast bacteria isolation strategies followed by a convenient microarray-based identification. Bead-based separation of bacteria by various micro particles with amino-functionalized surfaces from liquid matrices like drinking water or urine was performed. The enrichment protocols allow for the isolation of intact bacteria that can be further analysed either by culture- or nucleic acid-dependent methods. For the latter application time saving and easy-to-perform combinations of direct and asymmetric PCR were tested. Furthermore, we illustrate within this contribution the optimization of microarray-based bacteria identification to enable naked eye signal readout. Static as well as flow-through regimes for nucleic acid hybridization concomitant with immobilization of detection protein complexes were developed to significantly shorten the assay's operation time. The successful on-chip hybridization and immobilization of streptavidin-peroxidase complex to biotin-labelled DNA-hybrids is monitored by the deposition of silver nanoparticles (1,2).
Here, the presence of target DNA is easily traceable due to the enzymatically generated dark silver deposits, which represent a robust endpoint signal without signal fading over the time or the need of expensive signal readout devices.

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Acknowledgement
Funding of the research project 'WK BASIS' and the young research group 'Jenaer Biochip Initiative 2.0' within the framework 'Unternehmen Region - InnoProfile Transfer' from the Federal Ministry of Education and Research, Germany (BMBF) is gratefully acknowledged. The Thüringer Aufbaubank (TAB), Germany, financially supports the project "ROWDix".

DVP22

Identification of phospholipid fatty acyl compositions of *Legionella dumoffii*

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Introduction: *Legionella* are aquatic pathogens that are ubiquitously found in nature, in both antropogenic structures and environmental water. Among *Legionella* species that cause human pneumonia, *Legionella pneumophila* is the most common causative agent, while *L. dumoffii* is the fourth. Pneumonia caused by *L. dumoffii* is rapidly progressive and fulminant owing to the ability of this bacterium to invade and proliferate in human alveolar epithelial cells.
Materials and Methods: *Legionella dumoffii* strain ATCC 33279 was cultured on buffered charcoal-yeast extract (BCYE) agar plates for three days at 37 °C in a humid atmosphere and 5% CO_2. Lipids were isolated from bacterial cells using the Bligh and Dyer method: chloroform/methanol (1:2 v/v). Normal and reversed phase liquid chromatography coupled with mass spectrometric detection was used for characterization of phospholipid classes and the fatty acyl composition in phospholipid structures.
Results: On the basis of the characteristic fragmentation of the individual phospholipid classes, five groups of phospholipids were detected in *L. dumoffii*: phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidyl-N-monomethylethanolamine (MMPE),phosphatidyl-N,N-dimethylethanolamine (DMPE) and phosphatidylglycerol (PG). The identification of phospholipids and the fatty acids distribution were performed using ESI-MS and ESI-MS/MS spectra, in the positive and the negative ionization modes, obtained by LC/ESI-MS analyses. The fragmentation spectra of the investigated compounds, in the negative ion mode, were dominated by peaks corresponding to $[R_1COO]^-$ and $[R_2COO]^-$ ions which enabled to establish the *sn1* and *sn2* position in identified phospholipids. Among PCs C16:0/17:0, C15:0/18:0, C15:0/19:0 were found. For PEs, the following species were identified: C15:0/16:0, C14:0/17:0, C14:0/16:0. For PGs, the following species: C16:0/15:0, C17:0/15:0, C16:0/16:0 were distinctive.
Conclusions: The localization of fatty acids residues, in particular phospholipids, might be used as a taxonomic signature for *L. dumoffii*, helpful in identification of this species of bacteria.
This work was supported by National Science Centre research grant (N N303822640).

DVP23

Automated Isolation of HBV-DNA and HCV-RNA for quantitative Virus Detection from Human Serum Samples Using the MagNA Pure Compact System

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Introduction: To reduce the handling time and improve the workflow efficiency for HBV-DNA and HCV-RNA purification from human serum samples we aimed at conversion the currently used manual IVD certified HighPure System Viral Nucleic Acid Kit protocol into an automated purification protocol with the MagNa Pure Compact System.
Materials and Methods: Routinely tested samples for quantitative HBV (n=10) or HCV PCR (n=13) were collected and measured after automated preparation with the MagNa Pure Compact System in addition to preparation with the conventional manual method. Wherever applicable, the tests after automated preparation were carried out in triplicate (HCV) and duplicate (HBV). The virus load of the tested samples ranged between HCV (2,9E+02 - 5,6E+06 IU/mL)) and HBV (6,0E+00 - 1,2E+06 IU/mL). Additionally, reference samples from interlaboratory surveys executed by INSTAND e.V were probed for standardized evaluation.
Routinely, the specimen preparation was carried out manually with the High Pure System Viral Nucleic Acid Kit recommended by the manufacturer guidelines for the IVD certified PCR test-systems. The automated purification was performed with the MagNA Pure Compact Nucleic Acid Isolation Kit I- Large Volume and the Purification Protocol: Total_NA_Plasma_1000 (elution volume: 100µl). For quantification of the viral load we used the COBAS® TaqMan® HCV Test v2.0 for Hepatitis C virus (HCV) and COBAS® TaqMan® HBV Test for Hepatitis B virus (HBV). Both tests utilize real-time PCR technology for the detection and quantification by applying COBAS® TaqMan® Analyzer for automated amplification and detection.
Results and conclusion: For the MagNa Pure Compact System, so far, no isolation protocol was available neither for the preparation of HBV-DNA- nor for HCV-RNA. For laboratories with low to medium sample throughput it might be helpful to apply the MagNA Pure Compact System, a bench top solution, with allows processing of one to eight samples within a period of 20 to 50 minutes. Our findings provide evidence that the PCR results derived from manually isolated HCV-RNA (HBV-DNA) samples and samples processed by MagNA Pure Compact are congruent, precise, reproducible, and diagnostically reliable (correlation of the manual and automated processed sample results for the Log Titer IU/mL HCV R^2= 0,99 and for HBV R^2=0,98). Consequently automated isolation can support the workflow efficiency without losing accuracy in regard to the determination of the viral load for diagnosis and possible follow up after antiviral treatment.

DVP24

DNA-Array-based Typing of *Clostridium difficile*

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Clostridium difficile is a Gram-positive, rod-shaped, anaerobic bacterium that is able to form spores. It is a normal component of the colonic flora. When other bacteria in the colon are eliminated by antibiotics, the intrinsically more resistant *C. difficile* is able to multiply and to cause damage due to its production of several toxins. Resulting conditions are antibiotic-associated diarrhoea and pseudomembranous colitis. Transmissions within hospital settings are common, warranting molecular typing in order to trace chains of infections. A microarray-based assay was designed that included toxin genes (*tcdA/B*, *cdtA/B*), genes related to antimicrobial resistance (*cat, ermB, tetM*), known typing markers (*slpA*) as well as genes for which the analysis of published genome sequences showed either a variable occurrence, or the occurrence of distinct alleles indicating a possible use as typing markers. Completely sequenced reference strains 630, BI9, CF5, M120, CD196 and R20291 were used for protocol development and optimisation as well as for validation purposes. Besides that, a total of 249 clinical isolates were tested that originated mainly from the Dresden and Freiburg regions. After DNA preparation, labelling of the DNA was performed during the linear amplification step by incorporating dUTP-linked biotin. Amplicons were hybridised to DNA microarrays generating strain specific patterns as well as providing information on absence or presence of relevant, such as, toxin genes. For representative isolates, multilocus sequence typing (MLST) and ribotyping were performed.
Based on *slpA* alleles and overall hybridisation profiles (excluding possibly mobile or variable elements such as toxin genes and resistance markers), tested isolates clustered into 33 distinct patterns. If several isolates from one cluster were typed by MLST, they yielded identical or closely related sequence types. Occasionally, several ribotypes were observed within one cluster and some ribotypes were present in different, although similar or related clusters.
The array allows rapid and high-throughput genotyping of clinical *C. difficile* isolates including toxin gene detection and strain assignment. Further trials are planned to assess its performance under routine conditions.

DVP25

Detection of *mec* genes and characterization of terminal sequences of SCC elements towards *orfX*

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Staphylococcus aureus is a one of the major pathogens in hospitals and the environment. Methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with high morbidity and high mortality; and for infection control rapid molecular tests are needed. In this study we developed and optimized PCRs for methicillin-resistance genes *mecA* and *mecC*, for genes encoding the virulence factor Panton-Valentine-Leukocidin (PVL) and for the identification and characterization of the integration site of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) into the *S. aureus* genome. The PCRs were tested single- and multiplexed. The PCR products were labelled by incorporating Biotin-dUTPs and detected by subsequent microarray hybridization, scanning and analysis using IconoClust Software (Alere Technologies GmbH). PCRs were tested on DNA preparations from culture materials as well as from patient swab samples; and they yielded in serial dilutions sensitivities in an order of magnitude of about 10 copies per sample. Termini of the SCC elements towards *orfX* were characterized in about 300 clinical isolates and reference strains belonging to *ca*. 140 different epidemic strains of MRSA. The most common SCC terminal sequence was *dcs* (GenBank BA000017.4 [34173 to 34454], in 37% of tested strains) followed by FN433596.1 [34140-34456], with 30%. Nine other alleles for this site were sporadically found and irregular patterns in some isolates (namely, in Middle-Eastern CC22 MRSA) possibly indicated a presence of further unknown alleles. In one major strain (ST239-MRSA-III) a variability affecting SCC termini was noted.
These data will help optimizing multiplex amplifications with the aim of developing a point of care system for detection and preliminary genotyping of MRSA directly from patient samples.

DVP26

Phenotypic methods for detection of beta-lactamase production in *Staphylococcus lugdunensis*

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Introduction: *Staphylococcus lugdunensis* can cause infections like endocarditis, osteomyelitis, and severe skin and soft tissue infections. Penicillinase detection is important for treatment decisions, but can be challenging in staphylococci since the penicillin MIC or the penicillin inhibition zone in disk diffusion are not always strictly correlated with penicillinase production as has been demonstrated in *Staphylococcus aureus*. As no data regarding the performance of methods for penicillinase detection are available for *S. lugdunensis* the aim of this study was to compare different methods for penicillinase detection in this species.
Materials and Methods: A collection of 95 clinical non-copy strains of *S. lugdunensis* was tested. Reference method for penicillinase production in our study was a PCR targeting all four known *blaZ* variants in staphylococci. The results were compared to microdilution, cloverleaf test and disk diffusion according to EUCAST for penicillin (1 µg and 10 µg), mecillinam (10 µg), cephazolin (30 µg), amoxicillin (10 µg), amoxicillin/clavulanic acid (30 µg), piperacillin (30 µg) and piperacillin/tazobactam (30 + 6 µg). The inhibition zone characteristics for all antibiotic disks were recorded.
Results: Out of 95 *S. lugdunensis* strains 36 (38%) displayed a positive *blaZ*-PCR result. A sharp penicillin zone edge had a sensitivity and specificity of 100% for detection of penicillinase production. Inhibition zones for penicillin (1 µg) did not overlap between penicillinase positive (range 27 - 42 mm) and penicillinase-negative (range 7 - 18 mm) strains. Also using amoxicillin and penicillin disk the zone inhibition diameter could differentiate clearly between penicillinase positive and negative strains. The difference between inhibition zone diameters of amoxicillin and piperacillin alone and in combination with their respective beta-lactamase inhibitors displayed overlapping values for penicillinase-positive and -negative strains. Sensitivity and specificity of the cloverleaf test were 100% for penicillinase production. However the growth of the indicator strain *S. aureus* ATCC 25923 was inhibited by some *S. lugdunensis* strains making reading difficult. The MIC determination using Penicillin results in overlapping ranges for *blaZ*-negative (<0,015 - 0,5µg/ml) and *blaZ*-positive (0,5 - >16 µg/ml) strains. The EUCAST breakpoint of 0,12 µg/ml would have falsely classified 8 strains as beta-lactamase positive.
Conclusion: Determination of a sharp zone edge as well as disk diffusion using penicillin, amoxicillin and piperacillin and the cloverleaf test are the most reliable phenotypic method to detect penicillinase-production in strains of *S. lugdunensis*. However, reading of the cloverleaf test could be hampered by strain specific growth inhibition of the indicator strain. Penicillinase detection by penicillin microdilution was less reliable.

DVP27

New InterVening Sequence (IVS) elements as markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria

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Introduction: The taxonomic genus *Rickettsiella* (*Gammaproteobacteria; Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. Genomic analysis has revealed the presence of two insertion sequences within 23S ribosomal RNA encoding genes of these bacteria.
RNase III processed intervening sequence (IVS) elements have been identified at conserved positions within the rRNA operons of several gamma-proteobacteria as, e.g., *Salmonella*, but not of *Escherichia coli*. Based on sequence similarity relationships, bacterial IVS elements are generally grouped into several families and superfamilies.
Materials and Methods: Sequence comparison, secondary structure modelling and a plasmid replacement approach in the surrogate host *E. coli*

were used to investigate structure, function, and usefulness as diagnostic markers of putative *Rickettsiella* IVS elements.
Results: Secondary structure modelling shows that *Rickettsiella* 23S rRNA insertions fulfill the structural criteria for RNase III processed bacterial intervening sequence (IVS) elements. One of the insertion sequences occurs in 23S rRNA helix 25, i.e. at a position that is conserved with respect to deduced 23S rRNA secondary structures among gamma-proteobacterial IVS elements. The second insertion element is located at a previously unidentified insertion site within 23S rRNA helix 72.
Expression of the *Rickettsiella* 23S rRNA genes in the surrogate host *E. coli* by a plasmid replacement approach leads to rRNA fragmentation and thereby confirms that *Rickettsiella* insertion sequences at both sites can function as IVS elements. Given the lack of sequence similarity with current GenBank database entries, IVS25 and IVS72 give rise to two unprecedented IVS element superfamilies.
Whereas the IVS72 element is highly conserved across the full range of investigated *Rickettsiella* species and *Rickettsiella*-like bacteria, the sequence of element IVS25 strongly varies among different *Rickettsiella* strains. Using the sequence information available for both IVS elements, a PCR-based approach for the genus-specific identification and infra-generic characterization of *Rickettsiella* bacteria has been developed.
Discussion: Two new IVS elements have been identified in 23S rRNA genes of *Rickettsiella* bacteria. Due to their varying degrees of conservation throughout the genus, IVS elements can be used as markers for the differential diagnosis of these arthropod-associated bacteria.

DVP28

Applicability of Vitek MS Plus to differentiate between *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *bolletii*

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Introduction: *Mycobacterium abscessus* is a non-tuberculous mycobacterium, which is an important pathogen to cause chronic lung disease in patients with cystic fibrosis. *M. abscessus* can be divided into the subspecies *M. abscessus* supsp. *abscessus* and *M. abscessus* subsp. *bolletii*. There is evidence that treatment response rates are different between both subspecies in *M. abscessus* lung infections, so that the identification to the subspecies level may be of clinical interest. Both subspecies can be discriminated using molecular methods, but we aimed to study, if an identification to the subspecies level can also be performed with MALDI-TOF MS (Vitek MS Plus, BioMerieux).
Materials and Methods: 40 clinical strains of *M. abscessus* including 19 *M. abscessus* subsp. *abscessus* and 21 *M. abscessus* subsp. *bolletii* were cultivated in liquid medium using BACTEC™MGIT™960. Positive cultures were processed by a glass bead based method and mass spectra were recorded with Vitek MS Plus. Raw spectra were processed due to the pre-settings by BioMerieux. The peak lists were used as input data for hierarchical cluster analysis (HCA) and principal component analysis (PCA) (DataLab).
Results: HCA using the unweighted pair group method with arithmetic mean did not reproduce the grouping determined by the two subspecies. In contrast, the 3d-plot of the first three PCAs showed two clusters with only marginal overlap. One cluster contained *M. abscessus subspecies abscessus* and the other one *M. abscessus subspecies bolletii*.
Conclusion: Our study demonstrates that MALDI-TOF MS is sufficient for discrimination between *M. abscessus subspecies abscessus* and *M. abscessus subspecies bolletii* using principal component analysis.

DVP29

The fully automated DiaSorin LIAISON® in comparison to established ELISA and gold standard for stool diagnosis of *Clostridium difficile*

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Introduction: *C. difficile* is the most frequent cause of nosocomial, antibiotic-associated diarrhea. For diagnosis it is recommended to use a 2-step-algorithm with GDH and Toxin tests. To improve the laboratory efficiency through this sequential algorithm the fully-automated random-access DiaSorin LIAISON® based on chemiluminescence technology (CLIA) was evaluated against several ELISA systems.
Materials and Methods: Samples from the routine were used (n=10-219) in parallel for GDH and Toxins A&B tests. Available systems were the ELISA (EIA) of r-Biopharm Ridascreen® *C. difficile* GDH and Toxin A/B (EIA), Meridian Premier™ *C. difficile* GDH and Toxins A&B (EIA), Alere C. DIFF CHEK™-60 and *C. difficile* Tox A/B II (EIA), LIAISON® *C. difficile* GDH and Toxins A&B (CLIA) as well as the gold standard (culture). For the r-Biopharm and LIAISON® assays also a sequential workflow analysis was performed to provide information of a feasible algorithm and the efficiency of the different ways of work.
Results: The prevalence of positive stools confirmed by culture was 15.1%. The sensitivity and specificity of the LIAISON® GDH test was 100%, for the LIAISON® Toxin A&B test it was >90% and 100 %, respectively. The concordance for GDH was between CLIA and EIA at 94.8-98.3%, for Toxin A&B at 87.5-92.2%. In total, the LIAISON® assays were in terms of quality equal or better to EIA methods. Between CLIA and culture the concordance was 4.5% higher than of EIA with culture (87.2% vs. 82.7%). The LIAISON® needed ca. 40% of the time compared to EIA systems.
Discussion: The LIAISON® *C. difficile* GDH test is a sensitive screening method to identify negative stool specimens. The LIAISON® *C. difficile* Toxin A&B test has due to the used CLIA technology the same or even better quality as known ELISA. Automated re-flex testing of both LIAISON® assays, reduced hands-on time and short incubation times leads to a faster availability of the results in ca. 40% of ELISA time. In detail, for negative samples results are in C. difficile assays are suitable for the routine usage in diagnosis of *C. difficile* infection.

DVP30

Testing of 100 Total Positive and Negative Stool Samples using DiaSorin LIAISON® H. pylori SA, Oxoid Amplified IDEIA™ Hp StAR™, Oxoid RAPID Hp StAR™, r-biopharm RIDASCREEN® FemtoLab H. pylori and Meridian Bioscience, Inc. PREMIER PLATINUM HpSA® PLUS.

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Introduction: Several methods are used to diagnose H. pylori infection including the invasive method of gastric biopsy and non-invasive methods such as urea breath test (UBT) and stool antigen test. This study contains data comparing four H. pylori stool EIA/rapid products with the chemiluminescence (CLIA) LIAISON® H. pylori SA Stool Assay.
Materials and Methods: The DiaSorin LIAISON® is a fully automated random access analyser system based on CLIA technology. In the current study, 100 positive and negative samples were received from LabCorp (USA) and randomized mixed for testing with five different assays, DiaSorin LIAISON® H. pylori SA, Oxoid Amplified IDEIA™ Hp StAR™, Oxoid RAPID Hp StAR™, r-biopharm RIDASCREEN® FemtoLab H. pylori and Meridian Bioscience, Inc. PREMIER PLATINUM HpSA® PLUS. Before testing all on the same day, stool samples were mixed prior to testing.
Results: The overall agreement between LIAISON® H.pylori SA assay and the four kits was between 95-100%. The highest agreement (100%) was between LIAISON® and the Oxoid RAPID Hp StAR™, followed by the Oxoid Amplified IDEIA™ Hp StAR™ and r-Biopharm RIDASCREEN® FemtoLab H. pylori with 98% overall agreement. The Meridian Bioscience, Inc. PREMIER PLATINUM HpSA® PLUS showed an agreement of 95%, meaning five samples discordance with LIAISON® and Meridian. For the five results which are discordant between these two kits, the other four kits

support that the LIAISON H. pylori SA kit is correct in making a positive call.
Discussion: LIAISON® H. pylori SA kit appears to be well centred between the four EIA kits which were tested in this comparison study and can be used for routine diagnosis of H. pylori. The kit didn't gave false positive results, since other kits support confirmed the low positive results by the LIAISON® H. pylori SA. Switching from ELISA to a newer technology (CLIA) will have the same or even better quality.

DVP31
Correlation of IgG Immune Responses to selected *H. pylori* Proteins with disease status in different populations

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H. pylori colonizes half of the world's population, but only a minority of infected individuals develop associated diseases. Nevertheless, it is a major health problem because of its high worldwide prevalence and the link to ulcer, gastric cancer and MALT-lymphoma development. To date, it is not possible to non-invasively identify patients with progressed disease state. *H. pylori* virulence factors have been associated with disease development, but direct assessment of virulence factors up to now requires invasive methods to obtain gastric biopsies.
Our study evaluated a new prototype of diagnostic test for serological detection of a *H. pylori infection*, using an improved selection of virulence factors. Moreover, we compared the performance of this prototype to the commercially available *recom*Line Helicobacter, using serum samples from different cohorts across the world (Germany, Italy, China, Mexico). In combination, the humoral responses against these antigens correlate with the severity of disease among *H. pylori*-positive subjects. In all populations the new antigen combination showed superior test results compared to the *recom*Line Helicobacter. In future this new combination of antigens could replace the commercially available *recom*Line Helicobacter.

DVP32
Are susceptibility testing results for piperacillin-tazobactam in ESBL-producing *E. coli* and *K. pneumoniae* prone to errors due to an inoculum effect?

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Introduction: According to recent EUCAST recommendations piperacillin-tazobactam should be reported as found even in ESBL producing strains. EUCAST recommends a turbidity McFarland adjusted between 0.45 to 0.55 for susceptibility testing, but it might be challenging to ensure reproducible inoculum densities under the conditions of a routine, high throughput microbiological laboratory. Therefore it is of utmost importance to know, if susceptibility test for piperacillin-tazobactam by different methods is prone to inaccurate results due to an inoculum effect. We studied the effect of an intentionally wide inoculum density range on the susceptibility test results for piperacillin-tazobactam in ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*.
Materials and Methods: Eleven clinical non-duplicated strains of *E. coli* and *K. pneumoniae* with an ESBL phenotype were studied. Each strain was tested for piperacillin-tazobactam with an intentionally low (McFarland 0.27 to 0.5) and high inoculum (McFarland 0.79 to 2.1) by microbroth dilution, semi-automated susceptibility testing (Vitek2, AST-N 223), disk diffusion and gradient strips (Etest and Liofilchem) according to EUCAST.
Results: No MIC increase higher than one dilution step was observed when comparing the intentionally low and high inoculum. An increase of one dilution step was observed for microdilution in 18.2%, Vitek2 in 0%, gradient strip (Etest) in 9.1% and gradient strip (Liofilchem) in 27.3%, but did not result in a change of resistance category classification, except for one testing. The average decrease of the inhibition zone diameter using disk diffusion was 0.5 mm with a maximum decrease of 2 mm.
Discussion: In our strain collection we could not find a relevant inoculum effect when performing susceptibility testing for piperacillin-tazobactam by several methods with an intentionally large range of inoculum densities. In view of recent EUCAST recommendations with regard to reporting piperacillin-tazobactam results in ESBl strains, these results have high implications for the accuracy of *in vitro* susceptibility test Results: Additional studies with more diverse resistance mechanism are necessary.

DVP33
Development of a mass spectrometry-based workflow to rapidly classify human pathogens by identifying subspecies-specific tryptic peptide biomarkers

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A well accepted method for identification of microorganisms down to the species level uses Matrix-Assisted Laser Desorption/Ionisation Time Of Flight mass spectrometry (MALDI-TOF MS) coupled to analysis software which identifies and classifies the organism mostly according to its ribosomal protein spectral profile. The method, called MALDI biotyping, is widely used in clinical diagnostics and has partly replaced conventional microbiological techniques such as biochemical identification (time-to-result minutes vs. hours or days in classical phenotypic or genotypic identification). A limitation to this method, however, is that accurate identification on below-species level has not been achieved up to now, making further phylogenetic classification unreliable. The present work demonstrates the establishment of a novel workflow that allows rapid identification on below-species level. We combined High Intensity Focused Ultrasound (HIFU)-driven tryptic digestion of the acid/organic solvent extracted (classical biotyping preparation) and re-solubilized proteins with nano-liquid chromatography and subsequently identified the peptides by MALDI-TOF/TOF MS. The mass spectra were acquired using Bruker microflex and ultraflextreme mass spectrometers operating in positive ion mode. Data were evaluated using Bruker Biotyper, Compass and Excel Macros developed in-house. MS/MS data acquired through WARP-LC were searched on MASCOT using the Bruker ProteinScape software. As a proof of concept, using this targeted proteomics workflow we have identified subspecies-specific biomarker peptides for three *Salmonella* subspecies. Applying this workflow resulted in an extension of the mass range and type of proteins investigated as compared to classical MALDI biotyping. In particular, we have shown that HIFU improved re-solubilization of extracted proteins and reduced duration of the trypsinization step (Gekenidis et al., 2014). This method therefore offers a rapid and cost effective identification and classification of microorganisms at a deeper taxonomic level. The novel combined analytical method will contribute to a more accurate and more sensitive pathogen identification by increasing the discrimination power to subspecies level in a multitude of disciplines, such as clinical and veterinary diagnostics or in food safety control and outbreak tracking.

Reference

Gekenidis M-T, Studer P, Wüthrich S, Brunisholz R, Drissner D. 2014. Beyond the MALDI biotyping workflow: In search of microorganism-specific tryptic peptides enabling discrimination of subspecies. Applied and Environmental Microbiology, published online ahead of print on 2 May 2014, doi:10.1128/AEM.00740-14.

DVP34
Evaluation of the BD MAX StaphSR kit for use with additional sample types

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The BD MAX™ StaphSR kit allows detection of *Staphylococcus aureus* and methicillin resistance (MRSA) from nasal swabs with the fully automated BD MAX system. The kit has been approved for use with a limited number of swab types. We set out to evaluate the BD MAX StaphSR kit with additional sample types. Therefore we (i) tested a series of different swab types that are commonly used for sampling and (ii) also evaluated the test for detection from positive blood culture bottles.

First, we tested the performance of the BD MAX™ StaphSR Assay by mimicking sampling with six different swab types: (1) Liquid Stuart, (2) Liquid Amies, (3) dry, (4) Amies Gel without charcoal, (5) Amies Gel with charcoal (BD culture swabs) and (6) BD ESwab Collection Kit. Swabs were soaked in 50 µl of MRSA strain #NCTC10442 (SCC*mec* type I) bacterial suspensions of defined concentration. Afterwards the swabs were added back to the transport system for 20 minutes before placing the swabs into the BD MAX sample buffer tubes as recommended. For ESwabs 200µl of the liquid was used. Five for each swab. The analytical sensitivity (Limit of Detection or LoD) was determined for five bacterial concentrations in eight replicates. We calculated the LoD for the result "MRSA positive" by a two-parameter log-logistic model with lower limit at 0 and upper limit at 1 estimated by non-linear least squares. For 90% detection rate the LoD was 387 (97-1551) (estimate, 95%CI), 877 (238-3230), 986 (183-5287), 1292 (328-5078), 5848 (622-55021), 2400 (426-13518) CFU/swab for Liquid Stuart, Liquid Amies, dry, Amies Gel without charcoal, Amies Gel with charcoal and ESwab Collection swabs, respectively. The latter three swabs had a tendency to be slightly less sensitive but none of the differences was statistically significant. We did not observe inhibition of the internal control with any of swab system. We further evaluated the BD MAX™ StaphSR Assay for use with positive blood culture bottles (BACTEC Plus). Bottles were spiked with MRSA strain and placed into the blood culture system. Directly and 24h after flagging positive, 15, 50 and 100µl aliquots were tested with the assay. All samples produced positive results with Ct values well above the detection limit. No inhibition of the assay was observed.
The results show that the BD MAX™ StaphSR Assay can be used with different swab types. It can also be used for the analysis of positive blood culture bottles with a microscopy of gram positive cocci.

DVP35

Rapid and Economic DNA serotyping and antimicrobial resistance gene determination of *Salmonella enterica* with an oligonucleotide microarray based assay

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Salmonellosis caused by *Salmonella* (*S.*) belongs to the most prevalent food-borne zoonotic diseases throughout the world. Therefore, serotype identification for all culture-confirmed cases of *Salmonella* infection is important for epidemiological purposes. As a standard, the traditional culture method (ISO 6579:2002) is used to identify *Salmonella*. Classical serotyping takes 4-5 days to be completed, it is labor-intensive, expensive and more than 250 non-standardized sera are necessary to characterize more than 2,500 *Salmonella* serovars currently known. These technical difficulties could be overcome with modern molecular methods. We developed a microarray based serogenotyping assay for the most prevalent *Salmonella* serovars in Europe and North America. The current assay version could theoretically discriminate 28 O-antigens and 86 H-antigens. Additionally, we included 77 targets analyzing antimicrobial resistance genes. The *Salmonella* assay was evaluated with a set of 168 reference strains representing 132 serovars previously serotyped by conventional agglutination through various reference centers. 117 of 132 (81 %) tested serovars showed an unique microarray pattern. 15 of 132 serovars generated a pattern which was shared by multiple serovars (*e.g.*, *S.* ser. Enteritidis and *S.* ser. Nitra). These shared patterns mainly resulted from the high similarity of the genotypes of serogroup A and D1. Using patterns of the known reference strains, a database was build which represents the basis of new PatternMatch software that can serotype unknown *Salmonella* isolates automatically. After assay verification, the *Salmonella* serogenotyping assay was used to identify a field panel of 105 *Salmonella* isolates. All were identified as *Salmonella* and 93 of 105 isolates (88.6 %) were typed in full concordance with conventional serotyping. This microarray based assay is a powerful tool for serogenotyping.

DVP36

Isolation of OXA β-Lactamases mRNA by sequence specific probes immobilized on magnetic beads

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Introduction: An alarming emergence of Gram-negative bacteria expressing β-lactamases with extended substrate spectrum (ESBL) can be observed for the last decades. Here, OXA β-lactamases, that exhibit ESBL or carbapenemase resistance phenotypes gain in importance. OXA β-lactamases are a highly inhomogeneous group (class D) that can be encoded chromosomally or plasmidal. To date, sequences of more than 200 OXA variants with and without clinical relevance have been published. Due to their sequence inhomogeneity this class of β-lactamases is challenging for diagnostic approaches.
We focus on alternative extraction methods that i) allows a pre-purification of meaningful OXA β-lactamase genes or mRNA, ii) are suitable for direct purification and analysis from blood samples, and iii) can be easily automated.
Materials and Methods: Clinically relevant OXA variants were identified by a semantic search algorithm. Target specific probes against clinically relevant OXA variants were designed -one for each subgroup- to bind all OXA variants of the individual subgroup and were immobilized on magnetic beads. Spiked blood cultures containing various bacterial samples with different β-lactamases, including OXA variants served as samples. Bacterial RNA extraction was done by the Tempus™ Blood RNA Tube and probe was added for hybridization. Bound mRNA was magnetically isolated by beads and washed afterwards. Reverse transcription of the cDNA was conducted using the probe as primer. Determination was performed using PCR.
Results: Searching PubMed by a novel semantic algorithm identified 9 out of 13 OXA-subgroups to be of clinical relevance. The bead based sequence specific probes correctly detected and identified the clinically relevant OXA variants in all spiked blood samples. The experimental set-up using spiked blood samples revealed a detection limit between $3*10^4$ and $7*10^4$ CFU/ml. Removal of the magnetic beads was not necessary, since synthesis of cDNA followed by PCR could be done directly on the bead and no bead interference was observed.
Conclusion: The presented bead-based approach allows a fast detection and discrimination of OXA β-lactamases in blood samples containing various bacteria and β-lactamases. In addition, the semantic analysis was a useful tool to quickly determine OXA variants of current clinical relevance.

DVP37

Fast and standardized detection of carbapenemase activity using Maldi-TOF in combination with an optimized workflow using ready-to-use reagents and an automated interpretation software

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Introduction: Spreading of multiresistant bacteria has become an increasing problem worldwide and requires fast, sensitive and reproducible detection assay. MALDI-ToF can be applied as a fast method for detection of beta-lactamase positive strains including those with carbapenem resistance due to carbapenemase activity.
Materials and Methods: We optimized a standardized workflow for routine diagnostics using ready-to-use reagents and subsequent detection of ertapenem digestion profiles using Maldi-TOF. Additionally we established a bioinformatic tool for automated and standardized evaluation of ertapenem digestion profiles (Bionumerics). The requirements for routine testing were fast, easy, sensitive and reproducible detection of strains with carbapenemase activity. The results were compared to standard phenotypic methods (Hodge-test; ABCD70-test) in order to focus strenghts and limitations of the new method.
Results: The optimized protocol using ready-to-use reagents allows identification of carbapenemase positive isolates in <3 hours with limited

hands-on-time (15 minutes). In comparison to standard phenotypic methods as e.g. Hodge- or ABCD70-test (Mast Diagnostics) the turn-around time is shorter and the sensitivity and reproducibility seemed to be higher by the new MALDI-ToF-based enzymatic assay. This might be due to inter-test variety of bacterial growths and also due to the fact that interpretation of phenotypic tests is always a matter of subjectivity. However, this assumption remains to be confirmed by comprehensive analysis of clinical strains under routine conditions. For the optimized MALDI-ToF based carbapenemase activity assay the performance was improved using a new bioinformatic tool for standardized and objective evaluation of ertapenem digestion pattern. This is important for implementation of this assay into daily routine testing.

Conclusion: We established an optimized workflow using ready-to-use reagents and MALDI-ToF analysis as a fast and easy applicable method to detect carbapenem resistance in routine diagnostics. The advantage of our protocol is not only a significant reduction of time, but also a high level of sensitivity and reproducibility. This protocol might be also applied in a modified version to the detection of other beta-lactamases, e.g. as penicillinases and ESBL.

Figure 1

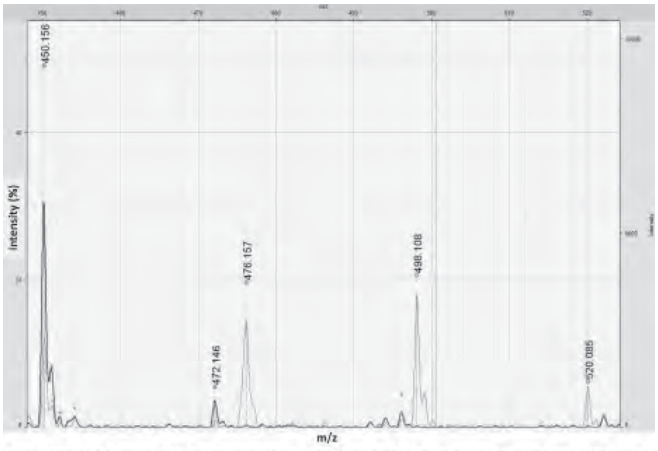

Figure 1: Hydrolysis of ertapenem by KPC-2 carbapenemase positive Klebsiella pneumoniae. Grey: Ertapenem (ETP) signals: ETP+H (476); ETP+Na (498) and ETP+2Na (520). Black: Ertapenem digestion procucts after 2h co-incubation with KPC-2 K. pneumoniae: ETP-hydr/decarb+H (450) and ETPhydr/decarb+Na (472).

DVP38

Characterisation of *Mycobacterium tuberculosis* resistance against later generation fluoroquinolones

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The tuberculosis (TB) incidence in Germany has steadily decreased over the last years. In the same time, the incidence of MDR (multidrug-resistant) TB, caused by *Mycobacterium tuberculosis* strains that are resistant to the first-line drugs isoniazid and rifampicin, is increasing. Fluoroquinolones (FQs) are presently used for the treatment MDR-TB. The aim of this study was to describe genetic mutations within *M. tuberculosis* isolates that are resistant against the later generation FQs moxifloxacin (MFX) and levofloxacin (LFX).

We analysed ofloxacin (OFX) resistant *M. tuberculosis* strains that were sent to the National Reference Laboratory for Mycobacteria, Research Centre Borstel, Germany between 2011 and 2013. Resistance mutations within the quinolone resistance determining region (QRDR) of *gyr*A and *gyr*B were detected by Sanger sequencing. To characterize the level of resistance to OFX, MFX and LFX, minimal inhibitory concentrations (MICs) were determined by the BACTEC MGIT 960 system together with the EpiCenter TBexist software.

In 40 of 48 ofloxacin resistant strains one out the mutations A90V, D94A or D94G was found within the *gyr*A QRDR. In one case, no mutations could be described. The MICs for most of the strains were much higher compared to the critical concentrations. While all strains are resistant to OFX and LFX, nine strain are susceptible to MFX. For the nine susceptible strains the MIC was identical to the critical concentration. The highest MICs (>16µg/ml for OFX, 20 µg/ml for LFX, 5 µg/ml for MFX) were detected for two strains that had a mutation at position 88 of the QRDR (G88S and G88C).

The results confirm earlier observations and provide new insights especially for the discussion about current critical concentrations of FQs. At present different levels of drug resistance are not regarded. Together with the knowledge of underlying mutations the MICs could be of great use for the improvement of strain-specific tuberculosis treatment in case of MDR-TB.

DVP39

Intracellular detection of *Mycobacterium gordonae* by Raman microspectroscopy

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Introduction: *Mycobacterium* genus includes a variety of pathogens like *M. tuberculosis* known to cause severe infections. For mycobacterial detection in patient samples, fluorescence microscopy and acid-fast technique are usually applied. Diagnosis of tuberculosis in its early stages is of high importance since it will allow a prompt treatment potentially avoiding the transformation of granulomatous bodies. Raman microspectroscopy, a novel technique, was successfully applied for the rapid detection and characterization of a wide range of bacterial species on a single cell level [1]. This technique offers the possibility to image complex biological systems and to follow metabolic processes inside cells [2, 3]. *M. gordonae* was reported to cause granulomatous inflammation and diseases in immunosuppressed and in immunocompetent individuals[4, 5]. In the scope of detecting intracellular *M. tuberculosis,* this study investigated the potentiality of Raman microspectroscopy to detect and localize intracellular *M. gordonae* as a model organism for invasive *Mycobacteria*.

Materials and Methods: As a model system, the murine macrophages cell lines J774A.1 were infected with *M. gordonae*. The sensitivity of bacterial detection inside the host cell by Raman microspectroscopy was investigated and compared with fluorescence microscopy.

Results: Viable *M. gordonae* were detected in murine cells. Moreover, two- and three dimensional chemical maps of the cellular microenvironment showed the localization and distribution patterns of bacteria within the cells.

Conclusion: The presented results demonstrated the potential of Raman microspectroscopy as a non-invasive and label-free technique for a fast and cultivation-free detection of *M. gordonae* in macrophages. Further outlooks would imply identifying *Mycobacterium* species in human macrophages and detecting various *Mycobacterium* species in a single infected cell.

Acknowledgement: We gratefully acknowledge the Free State of Thuringia and the European Union (EFRE) for financial support under support code 2013FE9057 (Fast-TB).

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DVP40

Evaluation of two new assays for the detection of anti-*Bordetella pertussis* (*B.p.*) Toxin (PT) antibodies

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Introduction: Since the introduction of the WHO 1st International Standard 06/140 clear recommendations for *B.p.* serology have been established, which should be considered during planning and development of respective assays by the manufacturers. This includes essentially choice of antigen and sample assessment criteria.
Materials and Methods: Bordetella-PT-IgG-ELISA medac and Bordetella-PT-IgA-ELISA medac were evaluated regarding precision, robustness, and diagnostic performance. Both assays are indirect ELISA using highly purified PT coated to the testplate, providing results in International Units by single-point quantitation (SPQ). Measuring ranges (IgG: 10-200 IU/ml; IgA: 2-30 IU/ml) inclose the recommended cut-points (40/100 IU/ml; 12 IU/ml). Precision experiments comprised determination of intra- and interassay variation, evaluation of linearity, and performance of SPQ. Accuracy was checked using the WHO Reference Reagent Pertussis Antiserum (Human) 06/142 (RR). Robustness measurements included comparisons between manually and automatically performed test runs using two different automatic devices (DSX, Evolis) and between serum and EDTA plasma samples. Diagnostic performance was evaluated using 215 samples from patients with suspected *B.p.* infection and 99 blood donors. Samples were investigated in comparison to commercial reference assays. Discrepant results were evaluated in the German Reference Laboratory for *B. p.* to define the respective nominal assessment. In addition 200 blood donor samples (BL) were investigated regarding prevalence.
Results: Determination of precision within the measuring range provided coefficients of variation (CV) below 10% (IgG) and 15% (IgA). Linearity investigations resulted in CV data calculated from dilution steps of 2-10% (IgG) and 1-10% (IgA). The SPQ performance evaluation provided coefficients of determination of 0.982 (IgG) and 0.988 (IgA). The RR was determined as 114.4 IU/ml (+7.9%, IgG) and 18.6 IU/ml (+3.3%, IgA). Comparison between manually and automatically performed test runs revealed the following coefficients of determination: 0.995 (DSX)/0.997 (Evolis) for IgG and 0.987/ 0.993 for IgA. Serum and EDTA plasma results were in very good agreement. Diagnostic performance evaluation showed an overall agreement with the nominal results of 95.5% (sensitivity = 100.0%, specificity = 95.2%) for IgG and 92.0% (sensitivity = 100.0%, specificity = 89.6%) for IgA. BL prevalence investigation showed 92.5% < 40 IU/ml, 5% 40-100 IU/ml, 2.5% ≥ 100 IU/ml for IgG and 95.5% <12 IU/ml and 4.5% ≥ 12 IU/ml for IgA.
Conclusion: The results of the evaluation experiments demonstrate that the new commercially available assays Bordetella-PT-IgG-ELISA medac and Bordetella-PT-IgA-ELISA medac are very well suited to perform state-of-the-art routine diagnostic according to the recommendations of the scientific societies.

DVP41

Development of a specific pre-enrichment procedure for extended-spectrum beta lactamase (ESBL) producing *E. coli* in manure of livestock husbandry and output samples of biogas plants

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Extended-spectrum beta lactamase (ESBL) producing *E. coli* are a health care problem today. Intensive application of antibiotics in livestock husbandry increases the occurrence of ESBL *E. coli* in livestock which can be released via manure into soil, ground and surface waters and enter the human food chain. Beside the direct application of manure on fields, manure used directly in biogas plants to produce biofuels. We developed an efficient pre-enrichment methods to detect ESBL *E. coli* in input and output samples of biogas plant to assess the effect of the biogas plant process on the release of ESBL *E. coli* into the environment.
Three quantities (0.1, 1 and 10 g) of input and output samples were pre-incubated in liquid medium in the presence of cefotaxim and ceftazidim and an aliquot was subsequently applied to CHROMagar ESBL (MAST Diagnostica, Paris, France). Colonies were screened of the presence of ESBL genes using a CTX-M, TEM and SHV targeting multiplex-PCR (Monstein et al., 2007). Positive colonies were further identified by 16S rRNA gene sequencing and characterized in more detail.
The pre-enrichment method was applied to detect ESBL *E. coli* in input and output samples of two German biogas plants at the four seasons in 2013/2014. While direct plating of serially diluted bacteria detached from input and output material on CHROMagar ESBL enabled the detection of only few ESBL *E. coli* in input and non in output samples, even in 0.1 g output material ESBL *E. coli* could be detected after pre-enrichment. Beside *E. coli, Pseudomonas*, *Acinetobacter*, and *Achromobacter* spp. were detected after pre-enrichment, but could be clearly distinguished from ESBL *E. coli* by coloration on CHROMagar plates. This was confirmed by 16S rRNA gene sequencing. A total of 296 isolates were screened and 136 ESBL *E. coli* were detected in input and output samples, respectively. Most carried CTX-M and/or TEM genes. MLST showed the presence of several ST types indicating a high clonal diversity of ESBL *E. coli* in livestock husbandry. Susceptibility testing to different antibiotic showed, that many of the detected *E. coli* were multi-drug resistant. In summary our study showed that ESBL *E. coli* were not eliminated in biogas plants and can be released into the environment.

Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K et al. (2007) Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. Apmis 115(12): 1400-1408.

DVP42

Comparison of a new chemiluminescence technology with the fully automated DiaSorin LIAISON® with the established ELISA technology of r-Biopharm for diagnostics *of C. difficile*

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Introduction: ELISA systems are mainly used for the diagnosis of *C. difficile* infections. The next evolution step for diagnosis is the usage of full automated chemiluminescence (CLIA) assays based on magnetic beat technology. The DiaSorin random access LIAISON® platform delivers fast and reliable results with less technical handling. In this study the LIAISON® *C. difficile* GDH und Toxin A&B assays was compared with the ELISA assays of r-Biopharm.
Materials and Methods: In the current study, 236 samples were taken out of the daily routine and tested in a 2-step-algorithem with the GDH assays as a screening parameter and with the Toxin A&B assays for confirmation following the ESCMID guideline. A direct comparison was done with r-Biopharm Ridascreen® *C. difficile* GDH (ELISA) and Toxin A/B (ELISA). The usage of *C. diffcile* culture and preliminary examination results was to clarify discrepant results between both systems and to define sensitivity and specificity.
Results: The prevalence of positive stools confirmed by culture was 11.2%. Overall concordance for the GDH assays was at 94%, for Toxin A&B assays at 82%. The specificity on GDH was for both methods similar with 98.3-98.9%, but the sensitivity of the GDH screening assay was with LIAISON® at 98% higher compared to ELISA at 90%. In the 2-step-procedure the sensitivity for Toxin A&B increased with LIAISON® to 100% and with ELISA to 77% while the specificity was at 90% and 100%. Putting the preliminary and culture data together the ELISA had 6.1% false negative/positive results while the LIAISON® showed only a rate of 1.7%.
Discussion: The DiaSorin fully automated random access analyser provides a rapid and enclosed sample processing. Very practical is the fact that samples can be tested as soon as they arrive in the laboratory according to the random access principle. The LIAISON® platform can be used in routine to detect *C. difficile* infections. The performance of CLIA showed high concordance with ELISA making a switch of the routine to the new technology more easy. Faster time to result and better workflow are also aspects to be taken into consideration.

EUKARYOTIC PATHOGENS

EKP01
Survival strategy of *Plasmodium spec.* in the vertebrate host

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Infections by eukaryotic organisms like *Plasmodium spec.* are different in principle from infections by prokaryotic pathogens. The quantitative analysis of the Plasmodium life cycle reveals features never observed in microbial infections. The development in the vertebrate host passes prepatency and patency like metazoic parasites: The propagation is linked with sexuality, and separated vegetative multiplications are genetically limited and regulated. The gamontogenesis is intensified during dry seasons and increases with the age of the human host compensating the parasite's age drift. Therefore human is the definitive and mosquitoes the intermediate host, the intestine of which outside world. Clinical symptoms shift between semi-immunity and manifestation but have to be boostered in continuity. Infection initiates immunological tolerance induced in the regional lymphnods and end spontaneously. Parasites prevent extinction controlling the host's population density.

Fig. 1: Life cycle of *Plasmodium falciparum* quantified.

Fig. 2. *Plasmodium*. Parasite-host balance. ***Left:*** *Genetically determined functions*: Limited merogonia in liver cells (LC) and regulated merogonia in the circulating blood control the multiplication for maintenance in the host. Gamogonia is positively correlated with the vector's seasonal population dynamics and increases with human age compensating for its natural losses (age drift). Both mechanisms obviate shortages in propagation. Short living *Plasmodium spec.* store hypnozoites against their disappearance. - - - - window of transmission.

Middle: *Directed sector of the vector population*: ~5% of the anthropophilic part of the vector population (100%) are multifactorially determined as being susceptible for the *Plasmodium.* This 5% portion will be genetically stabilized by the seasonally high vector population turnover. The gamonts develop into gametes inside the mosquito's gut lumen and unite to form the zygote. Of these, ~10% penetrate as ookinetes the peritrophic membrane. They create the oocysts in the gut epithelial cell in which the sporogonia is limited to 12 divisions. The sporozoites are transmitted by saliva at seasonally changing rates according to the vector contact rate.

Right: *Immunologically acquired interaction with the vertebrate host*: At high incidence, semi-immunity prevents pathogenic effects, saving the human population from excessive deficiencies. On abating due to a lack of boostering superinfections, the infection becomes manifest. Pathogenic effects reduce fitness and increase losses, preserving the host's resources. In summary, the shift controls the human population density and supports the simulzaneous survival of the *Plasmodium*. It prevents the host being killed by the *Plasmodium* (*above*) or an overpopulation of the host.

Wenk P, Renz A (2014) Malaria: Wettrüsten oder Selbstkontrolle? Nat. Rundsch. (subm.).

Figure 1

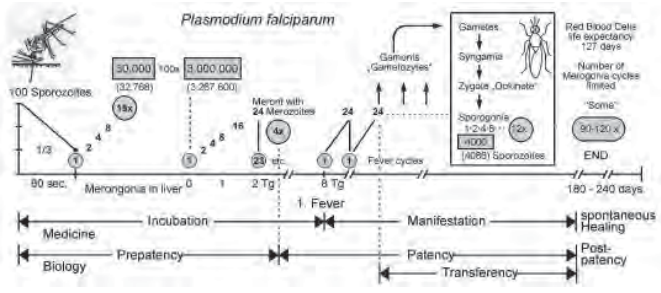
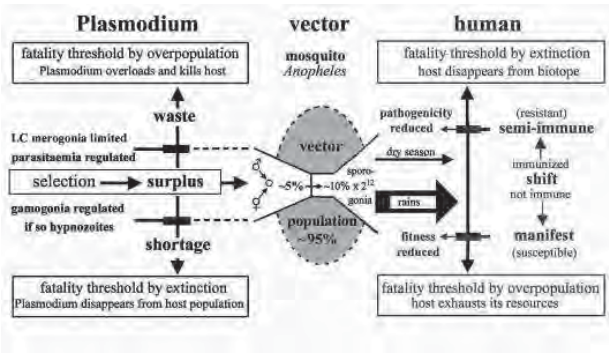


Figure 2



EKP02
Elimination of river blindness (*Onchocerca volvulus*): The survival strategy of eukaryotic parasites

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Parasitic infections of vertebrates caused by helminths like *Onchocerca volvulus* are profoundly different from infections by prokaryotic pathogens. In North Cameroon, 27 years of mass-treatment by the microfilaricide ivermectin have not eradicated the parasite *O. volvulus* in the human reservoir. This highlights the sophisticated mechanisms by which eukaryotic parasites establish a status of stable endemicity regardless of the intensity of transmission (Fig. 1). Their life-expectancy exceeds the latency of any immune-reaction and hence leads to a symbiosis of parasite and (vertebrate) host, which allows by a series of super- and re-infections the prolonged and repeated use of their hosts ("simultaneous strategy", Fig. 2). Immune reactions are used within the repertoire of density-dependent mechanisms as a means of self-limitation.

Even though ivermectin greatly reduces the numbers of microfilariae in the skin - which are responsible for pathogenicity - the transmission is much less affected. Much clearer than in human onchocerciasis, density-dependent regulation of a helminth parasitosis can be illustrated by the bionomics of the bovine homologue *Onchocerca ochengi* in African Zebu cattle. Its endemicity is very high (over 90 %), the individual worm load per host is very variable, but never presents a hazard to the health of the cattle host. In practice, these fundamental differences have their consequences for the immunology, epidemiology and control of helminths. We shall demonstrate this by comparing the attempts and results to control a helminth parasite versus the use of a highly lethal virus disease to eradicate rabbits in Australia.

Fig.1:
Fig. 1: Stable endemicity is established regardless of the number of *Simulium damnosum* Annual Biting Rates (ABR). The number of worms per person increases along with the Basic Reproductive Ratio (R_o 1 to 300), but is damped down to an effective ratio (R_{eff} = 1) at variable worm densities.

Fig.2:
Structure and function of survival strategies in parasites.
Opposing strategy:During incubation (white bar), the microbial pathogen propagates exponentially. As soon as specific symptoms appear (manifestation, black bar), either the host dies or it acquires immunity. The decision depends on non-specific factors. Surviving hosts create a new generation of susceptible hosts, to be reached via the infecting chain. Periodic epidemics regulate the population density and, in the long run, the infection becomes endemic. Pathogenicity is essential.
Simultaneous strategy: The parasite develops (prepatency - white bar) up until sexual maturity. During patency (black bar), the propagation stages are transmitted. Simultaneously, the host propagates and creates a new generation of hosts to be reached by the infecting chain. The parasite accumulates by superinfections, impairing the host's fitness and fecundity due to crowding effects. Instead of immunity, the limited life expectancy of the parasite terminates the parasitosis, and otherwise continues by reinfection.

Wenk P, Renz A (2012) Parasitism and evolution: opposing versus balancing strategies. Hist. Biology 25, 1-9
www.riverblindness.eu

Figure 1

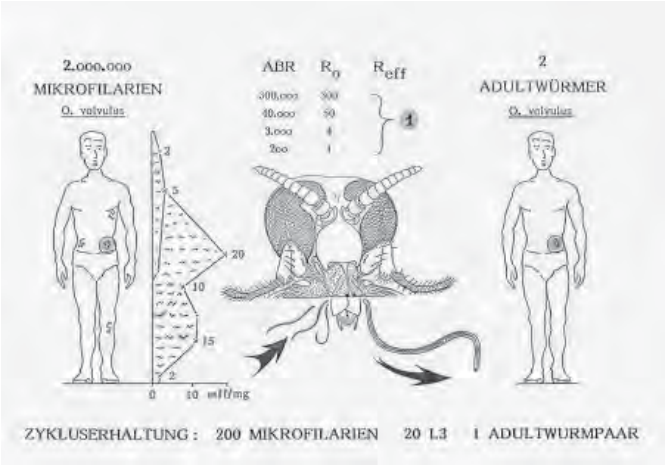
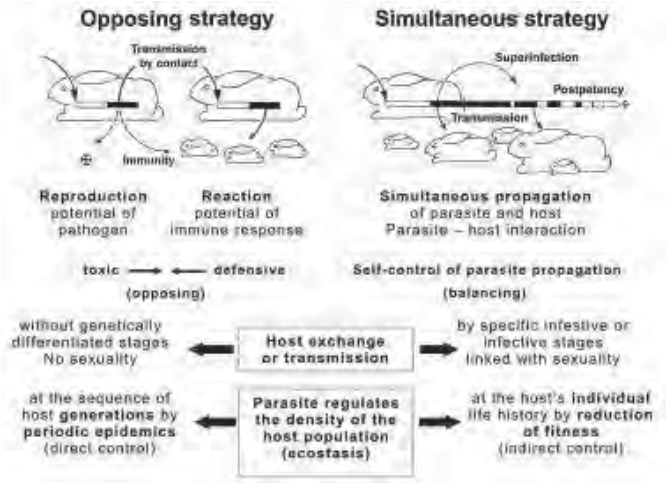


Figure 2



EKP03

Diagnostic accuracy and inter-laboratory comparison of microscopy and real-time PCR for detection of *Strongyloides stercoralis*

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Introduction: Human infections with the intestinal nematode *Strongyloides stercoralis* encompass a wide clinical spectrum, ranging from asymptomatic carriage to life-threatening disease. The diagnosis of *S. stercoralis* in stool is cumbersome and the sensitivity of conventional microscopic techniques is low, particularly in light infection intensity. Novel molecular tools have been developed to increase sensitivity, notably real-time PCR. We compared the diagnostic accuracy of PCR with that of microscopy for the detection of *S. stercoralis*, and investigated inter-laboratory agreement of real-time PCR in two European centres.

Materials and Methods: A cross-sectional epidemiological study was carried out in a rural part of south-central Côte d'Ivoire in mid-2009. Fresh single stool samples from 256 randomly selected individuals aged between 1 month and 74 years were examined in a field laboratory using three microscopic techniques (i.e. Kato-Katz, Koga agar plate and Baermann funnel) for detection of *S. stercoralis* and other intestinal helminths. Small aliquots of stool, fixed in ethanol (70%), were transferred to Europe and subjected to real-time PCR for *S. stercoralis* diagnosis in two laboratories.
Results: Considering any positive test result as 'true' positive, the overall prevalence of *S. stercoralis* was 21.9%. Real-time PCR revealed the highest prevalence (16.8%), followed by Baermann concentration (8.2%) and Koga agar culture (4.7%). About half of the *S. stercoralis* infections were detected exclusively by real-time PCR. All specimens that tested positive for *S. stercoralis* by both Baermann and Koga agar were confirmed by PCR. However, the diagnostic agreement between microscopy and PCR was considerably lower when only one microscopic technique was positive. The sensitivity of Koga agar, Baermann technique and real-time PCR for detection of *S. stercoralis* was 21.4%, 37.5% and 76.8%, respectively. The inter-laboratory comparison of real-time PCR yielded concordant results in 235 out of 256 samples, thus leading to a substantial diagnostic agreement (Cohen's kappa measure, κ=0.63, p<0.001).
Conclusion: Real-time PCR was more sensitive than a combination of the Baermann and Koga agar technique for the diagnosis of *S. stercoralis*, and the results obtained by real-time PCR in two European laboratories showed substantial agreement. Besides increasing sensitivity, PCR may also enhance specificity by preventing misdiagnosis of morphologically similar nematodes (e.g. *S. stercoralis* and hookworm larvae in Baermann funnel). A combined diagnostic algorithm using real-time PCR and the Baermann technique is recommended for detection of *S. stercoralis* with high diagnostic accuracy.

Figure 1

Table 1. Comparison of the diagnostic agreement between two microscopic techniques (Baermann technique, Koga agar) and real-time PC (performed in two European laboratories) for the diagnosis of *S. stercoralis* in 256 stool samples obtained from a cross-sectional communit survey in south-central Côte d'Ivoire.

n	Microscopy		PCR in laboratory 1			PCR in laboratory 2			Diagnostic concordanc between laboratory 1 a laboratory 2
	Baermann	Koga	Positive	Diagnostic agreement with microscopy	Median Ct value	Positive	Diagnostic agreement with microscopy	Median Ct value	
5	+	+	5	100%	32	5	100%	37	100%
16	+	–	8	50%	31	8	50%	34	100%
7	–	+	1	14.9%	32	1	14.9%	39	71.4%
228	–	–	17	92.5%	34	20	91.2%	38	91.7%

EKP04

Approaching Elimination phase of Urinary Schistosomiasis in Iran

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Since five decades ago urinary schistosomiasis was seriously taken into account by Iranian health authorities aiming to prevent and control the infection transmission in Khuzestan province the only known endemic area for bilharziasis in the country. More than 45000 cases were annually reported from seven hot spots in southwestern Iran with the highest rate of 8.3% in epidemic periods in 1970. The initial program was successfully implemented in the endemic area based on, case finding, treatment, health education and environmental control targeting the habitat of *Bulinus truncatus*. Until recently urine examination was an intensive task covering about 80000 of school age children throughout the endemic rural areas annually. Thanks to restless health workers with regards to significant growing of social determinant of health standards, positive cases were dramatically declined over the years of field activities program. The last confirmed case of urinary schistosomiasis was seen in 2001.From that time our national health system could not detect any egg passer patient within the endemic area of Khuzestan. Since the elimination phase is approaching in the country, schistosomiasis is under close watch through annual screening of direct urine examination based on our updated surveillance program. Common border between Khuzestan and Iraq where bilharziasis is still reported endemic is regarded as the main risk factor for re-introduction of disease transmission in Iran. In present abstract WHO comments and suggestions towards urinary schistosomiasis elimination in Iran is elaborated and discussed.

EKP05

The physiological importance of mitochondrial division during the life cycle of *Aspergillus fumigatus*

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Aspergillus fumigatus is a mold and opportunistic pathogen that causes invasive aspergillosis in the immunocompromised host. Due to limited therapeutic options this systemic infection is associated with high mortality rates. Similar to other eukaryotes, fungi depend on functional mitochondria. The morphology of mitochondria within a cell is highly dynamic and depends on regular mitochondrial fusion and fission events. Notably, the machinery that facilitates mitochondrial fission is only partially conserved from fungi to mammals. In this study, we investigated the importance of mitochondrial fission for growth and viability of *A. fumigatus*. Growth of an *Aspergillus* fission mutant is strikingly reduced when compared to wild type. We visualized the mitochondrial morphology within *Aspergillus* hyphae by expressing a mitochondrially targeted GFP. Disruption of mitochondrial fission results in a significant alteration of the mitochondrial morphology. Interestingly, the *Aspergillus* fission mutant produces significantly less conidia than a wild type, suggesting that mitochondrial fission is important during sporulation.

EKP06

An alternative approach to combat *A. fumigatus* infections by activating the High Osmolarity Glycerol pathway.

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Aspergillus fumigatus is the causative agent of invasive aspergillosis, a life-threatening disease that menaces severely immunocompromised patients. The unacceptable high mortality of this disease is a consequence of shortcomings at the levels of diagnostics and therapy. Antifungal agents that are currently available for clinical use either attack the fungal membrane (azoles and polyenes) or the fungal cell wall (echinocandins). In this study, we have explored an alternative approach by activating the type III two-component sensor kinase TcsC and thereby targeting the fungal-specific High Osmolarity Glycerol (HOG) pathway. The HOG pathway enables molds to adapt to hyperosmotic environments. Compounds, like fludioxonil and iprodione, activate TcsC and thereby cause a dramatic swelling of hyphal cells that finally culminates in lysis. Dramatic changes in the cellular organization occur in swollen hyphae, such as an increased number of nuclei per compartment. Live-cell imaging furthermore revealed an immediate growth arrest by fludioxonil and iprofione that clearly precedes cellular swelling. In conclusion, we have obtained evidence that fludioxonil and iprodione trigger distinct cellular responses that paralyze and finally kill *A. fumigatus*. Similar effects have been observed for other clinical relevant molds, whereas *Candida* species appear be completely resistant since they lack the relevant type III two-component sensor kinase.

EKP07

Species Identification of Acanthamoeba isolates from keratitis patients by PCR-RFLP method

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Question: *Acanthamoeba* is an opportunistic protist that is ubiquitously distributed in the environment. The infections with *Acanthamoeba* spp. threat human health, such as *Acanthamoeba keratitis*, that threat human eye vision. This study aimed to identify the species of Acanthamoeba, isolated from cornea of keratitis patients referred to eye center.
Materials and Methods: Ten isolates were subjected for identification by PCR-RFLP method and the commercially available restriction enzymes NlaIV, BseDI and HpyCH4IV were used.
Results: Results showed that six samples belonged to *A. culbertsoni* and the rest were found to be *A. palestinensis*, thus all strains considered to be pathogen. To the authors' knowledge, this is the first report of species identification by PCR-RFLP method from Iran.
Conclusion/Discussion: These microorganisms are found in soil, water and dust, as well as in nasal cavity. Contact lenses wearers and Immuno compromised individuals are more susceptible to infection with *A. keratitis*. Thus properly disinfection of the lenses is recommended, because the Acanthamoeba can inter to the corneal tissue and survive in the space between the lens and the eyes, therefore *A. keratitis* will occur. Thus ophthalmologists must consider clinical symptoms especially in this group because early diagnosis could help in early treatment.

Figure 1

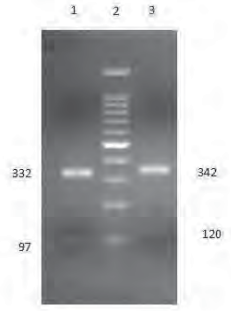

Fig. 1- A. culbertsoni, Restriction enzymes pattern: 1- BspLI(NlaIV) Restriction enzyme (332 bp, 97 bp), 2-100 bp DNA Ladder, 3-HpyCH4V Restriction enzyme (342 bp, 120 bp)

Figure 2

ion enzymes pattern: 1-HpyCH4V Restriction e
NlaIV) Restriction enzyme (305 bp, 97 bp), 4- ur

EKP08

The deletion of Candida albicans CZF1 influences the hypersensitivity of eed1Δ to farnesol

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Introduction: The polymorphic fungus *Candida albicans* is both a commensal and facultative pathogen that can cause superficial and life-threatening systemic infections. The morphological transition between yeast and hyphal growth is tightly controlled by a regulatory network. The quorum sensing molecule farnesol inhibits the filamentation by affecting different pathways. The *EED1* gene of *C. albicans* is important for hyphal maintenance. Mutants lacking *EED1* can still form germ tubes but return to yeast growth without forming extended hyphae. We furthermore observed that the *EED1* deletion mutant (*eed1*Δ) was hypersensitive to inhibition of filamentation by farnesol. Thus, the aim of this project is to elucidate the molecular mechanisms mediating this effect.
Materials and Methods: As the transcription factor *CZF1* is required for farnesol-mediated inhibition of filamentation under specific conditions, we investigated the role of *CZF1* for the hypersensitivity of *eed1*Δ to farnesol. Therefore, heterozygous and homozygous deletion mutants of *CZF1* in wild type and *eed1*Δ backgrounds were constructed. These strains were analyzed for filamentation under hypha-inducing conditions on plastic and agar surfaces and when embedded in an agar matrix.

Results: Farnesol suppressed hyphae formation in the wild type. Under embedded conditions, this effect was more prominent at 25°C than 37°C and was neutralized by the deletion of *CZF1*. In contrast, the effect of farnesol was not affected by deletion of *CZF1* in the other conditions tested. Furthermore, we found that *eed1*Δ is able to form true hyphae under embedded conditions after prolonged periods of incubation, which was inhibited by farnesol. Under these conditions, the additional deletion of both *CZF1* alleles fully alleviated the sensitivity of *eed1*Δ to farnesol, whereas the heterozygous *czf1* mutant (*CZF1*/*czf1*) in the *eed1*Δ background showed an intermediate filamentous phenotype. In contrast, deletion of *CZF1* in *eed1*Δ had no effect on hypersensitivity to farnesol on plastic and agar surfaces.
Discussion: Our results indicate that the sensitivity of *C. albicans eed1*Δ to farnesol under embedded conditions is mediated by *CZF1*. This appears to be specific for embedded conditions, as the inhibitory effects of farnesol in *eed1*Δ under other hyphae inducing conditions were not affected by the absence of *CZF1*. Factors that have been shown to contribute to farnesol effects and therefore might contribute to farnesol hypersensitivity in *eed1*Δ under other conditions include the transcription factors Efg1 and Tup1 as well as members of the PKA and MAPK pathways. We currently investigate the role of these factors for the *eed1*Δ phenotype. Furthermore, the observed filamentation of the *eed1*Δ mutant under prolonged embedded conditions suggests the existence of an Eed1-independent pathway supporting hyphal extension under complex and prolonged stimulation of filamentation.

EKP09

Functional analysis of molecular components of the habitat of *Leishmania*

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Leishmania spp. are medically important protozoan parasites that are transmitted as flagellated extracellular promastigotes by sandfly vectors during blood sucking to vertebrate hosts. *Leishmania* spp. cause a spectrum of diseases called leishmaniases that threaten more than 350 million people worldwide with 2 million individuals infected annually. It has been demonstrated that serum cholesterol as well as cholesterol located in the plasma membrane of infected host cells plays an important role during *Leishmania* infection. Nevertheless, little is known about the intracellular distribution of cholesterol in macrophages infected with *Leishmania* parasites. Pulse-chase experiments revealed a correlation between *Leishmania* infection and an increased retention of cholesterol within the parasitophorous vacuole, which represents the intracellular habitat of *Leishmania* parasites in macrophages. Confocal microscopy and proteomics analyses showed that cholesterol retention coincides with a reduced detection or absence of proteins that regulate cholesterol export in parasite-harboring compartments compared to phagosomes containing latex beads. Furthermore, during *Leishmania* infection, we detected an increased accumulation of filipin-stained free cholesterol around the internalized parasites, indicated as cholesterol halo. Labelling of the host cell cholesterol pool by fluorescent cholesterol species and following this pool in infected cells indicated that this pool too is trafficked to the parasitophorous vacuole, but it becomes incorporated into parasites and seems not to contribute to the halo detected by filipin. This two-way cholesterol sequestration correlates with the up-regulation of genes encoding proteins required for host cholesterol biosynthesis. These findings suggest that *Leishmania* amastigotes affect host cholesterol-dependent processes in order to survive in the harsh environment of the parasitophorous vacuole.

EKP10

Regulation of the carboanhydrase gene *NCE103* in *C.glabrata* and *S.cerevisiae*

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CO_2 is a key molecule in metabolism of different fungal species and plays an important role in host colonization and fungal proliferation and survival. CO_2-adaptation was investigated in a wide range of fungi, including *C.albicans*, *C.neoformans* and *C.glabrata.* For fixation of CO_2, expression of carboanhydrase *NCE103* leads to $HCO3^-$ generation and is therefore essential for fungal growth in the absence of CO_2. In *C.glabrata, NCE103* expression is regulated by Rca1 (Regulator of carbonic anhydrase 1), in *S.cerevisiae* by Rca1 homolouge Cst6, which are phophorylated under high CO_2 leading to repression of *NCE103* expression. Noteworthly, not only this mechanism but also the binding motif of Cst6 and Rca1 is conserved in *S.cerevisiae* and *C.glabrata*. To clarify the corresponding signal cascade, the aim of this work is to identify the Rca1-specific kinase and phosphatase.
Because of the phylogenetically close relation of *C.glabrata* and *S.cerevisiae*, experiments were predominantly carried out in *S.cerevisiae* using the powerful tools available for this model organism. A phosphatase/kinase mutant library of *S.cerevisiae* was screened by qRT-PCR to analyze *NCE103* expression under high and low CO_2 conditions to reveal candidates for phosphorylation and dephosphorylation of Rca1. The most promising kinase candidates, as well as Nce103 and Cst6, were recombinantly expressed in order to produce specific antibodies. To verify phosphorylation *in vivo*, *CST6* was overexpressed in *S.cerevisiae* under control of different inducible and non-inducible promoters and analyzed by qRT-PCR . Protein levels were detected via western blot. Within the *S.cerevisiae* mutant library screening, Sch9 and Bud32 were identified as most promising kinase candidates: Δ*sch9* showed exactly the expected *NCE103* expression pattern under high and low CO_2, and Δ*bud32* exhibited the highest overall upregulation. *BUD32*, as well as *CST6* and *NCE103* were cloned, expressed recombinantly in *E.coli* and purified. For further studies specific rabbit antibodies against these antigens will be produced. Concerning the *CST6* overexpression in yeast, the highest expression was reached under control of *GAL1* promoter. This could also be shown at protein levels. Within this project, is was already possible to identify 2 promising candidates for phophorylation of Rca1/Cst6. The production of specific antibodies will enable further investigation of protein interactions. In future experiments, phosphorylation of Rca1/Cst6 will be addressed in *in vitro* phosphorylation assays using purified, recombinant proteins.

EKP11

Development of a novel Malaria Antibody assay utilizing antigens from all 5 human pathogenic Plasmodium species

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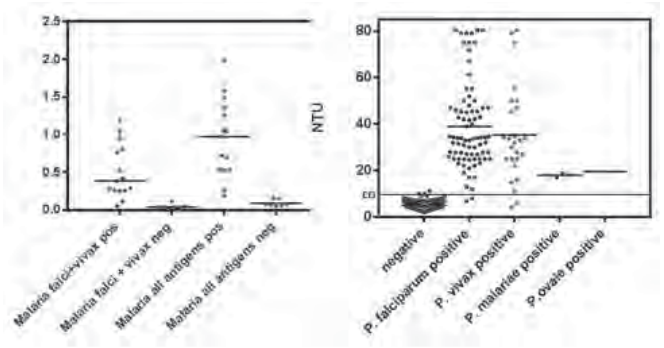
The proper diagnosis of Malaria disease is essential to provide early treatment and improve the prognosis of patients. Serological methods often fail in diagnosing new borns due to their altered immune system, resulting in a need for new diagnostic methods reliably working with sample from patients ranging from 0-12 month of age. Transfusion-transmitted Malaria is rare, but it may produce severe problems in the safety of blood transfusion and blood related products due to the lack of reliable procedure to evaluate donors potentially exposed to malaria. Microscopy, still considered the gold standard for diagnosing malaria. It is time consuming and requires trained expertise. Moreover, errors occasionally occur especially at low parasitaemia, limiting its use in blood banking and screening of populations. PCR shows a high sensitivity even at low parasitaemia and can distinguish between different Plasmodium species, but it is expensive and a state of the art laboratory is needed. ELISAs are known to be ideal for high throughput screening with high sensitivity and specificity, but it also requires trained personal and an equipped laboratory. Line Blots are often used as confirmatory tests since they provide high sensitivity and specificity. There is nearly no lab equipment needed to perform this kind of assay. In addition, blots can also be used in automated processes for high throughput screening. In our study, blots seem to be a good tool for diagnosing malaria in newborns. Here we show an improved diagnostic performance of the new

antibody detection Systems (ELISA and Lineblot) utilizing early and late antigens of all 5 human pathogenic Plasmodium species (P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi) compared to test systems only relying on antigens derived from one or two Plamsodium species. #IMG:Performance# The novel Lineblot is able to discriminate between all 5 parasite species. Assays with a limited number of antigens often fail to detect antibodies from certain regions of the world. For evaluation purpose, we collected samples from all over the world, including samples from newborns. We evaluated the performance of ELISA and Lineblot directly in endemic countries with samples of patients who presented symptoms akin to malaria infection in local hospitals.

Figure 1



EKP12

Performance Evaluation of Enzyme Linked Immunosorbent Assay and Lineblot for Serological Diagnosis of Chagas (Trypanosoma Cruzi) Disease

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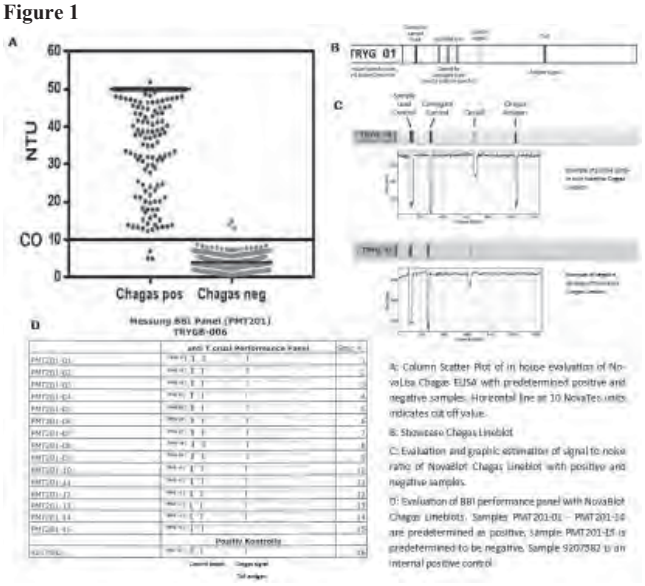
Chagas disease, caused by infection with the protozoan parasite Trypanosoma cruzi, affects 8-11 million individuals worldwide. It is endemic from the south of the US to Central America and South America. The disease is commonly transmitted by an insect vector but may also be spread through blood transfusion and organ transplantation, ingestion of food contaminated with parasites and from a mother to her fetus. Without specific treatment, the mortality rate among such children is high. Early diagnosis is essential so that etiological treatment can be administered. Screening of donated blood, blood components, and solid organ donors, as well as donors of cells, tissues, and cell and tissue products for *T. cruzi* is mandated in all Chagas-endemic countries and has been implemented. Due to travelling to endemic regions and immigration from endemic regions Chagas can be found also in Europe.
Serodiagnosis of new borns is often difficult due to their altered immune system and maternal immunoglobulin still present in the blood. Here we describe a new Novalisa ELISA and lineblot for diagnosis of Chagas disease. These test systems are taking advantage of the chimeric multiepitope antigen TcF (IDRI). The test was evaluated in endemic countries like Colombia and Guatemala with chronic patients (symptomatic and asymptomatic), pregnant women and newborns and a Chagas negative control group. Overall performance of both testsystems, ELISA and blot, was excellent. In addition both new testsystems were the only one who could reliably detect congenital transmission of Chagas from a mother to the child, making it essential for screening of new borns in endemic countries to provide treatment as early as possible.
In our hands NovaTec Immundiagnostica ELISA and blots can reveal positive patients that currently used in house tests can not detect. Results were confirmed by qRT-PCR and compared with results of other commercial kits.

Figure 1



EKP13

Analysis of a fungus-specific transcription factor family, the *Candida albicans* zinc cluster proteins, by artificial activation

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The zinc cluster proteins are family of transcription factors (TFs) that are unique to the fungal kingdom. In the pathogenic yeast *Candida albicans*, zinc cluster TFs regulate a variety of virulence-associated traits, like adhesion, morphogenesis, and biofilm formation. Three of these TFs, namely, Upc2, Tac1, and Mrr1, are key regulators of azole resistance and control the expression of ergosterol biosynthesis genes and multidrug efflux pumps, respectively. Gain-of-function mutations in these TFs, which result in their constitutive activity, are responsible for drug resistance in many clinical *C. albicans* isolates. We found that Mrr1, Tac1, and Upc2 can also be rendered constitutively active by C-terminal fusion with the heterologous Gal4 activation domain, suggesting that this may represent a general strategy for the artificial activation of zinc cluster TFs. We therefore created a complete library of all 82 *C. albicans* zinc cluster TFs in a potentially hyperactive form, which were expressed in the wild-type reference strain SC5314. Screening of this library resulted in the discovery of novel regulators of morphogenesis and resistance to drugs and oxidative stress. Among these were Mrr2, which controls the expression of the major multidrug efflux pump *CDR1*, as well as several transcription factors that conferred increased resistance to hydrogen peroxide. In many cases, overexpression or deletion of the wild-type gene, two commonly used methods to analyse gene function, did not cause a corresponding phenotypic alteration. Artificial activation is therefore a highly useful and complementary method to elucidate the role of zinc cluster transcription factors of unknown function in *C. albicans* and other fungi.

EKP14

Influence of hypoxia on interaction of *Candida albicans* with intestinal epithelia cells

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Introduction: The opportunistic fungal pathogen *Candida albicans* frequently occurs as commensal in the gastrointestinal tract of humans. If the intestinal barrier is disturbed, *C. albicans* can translocate and disseminate, potentially leading to life-threatening systemic infections. While it is known that intestinal epithelial cells are highly sensitive to ischemic hypoxia, the role of hypoxia-mediated damage in the translocation of *C. albicans* through the intestinal barrier is unclear. Thus, to evaluate the role of hypoxia on *C. albicans* translocation, we characterised enterocyte-*C. albicans* interactions under normoxic and hypoxic conditions.
Materials and Methods: Caco-2 cells were incubated at 37°C, 5 % CO_2 and different O_2 levels. At distinct time points of differentiation enterocytes were infected with *C. albicans* wild type and the mutant *eed1*ΔΔ (defective in

hyphae maintainance). The measurement of lactate dehydrogenase (LDH) levels 48 h postinfection was used to determine the Caco-2 damage. In parallel, fungal growth and morphology was assessed.
Results: Infection of enterocytes with *C. albicans* wild type led to comparable levels of damage if cells were incubated at ambient O_2 (~ 21 %) and under normoxic conditions (15 % and 12.5 %). At O_2 levels ≤ 10 %, *C. albicans* mediated damage significantly increased, while hypoxia-mediated damage of cells was only observed at ≤ 1 % O_2. As expected, the filament-deficient *eed1*ΔΔ mutant caused less damage than the wild type under normoxic conditions. However, at O_2 levels ≤ 10 %, LDH release by the mutant was comparable to the wild type. The *Candida* morphology during infection was comparable at all O_2 levels tested: As expected the wild type formed pronounced filaments while the *eed1*ΔΔ mutant grew as yeasts.
Conclusions: Although no gross damage by hypoxia was observed at 7.5 % and 10 % O_2, intestinal cells grown under these conditions were hypersensitive to *C. albicans*-mediated damage. This suggests, that ischemic conditions might enhance *C. albicans* translocation even in the absence of direct epithelial damage by hypoxia. In ongoing experiments we aim to further characterise the alterations that are induced by hypoxia and promote *C. albicans* invasion and damage. This includes detailed characterisation of epithelial barrier function, immune response and the identification of the fungal factors involved.

EKP15
Characterization of a putative *Aspergillus fumigatus* cell wall stress sensor important for antifungal resistance.
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The mold *Aspergillus fumigatus* is a major fungal pathogen and causative agent of invasive aspergillosis, a fatal systemic infection in immunocompromised patients with a significant mortality rate. A promising drug target for antifungal chemotherapy is the fungal cell wall. Its composition, biogenesis and adaptation to environmental stress are regulated by the cell wall integrity (CWI) pathway. We have previously shown that deletion of genes encoding the cell wall stress sensors MidA, Wsc1 and Wsc3 severely effects antifungal drug resistance, growth behavior and virulence[1]. However, our previous findings also indicated that additional cell wall stress sensors could exist. Here we characterized the role of a mucin family adhesin-like protein, MsbA, with similarity to *Saccharomyces cerevisiae* Msb2 and *Candida albicans* Msb2. An *A. fumigatus* Δ*msbA* mutant shows increased susceptibility against cell wall stress and against the echinocandin antifungal caspofungin. However, deletion of *msbA* does not affect activation of CWI effector proteins upon stress. Compared to wild type, the Δ*msbA* is more susceptible to fludioxonil, a fungicide causing hyper-activation of the high osmolarity glycerol pathway. However, no increased SakA phosphorylation was observed in the Δ*msbA* mutant.

EKP16
Survay of Chronic Fungal Rhinosinusitis in Infected Patients
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Introduction: The incidence of rhinosinusitis has been increasing steadily over the last decade. Fungi can be a causative agent of this infection, nasal polyps and allergic fungal rhinosinusitis. The aim of this study is to determine the prevalence of fungal rhinosinusitis among sinus and polyps biopsy of immunocompetent patients.
Materials and Methods: In this study biopsies from 98 patients underwent functional endoscopic sinus surgery were collected in sterile tubes with normal saline and referred to professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Science, Shiraz, Iran. Small pieces of tissue used for preparation of KOH smear and examined by microscope. Remained specimens were cultured on Sabouraud *dextrose* agar and incubate in room temperature for 14 days.
Results: The mean age of patients were 39 years and 57% were male. Among these 9.2% of patients positive culture for fungal elements. The etiologic agents were 3 *Aspergillus fumigatus* , 2 *Aspergillus flavus* , 1 *Alternaria* spp. and 3 *Penicillium*.
Conclusion: Fungal rhinosinusitis in immunocompetent hosts that suffer from chronic sinusitis is noticeable and early detection can be helpful for managment and treatment of patients.

EKP17
Antifungal Susceptibility of *Aspergillus* species Isolated from Immunocompromised Patients
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Introduction: Fungal infection may cause many severe infections with high morbidity and mortality particularly in immunocompromised patients.The mortality rate in infected patientsis high even the availability of antifungal therapy(>50%). The effective surveillance of these patients depends on early and suitable antifungal treatments. Since resistance to the antifungal drugs is seen in patients, susceptibility testing can then be helpful in management of the patients. The aim of this study was to investigate the susceptibility patterns of *Aspergillus* species, isolated from the patients by CLSI reference broth microdilution (MD) assay.
Materials and Methods: The MICs of various antifungal agents (ketoconazole, itraconazole, posaconazole,voriconazole, caspofungin and amphotericin B) for 48*Aspergillus* speciesisolated from the clinical samples of patients were determined by CLSI M38-A broth microdilution according to standard protocole.
Result: The MICs of the isolated fungi (27 *A. flavus*, 11 *A. fumigatus*, 10 *A. niger*) varied according to the species and resistance to amphotericin B and itraconazole was observed in *Aspergillus* species. The MIC_{90} of isolated for amphotericin B was 1.3, ketoconazole 6.5, itraconazole 0.9, posaconazole 0.3, voriconazole 0.24 and caspofungin 0.07 µg/ml.
Conclusion: To develop more efficient management of the immunocompromised patients, investigation of the susceptibility pattern of isolated to antifungal agents are necessary.

EKP18
Generation and characterization of monoclonal antibodies reactive with the surface of *Aspergillus fumigatus* hyphae.
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Aspergillus fumigatus is currently the most important air-borne fungal pathogen in humans. During infection, host-pathogen interactions take place on the fungal surface. Components of the fungal cell wall constitute the armor that protects the fungus, but some of them, e.g. β-1,3-glucan, are also recognized by host immune receptors. Apart from major polysaccharides, such as chitin, glucans and galactomannan, we know surprisingly little about the components and molecules that are present on the fungal surface. In this study, we have immunized mice with *A. fumigatus* germlings and screened for hybridoma cells producing antibodies with reactivity to the fungal surface. A panel of antibodies with distinct staining patterns was obtained and selected hybridoma cells were further characterized and cloned. As expected, most antibodies belong to the IgM subclass and appear to recognize carbohydrate antigens. Several of them are specific for galactomannan, but some have clearly distinct properties. Monoclonal antibody AB290-E2 is of particular interest; it shows a strong and uniform surface labeling of hyphae, but only a weak binding to the surface of resting and swollen conidia. The overall distribution of the AB290-E2 antigen resembles that of galactomannan, but AB290-E2 belongs to the IgG1 subclass and appears to recognize a novel protein antigen. IgG antibodies recognizing the hyphal surface of *Aspergillus* are extremely rare and may provide valuable tools to combat this important fungal pathogen.

EKP19
Prevalence of sporozoan protozoa and enteroparasites in the gastroenteritic patients referring to the health centers of seven provinces of Iran
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[2] Center for Diseases Control (CDC), MOHME, Tehran, Iran

Introduction: Sporozoan entero-parasites cause gastroenteritis and they are major cause of self limiting diarrhea in immunocompetents, however they cause serious diseases in immunosuppressed pateints. The aim of this study was to identify prevalence of sporozoa and entero-parasites among patients with gastroenteritis among referral health centers in seven provinces of Iran.
Methodology: In this cross sectional study, 4200 random stool samples were collected from patients with gastroenteritis in selected hospitals of

Gilan, East Azarbayjan, Qazvin, Kordestan, Mazandaran, Tehran and Khorassan-e Razavi provinces during one year. Primarily samples were examined directly for enteroparasites. The samples were then filtered and concentrated using Paraseb Kit. The pellets were fixed, stained by different assays including Acid Fast staining, Auramin Phenol Fluorescence, Geimsa and light microscopy.
Results: The results revealed the overall rate of infection was 3.86% (163 cases), as an indicator of infection to parasitic enteropathogens in Iran. Among the provinces, Khorassan-e Razavi with 8.83% (53 cases) had the highest rates of infection and Mazandaran with 0.34% (2 cases) showed the lowest rates of infections. The frequency of sporozoa including Cryptosporidium, Microsporidium, Isospora and Cyclospora was 0.1%, 0.1%, 0.07% and 0.02% respectively. Among parasites, Giardia lambelia with 1.78% reprsented the higest rate, Taenia saginata and Hook worms with 0.02% presented the lowest rates of infection. According to age groups, the higest rate of infection was in 0-10 year group (48%) and the least frequency was for 41 years old and above (6.7%).
Conclusion: Despite relatively low prevalence of sporozoa, Giardiosis is the most prevalent agent for gastroenteritis amongst 3.86% of parasitic infection in Iran. This study confirms abundance of infection in warm and wet seasons, and more frequency of infection among children than adults. Meanwhile, geographical and agricultural conditions, seasonal rainfall, abundance of water and animal contact are key factors affecting sporozoa infections.

FREE TOPICS

FTP01
Thaxtomin and non-thaxtomin producing potato pathogenic Streptomyces from Iran

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Several Streptomyces species are reported from the potato growing area in the world which cause potato scab diseases. Pathogenicity genes of them are nec1 and thaxtomin biosynthesis genes including txtA, txtB, txtC and txtD and the main produced phytotoxins are thaxtomin, concanamycin and a compound named as FD-981. As potato scab disease is an important disease in Iran, samples which shown scab symptoms including raised, netted and deep or shallow-pitted lesions collected from the main potato fields. The causative agent Streptomyces strains were isolated, which they were very heterogeneous belong to four main groups: S. scbies, S. acidiscabies, S. turgidiscabies and Streptomyces sp. They were pathogenic on potato, parsnip, horseradish, carrot and some other tested plants. Raised and netted potato scab-inducing strains produced thaxtomin determined by thin layer chromatography, but this phytotoxin could not be detected in the pitted lesion-inducing strains. Selected strains were examined for the presence and situation of the pathogenicity related genes as they induced variable disease symptoms under field and greenhouse condition. Pulsed field gel electrophoresis technique revealed that most of the tested strains carried a linear plasmid. Amplification of the pathogenicity gene fragments and Southern hybridization analysis showed that only some tested trains harbor nec1 and txt genes. A total of 20 representative strains was grown in modified oatmeal medium and extracted with ethyl acetate. It was shown that some strains produced pathogenicity compound (s) other than thaxtomin which induced pitted lesion on potato tuber slice.

FTP06
Baeyer-Villiger type monooxygenases in *Comamonas testosteroni* KF-1

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Members of the genus *Comamonas* are known specialists for the degradation of aromatic and xenobiotic compounds [1]. *Comamonas testosteroni* strain KF-1 is an efficient degrader of sulfophenylcarboxylates (SPCs), which are degradation intermediates of xenobiotic laundry surfactant linear alkylbenzenesulfonates (LAS) [2]. The mineralization of SPCs by strain KF-1 involves a type I family (FAD- and NADPH-dependent) Baeyer-Villiger monooxygenase (BVMO) and an esterase for a conversion of 4-sulfoacetophenone to 4-sulfophenyl acetate, and of 4-sulfophenyl acetate further to 4-sulfophenol and acetate, respectively; the enzymes have been identified, heterologously expressed, and characterized [3]. However, strain KF-1 encodes also three other candidate genes for type I family BVMOs [3], and these BVMO candidates were also cloned, heterologously expressed, and the substrate range of the purified enzymes determined. It turned out that only one of the enzymes is able to convert progesterone to testosterone acetate, hence, is a steroid BVMO [4], and that only this BVMO gene is inducibly transcribed during growth of strain KF-1 with progesterone; a corresponding testosterone acetate esterase was also identified. One of the other BVMO candidates exhibited activity only with an aliphatic ketone, 2-decanone, the aromatic ketone phenylacetone, and the carbothioamid and prodrug ethionamide, as substrates [5], whereat the last BVMO candidate showed no activity with any of the substrates tested thus far. In conclusion, we identified physiological functions for two of the four BVMO candidates in *C. testosteroni* KF-1, for a metabolism of SPC (4-sulfoacetophenone BVMO; [3]) and for a metabolism of progesterone (steroid BVMO), respectively. The third BVMO might have a role in a degradation pathway for aliphatic ketones in strain KF-1, whereat the substrate(s) and physiological function of the fourth BVMO candidate remain unknown.

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FTP07
Common aerobic bacterial species isolated from diabetic foot ulcer and their antibiotic susceptibility testing.

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Introduction: Magnitude of diabetes mellitus is increasing globally at an alarming rate. About 150-170 million populations are suffering from this diseases worldwide and the prevalence of diabetes will be double by 2025 as per WHO reports. Foot infections in diabetes are rarely due to a single organism. Aerobic bacteria (*Staphylococcus* spp.,*Streptococcus* spp., and *Enterobacteraciae*), anaerobic flora (*Bacteroides* spp., *Clostridium* spp., and *Peptostreptococci* spp.), and fungi are the organisms that are isolated most often.
Objectives: To investigate the aerobic bacteriological profile of diabetic foot ulcers and to find out the antimicrobial susceptibility of the isolated bacteria.
Materials and Methods: The study included 50 wound samples were collected from patients suffered from type 1 insulin dependent diabetes mellitus, and type2 non-insulin dependent diabetes mellitus admitted to Alkums General Hospital during the period of June to December 2013. Swab was collected from ulcer and has been cultured in media of blood agar and MacConkey agar and identification of the isolated bacteria depends on biochemical tests and indicators.
Results and discussion: The results indicate that common pathogen isolates from the diabetic pus included *Pseudomonas aeruginosae, E. coli* followed by *Proteus mirabilis* and *Staphylococcus aureus*. Antibiotic susceptibility according to Kirby-Bauer disc diffusion method to seven commonly used antibiotics. It was obvious that, Ciprofloxacin and rifampicin is more active against *E. coli* followed by gentamycin . Ofloxacin is more active against *Staphylococcus aureus* followed by gentamycin and amipcillin. Amikacin *is active against Proteus mirabilis* followed by gentamycin and erythromycin. *Pseudomonas aeruginosae* isolates were highly resistant to antibiotics such as ampicillin, rifampicin, cephalosporins and erythromycin.
Conclusion: Appropriate antibiotic therapy is an essential part of diabetic foot ulcer management.

FTP08

Characterization of *Vibrio cholerae* non-O1, non-O139 strains isolated from German and Austrian patients

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Introduction: *Vibrio cholerae* belonging to the non-O1, non-O139 serogroups are present in coastal waters of Germany and in some German and Austrian lakes. These bacteria can cause gastroenteritis and extraintestinal infections and are transmitted through contaminated food and water, however, non-O1, non-O139 *V. cholerae* infections are rare in Germany. We studied strains from German and Austrian patients with diarrhea or local infections for their virulence associated genotype and phenotype to assess their pathogenic potential.
Materials and Methods: Eighteen clinical non-O1, non-O139 strains and five toxigenic strains of the serogroups O1 and O139 were confirmed as *V. cholerae* by partial sequencing of the *rpoB* gene, MALDI-TOF MS, *toxR* PCR and biochemical confirmation. All strains were examined for the presence of genes encoding cholera toxin and toxin correlated pilus as well as other virulence associated factors or markers including hemolysins, RTX toxins, *Vibrio* seventh pandemic islands VSP-1 and VSP-2, and the type III secretion system (TTSS). Phenotypic assays for their hemolytic activity, serum resistance and ability to form a biofilm were also performed.
Results: Phenotyping and genotyping of putative pathogenicity associated traits were combined in a binary table to characterize the strains. The cluster analysis clearly separated the toxigenic strains from the non-O1, non-O139 strains, but also revealed genetic differences of the latter strains correlating with the nature of the clinical infection. Non-O1, non-O139 strains from diarrheal patients possessed TTSS and/or the MARTX toxin which were not found in strains isolated from ear or wound infections. Routine MALDI-TOF mass spectrometry analysis of all strains provided reliable identification of the species but failed to differentiate subtypes.
Discussion: The results of this study indicate the need for continued surveillance of *V. cholerae* non-O1, non-O139 in Germany, as the growing prevalence of *Vibrio* spp. in the environment due to climate change could lead to an increase of *Vibrio* caused infections.

FTP09

Antimutagenic activity of bee venom by Ames Salmonella/microsome mutagenicity assay

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Introduction: Honey bee venom (BV) is an active product that is produced by venom glands associated with the sting apparatus of honey bee workers and their queen. Injection of BV during the stinging process is the defense system for honey bees. The history of apitherapy traces back 5000 years. BV is a very complex mixture of active enzymes, peptides and amines. Its most important components are Melittin and phospholipase A_2. Several *in vitro* and in vivo studies revealed that BV has anti-inflammatory, cytotoxic and antibacterial effects. The aim of this study was to determine the antimutagenic activity of bee venom against sodium azide by Ames assay in presence and absence of rat microsomal liver enzymes (S9). Ames Salmonella/microsome mutagenicity assay is a convenient and short-term bacterial reserve mutation for the screening of substances that are able to produce gene damage and mutation.
Materials and Methods: Isfahan honey bee venom was purchased from science and research Islamic Azad University as powder. The histidine dependent strain of *S. typhimurium* TA100, developed by Dr. Ames of the University of California, Berkeley, USA, was cultured in a nutrient broth (Sigma, America). The Ames test was performed for 1-7 mg/ml concentrations of BV in the presence and absence of S9. The prevention percentage was calculated according to the following formula: [1-T/M] ×100. The result was assessed by one-way ANOVA and also in combination with a Tukey test for pairwise comparison. Values less than 0.05 were considered significant.
Result and Conclusion: The Ames test results indicated that bee venom can inhibit a mutagenic agent of sodium azide. Antimutagenic activity was increased significantly in the presence of S9. Bee venom, concentrated to 7 mg/ml, with the inhibition of 63.08% sodium azide showed high potential in decreasing mutagenic agents. As such, the findings reveal the antigenotoxic potential of bee venom both in the absence and presence of metabolic activation (S9 mix) systems.

FTP10

Actinobacillus equuli ssp. haemolyticus in a semi-occlusively treated horse bite wound in a 2-year-old girl.

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Introduction: We report on the isolation of *Actinobacillus equuli ssp. haemolyticus* from wound smears of a 2-year-old girl who was admitted to our hospital due to partial amputation of the distal phalanx of her right middle finger which was caused by a horse bite. *A. equuli* typically causes diseases in horses and only very few reports describing human infections (mostly associated with wounds) exist. Despite repeated isolation of the bacteria in consecutive smears, there were no signs of advancing infection or inflammation. Moreover, the fingertip regenerated after 74 days under semi-occlusive dressings with very pleasant Results:
Materials and Methods: Gram staining showed Gram-negative cocci. 24 hours after incubation at 37ºC and 5% CO_2 small grey-white bacterial colonies grew on Columbia blood agar. The strains were subsequently identified using Vitek 2, MALDI-TOF MS and 16s rDNA. The antimicrobial susceptibility testing was performed according to the CLSI criteria for *Pasteurella spp.* and additionally according to "EUCAST".
Results: Vitek 2 identified the strain as *Pasteurella pneumotropica*. In contrast to this, MALDI-TOF MS analysis revealed "*A. equuli*" which was confirmed by 16S rDNA sequencing (*Actinobacillus equuli ssp. haemolyticus*). Antimicrobial testing using the CLSI criteria showed susceptibility towards ampicillin, amoxicillin/clavulanic acid, moxifloxacin, trimethoprim/sulfamethoxazole and tetracycline but resistance against erythromycin. According to EUCAST-criteria our strain was susceptible towards ampicillin, amoxicillin/clavulanic acid, cefotaxim and ciprofloxacin. However, breakpoints for trimethoprim/sulfamethoxazole and tetracycline were not jet defined.
Discussion: Two independent methods (MALDI TOF MS and 16s rDNA sequencing) revealed *Actinobacillus equuli* as the correct identification whereas Vitek 2 misidentified our strain as *Pasteurella pneumotropica.* This identification error might result from the fact that discrimination between these two bacteria is based only on a few different biochemical reactions. Antibiotic susceptibility testing can be reliably performed according to both, CLSI and EUCAST guidelines. Moreover, due to the very convincing results obtained by keeping the finger under semi-occusive conditions plus the absence of any inflammation or infection, we assume that wound healing may have been positively influenced by the
bacteria growing under these conditions.

FTP11

FluBpH : A new ratiometric FRET-based biosensor for pH imaging

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The intracellular pH is an important modulator for various biological processes such as enzymatic activities, cellular signaling and transport processes. However, in general the intracellular distribution of protons is not uniform but depends on the nature of the observed tissues, cells and subcellular compartments. Dysfunction of cellular processes, in turn, is often associated with altered proton distributions and intracellular pH values. Consequently, accurate monitoring of intracellular pH allows to elucidate pH-dependent physiological or pathogenic processes. In this context, genetically encoded pH-biosensors represent a powerful tool to determine intracellular pH values non-invasively and with high spatial and temporal resolution. Remarkably, about 70 % of the so far described genetically encoded biosensors use the principle of Förster resonance energy transfer (FRET). Prominent examples for genetically encoded pH sensors are the pHlameleons, where the fluorescence of the FRET-acceptor domain (YFP) is quenched upon acidic pH due to chromophore protonation whereas the brightness of the FRET-donor domain (CFP) is almost unaffected.
Here, we present a new FRET-based pH biosensor toolbox (FluBpH), which utilizes the FMN-based fluorescent protein (FbFP)[1,2] as an alternative FRET

donor and different EYFP variants as FRET acceptors. In contrast to CFP, FbFP exhibit a remarkable tolerance towards acidic pH (p*Ka* < 3), it does not need molecular oxygen for chromophore synthesis and its fluorescence signal is not affected by halide ions. To cover a broad range of physiological relevant cellular pH values, we decided to use three EYFP variants as FRET acceptor domains exhibiting p*Ka* values of 5.7, 6.1 and 7.5. The three resulting FRET-based biosensors FluBpH 5.7, FluBpH 6.1 and FluBpH 7.5, were calibrated in different pH buffer systems with varying chloride concentrations to accurately evaluate their pH indicator properties. The ratiometric readout of the EYFP (λ_{max}=527nm) and FbFP (λ_{max}=495nm) specific fluorescence revealed that FluBpHs can be used to determine pH values from pH 5 to 9 with a superior dynamic range.

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FTP12
Development of a rapid detection system for *Clostridium sordelli* and *Clostridium difficile* in biogas reactors based on quantitative real-time PCR

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In the last decade, the question arose whether the increasing number of biogas plants can lead to the proliferation and distribution of microorganisms pathogenic for humans and animals. In order to clarify this question, a rapid and robust screening method is required which enables monitoring of the whole biogas process chain. Especially, endospore-forming bacteria such as Clostridia are able to outlast under harsh conditions, which can be found in biogas reactors *(*1). Indeed, clostridial toxin-associated protein families have been detected within biogas reactors by metagenome analyses (2). Therefore, our objective was to establish a quantitative real-time PCR (qPCR)-based system allowing the detection of pathogenic Clostridia within substrate, biogas reactor samples and digestate. Initially, pathogens relevant under project conditions (substrate grass silage, inoculation by cattle manure) were filtered out based on literature research. Further, only species which are pathogenic for human, animals or plants and able to persist biogas processes, were of interest. *Clostridium sordelli* and *Clostridium difficile* were selected as representatives of pathogenic Clostridia. For these microorganisms, an appropriate DNA-extraction method from silage, biogas reactor samples and digestative was established and the successful isolation of clostridial DNA was shown by spiking experiments. Furthermore, marker regions which are suitable for qPCR detection were searched and only virulence associated genes were considered. Thus, primer and probes were designed within the toxin genes of *C. sordelli* and *C. difficile* and tested for qPCR. In addition, the discrimination of both species by probe detection has been shown. Finally, specificity and sensitivity of the assay was successfully tested. In conclusion, we established a qPCR which facilitates the monitoring of the biogas process in regard to the potential contamination with pathogenic microorganisms. Potential threats caused by biogas plants can quickly be assessed, which contributes to improve security standards.

1)Dohrmann et al. 2010 Bacterial community structure in experimental methanogenic bioreactors and search for pathogenic clostridia as community members

Kalia 2011 et al. 2011 Analysis of the unexplored features of rrs (16S rDNA) of the Genus Clostridium

Bagge 2009 Hygiene Aspects of the Biogas Process with Emphasis on spore-forming bacteria

2)Eikmeyer et al. 2013 Detailed analysis of metagenome datasets obtained from biogas-producing microbial communities residing in biogas reactors does not indicate the presence of putative pathogenic microorganisms

FTP13
Initial steps of violacein biosynthesis

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Violacein is a purple chromobacterial pigment with a wide range of anti-bacterial and cytotoxic activity. It is produced and secreted by several Gram-negative bacteria mainly by the tropical bacterium *Chromobacterium violaceum* [1]. The biosynthesis of this antibiotic arises by enzymatic oxidation and coupling of two molecules of L-tryptophan. The resulting pyrrolidone-ring represents the scaffold of the final molecule [2]. The first step of tryptophan oxidation to indole-3-pyruvic acid (IPA) imine is catayzed by the flavoenzyme VioA. Subsequently, the hemeprotein VioB couples two IPA imine molecules to an IPA imine dimer. The present work is focusing on the biochemical and structural characterization of these initial enzymes of violacein biosynthesis from *C. violaceum*. Different recombinant *E. coli* production systems for VioA and the codon-optimized VioB protein have been established. UV-Vis-spectroscopic analysis of both purified proteins confirmed the presence of a FAD cofactor for VioA and a heme B cofactor for VioB. The crystal structure of VioA could be determined by heavy atom soaking of VioA crystals. In combination with small angle x-ray scattering the structure could be identified as a dimeric protein. Based on the VioA crystal structure a protein interaction with VioB is most likely.

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FTP14
Characterization and regulation of the RND-type multidrug efflux pumps AcrD, MdtABC and MdtUVW from *Erwinia amylovora*

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The Gram-negative bacterium *Erwinia amylovora* is the causal agent of fire blight, a destructive disease in rosaceous plants such as apple and pear. In order to colonize its host, *E. amylovora* must be able to circumvent the toxic effects of antimicrobial plant compounds. Multidrug efflux pumps, which mediate resistance towards structurally unrelated compounds, might confer increased tolerance to these plant antimicrobials. We identified and characterized three resistance-nodulation-cell division (RND) transporters, AcrD, MdtABC and MdtUVW, from *E. amylovora*. AcrD has been shown to be involved in efflux of highly hydrophilic aminoglycosides in *E. coli*. MdtABC and MdtUVW are heterotrimeric RND systems containing two different RND proteins forming a functional pump. The substrate specificities of the efflux systems were studied by overexpression of the corresponding transporter genes in a hypersensitive *E. amylovora* mutant lacking the major multidrug efflux pump AcrB. AcrD mediated resistance to several amphiphilic compounds including clotrimazole and luteolin, two compounds hitherto not described as substrates of AcrD in other enterobacteria. However, AcrD from *E. amylovora* was not able to expel aminoglycosides. The MdtABC and MdtUVW transporters mediated resistance to several flavonoids, fusidic acid and novobiocin. Additionally, MdtABC mediated resistance towards josamycin, bile salts and silver nitrate, and MdtUVW towards clotrimazole.
The ability of *mdtABC*- and *mdtUVW*-deficient mutants to multiply in apple rootstock was reduced, whereas an *acrD*-deficient mutant exhibited full virulence. Quantitative RT-PCR analyses revealed that the expression of the transporter genes was induced during infection of apple rootstock. Finally, we found that the expression of the *acrD* and *mdtABC* genes is regulated by the two-component regulator BaeR suggesting a role of these RND transporters in the cell envelope stress response of *E. amylovora*.

FTP15
The physiological role of AtpI in *Escherichia coli*

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The *atp* operon encodes the 8 structural subunits a, c, b, δ, α, γ, β and ε of the F_1F_o-ATPase and subunit i, the only non-essential and non-permanent part of the complex (3,5). F_1F_o-ATPase consists of a membrane integrated F_o part that translocates protons and a soluble F_1 part containing the catalytic sites for ATP synthesis from ADP and P_i using the electrochemical proton gradient (2,4). The function of AtpI is unknown although a chaperone-like function in c-ring assembly has been suggested for Na^+-translocating ATPases (1,6). In this study we used co-purification experiments and the BACTH (Bacterial Adenylate Cyclase-based two hybrid) system to investigate the interaction of AtpI with the subunits of the membrane integrated F_o complex. Further, we used shortened constructs and site-directed mutagenesis to describe the interaction interfaces.
Our results demonstrate for the first time the interaction of AtpI with the c-subunit of an H^+-translocating F_1F_o-ATPase. Furthermore, we were able to identify the interaction interfaces of both proteins. Additionally, we demonstrate that this interaction takes place either in the cytoplasm or at the cytoplasmic site of the cell membrane. Taken together the results of our investigation strongly support the model of AtpI as a chaperone-like protein. It directly interacts with the c-subunit of the F_o complex prior to the assembly of the c-ring and despite the fact that it has been shown to be widely dispensable for F_1F_o-ATPase synthesis and assembly it might play a role in localization or quality control of the complex.

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FTP16
Identification of phytoplasma species associated with Yellow Frond Disease on ornamental palms in Malaysia

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Introduction: Phytoplasmas are prokaryotes within the class *Mollicutes*, which have diverged from low-GC gram-positive bacteria. Lack of a cell wall placed these pathogens close to mycoplasmas and spiroplasmas. Phytoplasmas have been associated with over 700 diseases in different plant species including agricultural crops and ornamentals universally. Detection and characterization of phytoplasmas are very difficult because of the inability to culture them *in vitro*. In Malaysia, phytoplasma caused yellowing symptoms similar to coconut yellow decline on ornamental palms. Popular evergreen ornamental palms which are infected by phytoplasma not only lose their green and vivid appearance as decorative and landscape uses trees, but become a harbor for this pathogen as a source for next infection. It means detecting and characterization of phytoplasma associated with yellow disease on suspected palms is important before it is highly spreads.
Materials and Methods: Samples were collected from 20 symptomatic fronds and crown leaves of ornamental and coconut palm trees and two samples were chosen from healthy plants as a negative control. Total genomic DNA was extracted using CTAB method. Phytoplasma infection was investigated initially by nested PCR assays using phytoplasma-universal primer pair P1/P7 followed by R16F2n/R16R2 and fU5/rU3. The 16SrDNA nested PCR products were cloned and sequenced commercially. Representative sequences were compared with the phytoplasma sequences available in the GenBank database using BLASTn and submitted to NCBI. 16S rDNA sequences were aligned and a phylogenetic tree was constructed with the neighbor-joining method using MEGA 4.0 program. Virtual RFLP analyses of 16S rRNA gene F2nR2 fragments were performed with 17 distinct restriction enzymes using *i*Phyclassifier software.
Results and Discussion: Phytoplasma was detected by PCR assays and nested PCR products of the expected size (1250bp). Sequence analysis of the 1250 bp of ribosomal RNA gene revealed the phytoplasma as causal agent of yellow decline disease on foxtail (Wodyetia bifurcate), lipstick (Cyrtostachys renda), royal (Roystonea regia) and coconut palm trees. Detected phytoplasmas were belonging to 16SrI and 16SrXIV groups with accession numbers: KC924727, KC924728, KF803561, KC751560 & KC751561) (Naderali et al., 2013a, b). Virtual RFLP of rRNA operon sequences corroborated the sequencing results and revealed that phytoplasma strains were belonging to subgroup 16SrI-B and a possibly new 16SrI subgroup. Phytoplasmas in current study were showed highest 99% homology firstly with phytoplasma causing Bermuda grass white leaf (AF248961) and coconut yellow decline (EU636906), members of the 16SrXIV 'Candidatus Phytoplasma cynodontis' group, and secondly with Onion yellows phytoplasma OY-M strain (NR074811), from the 16SrI-B 'Candidatus Phytoplasma asteris' group.

Figure 1

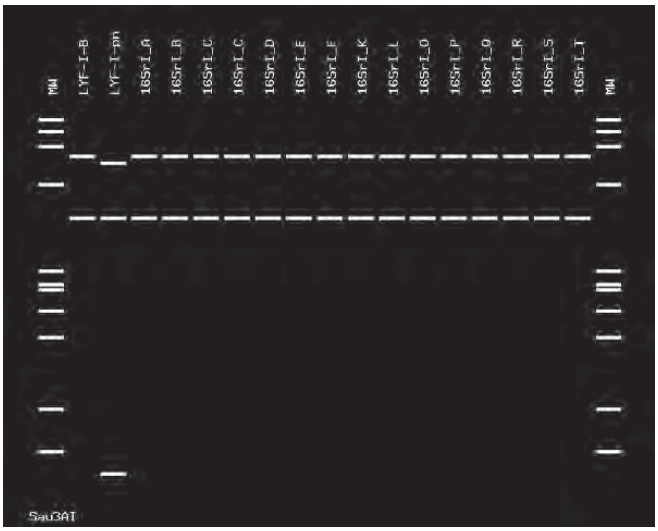
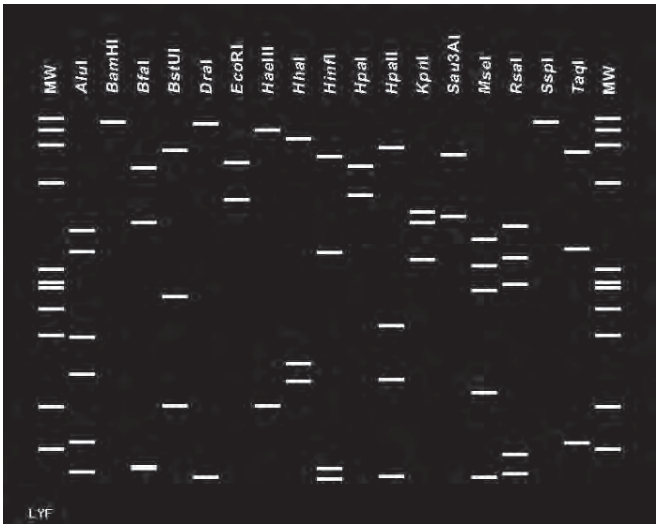


Figure 2



FTP17
Continuous fluorescence based measurement of redox driven sodium ion translocation

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Investigation of the mechanism of sodium ion pumping enzymes requires methods to follow the translocation of sodium ions by the purified and reconstituted proteins *in vitro*. Here, we describe a method that allows following the accumulation of Na^+ in proteoliposomes by the Na^+ -tanslocating NADH:quinone oxidoreductase (Na^+ -NQR) from *Vibrio cholerae* using a fluorescence based assay. The sodium sensitive fluorophor, sodium green, is frequently being used to detect changes in sodium ion concentrations in living cells [1]. By incorporation of sodium green into proteoliposomes, we were able to monitor sodium ion transport by reconstituted Na^+ -NQR. As a prerequisite for the accumulation of sodium ions in the lumen of the proteoliposomes, it was necessary to introduce a

regenerative system for one of the substrates, namely NADH. In the presence of lactic acid and lactate dehydrogenase, which continuously regenerates NADH from NAD^+, the Na^+ -NQR is able to accumulate sufficient amounts of Na^+ in the proteoliposomes which is visible as a change in fluorescence. We anticipate that this method will be very useful for the biophysical characterization of Na^+ translocating membrane proteins in general.

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FTP18
Preliminary proteomic analysis of porcine commensal *Escherichia coli* revealed clues to its response to zinc

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Introduction: Prohibition of feeding antimicrobial agents as growth promoters led to increased use of the dietary trace mineral zinc. In piglets, zinc reduces post weaning diarrhea caused by intestinal pathogenic *E. coli* (InPEC) and enhances growth performance. However, a great part of gut microbiota is commensal *E. coli*. To understand the influence of zinc on these commensal strains, we investigated the protein expression pattern of one commensal *E. coli* strain (IMT29408) that was isolated from 50% of the piglets during the zinc feeding trials.
Materials and Methods: Bacterial cultures and zinc exposure (1 mM $ZnCl_2$) were carried out in a bioreactor simulating anaerobic gut conditions. Bacteria were harvested after 2 h and 5 h of exposure, whole cell protein extracts were separated using 2-dimensional difference gel electrophoresis (2D-DIGE). The differentially expressed protein spots were excised, proteins were digested with trypsin and identified using MALDI-TOF MS.
Results: Zinc exposure did not display any significant effect on growth rate at both time points. Decodon software analysis detected 544 protein spots on the gel, among which 33 spots (zinc vs. control, >1.5 fold change, $p<0.05$) were differentially expressed out of which 19 proteins were identified. These proteins were found to be mainly involved in metabolic processes and signaling pathways. Interestingly, serine endoprotease (DegP), up-regulated at both time points, is reported to be involved in cell survival by degradation of denatured proteins after oxidative stress. 50S ribosomal protein L4 (RplD) which might play a role in stress response, was found to be up-regulated at 5 h of zinc exposure.
Conclusion: A commensal *E. coli* strain isolated from zinc feeding trials appeared to be resistant against zinc treatment in terms of growth rate. The present study revealed over expression of proteins that might cope with zinc induced stress.

FTP19
Do genome studies of streptophages enable new insights into soil microbiology and biotechnological solutions?

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The continual increase in heavy metal contaminated soils asks for new concepts for handling and remediation. In this framework, microbially assisted phytoremediation has been proposed to alter metal bioavailability which can be applied to reduce concentrations and toxic effects. A major group of bacteria involved in this processes are aerobic, gram-positive and filamentous *Streptomyces* species, a dominant group of soil bacteria specifically enriched at metal containing sites. Their growth, distribution and activity is controlled mainly by specific bacteriophages, making investigations of major ecological influences of phages on the diversity of soil microbial populations necessary to evaluate the impact of streptomycetes on bioremediation approaches. In addition, phages are the largest genetic pool on Earth, and therefore can be used as tools for genetic and biotechnological processes, especially since they are naturally involved in horizontal gene transfer. In consequence, streptophages (infecting species of *Streptomyces*) may be active in distributing important metal resistance genes such as methallothioneins, chelators (e.g. siderophores) or efflux transporters among *Streptomyces* species. To better understand the processes active between members of *Streptomyces* and their specific phages, three lytic streptophages were investigated. The polyvalent phage S7, originally isolated from compost, the narrow host range phage S3 from garden soil, and phage L44 from forest soil were sequenced and genes useful for genetic manipulation of *Streptomyces*, e.g. with a streptophage-*Streptomyces* transduction system, were identified, opening up possibilities to investigate the ecological role in metal resistance of streptomycetes. In addition, morphological investigations of the life cycle and phage size measurements allowed a taxonomic classification of the streptophages.
Our investigations are adding to the understanding of the role of phages for soil microbiology in general, as well as with specific impact for metal contaminated site ecology and bioremediation. They also allow to evaluate future use of streptomycetes for biotechnological applications, enhancing the already high versatility of *Streptomyces* for natural product production.

FTP20
The human T cell response to *S. aureus*

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Successful vaccination relies on immune memory, a function performed by T cells, B cells and antibodies. The natural T cell response to *S. aureus* is, therefore, of great interest, especially since T cell deficiencies increase the susceptibility to *S. aureus* infection. This indicates a protective role of T cells in human encounters with *S. aureus*. Moreover, it is well documented that most adults harbor a broad spectrum of *S. aureus*-specific antibodies. The fact that most B cells require the help of T cells for antibody production implies a large *S. aureus*-specific T cell pool.
The present study quantified human *S. aureus*-specific T cells in healthy adults and analysed their response to different bacterial antigens. First, extracellular *S. aureus* proteins were extracted from cultures of superantigen-negative *S. aureus* strains. They elicited vigorous proliferation of human T cells, which was much stronger than that triggered by intra-cellular bacterial proteins or by heat-killed bacteria. Next, the human T-cell response to recombinant *S. aureus* proteins (lipases, phospholipases, α-hemolysin, lipoproteins) and to wall teichoic acids (WTA) was examined. All antigens were used at concentrations which induced a robust response in peripheral blood mononuclear cells. For each antigen, cells from ten healthy donors, five *S. aureus* carriers and five non-carriers, were tested. The frequencies of *S. aureus*-specific T cells were determined by limiting dilution assays. In most cases, between 1 out of 200 and 1 out of 20,000 T cells responded to each antigen with no difference between *S. aureus* carriers and non-carriers. The *S. aureus*-reactive T cells were similar in frequency to those responding to typical recall antigens such as tetanus toxoid and cytomegaly virus lysate. This reflects robust T-cell memory of *S. aureus* in all tested individuals. Most T-cell clones reacted to the bacterial antigens with a pro-inflammatory cytokine profile, but with considerable variability. Extrapolating from the frequencies of T cells responding to the selected antigens, we estimate that at least 1% of all peripheral human T cells specifically recognize *S. aureus* antigens, which corresponds to around 10^{10} T cells in total. This large number of *S. aureus* antigen-reactive, memory T lymphocytes must shape the disease course in *S. aureus* infections.

FTP21
Pertussis toxin influences brain endothelial and immune cells to enhance *E.coli* RS218 transmigration

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The exotoxin Pertussis Toxin (PTx) is a major virulence factor of the Gram-negative bacterium *Bordetella pertussis* which causes whooping cough. PTx mediates the bacterial colonization of the respiratory tract and is responsible for the establishment of the infection. Especially in infants severe complications such as encephalopathies are observed leading to neurological disorders. These sequelae have been associated with PTx which is capable of disrupting the integrity of the blood-brain-barrier (BBB) as shown in different *in vitro* models (Brückner *et al.*, Kügler *et al.*, 2007; Seidel *et al.*,

2011). As a result, bacterial secondary infections of the central nervous system are benefited, above all by the pathogenic *Escherichia coli (E. coli)* strain *E. coli* K1 (Loh and Ward, 2012).
HBMEC and TY10 cells were used in a two-compartment tissue culture model to investigate the cellular and molecular effects of PTx such as invasion and translocation rates of meningitis-causing *E. coli* RS218 (O18:K1). Co-immunoprecipitations, western blot studies and qRT-PCR of proinflammatory cytokines were performed to investigate molecular changes in signaling and protein transcription after PTx (200ng/ml) incubation and *E. coli* RS218 infection.
Here we show that incubation of HBMEC and TY10 cells with PTx increases the invasion and translocation rates of *E. coli* RS218. The increase is not caused by direct cytotoxic effects of the bacteria or PTx itself, as shown by LDH and MTT assays. Instead PTx is able to activate signaling cascades which are described to be essential for E.coli RS218 invasion and translocation. Additionally we find a downregulation of inflammatory cytokines (TNFα, Il-1β and Il-8) on the transcriptional level.
In summary, we could show that PTx is acting at several key points to increase the permeability of the BBB ranging from downregulation of proinflammatory cytokines to relocalisation of TJ and AJ proteins.

FTP22
Antiangiogenic Activity of Girinimbine Isolated from *Murraya koenigii*

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Angiogenesis, the development of new blood vessels from existing vasculature, is a hallmark of over 50 different disease states including cancer. It is generally accepted that the blocking of angiogenesis is an attractive therapeutic strategy for the treatment of wide varieties of human cancers. In drug discovery, natural products hold much potential due to their active substances with chemotherapeutic properties. *Murraya koenigii* is an edible herb from the Rutacea family which is native in most parts of Asia including Malaysia, China and India. Girinimbine is a carbazole alkaloid isolated from the stem bark of *M. koeinigii.* Previous studies have established the anticancer potential of carbazole alkaloids against various cancer cell lines. The anti-angiogenic activities of girinimbine have not yet been reported*. In vitro*,, girinimbine exerted selective cytotoxicity on human umbilical vein endothelial cells (HUVECs) with compared to human normal colon epithelial cells. Significant time- and dose-dependent inhibition by girinimbine was also observed through endothelial cell migration, invasion and tube formation assays. Moreover girinibine mediates its anti-angiogenic activity through up- and downregulation of angiogenic and anti-aniogenic proteins *In vivo*, zebrafish embryos at 20 hpf were exposed to girinimbine for 24 hrs, and monitored daily up to 72 hpf for changes in morphology and blood circulation. Intersegmental vessles (ISV) were then visualized *in situ* by endogenous alkaline phosphatase (ALP) staining. Both *in vivo* and *in vitro* outcomes suggest anti-angiogenic properties for girinimbine, making it a possible anti-angiogenic agent in cancer therapy.

FTP23
SMOB, the NADH:FAD oxidoreductase of the two-component styrene monooxygenase system in *Acinetobacter baylyi* ADP1: biochemical and fluorimetric properties

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Flavin-dependent monooxygenases are involved in a multitude of catabolic reactions as it is the case for styrene monooxygenases (SMOs) which catalyse the stereospecific epoxidation of styrene into [*S*]-styrene oxide.
SMOs can be classified either as a common type of two-component monooxygenase (e.g. StyA/StyB of pseudomonads), or as a so called self-sufficient SMO (e.g. StyA1/StyA2B of *R. opacus* 1CP). The latter type can be easily recognized since the NADH:FAD oxidoreductase "StyB" is fused to another copy of the oxygenase subunit "StyA2". Moreover, proteins of both types yield different clades within a dendrogram which indicates that *styA*/*styB* are not the origins in the evolutionary event of gene fusion leading to *styA2B*. With the very recent identification of *smoA*/*smoB* within the genome of *Acinetobacter baylyi* ADP1 we could give first evidence for such an evolutionary link. In an attempt to identify the functional effects during the evolution of StyA/StyB → SMOA/SMOB → StyA1/StyA2B we focus here on the kinetic properties of SMOB and compare them to the ones of StyB (*Pseudomonas* sp. VLB120) and StyA2B (*R. opacus* 1CP).
For that purpose the oxidoreductase gene of SMOB was recombinantly expressed, purified by IEX, and subjected to various spectrophotometric studies.
Similar to StyB and StyA2B, SMOB activity is restricted towards NADH as the substrate. A comparable tolerance was found towards flavin substrates since riboflavin, FMN, and FAD could be used as substrates. With $k_{cat(FAD)}$ and $K_{m(FAD)}$ values of 56 s^{-1} and 4.4 µM, respectively, SMOB behaves like StyB of strain VLB120 (47 s^{-1}; 11.6 µM) but differs from StyA2B (5.2 s^{-1}; 26 µM). Fluorimetric studies showed that interaction SMOB to FAD quenches the specific fluorescence of that cosubstrate ($K_{d(FAD)}$ = 1.8 µM). In contrast, StyB of strain VLB120 increases the fluorescence of bound FAD ($K_{d(FAD)}$ = 2.3 µM) significantly. Since the above value was determined to be in the µM range, FAD is not bound as a prosthetic group as it is the case for PheA2 of *Geobacillus thermoglucosidasius* A7 ($K_{d(FAD)}$ = 9.8 nM). The presented results strongly indicate that SMOB of *A. baylyi* ADP1 is representing indeed a transitioning state between the classical two-component and the fused SMOs. In future, it would be interesting to extend these studies on the oxygenase subunit SMOA.

FTP24
PA3911 - a potential lipid carrier protein in *Pseudomonas aeruginosa*

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The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen which is colonizing the lung of patients suffering from the genetic disorder cystic fibrosis. In the chronic state of infection, *P. aeruginosa* grows as a biofilm in the airways of cystic fibrosis patients and possesses a high resistance to antibiotics [1]. A proteomic approach revealed that protein PA3911 from *P. aeruginosa* PAO1 is one of the most up-regulated proteins under anaerobic biofilm conditions of the cystic fibrosis lung. To date no biological function for PA3911 is described in the literature. From theoretical analysis a potential function as a lipid carrier protein was proposed. Three different vector systems (N-terminal GST-tag, thioredoxin-His_6-S-tag or *Strep*II-tag) for the heterologous overproduction of the codon-optimized PA3911 protein in *E. coli* were constructed. The PA3911 protein was purified *via* affinity chromatography and the specific lipid-binding capacity was analyzed using commercially available membrane lipid strips™ (Echelon® Biosciences Incorporated). The purified PA3911 protein binds to phosphatidic acid, different phosphatidylinositol derivatives and 3-sulfogalactosylceramide. All these compounds possess a common core structure. Phosphatidic acid is an important precursor for the biosynthesis of numerous phospholipids [2]. Accordingly, a central role of PA3911 for the *de novo* lipid biosynthesis and/or for *P. aeruginosa* lipid homeostasis was concluded.

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FTP25
Bacterial effector protein-derived cell-penetrating peptide (CPPs) as delivery system for antimicrobial agents

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Infectious diseases represent one of the major threats to human health worldwide and are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Among them, intracellular pathogens constitute a challenge for conventional antimicrobial therapies, because intracellular therapeutic levels are difficult to achieve. Drugs and therapeutic molecules (like oligonucleotides, proteins or organic molecules) generally lack a membrane penetrating capability and are not able to translocate into the

cytoplasm. Attempts to bind these drugs to polymers resulted in degradation and/or loss of activity. As cell-penetrating peptides (CPPs) are capable to mediate the internalization of biologically active molecules through plasma membranes they might also be employed to translocate antimicrobials into infected host cells. In this study, we characterized two domains derived from the *Y. enterocolitica* effector protein YopM, α_2H and 2αH, which previously showed cell-penetrating ability. These CPPs as well as the Tat peptide derived form HIV are currently investigated for their capability to deliver antimicrobial agents, such as antibiotics or siRNAs, to target intracellular bacteria and to inhibit Influenza A virus replication.
We showed that YopM-derived peptides possess penetration ability to the same extent as the Tat peptide. By flow cytometry and fluorescence microscopy, we found that all CPPs are able to deliver siRNAs, which accumulate in the cytosol of human lung epithelial A549 cells.
Furthermore, CPP-antibiotic conjugates reduced the load of intracellular pathogenic bacteria such as *E. coli* K1, *Salmonella* and *Shigella* in infected cells, as demonstrated by an invasion assay. Taken together, these data underline the potential of CPPs as delivery vehicles for antimicrobial agents. This might open the possibility for a remarkable new tool for the treatment of infectious diseases, especially those involving intracellular pathogens.

FTP26

Extracellular structures of pelagic *Formosa*

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Flavobacteria are known for their ability to degrade complex organic matter, in particular proteins and polysaccharides. During the decay of a spring phytoplankton bloom in the German Bight of the North Sea near Helgoland in 2009 a successive occurrence of different *Flavobacteria* clades was observed, which represented up to 60% of the bacterial community [1]. New strains of the genus *Formosa*, belonging to the marine clade of *Flavobacteriaceae*, were recently isolated from surface seawater off the coast of Helgoland [2]. Two of the strains possessed extracellular structures [2]. As only a few extracellular structures have been described within the family of *Flavobacteriaceae* and their function is still unclear, the description and analysis of such appendages is promising to give new insights into their biological role within the pelagic bacterioplankton. The two appendaged strains and the type strain *Formosa agariphila* were cultivated in HaHa_100 medium [2] and were examined for extracellular structures using light microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The cells of *Formosa* clade A exhibited segmented chain-like appendages with a width between 40 to 100 nm analyzed by TEM. To characterize the appendages, *Formosa* clade A cells were disintegrated by treatment with a French® pressure cell press and the cell debris was fractionated by differential centrifugation. Protein fractions were analyzed by SDS-PAGE and MALDI-TOF-MS. Identified proteins included many TonB-dependent receptors and an OmpA protein. In addition, one SusD-like protein and two hypothetical proteins of the outer membrane protein family were detected in an outer membrane enriched fraction. It is yet unclear whether the hypothetical proteins are associated with the appendages or represent outer membrane proteins involved in oligo- or polysaccharide degradation and transport.

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FTP27

Dye adsorption-based biomass quantification: a standardized method for facile and quantitative analysis of *Streptomyces* growth and physiology.

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Like other filamentous microorganisms streptomycetes perform tip growth with hyphal branching and hence form mycelial aggregates. This hinders simple and rapid growth determination by turbidimetric methods, like optical density measurement, typically used for unicellular microbes. For the soil dwelling gram-positive high GC bacteria of *Streptomycetales* biomass quantification is usually performed by determination of cell dry weight. This method requires relatively large culture volumes and incurs a time delay of hours before measurement can finally take place. In physiological studies it is often necessary to define biomass amount immediately after cultivation. Development of a suitable dye-based technique that reduces sampling times and culture volumes, while retaining resolution would be of great benefit in quantifying growth of filamentous microbes such as actinobacteria of the genus *Streptomyces*. The adsorption of cationic dyes to negatively charged cell components provides a suitable method to quantify biomass gain. Methylene blue is a well-known dye suitable for different scientific applications and it turns out to be an excellent dye for adsorption measurements of *Streptomyces*. A cell amount of 1 mg mycelial biomass (dry weight) adsorbs an equivalent of ca. 1000 nmol dye from an aqueous methylene blue solution [(1)]. This derived Cell Amount Equivalent (CAE) was used for monitoring growth of *Streptomyces* starting from spores up to the stationary phase in small-scale (1-2 ml) cultures. Validation of the correlation between dye adsorption and dry weight measurements reveals there is no growth-dependent change in CAE ratios. This method was also demonstrated to work well for other microorganisms including the unicellular *Escherichia coli*. The methylene blue-based determination of the cell amount by adsorption (MBA) needs only a small mycelial probe with 0.025 - 0.25 mg (dry weight). The measurement procedure is rapid (15-30 min) and gives highly reproducible values with a good resolution. Due to several advantages, MBA with its CAE unit is predestined as a new standard reference measure for *Streptomyces* growth and the study of its physiology. Beyond the proof-of-principle and the validation of the method, we demonstrate its utility in the study of oxygen-dependent growth, in nitrite toxicity investigations and in respiration studies.

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FTP28

Influence of alkali cations and pH on electron transfer and voltage generation by the Na^+ - translocating NADH:quinone oxidoreductase (Na^+ -NQR) of *Vibrio cholerae*

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The Na^+ -translocating NADH:quinone oxidoreductase (Na^+-NQR) is the major entry site for electrons into the respiratory chain of *Vibrio cholerae,* the causative agent of Cholera disease. This respiratory complex couples the electron transfer from NADH to quinone to the translocation of sodium ions across the membrane [1]. Until now it is not known where Na^+ crosses the membrane-bound part of the complex, and which electron transfer step is essential for ion translocation. Here we characterized electron transfer and sodium motif force (SMF) generation catalyzed by the Na^+-NQR. Since the quinone substrate undergoes protonation after reduction, the influence of pH (neutral - alkaline) was studied. In addition, the effect of rubidium and lithium ions (chloride salts) was investigated. In a previous study, Li^+ was shown to slightly stimulate Na^+ -NQR activity, whereas Rb^+ was reported to be inhibitory [2]. To determine the electron transfer activity of Na^+-NQR, the reduction of quinone was followed by UV-VIS spectroscopy using purified Na^+-NQR solubilized in dodecyl-β-D-maltoside [1]. The Na^+ transport was monitored with Na^+ -NQR reconstituted into liposomes using the voltage-sensitive dye Oxonol VI [1]. Activities of the Na^+-NQR (both electron transfer and SMF generation) were most pronounced around pH 7.5 and 8.0. A decrease in proton concentration led to diminished electron transfer and Na^+ translocation activities, suggesting a critical role for protons during the catalytic cycle. At pH 9, the relative decrease of SMF generation

compared to the optimum value was as pronounced as the relative decrease of quinone reduction activity. This indicates that electron transfer and sodium transport were still coupled. The exposure to Li^+ led to a reduction of electron transfer activity of Na^+-NQR compared to activity measured in the presence of Na^+ as also previously reported [2]. Barquera and coworkers [2] reported that Rb^+ inhibited electron transfer of the Na^+ -NQR, a finding which we could not reproduce with our preparation of the enzyme. Exposure of the Na^+-NQR to Rb^+ increased the NADH oxidation and quinone reduction activities. This suggests that the Na^+ -NQR, in addition to Na^+ and Li^+, specifically interacts with Rb^+. Further investigations will address if Rb^+ acts as a coupling cation for the Na^+ -NQR.

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FTP29

Novel microfluidic biosensor for online monitoring of biofilm formation

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Controlling and monitoring of biofilm formation are still demanding tasks. Established biofilm research methods mainly provide destructive end-point analysis. Therefore, we developed a new sensor system for characterizing biofilm formation online in a microfluidic flow system by two parameters: attached biomass and biofilm activity. Our newly developed microfluidic biosensor is based on electrical impedance spectroscopy and parallel measurement of amperometric current which allows the real-time monitoring of biofilm formation processes. Biofilm biomass and activity are recorded in a non-destructive manner. Thereby increasing impedance correlates with an increase in biomass attached to the electrode and increase in amperometric measured current corresponds to a higher respiratory activity of the biofilm. These features were proven by microscopic time-lapse experiments and exo-enzymatic activity measurements. Integration of a reference channel allows minimizing environmental oscillations. The microfluidic properties of the senor enable parallel screening of different bacteria as well as biofilm affecting substances by providing 48 parallel flow channels with each containing two electrodes. A direct RNA extraction out of the channels allows transcriptome analysis on desired time-points. Using this setup we were able to monitor biofilm development of different Gram-positive and Gram-negative bacterial species including *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Staphylococcus aureus, Bacillus subtilis*, and even complex waste water biofilms. Screening of strain collections identified different biofilm formation potentials of the strains. Furthermore, the device enables monitoring of agents with biocidal or biofilm destabilizing effects. Loosening of the EPS matrix leads to a destabilized biofilm which can be monitored online by a decrease in impedance signal. Toxicity effects of biocides cause inactivation of the biofilm and therefore a decrease in the amperometric signal is observed. Regrowth after treatment reveals the presence of persister cells and is indicated by a recovery of both signals. Overall, this sensor provides a tool for real-time monitoring biofilm formation and allows a rapid screening of biofilm influencing substances in a microfluidic system. Ongoing development of the sensor targets the implementation into technical systems, where biofilm formation is a demanding problem and early on monitoring is necessary.

FTP30

Deletion of the ZIP-transporter ZupT influences zinc pools in *Cupriavidus metallidurans* CH34

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Cupriavidus metallidurans CH34 accomplishes a high level of transition metal resistance by a combination of unspecific transition metal import and controlled efflux. Using the plasmid-free strain AE104 that possesses only a limited number of metal efflux systems, cellular metal pools were identified as counterparts of these transport reactions. At low zinc concentrations AE104 took up Zn^{2+} until the zinc content reached an optimum level of 70.000 Zn^{2+}/cell in the exponential phase of growth, whereas a Δ*zupT* mutant lacking the zinc importer ZupT contained only 20.000 Zn^{2+}/cell, possibly the minimum zinc content. By incubation with 100 µM zinc the mutant and parent cells accumulated up to 125.000 Zn^{2+}/cell. In comparison the AE104 Δe4 mutant strain, which is lacking the known genes for zinc efflux and Δe4*zupT*, contained 250.000 Zn^{2+}/cell when cultivated in the presence of 10 µM zinc, which is close to the upper tolerance level. This seems to be the maximum zinc content in *C. metallidurans* cells. Even when the cells contained zinc, the zinc importer ZupT was required for important cellular processes, indicating the presence of a pool of tightly bound zinc ions, which depends on ZupT for replenishment. Absence of ZupT led e.g. to formation of inclusion bodies and decreased efficiency in synthesis of the zinc-dependent RNA-polymerase subunit RpoC, leading to RpoC accumulation (1). Two zinc pools appear to exist in *C. metallidurans*: one pool of at least 20.000 tightly bound Zn^{2+} ions, in addition to a second pool of up to 200.000 cations bound with low substrate specificity.

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FTP31

Effect of long-term starvation and temperature on survival and outer membrane proteome of *Acinetobacter baumanii*

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Introduction: The factors contributing to colonization, invasion and persistence of *A. baumannii* are still poorly defined, but it is apparent that this opportunistic pathogen has developed several strategies that play a role in its persistence and spread under hostile conditions. Study and characterization of the changes taking place in *A. baumannii* cells during the long-term starvation process can contribute to a better understanding of how this pathogen survives and persists in the environment.

Materials and Methods: *A. baumannii* ATCC 19606 cells from the stationary phase were incubated under nutrient deprivation at 20 and 37ºC in sterile saline solution. Aliquots were periodically withdrawn to (i) enumerate total viable and culturable bacteria, (ii) determine the changes in the outer membrane subproteome and and (iii) analyze the cell's appearance by scanning electron microscopy (SEM).

Results: The cells incubated at 20ºC remained viable and culturable during at least 15 days. In contrast, the viability and culturability of the cells incubated at 37ºC decreased gradually by a factor of 2-2.5 log. Moreover, SEM analysis revealed some cells with altered cell morphology (distorted cells) at this temperature. We also found that during the survival process, the outer membrane proteome underwent few changes caused by starvation and/or temperature. These changes mainly affected the level of proteins involved in transport and membrane permeability. For instance, although the outer membrane protein OmpW was practically undetectable in the membrane fraction of the cells present in the initial inoculums, its level dramatically increased during the survival process.

Conclusion and Discussion: Our results suggest that the loss of viability and culturability in *A. baumannii* was mainly dependent on temperature and was nearly unaffected by the lack of nutrients. Moreover, the percentage of the survived cells was considerably higher when they were incubated at 20ºC, i.e. below the temperature normally sustaining the maximal growth in standard growth media. Nevertheless, despite its effects on cell viability, temperature variations had little impacts on outer membrane subproteome.

BIOLOGY OF FILAMENTOUS FUNGI

FUP01

Modeling substrate specificity of cyclohexadepsipeptide producing synthetases by swapping a highly promiscuous A-domain to generate new bioactive compound libraries

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Introduction: Highly homologous nonribosomal peptide synthetases of filamentous fungi produce a broad range of bioactive cyclodepsipeptides with antibacterial, anthelmintic, insecticidal and anti-cancer properties [1]. As the A-domain of PF1022 synthetase is well-known for its rather flexible substrate specificity we aimed to expand structural diversity of medical relevant cyclodepsipeptides through swapping the highly promiscous A-domain containing module into other NRPS backbones [2, 3].
Materials and Methods: Combinatorial biosynthesis by genetic recombination of multienzyme complexes for generation of hybrid NRPS verified the production of new cyclohexadepsipeptides with LC/MS/MS. Extension of a bioactive compound library using precursor directed biosynthesis in heterologous hosts like *Escherichia coli* and *Aspergillus niger*.
Results: Swapping a whole C-A-T module of PF1022 synthetase with a promiscuous A-domain into enniatin and beauvericin synthetase led to advanced hybrid cyclohexadepsipeptides. From this biocombinatorial approach we obtained cyclic hexapeptides confirming earlier investigations on ring size at the C-terminal module of cyclooligomer depsipeptide synthetases with an iterative manner [4]. Furthermore we gained novel non-natural peptide derivatives by feeding synthetic substrates into *E. coli* and *A. niger* that differ in ability of absorption, intracellular access and thus incorporation into the cyclic backbone.
Conclusion: In our work we developed novel hybrid synthetases by combining partial gene sequences coding for the production of cyclohexa- and cyclooctapeptides of fungal origin. These functional enzymes lead to manifold opportunities for incorporation of diverse d-hydroxy carboxylic acids. With high yield heterologous expression in *A. niger* we were able to isolate great amounts of pure new substances for bioactivity testings.

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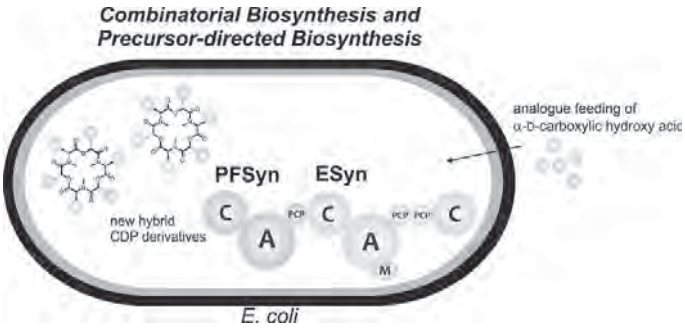
Figure 1



FUP02

Stress signaling in *Botrytis cinerea:* The response regulator BcSkn7

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In the course of infection the pathogenic grey mould fungus *Botrytis cinerea* triggers an oxidative burst as early plant defense reaction. This leads to an active release of reactive oxygen species (ROS), which are on the one hand known to be responsible for molecular damages of biological molecules, but on the other hand they are also involved in cell signaling pathways. Several components of these pathways are necessary for the signal transfer and therewith the regulation and induction of a specific stress response, which is important for all physiological processes.
Two key players in in the oxidative stress response are the transcription factors Bap1 (*Botrytis* activator protein 1)[1] and BcSkn7 (suppressor ofkrenull 7). A characterization of Δbcskn7 and ΔΔbap1bcskn7 included analysis of differentiation, development, virulence and gene expression. Δbcskn7 shows strongly reduced vegetative growth and conidia formation. Furthermore, the deletion mutant is highly sensitive to different types of stress, including oxidative, osmotic, cell wall and membrane stressors. Contrary, an enhanced secretion of ROS in comparison to the WT could be detected. Although, BcSkn7 seems to be an important regulator in stress response the influence on virulence is only minor. The effect of the double deletion mutant is even more severe. This additive effect shows that the regulators act in different pathways.
Expression analyses revealed a strong influence of Bap1 and BcSkn7 on the regulation of oxidative stress responsive genes. In a Y1H approach a direct binding to the promotors of *gsh1* and *grx1* by Bap1 and of *glr1* by BcSkn7 could be verified.
Future tasks include the verification of a possible interaction between Bap1 and BcSkn7 as they seem to work in concert. Moreover, investigations about a putative connection to calcium signaling via thecalcineurin-responsivezinc-finger transcription factor Crz1 are ongoing.
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FUP03

Mining the genome of the filamentous fungus *Fusarium fujikuroi* for new secondary metabolites

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The ascomycete *Fusarium fujikuroi*, a member of the *Gibberella fujikuroi* species complex, is a plant pathogen leading to the bakanae disease in rice. Disease symptoms include etiolation as well as hyper-elongation caused by fungal secretion of the secondary metabolite gibberellic acid. Additional known metabolites from *F. fujikuroi* include bikaverin, fusarubin, fusarin C as well as fusaric acid. Genome sequence analysis revealed a total of 46 putative secondary metabolite gene clusters, with the majority being silent under laboratory conditions and without an assigned product or clear cluster borders[1].
To identify the products of these cryptic gene clusters, we use deletion or overexpression mutants of global transcription regulators such as histone acetyltransferases and deacetylases as well as GATA transcription factors, e.g. the homologue of the sexual regulator NsdD from *Aspergillus nidulans*[2] (temporarily denoted Nsd1) or the global nitrogen regulator AreA[3]. By using a genome-wide microarray approach, we were able to identify differentially expressed gene clusters as well as assign cluster borders based on similar expression patterns of the adjacent genes. For example, comparing the Δ*nsd1* mutant with the wild type under various nitrogen conditions, three uncharacterized gene clusters were found to be down-regulated, while five previously silent metabolite clusters were awakened in the deletion mutant.
To identify the new metabolites, we performed software-aided comparative analysis of culture filtrates from both the mutant and wild type *via* HPLC-HRMS. Combining the genome-wide expression analysis and the metabolic data, we selected the gene cluster of the sequiterpene cyclase 1 as a promising candidate for further analysis. This includes a thorough molecular characterization as well as structure elucidation of the compound.

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FUP04
BcLTF2 (light-responsive transcription factor 2) is the master regulator of asexual reproduction in the gray mold fungus *Botrytis cinerea*

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The ascomycetous fungus *Botrytis cinerea* has a worldwide distribution and is the causal agent of gray mold diseases in more than 235 plants species including high-value crops such as grape vine and strawberry. The fungus may reproduce asexually by forming macroconidia for dispersal (summer cycle) and sclerotia for survival (winter cycle); the latter also participate in sexual reproduction by bearing the apothecia after fertilization by microconidia has taken place. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. Field populations are highly diverse with regard to different phenotypic traits and can be divided into two groups: strains that undergo photomorphogenesis (*LIGHT-RESPONSIVE*) and *BLIND* strains that produce either sterile mycelia (*FLUFFY*) or the same reproductive structures under different light conditions (*ALWAYS CONIDIA, ALWAYS SCLEROTIA)*. However, the inability to form certain reproductive structures such as conidia as the major source of inoculum or sclerotia as survival structures and as prerequisite for sexual recombination is expected to decrease the overall fitness of the pathogen, and thus, it is questionable why *BLIND* strains are abundantly found in the field. In fact, the *BLIND* phenotype may be accompanied by reduced virulence, as shown for the *ALWAYS CONIDIA* phenotype of strains T4 and 1750 that is caused by single nucleotide polymorphisms (SNPs) in the gene *bcvel1* (Schumacher et al. 2012, 2013). In a recent study, 293 light-responsive genes including six transcription factor-encoding genes (BcLTF1-6) were identified in *B. cinerea* strain B05.10 (Schumacher et al. 2014). *Bcltf2* encodes a C2H2-TF which is the ortholog of *Neurospora crassa* SAH-1. Expression levels of *bcltf2* are increased in mutants exhibiting the *ALWAYS CONIDIA* phenotype such as deletion mutants of BcLTF1 and BcVEL1, suggesting a role of BcLTF2 in conidiation. Preliminary data indicate the function of BcLTF2 as a regulator of conidiation because its overexpression (OE::*bcltf2*) is sufficient to induce conidiation in the dark. Accordingly deletion mutants (Δ*bcltf2*) do not form conidia; however, they form sclerotia in light and darkness. Thus, we regard BcLTF2 as the master regulator of asexual reproduction (conidiation vs. sclerotial development) in the gray mold fungus.

FUP05
Analysis of SCI1, a potential new interaction partner of the STRIPAK complex

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The "striatin interacting phosphatase and kinase" (STRIPAK) complex is a conserved multi-protein complex, which is found in mammals and in filamentous fungi but not in prokaryotes, and plants. Homologs of STRIPAK complex proteins are also found in the budding yeast *S. cerevisiae* and in the fission yeast *Schizosaccharomyces pombe* [4;5]. However, some components known in *S. cerevisiae* are lacking in *S. pombe* and in metazoa. Therefore, distinct functions of the STRIPAK complex in different organisms are assumed to be dependent on the proteins that come together in the STRIPAK complex [3]. Diverse signaling pathways are directed by the STRIPAK complex. In the filamentous ascomycete *Sordaria macrospora* the STRIPAK complex proteins SmPP2AA, SmMOB3 and PRO11 are involved in the control of hyphal fusion and fruiting-body development [1;2;6]. PRO11 a homolog of the mammalian striatin forms homodimers and is thought to act as scaffold for the binding of other proteins in the STRIPAK complex. A putative calmodulin-binding domain of PRO11 indicates that PRO11 might function in calcium-dependent signaling pathways [2;6]. The putative kinase activator SmMOB3 contains a conserved MOB domain and further shares homology with a subunit of the clathrin-adaptor protein complex [1]. The scaffolding subunit of the protein phosphatase PP2A (PP2AA) interacts with regulatory B subunits that associate with the STRIPAK complex for the recruitment of substrates and the targeting to subcellular compartments [2].
GFP-Trap and Mass spectrometry was used to identify new STRIPAK complex components in *S. macrospora*. Up to now, no homologs of the STRIPAK complex components like CCM3 or kinases of the GCK III family could be found in the GFP-Trap experiments. However, known interaction partners of PRO11 like SmMOB3 and PRO22 could be identified. Interestingly, an unknown protein, which we termed SCI1 (STRIPAK complex interactor 1) and which seems to be conserved in fungi, was found using PRO11 as bait. The SCI1 protein with 298 AAs was predicted to contain 3 coiled-coil regions and a low complexity region. Here we present data of the functional analysis of *S. macrospora* protein SCI1 and characterize the interaction of SCI1 with STRIPAK components in *S. macrospora*.

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FUP06
Comparing the signaling in ecto- and endomycorrhizal symbioses

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The availability of genome sequences from both endo- and ectomycorrhizal fungi as well as from their hosts has, together with elegant biochemical and molecular biological analyses, provided new information on signal exchange between the partners in mycorrhizal associations. The progress in understanding cellular processes has been faster in endomycorrhizal symbiosis, due to the similarities of early processes with *Rhizobium*-legume symbiosis. In ectomycorrhiza, the role of auxin and ethylene produced by both symbionts, fungus and host plant, starts to be unraveled at the molecular level, although the actual ligands and receptors leading to ectomycorrhizal symbiosis have not yet been discovered. For both systems, the functions of small effector proteins secreted from the respective fungus and taken up into the plant cell may be pivotal in understanding the attenuation of host defense. The present advance of knowledge warrants a review of the signaling processes known. Here, we endeavor to add by comparing cross-talk between fungal and plant partners during formation and in established of endo- and ectomycorrhizal symbioses.

FUP07
Selective autophagy in the filamentous fungus *Sordaria macrospora*

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Autophagy is a conserved ubiquitous degradation process in eukaryotic cells, which is responsible for nutrient recycling and non-selective digestion of proteins and organelles [1]. Typically, it is induced by starvation or stress conditions. Autophagy can be discriminated in macroautophagy, the uptake of cell content via a double membrane structure called autophagosome and microautophagy, a direct invagination of cell components by the vacuole/lysosome. In yeast non-selective macroautophagy is extensively studied and can be divided in five consecutive steps: induction, nucleation, expansion, fusion and breakdown. After induction the autophagosomal membrane engulfs random cytosolic cell content and delivers it to the vacuole for degradation. During nutrient-rich conditions in yeast, but not in filamentous fungi, a cytoplasm-to-vacuole targeting (cvt) pathway has been described. Besides these autophagy processes, selective autophagy of proteins or surplus organelles such as peroxisomes, mitochondria, ribosomes and nuclei is referred to as aggre-, pexo-, mito-, ribo-, and nucleophagy, respectively. In filamentous fungi, it has been found that autophagy has an impact on growth, morphology and pathogenicity [2]. *Sordaria macrospora* (Sm) is a homothallic filamentous ascomycete that can complete its life cycle within seven days and the genome is completely sequenced. Many

components of the autophagic system seem to be conserved in *S. macrospora* [3], however, cargo receptors for selective autophagy have not been described yet. In all eukaryotes autophagy related (ATG) protein ATG8, a homologue of the mammalian LC3-family protein, is essential for the autophagosomal membrane formation during expansion. ATG8 is conjugated to phosphatidyl-ethanolamine (PE) and localizes to the autophagosomal membrane [4]. Since ATG8 is also involved in selective autophagy, interaction partners of SmATG8 were identified by GFP-Trap and MS analysis in *S. macrospora* [5]. One of the identified proteins, SmNBR1, is a putative homolog of the human NBR1 (neighbor of BRCA1 gene1) and habours a conserved LC3-interacting region (LIR). In *Homo sapiens* NBR1 has been discribed as a receptor for ubiquitinylated targets and pexophagy [6]. The interaction of SmATG8 and SmNBR1 was confirmed by means of yeast two-hybrid experiments and fluorescence microscopy. A ΔSmnbr1 mutant could be generated which is impaired in vegetative growth and fruiting-body development. The role of SmNBR1 in autophagy and the analysis of its interaction with SmATG8 will be investigated in further studies.

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FUP08

Development and application of molecular genetic tools for the cephalosporin C producer *Acremonium chrysogenum* to analyze arthrospore formation

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Acremonium chrysogenum is the natural producer of the β-lactam antibiotic cephalosporin C. Due to its antibacterial activity against gram-positive and gram-negative bacteria, this β-lactam antibiotic is of great biotechnological and medical relevance. To optimize cephalosporin C production efficiently, continuous and directed improvement of industrial strains is required. A typical morphological feature of *A. chrysogenum* is the fragmentation of vegetative mycelium into arthrospores. These are uni- or binuclear cells, which develop during a prolonged cultivation under limited nutrient supply. Due to the known correlation of cephalosporin C production and arthrospore formation, we are interested to identify specific regulatory factors affecting both, cephalosporin C biosynthesis and morphological development. However, the genetic manipulation of *A. chrysogenum* is still rather difficult because it lacks a known sexual cycle and produces only few conidiospores. Here, we demonstrate the development of several molecular tools for the genetic engineering of *A. chrysogenum*. Besides the generation of an *Acku70* deletion strain for homologous recombination, the functionality of a xylose-inducible promoter from *Sordaria macrospora* (*Smxyl*) was demonstrated using *egfp* as reporter gene. In further studies, the *Smxyl* promoter was used to establish a one-step FLP/*FRT* recombination system in *A. chrysogenum*. The combined use of all molecular tools resulted in the construction of a strain, lacking the formin gene. Formin proteins act as nucleators within the assembly of actin filaments and have a controlling function in the dynamic remodeling of the actin cytoskeleton. They are further involved in septation processes of fungal hyphae and might consequently act as putative regulators of arthrospore formation.

FUP09

Gene silencing on demand: Establishment of the Tet-off system for Aspergillus niger

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The tetracycline-expression system is a versatile tool to control and fine-tune gene expression in eukaryotic cells in a metabolism-independent manner. By the addition of doxycycline, genes can either be switched on (Tet-on system) or switched off (Tet-off system). Recently, the Tet-on system has successfully been established for *Aspergillus niger* (Meyer et al., 2011). In the current study, we tested and evaluated different variations of the Tet-off system for use in *A. niger* by using luciferase as a reporter gene. Transformants with a single copy of the Tet-Off system were generated and their expression levels determined and compared with a respective control strain. By adding various concentrations of doxycycline to the cultivation medium, it could be shown that the expression of the reporter gene could indeed be down-regulated in a concentration-dependent manner. Most importantly, it was possible to completely shut down luciferase expression.

FUP10

A New and Reliable Method for Live Imaging and Quantification of Reactive Oxygen Species in *Botrytis Cinerea*

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Reactive oxygen species (ROS) are produced in conserved cellular processes either as byproducts of the cellular respiration in mitochondria or as a support for defense mechanisms, signaling cascades or cell homeostasis. They have a Janus-faced nature because of their high damaging potential for DNA, lipids and other molecules and due to their indispensability for signaling and developmental processes. In filamentous fungi, the role of ROS in growth and development has been studied in detail (Heller and Tudzynski, 2011); these analyses were often hampered by the lack of reliable and specific techniques to monitor different reactive oxygen species in living cells.
Our recent studies focus on the suitability of a new method for live cell imaging of ROS in filamentous fungi. We demonstrate that by using a mixture of two fluorescent dyes it is possible to monitor H_2O_2 and superoxide specifically and simultaneously in distinct cellular structures during various hyphal differentiation processes. In addition, the method allows a reliable fluorometric quantification of ROS. We show that this can be used to characterize different mutants with respect to their ROS production/scavenging potential.
Another approach to learn more about the role of ROS as messenger and answer to external stimuli is the monitoring of the ROS producing capacity of *B. cinerea* in response to chemicals. The obtained results in combination with studies concerning the visualization of the redox status by the redox sensitive GFP2 (Heller et al., 2012) may contribute to an enhanced understanding of signaling cascades and cellular mechanisms in filamentous fungi.

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FUP11

Depolymerization of Polystyrene Sulfonate by the Brown-Rot Fungus *Gloeophyllum trabeum*

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The accumulation of anthropogenic plastic polymers in the environment is cause for serious concern. As the backbones of conventional hydrocarbon polymers (like polyethylene, polypropylene, or polystyrene) are made up by non-hydrolyzable C-C bonds, they are considered highly resistant to any biological degradation [1]. In this study, we focused on the water-soluble polystyrene analogon polystyrene sulfonate (PSS) in order to investigate the ability of the brown-rot fungus *Gloeophyllum trabeum* DSM 1398 to depolymerize recalcitrant hydrocarbon polymers.
G. trabeum was pregrown in liquid malt extract medium and transferred to a mineral medium devoid of any C source to initiate degradation of PSS. Various compounds were added or omitted to investigate the mechanism of depolymerization. PSS molecular weight (MW) distribution was analyzed by size exclusion chromatography. *G. trabeum* was found to reduce the MW of PSS from 70 kDa to 6.8 kDa within 20 days. None of the typical lignin-degrading enzymes of fungi (laccase, manganese peroxidase, lignin peroxidase) could be detected in the culture supernatant during cultivation. PSS depolymerization was found to be highly dependent on the presence of catalytic Fe(II) in the medium, while it was inhibited by the known hydroxyl radical scavenger mannitol and oxalate. Addition of 500 µM 2,6-dimethoxy-1,4-dihydroquinone massively sped up depolymerization, resulting in an average MW of 6 kDa after only three days. Abiotic Fenton reagent (H_2O_2 + Fe(II)) caused similar a degradation pattern. Our data indicate that depolymerization of PSS by *G. trabeum* proceeds via a Fenton-type reaction

driven by quinone redox cycling. Hydroxyl radicals produced in this system are the most likely agents to attack and cleave C-C bonds in the backbone of PSS, similar to what has already been demonstrated for the polyether poly(ethylene oxide) [2]. These results highlight the ability of *G. trabeum* to degrade highly recalcitrant synthetic polymers, a feat few fungal strains are capable of. In the next steps, we will investigate the ability of the strain to degrade solid plastic polymers.

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FUP12

Sugar degrading enzymes in the host-pathogen interaction of Claviceps purpurea and rye

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The biotrophic filamentous fungus *Claviceps purpurea* infects more than 400 monocotyledonous plant species including crop plants such as rye and wheat. Broad infections cause toxification of the harvest and disable its use as food and feed. The ovary-based infection process is very unique and features the so called honeydew production. Several days after the successful infection a viscous sugar rich fluid appears on the flower. The mechanisms of the honeydew formation are poorly understood but it is assumed that it is part of the host-pathogen interaction and essential for a successful infection. The main source of sugar for the fungus is the plant phloem which mainly contains sucrose. The main sugar components of the honeydew are typical products of invertase activity on sucrose. Seven putative invertase encoding genes were found in the genome of *C. purpurea*, six of them show high homology (70-80 %) and four of these are located in a cluster. qRT-PCR based in planta expression data revealed the highest expression for *cpinv4* during honeydew production, it was therefore chosen for functional analysis. Tagging with mCherry indicated an extracellular localization in accordance with a predicted secretion signal. These findings and studies on invertase-regulated phloem unloading and nutrient distribution in plant-pathogen interactions indicate a role in host manipulation to obtain sufficient nutrients for the honeydew production. A knockout approach was initiated. To maintain the phloem flow from the plant to the fungus another group of sugar degrading enzymes might be important: β-1,3-glucanases which degrade callose. This plant polymer is assumed to locally block pathogen invasion and to regulate the phloem flow by deposition in the sieve plates. Reduced amounts of callose in infected ovaries and extracellular β-1,3-glucanase activity of the fungus lead to the "phloem unblocking hypothesis" (Tenberge *et al*., 1999). It postulates that a secreted β-1,3-glucanase is essential for the infection to enable hyphal growth and phloem unblocking. The encoding gene (*cpbgn1*) was identified in the recently sequenced genome (Schardl *et al*., 2013) and functionally characterized. The phenotype of the deletion mutant was undistinguishable from the wildtype regarding pathogenicity and morphology. However, because of the presence of additional putative β-1,3-glucanase encoding genes in the genome it cannot be excluded that extracellular β-1,3-glucanase activity is essential for the infection process to fulfill the sugar demand of the fungus which is massively increased due to the honeydew secretion.

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FUP13

Genome miners at work - Characterization of cryptic secondary metabolite gene clusters in the rice pathogenic fungus *Fusarium fujikuroi*

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Fusarium fujikuroi is well-studied due to its ability to produce highly bioactive plant hormones, gibberellic acids, that cause the *bakanae* disease of rice plants. In addition, *F. fujikuroi* possesses the powerful potential to synthesize a vast range of other secondary metabolites (SMs), with 46 SM key enzymes being encoded by the fungal genome. More precisely, bioinformatic analysis revealed the presence of 17 polyketide synthases (PKS), 16 non-ribosomal peptide synthetases (NRPS), eleven terpene cyclases (TC) as well as two dimethylallyltryptophan synthases (DMATS). However, the majority of the corresponding products is not known to date, particularly due to gene clusters being silent under standard laboratory conditions (Wiemann *et al*., 2013).
The activation of cryptic gene clusters in *F. fujikuroi* is achieved by genetic manipulation of SM biosynthesis on different levels. Next to the **overexpression of the biosynthetic key gene** of a putative cluster, the modulation of **pathway-specific regulation** represents a powerful tool. By overexpressing the pathway-specific transcription factor (TF) gene within the NRPS31-cluster, a novel, species-specific SM, apicidin F, was isolated and its structure elucidated (von Bargen *et al*., 2013).
While some gene clusters encode such a pathway-specific TF, activation can also be achieved through interference with global regulatory processes. On this higher hierarchical level, the manipulation of **chromatin-mediated regulation** was shown to result in a strongly influenced SM profile in *F. fujikuroi*. Among the analyzed complex partners, there are members of the fungal-specific *Velvet* complex, a global regulator of differentiation as well as secondary metabolism (Wiemann *et al*., 2010). Furthermore, members of the histone-modifying **com**plex of **p**roteins **as**sociated with **S**et1 (COMPASS) were shown to have a strong impact on secondary metabolism. Notably, COMPASS confers H3K4 methylation, a mark generally associated with euchromatin and thus, transcriptional activation.
The use of different genome mining approaches in order to activate silent gene clusters is a highly encouraging field of study concerning the isolation of yet unknown, fungal SMs with possibly medical or biotechnological application. In this context, the newly identified SM apicidin F produced by *F. fujikuroi* represents a histone deacetylase inhibitor that exhibits promising activity against *Plasmodium falciparum* as well as human liver carcinoma cells *in vitro* (von Bargen *et al*., 2013, unpublished).

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FUP14

The transcriptome of *Pleurotus sapidus*.

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The ability of basidiomycetes to degrade wood is of great importance for renewable resources-based biotechnology. In the last decades there have been discoveries of novel enzymes involved in this process. The white-rot fungus *Pleurotus sapidus* is known for producing a broad spectrum of lignin degrading enzymes [1, 2]. The development on next generation sequencing techniques led to numerous genomic and transcriptomic studies of model organisms in recent years. The constantly falling costs for RNA-Seq turned this method into a good alternative to microarrays, especially for studies which involve non-model organisms with not yet determined genomes [3]. The objective of the current study was the exploration of the changes in the transcriptome of *Pleurotus sapidus* cultures during growth with rapeseed straw as sole carbon source. Mycelia from submerged (SmF) and emerged (SSF) cultures of different cultivation days were used for RNA extraction followed by mRNA purification, cDNA library synthesis and sequencing via MiSeq™ sequencing system from Illumina. We obtained 20.58 million 150 bp paired-end reads. Due to the lack of a reference genome, a *de novo* assembly of the transcripts was carried out using Trinity [4] and Velvet/Oases [5]. For data analysis the Sequence Analysis and Management System "SAMS" [6] (http://www.cebitec.uni-bielefeld.de/comics/index.php/sams) was applied. Out of 30,680 contigs 4,551 showed an altered expression pattern during different cultivation conditions and/or different time points of cultivation. Based on their expression pattern, these differentially expressed contigs were assigned to one of 64 clusters. Carbohydrate active enzyme transcripts have been explored via the CAZy database (http://www.cazy.org) and more than 1,500 contigs could be allocated to one CAZy class: e.g. five manganese, three versatile and four dye-decolorizing peroxidases, as well as ten laccases and one aryl-alcohol oxidase have been predicted among the discovered contigs. Some lignolytic enzymes were expressed similarly in all experimental setups, whereas others were preferably expressed in SSF or SmF cultures.

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GASTROINTESTINAL PATHOGENS

GIP01
Pathogenic enteric viruses and fecal indicator bacteria in Korean urban river water

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High number of gastroenteritis patients occurs every year in South Korea. According to the Korea Centers for Disease Control and Prevention (KCDC), approximately 18,731 patients were suspected to be infected by waterborne viruses and 3,270 patients among them showed positive for NoV in 2012. Many enteric viruses including NoV take fecal-oral infection route, having surface water as one of major infection media. When enteric viruses are excreted through patients' feces, they may flow into the surface water, even after the wastewater treatment, and infect other persons through vegetables, shellfishes, and various water resources. Therefore, monitoring of surface and ground water for surveillance of pathogenic enteric viruses is important in understanding the life cycle of enteric viruses and prevention of further spread of the disease. From December 2013 to March 2014, 3 sets of samples were collected from Hwang-gu-ji River (HGJ River) upstream and downstream, and Suwon Wastewater treatment plant (WWTP) effluent which is located in between the two sampling sites of HGJ River. These sites are located in Suwon metropolitan area of Korea. For these samples, NoV genotypes I and II and adenovirus (AdV), which are known to be most prevalent in Korea, were enumerated using quantitative PCR analysis. Also, five different fecal indicator bacteria (FIB), total coliform (TC), fecal coliform (FC), *Enterococci* (EC), *E. coli* and *Bacteroides* spp. were enumerated. From all the samples, high number of enteric viruses and FIBs were detected. When surface water samples were compared with the WWTP effluent sample, high number of FIBs in the effluent samples seemed to be contaminating the downstream of the HGJ River. Same was observed with enteric viruses. High number of NoVs was detected from both WWTP effluent and the downstream of the HGJ River while there were no detection of them from upstream. This indicates the contamination of urban surface water by the WWTP effluent. From previous studies, infectious dose of NoV is known to be 18 viral particles, which is about 1,015 genome copies. From this study, 1,099 genome copies of NoV GI and 4,661 genome copies of NoV GII, at most, were detected from the downstream of HGJ River, which exceeds the infectious dose of NoV. Considering the fact that HGJ River is located in the middle of highly populated city and some residents visit this river for leisure and fishing, secondary infection of enteric viruses through direct contact were suspected. Also, WWTP with standardized facility seem not to be adequately removing both FIBs and enteric viruses, increasing the susceptibility to pathogenic viruses and vulnerability of gastroenteritis outbreaks.

Figure 1. Fecal indicator bacteria detected from Hwang-gu-ji River (HGJ River) and Suwon wastewater treatment plant (WWTP) effluent in December 2013. Total coliform, *Enteroccoci,* Fecal coliform, *E.coli* and *Bacteroides* spp. were enumerated and compared.
Figure 2. Enteric viruses detected from Hwang-gu-ji River (HGJ River) and Suwon wastewater treatment plant (WWTP) effluent in December 2013. Norovirus GI and GII and adenoviruses were enumerated and compared.

Figure 1

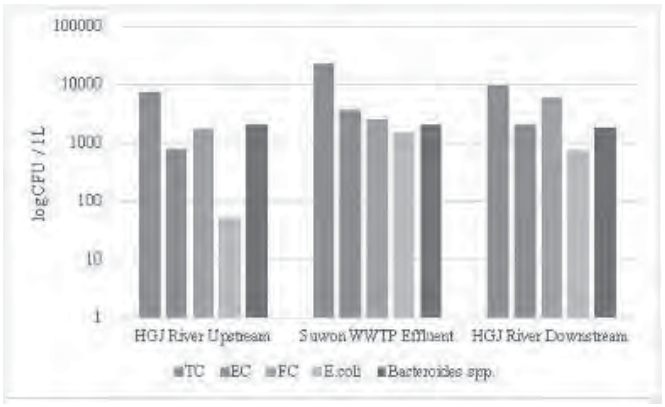
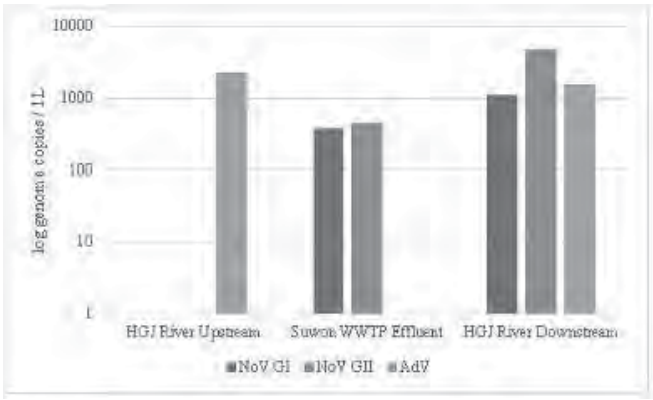


Figure 2



GIP02
Involvement of the *Helicobacter pylori* plasticity region genes in the development of gastroduodenal diseases in India

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Infection by *Helicobacter pylori* is associated with the development of gastritis, peptic ulcer and gastric adenocarcinoma. Although a number of putative virulence factors have been reported for *H. pylori*, there are conflicting results regarding their association with diseases. Recently,there has been a considerable interest in strain-specific genes outside the *cag* pathogenicity island, especially genes in the plasticity regions. Recent studies showed that certain genes in this region may play important roles in the pathogenesis of *H. pylori*-associated diseases.Our aim was to assess the role of selected genes(*jhp0940*, *jhp0945*, *jhp0947*and*jhp0949*) in the plasticity region in relation to risk of *H. pylori*-related diseases in Indian population. A total of 113 *H. pylori* strains isolated from duodenal ulcer (DU) [n=61] and non-ulcer dyspepsia (NUD) subjects (n=52) were screened by PCR and Dot-Blot to determine the presence of these genes.PCR and Dot-Blot results indicated presence of *jhp0940*, *jhp0945*, *jhp0947* and *jhp0949*in 9.8%, 47.5%, 50.8%, 40.9% and 17.3%, 28.8%, 26.9%, 19.2% of *H. pylori* strains isolated from DU and NUD, respectively.IL-8 production and apoptotic cell deathwere significantly higher in *H. pylori* strains containing *jhp0945*, *jhp0947* and *jhp0949 than the strains lacking those genes.* So, our study showed that the presence of *jhp0945*, *jhp0947* and *jhp0949* were significantly associated with symptomatic expressions whereas *jhp0940* seems to be negatively associated with the disease status. These results suggest that *jhp0945*, *jhp0947* and *jhp0949*could be a useful prognostic markers for the development of peptic ulcer in Indian subcontinent.

GIP03

Prevalence of *Dientamoeba fragilis* in elderly residents of a nursing home in Iran

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Introduction: *Dientamoeba fragilis* is a pathogenic human intestinal protozoan parasite which has emerged as an important cause of gastrointestinal disease. Prevalence rates range between 0.5% and 50% throughout the world. The purpose of this study was to determine the prevalence of *D. fragilis* in elderly residents of a large nursing home in Tehran, Iran.
Materials and Methods: In this cross-sectional study, 300 triple fecal samples were collected from residents of Kahrizak nursing home, Tehran, Iran from September 2012 to February 2013. The specimens were investigated by trichrome staining and polymerase chain reaction (PCR) assay targeting the 5.8S rRNA gene of *D. fragilis*. The relationship between prevalence of *D. fragilis*, clinical symptoms, and demographics of the residents was examined. Participants were grouped in six age categories: 61-65, 66-70, 71-75, 76-80, 81-85, >85.
Results: Trichrome staining were revealed 17 (6%) of participants infected with *Dientamoeba.* Amplification of 5.8S rRNA gene detected 105 (35%) cases of infection. There was no statistically significant relationship between sex, gastrointestinal symptoms, and infection with *Dientamoeba* in the sample studied. However, age affected the prevalence of *D. fragilis.* The highest prevalence of infection (50%) was detected in participants between 66-70 years of age.
Conclusion: The high prevalence of *D. fragilis* (35%) in the investigated population suggest that better personal care and social health policies should be considered in such care facilities for elderly. Higher prevalence of infection in a specific age group warrants further investigation.

GIP04

LewisB binding and *babA* sequence variation during chronic *Helicobacter pylori* infection in humans

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Introduction: Adhesion plays an important role in the colonization and long-time persistence of *Helicobacter pylori* in the hostile environment of the human stomach. One of the best characterized adhesins of *H. pylori* is the blood group antigen-binding adhesin (BabA), which mediates binding to the fucosylated LewisB (LeB) and related histo-blood group antigens located on the surface of gastric epithelial cells. Previous studies in different animal models demonstrated frequent loss of the LeB binding ability of *H. pylori* in the course of chronic infection. This led us to investigate the dynamics of LeB binding and sequence variation of *babA* during chronic infection in humans.
Materials and Methods: We analyzed 47 sequential *H. pylori* isolates obtained from 23 chronically infected individuals during two clinical trials in Louisiana/USA and Colombia, respectively. Twenty-three strain pairs were isolated at intervals ranging from three months to four years; for one individual a third isolate obtained 16 years after the initial biopsy was available. Binding of all *H. pylori* isolates to purified LeB glycoconjugate was determined using an ELISA-based procedure. In addition, all strains were analyzed for BabA expression and genotyped to identify *babA* containing loci for subsequent sequencing. To characterize epitopes within the BabA protein important for protein stability and ligand binding, we generated a set of recombinant *H. pylori* strains harboring chimeric *babA* genes and determined the LeB binding properties conferred by these mosaic alleles.
Results: Twenty seven strains adhered to LeB, but with marked differences in their binding affinities. The LeB binding phenotype was found to be relatively stable among sequential isolates; only 5 out of 23 sets showed a statistically significant reduction or complete loss of LeB binding in the course of infection. Subsequent *bab* genotyping identified 36 strains that harbored at least one *babA* copy and from 33 of these strains we obtained *babA* sequences. Comparison of deduced BabA protein sequences detected most differences in the predicted N-terminal extracellular adhesion domain, while the C-terminus was highly conserved. The subsequent analysis of *babA* chimeras demonstrated the importance of the C-terminus for protein stability.
Discussion: The high sequence diversity in the predicted adhesion domain of BabA is in agreement with its role in mediating specificity and affinity in LeB binding. Less sequence variation has been observed in the C-terminus, which is predicted to encode a transmembrane domain and recognition of which is important for assembly into the outer membrane.

GIP05

Two novel EHEC/EAEC hybrid strains isolated from human infections

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Introduction: The so far the highest number of life-threatening hemolytic uremic syndrome was associated with a food-borne outbreak in 2011 in Germany which was caused by an enterohemorrhagic *Escherichia coli* (EHEC) of the rare serotype O104:H4. Most importantly, the outbreak strain harbored genes characteristic of both EHEC and enteroaggregative *E. coli* (EAEC). Such strains have been described seldom but due to the combination of virulence genes show a high pathogenicity potential. To evaluate the importance of EHEC/EAEC hybrid strains in human disease, we analyzed the EHEC strain collection of the German National Reference Centre for Salmonella and other Enteric Bacterial Pathogens (NRC).
Materials and Methods: The search for EHEC/EAEC strains and their subsequent analysis included the following methods: PCR or Southern blotting for the detection of EHEC (such as *stx* and *eaeA*) and EAEC marker genes (such as *aatA*) as well as for aggregative adherence fimbriae (AAF), characterization of adherence pattern and cytotoxicity, analysis of antibiotic resistance profile, macrorestriction analysis / pulsed-field gel electrophoresis, multi locus sequence typing and *stx* sequence analysis.
Results: After exclusion of O104:H4 EHEC/EAEC strains, out of about 2400 EHEC strains sent to NRC between 2008 and 2012, two strains exhibited both EHEC and EAEC marker genes, specifically were *stx2* and *aatA* positive. Like the 2011 outbreak strain, one of the novel EHEC/EAEC harbored the shiga toxin gene type stx2a. The strain was isolated from a patient with bloody diarrhea in 2010, was serotyped as O59:H-, belonged to MLST ST1136, and exhibited genes for type IV AAF. The second strain was isolated from a patient with diarrhea in 2012, harbored stx2b, was typed as Orough:H-, and belonged to MLST ST26. Although the strain conferred the aggregative adherence phenotype, no known AAF genes corresponding to fimbrial types I to V were detected.
Conclusion: So called mixed *E. coli* pathovars or hybrid strains have been seldom described and show a high virulence potential. Therefore, we searched for yet undetected EHEC/EAEC strains and indeed found two novel strains isolated from human disease cases in Germany in 2010 and 2012. Those strains belong to MLST sequence types and/or serotypes seldom associated with human disease and in addition to stx2 harbor EAEC characteristics which qualify them to cause severe disease.

GIP06

Systematic analysis of phosphotyrosine antibodies recognizing single phosphorylated EPIYA-motifs in CagA of Western and East Asian type *Helicobacter pylori* strains

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Introduction: Highly virulent *Helicobacter pylori* strains encode a type IV secretion system (T4SS) that delivers the effector protein CagA into gastric epithelial cells. Translocated CagA undergoes tyrosine phosphorylation by members of the oncogenic c-Src and c-Abl host kinases at EPIYA-sequence motifs A, B and C in Western-type strains or EPIYA-motifs A, B and D in East Asian-type strains. Phosphorylated EPIYA-motifs can then mediate interactions of CagA with host signaling factors - in particular various SH2-domain containing human proteins - thereby hijacking multiple downstream signaling cascades.
Research question: Detection of tyrosine-phosphorylated CagA is mainly based on the use of commercial pan-phosphotyrosine antibodies, which originally were selected to detect phosphotyrosines in mammalian proteins.

Specific anti-phospho-EPIYA antibodies for each of the four sites are not forthcoming. This study was therefore designed to systematically analyze the detection preferences of each phosphorylated EPIYA-motif by seven different pan-phosphotyrosine antibodies and to determine a minimal recognition sequence.
Materials and Methods: We first synthesized a series of phospho- and non-phosphopeptides derived from the EPIYA-A motif exhibiting the phosphotyrosine residue in the middle +/- five, four, three or two flanking amino acids, including the STEPIYAKVNK (11-mer), TEPIYAKVN (9-mer), EPIYAKV (7-mer) and PIYAK (5-mer) sequences, and determined the recognition patterns by pan-phosphotyrosine antibodies in Western blots. We compared these results with those from phospho- and non-phosphopeptides representing each predominant Western and East Asian CagA EPIYA-motif B, C and D and also performed infection studies with diverse representative worldwide *H. pylori* strains.
Results: Our results show that a total of 9-11 amino acids containing the phosphorylated EPIYA-motifs are necessary and sufficient for specific detection by these antibodies, but revealed great variability in sequence recognition. Three of the antibodies recognized phosphorylated EPIYA-motifs A, B, C and D similarly well; whereas preferential binding to phosphorylated motif A was found with two antibodies. In addition, phosphorylated motifs A and C or A and D was found were found to be recognized by a sixth anti-phosphotyrosine antibody, and the seventh antibody did not recognize any phosphorylated EPIYA-motif. Controls showed that none of the antibodies recognized the corresponding non-phospho CagA peptides or non-phospho CagA, and that all of them recognized phosphotyrosines in mammalian proteins.
Conclusions: We unraveled the recognition preferences by seven different anti-phosphotyrosine antibodies in all four described phospho-EPIYA-motifs of CagA. These data are valuable in judicious application of commercial anti-phosphotyrosine antibodies in general and in particular for the characterization of CagA phosphorylation events during infection and disease development. A model for successive CagA phosphorylation steps at the EPIYA-motifs is also presented.

GIP07

A novel reporter assay to monitor Cytotoxin-associated gene (Cag) type IV secretion in *Helicobacter pylori*

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Helicobacter pylori is a bacterial pathogen with a worldwide infection rate of about 50 %. It colonizes the gastric mucosa persistently, causing chronic gastritis, gastric or duodenal ulcers as well as gastric cancer and MALT lymphoma. The development of these malignant disorders is mostly due to the *H. pylori* Cag type IV secretion system. This molecular machine specifically transfers the bacterial protein CagA into gastric host cells. Once inside the target cell, CagA becomes phosphorylated at several tyrosine residues and finally leads to changes in eukaryotic cell morphology and gene expression. CagA translocation is routinely analyzed by Western blotting to determine the level of phosphorylated CagA protein. Although this method has been used since years, it is time-consuming, labor-intensive and unsuitable for high-throughput screenings. Here we present a novel reporter system which is based on a CagA protein fused to a β-lactamase (TEM-1). This assay permits to monitor TEM-1-CagA translocation into target cells by enzymatic conversion of the fluorescent β-lactam derivative CCF4 by TEM-1. The CCF4 substrate and cleavage product differ in their fluorescence emission spectra, thus allowing ratiometric analysis. This fast, sensitive and highly specific method enables CagA translocation analysis by fluorescence microscopy, flow cytometry and fluorescence-assisted plate reading for high-throughput screening approaches. Hence, this assay can be used to screen for small molecules that inhibit the CagA translocation process. Moreover, it will contribute to the elucidation of the poorly understood type IV signal recognition and secretion process.

GIP08

Cyclic-di-GMP signaling and biofilm-related properties of the Shiga toxin-producing 2011 German outbreak *Escherichia coli* O104:H4

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Introduction: In 2011 nearly 4000 persons in Germany were infected by a Shiga toxin (Stx)-producing *Escherichia coli* O104:H4 with >20% of patients developing hemolytic uremic syndrome (HUS). Genome sequencing showed the outbreak strain to be an enteroaggregative *E. coli* (EAEC), suggesting its high virulence results from EAEC-typical strong adherence and biofilm formation combined to Stx production (Muniesa *et al*. 2012. Appl Env. Microbiol. 78: 4065-73). It has been observed that the outbreak strain produces thick submerged biofilms *in vitro* and has strong expression of both the EAEC virulence regulator *aggR* and *pgaA* (essential for the production of the biofilm-associated exopolysaccharide PGA). Bacterial adherence and biofilm formation is almost universally stimulated by the second messenger c-di-GMP. The intracellular level of c-di-GMP is in turn regulated by two sets of proteins: GGDEF domain containing diguanylate cyclases (DGCs) which synthesize, and EAL domain containing phosphodiesterases (PDEs) which degrade c-di-GMP, respectively. C-di-GMP, also promotes the expression of CsgD, a biofilm regulator essential for the production of two biofilm matrix components: amyloid curli fibers and cellulose. Since the outbreak O104:H4 strain is an excellent biofilm former, this prompted us to analyze GGDEF/EAL domain-encoding genes in the O104:H4 genome in comparison to other EAEC, EHEC and *E. coli* K-12 strains.
Materials and Methods: A bioinformatic genome analysis was performed for five strains (LB226692, HUSEC041, 55989, EDL933 and W3110) and the data were correlated with the variation in morphology, biofilm matrix component content, and CsgD expression.
Results: The O104:H4 outbreak strain, its close relatives HUSEC041 and EAEC 55989 all contain additional diguanylate cyclases (DgcX and YneF). DgcX shows higher expression than any other known *E. coli* diguanylate cyclase at both 28°C and 37°C. The outbreak strain expresses CsgD and amyloid curli fibres at 37°C, but is cellulose-negative. Moreover, it constantly produces derivatives with further increased and deregulated production of CsgD and curli. Since curli fibres are strongly proinflammatory, with cellulose counteracting this effect, high c-di-GMP and curli production by the outbreak O104:H4 strain may boost not only adherence but may also contribute to inflammation thereby facilitating entry of Stx into the bloodstream and to the kidneys where Stx can cause HUS.
Conclusion: The O104:H4 outbreak strain shows a unique combination of biofilm-related properties not observed in any pathogenic *E. coli* before that can contribute to explaining its high virulence.

GIP09

Incidence of astrovirus and sapovirus in norovirus negative pediatric stool specimens in Switzerland

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Objectives: Gastroenteritis is a common cause of morbidity in children aged Real-time PCR allows for the simultaneous detection and differentiation of a variety of viral gastrointestinal pathogens. Therefore, the incidence of astrovirus and sapovirus in samples previously tested negative for norovirus were evaluated in a Swiss private laboratory.
Materials and Methods: For real-time RT-PCR analysis, 141 stool specimens from children aged 0-5 years were isolated with the NucliSENS® easyMag™ (bioMérieux). Norovirus detection was performed either with norovirus real time RT-PCR Kit (AnDiaTec®, 61 samples) or with xTAG GPP ® (Luminex, 80 samples). Upon negative results for norovirus, RNA was further analyzed with the RIDA®GENE Viral Stool Panel II, detecting adenovirus, astrovirus and rotavirus, and with the RIDA®GENE sapovirus real-time RT-PCR assay on the LightCycler® 480 (Roche). Sapovirus positive samples were confirmed by a second independent RT-PCR method.
Results: In 141 samples negative for norovirus, the additional testing allowed the detection of astrovirus in 13 (9.2%) samples, and further 16 (11.3%) samples were positive for sapovirus. In 23 (16.3%) samples, adenovirus was detected, without discrimination of enteric or respiratory pathogens. Rotavirus was found in 9 (6.4 %) samples. Double infection was confirmed in 11 (7.8%) samples.

Conclusion: In Swiss children aged
The simultaneous testing for four viruses allows a faster detection of the pathogen and therefore a rapid decision about the adequate therapy.

GIP10

Antibacterial activity of Thai medicinal plant extracts against oral and gastrointestinal pathogenic bacteria and prebiotic effect on the growth of *Lactobacillus acidophilus*

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Introduction: Gastrointestinal tract infections are the most common gastrointestinal disorders. Pathogenic bacteria related to the pathogenesis include *Porphyromonas gingivalis* (an oral pathogen), *Helicobacter pylori* (a stomach pathogen) and other pathogenic bacteria, *Bacillus cereus*, *Escherichia coli*, *Enterobacter aerogenes*, *Listeria monocytogenes*, *Salmonella* Rissen, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Yersinia enterocolitica*. To alleviated these problems, antibacterial activity of plant extracts against these bacteria should be explored.
Materials and Methods: The 25 crude methanolic extracts of Thai medicinal plants were tested for their antibacterial activity against the bacterial strains as mentioned above using agar diffusion test and minimum inhibitory concentration (MIC) determination. Their phytochemicals and prebiotic properties were also determined.
Results: Rhubarb (*Rheum palmatum*) root, wild turmeric (*Curcuma aromatica*) rhizome, aleppo oak (*Quercus infectoria*) gall and ringworm bush (*Cassia alata*) stem extracts possessed strong antibacterial activity. At 0.32 mg/ml MIC, aleppo oak and wild turmeric extracts inhibited the growth of *Y. enterocolitica* while rhubarb, wild turmeric, aromatic ginger (*Kaempferia galanga*) rhizome and ringworm bush extracts inhibited *P. gingivalis* growth. The aleppo oak gall extract had highest phenolics and tannins (672.13 mg gallic acid equivalents (GAE)/g extract and 884.79 mg tannic acid equivalents (TAE)/g extract, respectively) whereas the copper pod (*Peltophorum pterocarpum*) stem bark extract had highest flavonoids (5,293.60 mg quercetin eqivalents (QE)/g extract). Five plant extracts with high water soluble carbohydrate were tested for prebiotic properties. Their indigestible polysaccharide content and stimulatory effect on the growth of *Lactobacillus acidophilus* in MRS broth were determined. Of all, mangosteen (*Garcinia mangostana*) fruit peel and gac (*Momordica cochinchinensis*) root extracts had high indigestible polysaccharide contents, and exhibited good stimulatory effect to the growth of *L. acidophilus*.
Conclusion: Rhubarb root, wild turmeric rhizome, aleppo oak gall and ringworm bush stem could be used in combination with mangosteen fruit peel and gac root for treatment of gastrointestinal tract infections.

GIP11

Photodynamic inactivation of Helicobacter pylori - In-vitro studies of lab and patient strains using Chlorin e6

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Introduction: *Helicobacter pylori* (HP) is one of the most common pathogenic bacteria in the stomach of humans, whereby more than half of the world population is nowadays infected. Due to the emergence of antibiotic resistance, Photodynamic Inactivation (PDI) of bacteria presents a new approach to treat chronic bacterial stomach infections.
Materials and Methods: In-vitro experiments were performed with different strains (CCUG 38770, ATCC 43526, ATCC 43054) and HP isolated from patient specimen. HP were cultured under microaerophilic conditions, high air humidity and a temperature of 37 °C on HP selective agar. Then bacteria were suspended in 0.9 % NaCl to 1.0 - 3.0 McFarland (+/- 0.1) and incubated with the photosensitizer Chlorin e6 (0.1 - 1000 µM). After the samples were twofold centrifuged, washed and resuspended in 0.9 % NaCl to remove unbound Chlorin e6, the uptake was measured by fluorescence spectroscopy. Alternatively, the samples were irradiated for different times, using high power LEDs with a wavelength of 660 nm and an intensity of 9 mW/cm^2. The quantification was performed by counting the number of grown colonies after recultivation.
Results: A 6-log reduction was achieved within 30 seconds of irradiation and a Chlorin e6 concentration of 100 µM for the lab strains. After two seconds, already 90 % of the lab strains were killed, whereby some patient strains were significantly more resistant. A total inactivation was shown for all tested strains after an irradiation of three minutes using Chlorin e6 concentrations in the range of 100 µM.
Conclusion: PDI of HP using Chlorin e6 shows an efficient and desired elimination rate. Especially for multi-resistance variants of HP, PDI may be a promising approach for infections in contrast to antibiotics.

GIP12

Clostridium difficile ribotype distribution indicated different sources of infection in the various age groups

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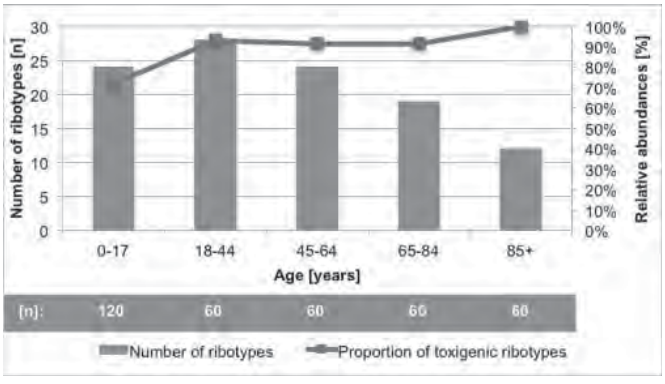
Introduction: C*lostridium difficile* is the most common cause of hospital-acquired diarrhea. Since 2000 the number of nosocomial infections increased continuously for pediatric as well as for adult patients. However, little is known about the age-specific distribution of ribotypes and their toxin-status.
Materials and Methods: We analyzed 360 *Clostridium difficile* isolates of patients in different age-groups (0-17, 18-44, 45-64, 65-84, ≥85 years). All isolates derived from hospitalized patients with diarrhea who were treated at the University of Saarland Medical Center. Genotyping was performed by PCR-ribotyping whereas the toxin-status (tcdA, tcdB, cdtA and cdtB) was investigated by multiplex-PCR.
Results: Highest diversity of ribotypes (n = 28) was detected in young adults (18-44 years) followed by children (0-17 years) and adults (45-64 years). In older age groups, ribotype diversity steadily decreased down to twelve ribotypes in the oldest age group. Ribotype distribution was dominated in older age groups by ribotype 001, 027 and 014. Accordingly, the proportion of isolates with antibiotic resistance to frequently used antibiotics (e.g. fluoroquinolones and macrolides) was higher in older patients. Age-dependency was revealed also for the presence of toxigenic and non-toxigenic *C. difficile* isolates. While non-toxigenic strains were present in 28% of *C. difficile* infected children, the rate decreased to 7-8% in adults and older patients (18-84 years). No single patient with non-toxigenic infection was detected in the oldest age group.
Discussion and Conclusion: Age dependence of the prevalent *C. difficile* ribotypes suggests that the reservoir of *C. difficile* for CDI is different between children, adults and elderly people. Alternatively, or in addition, susceptibility to various ribotypes may be age-dependent. While ribotypes of *C. difficile* infected elderly patients resembled hospital-associated strains, the high variety of ribotypes in childhood and younger adults are supposed to represent infections by environmental strains in the community.

Figure 1



GIP13

TsdA from Campylobacter jejuni: Biophysical properties of an unusual sulfur-metabolizing cytochrome in a human gut pathogen

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Evidence is constantly increasing that inorganic sulfur compounds play an important role for the human gut microbiota. One prominent example is the microaerophilic mucosal pathogen *Campylobacter jejuni.* Tetrathionate stimulates growth of this bacterium under oxygen-limited conditions and recently a novel bifunctional tetrathionate reductases/thiosulfate dehydrogenase was identified in *C. jejuni* [1]. This soluble diheme *c*-type cytochrome is distinct from the well-described octaheme tetrathionate reductase from *Shewanella oneidensis* and the membrane-bound iron-sulfur molybdoprotein TTR from *Salmonella typhimurium.* Sequence comparisons and initial EPR spectroscopic characterization of the protein from the purple sulfur bacterium *Allochromatium vinosum* indicated that TsdAs generally contain one heme with unusual axial histidine-cysteine iron coordination [2]. Such cytochromes appear to play a pivotal role in sulfur-based bacterial energy metabolism, however, in-depth studies of their spectroscopic and electrochemical properties are scare and reaction mechanisms still need to be clarified. We started to fill this gap and performed detailed UV-vis spectroscopic and enzyme kinetic studies on recombinant *C. jejuni* TsdA and derivatives thereof carrying single amino acid substitutions of the residues potentially acting as the sixth axial ligands of the two heme iron atoms, cysteine$_{138}$ (heme 1) and methionine$_{255}$ (heme 2), respectively. Replacement of the cysteine by glycine resulted in a blue shift of the Soret band by 5 nm and appearance of a charge transfer band between 620 and 630 nm for the oxidized protein. The latter indicated presence of high-spin heme, thus proving Cys$_{138}$ as an axial ligand of heme 1. Presence of methionine or histidine at this position did not give rise to significant high-spin heme absorption, led to small blue-shifts of the Soret band and enzyme activity was abolished. Together these findings imply methionine and histidine as functional though somewhat weaker heme iron ligands. Stepwise reduction of oxidized wildtype CjTsdA resulted in intermediary appearance of a spectral feature at 629 nm, indicating that the reaction mechanism involves intermediate replacement of the heme-ligating cysteine from the heme iron. Heme 2 in CjTsdA is clearly ligated by Met$_{255}$, because replacement of this residue by glycine led to high-spin heme. Obviously, Met$_{255}$ cannot be effectively replaced by other nearby residues with the principal ability of heme ligation.

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GIP14

Toxin production of enteropathogenic Bacillus cereus is enhanced by CaCo-2 cells

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Bacillus cereus has become one of the major problems in food hygiene and is associated with two types of gastrointestinal diseases, the emetic and the diarrheal syndrome [1]. The emetic disease is caused by the dodecadepsipeptide cereulide, which is preformed in food. The diarrheal form occurs after ingestion of viable bacteria or spores, which produce enterotoxins in the small intestine. These enterotoxins are the single protein CytK (cytotoxinK) as well as hemolysin BL (Hbl) and the non hemolytic enterotoxin Nhe, each consisting of three components (Hbl L2, L1 and B and NheA, B and C). The *nhe* operon can be detected in 99 % of all enterotoxin producing *B. cereus* strains, while approximately 50 % additionally harbor the *hbl* operon [2]. The toxic potential varies intensely among enteropathogenic *B. cereus* strains. Some strains cause severe food infections and have even been responsible for deaths, others are harmless and are even used as probiotics. So far, the mechanisms which lead to such diverging forms of enterotoxin production and thus toxicity are only poorly understood. In this study, 19 enteropathogenic *B. cereus* strains were selected according to their enterotoxin gene profile (*nhe*, *hbl* and *cytK2*). The occurrence of further putative virulence factors such as sphingomyelinase, metalloproteases or hemolysinII was also considered. The selected strains were isolated from food or food poisoning outbreaks, preferably showing very high or very low toxic potential.
Growth and toxin production of all strains were compared 1. under standard growth conditions (full medium, 30°C, agitation) and 2. under simulated intestinal conditions (RPMI 1640 medium, 37°C, 7% CO_2, preincubated with CaCo-2 cells). No correlation between growth behavior and toxic activity was observed, although growth of all strains was clearly reduced under simulated intestinal compared to standard growth conditions. Toxin production was measured by detection of the NheB component in ELISA. In relation to the slow growth rate, NheB titers were significantly increased under simulated intestinal conditions. Here, comparably high amounts of NheB were detected after only two hours of growth. In further studies the *B. cereus* strains were cultivated under the same conditions in the presence or absence of the human colon carcinoma cell line CaCo-2, showing that co-cultivation enhances toxin production.
This research project was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project AiF 17506 N.

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GIP15

Response of *Vibrio cholerae* towards the catecholamine hormones epinephrine and norepinephrine

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For centuries it has been known - based on empiric observations more than scientific experiments - that stress enhances the probability to get infected with pathogenic microorganisms. Under stress the body produces the catecholamine hormones epinephrine (E) and norepinephrine (NE) which trigger the *fight or flight* reaction in humans. The influence of E and NE on growth and motility is well characterized in pathogenic *Escherichia coli* [1] and *Salmonella enterica* [2, 3]. Here we investigated the influence of E and NE on the human pathogen *Vibrio cholerae* 0395-N1 and elucidated the fate of these compounds during bacterial cultivation.
We followed growth of *V. cholerae* in liquid culture and studied motility on swarming plates under the influence of E or NE. To elucidate the fate of these catecholamine compounds, bacterial supernatants with either E or NE were analyzed using HPLC. Changes in transcript levels of two target genes (*pomB* - as component of the flagellar stator and a *qseC*-like gene - a putative sensor for E and NE) were analyzed using qRT-PCR.
In a previous study it has been shown that *V. cholerae* does not respond to NE [4]. Here, it could be demonstrated that growth and motility of *V. cholerae* was stimulated after the addition of either E or NE. Sequence alignments revealed that *V. cholerae* posesses a sensor kinase which is homologous to QseC - a receptor for E and NE in *E. coli* [1]. Transcript levels of the flagellar associated gene *pomB* and the putative sensor kinase *qseC* were elevated after E and NE influence. HPLC measurements of bacterial supernatant revealed that *V. cholerae* partially stabilized E and NE when compared to cell-free controls. A fraction of these catecholates was converted to yet unidentified compounds. It is proposed that the specific response of *V. cholerae* towards NE and E modulates its pathogenicity.

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GIP16

Integrating conjugative elements (ICEs) in *Helicobacter pylori*: Chromosomal integration and horizontal gene transfer

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Similar to many other pathogenic bacteria, the human gastric pathogen *Helicobacter pylori* harbours different mobile genetic elements in its genome. One example is the *cag* pathogenicity island, which encodes a type IV secretion system and the translocated effector protein CagA, a well-characterized risk factor for cancer development. Other mobile elements include various cryptic plasmids, but also further genome islands with possible correlation to ulcer development. We have recently shown that these genome islands, often referred to as "plasticity zones", have typical features of integrating conjugative elements (ICEs). For example, they integrate into particular sites within their host's chromosomes, and they contain independent type IV secretion systems that may enable self-transfer between bacterial cells. Here, we have performed a comprehensive analysis of ICE sequences from about 50 *H. pylori* strains, and we have characterized excision and transfer capabilities of one ICE (ICE*Hptfs4*), using molecular genetic techniques. Comparative analysis of ICE sequences and chromosomal integration sites showed that their gene content is rather fixed, and that they integrate with an intermediate specificity which is lower than that of most genome islands, but higher than that of typical conjugative transposons. We show that recombinase-mediated excision uses a characteristic sequence motif to generate circularized intermediates. We also see transfer of the ICE*Hptfs4* element from donor to recipient cells; however, measuring transfer rates is complicated by the potent natural transformation (ComB) and homologous recombination (HR) systems of *H. pylori*, which result in a high efficiency of HR-mediated exchange of ICE fragments. Transfer of the complete element seems to be very rare under laboratory conditions. Interestingly, we found that the ComB system supports, but is not essential for, ICE gene uptake.
In conclusion, we show that *H. pylori* ICEs are horizontally transferable elements which integrate into the genome with intermediate site selectivity, rather than being site-specific. Which impact ICE transfer and ICE gene expression have on *H. pylori* pathogenicity, remains to be shown in further studies.

GIP17

Multiplexed molecular detection of gastrointestinal pathogens, including parasites, viruses and bacteria, in human stool samples from Cote d´Ivoire

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Aim: In the contest of a multidisciplinary study proposed to investigate the gut microbiotain Sub-Saharan Africa, we analyzed 15 human fecal samples collected in the Clinical Laboratory of the Don Orione Centre in Bonoua (Côte d´Ivoire).To complete the analysis of microbiological gut composition,15 of the most common human enteric pathogenswere analyzed concurrently using the xTAG GPP from Luminex, a qualitative bead-based multiplexed molecular diagnostic test (Figure 1a). To our knowledge this research represents one of the first attempts to apply this technology to patients coming from developing countries.
Materials and Methods: After collection, the samples were transported to Germany for the subsequent extraction of the nucleic acids using VERSANT® kPCR. The xTAG GPP workflow is described in Figure 1b. In order to confirm the reliability of the xTAG GPP and the preservation of the viral nucleic acids during the transport to Europe the samples were parallel analyzed by rota-/adeno-/astrovirus antigen tests as well as by real-time PCR for the detection of noro-, adeno-, entero-, parecho- and hepatitis E viruses. We performed also the molecular characterization of *G. duodenalis* and *Entamoeba* spp. isolates by conventional PCR.
Results: Overall, it was detected at least one pathogen in 9 out of 15 samples. All positive samples from patients under 12 years revealed a mixed infection (Table 1). The xTAG GPP identified 8 positive samples. The viral isolates detected by xTAG GPP were confirmed by the other methods, with the exception of the sample M18, resulting positive for adenovirus in real-time PCR. Six isolates of *Giardia* were detected by xTAG GPP as well as by conventional PCR and molecularly assigned to Assemblage A or B. Two *Entamoeba* isolates were assigned to the non-pathogen *coli* or *dispar* species.
Conclusions: The present data highlights the potential of xTAG GPP to detect simultaneously in a single test 15 gastrointestinal pathogens, including parasites, bacteria and viruses. Considering the burden of the environmental enteropathies in developing countries with a very high rate of co-infections in particular in the infant population, this study let´s hope the wider application of xTAG GPP in endemic areas. The application of this technology allowed us to deepen knowledge of gut microbiota composition in people living in the south of the Cote d'Ivoire, to better understand the multiplex interactions between microbiota, host and pathogens.

Figure 1

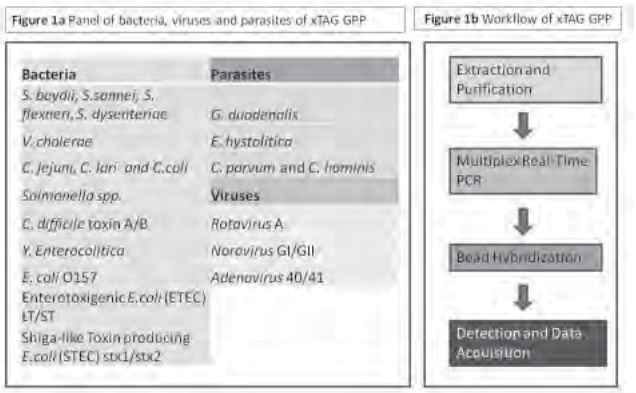

Figure 2

ID	Age (years)	xTAG GPP	Real-time PCR	*Giardia* PCR and genotyping	*Entamoeba* PCR and genotyping	Rota-/adeno-/astrovirus antigen test
M1	8	-	-	-	-	-
M5	8	*E.coli* 0157 *G.duodenalis*	-	*G.duodenalis* Assemblage A	-	-
M7	10	*E.coli* 0157 ETEC *G.duodenalis*	-	*G.duodenalis* Assemblage B	E. coli	-
M8	5	*Campylobacter* ETEC *G.duodenalis*	Enterovirus 116* Coxsackievirus B3*	*G.duodenalis* Assemblage A	E. dispar	-
M9	3	*G.duodenalis*	Coxsackievirus A13* Parechovirus	*G.duodenalis* Assemblage B	-	-
M11	9	-	-	-	-	-
M12	40	-	-	-	-	-
M13	30	-	-	-	-	-
M14	12	Norovirus GI *G.duodenalis*	Norovirus GI Enterovirus 119*	*G.duodenalis* Assemblage A	-	-
M15	12	-	-	-	-	-
M16	39	Norovirus GII Rotavirus A	Norovirus GII	-	-	Rotavirus
M17	50	*G.duodenalis*	-	*G.duodenalis* Assemblage B	-	-
M18	41	-	Adenovirus	-	-	-
M19	67	-	-	-	-	-
M21	24	Shigella	-	-	-	-

Table 1
Summary of results obtained by xTAG GPP, real-time PCR (adeno-/noro-/entero-/parecho-/hepatitis E virus), *Giardia* and *Entamoeba* PCR and genotyping, rota-/adeno-/astrovirus antigen test.
*Enterovirus isolates were molecularly characterized at the National Reference Centre for Poliomyelitis and Enteroviruses (Robert Koch Institute).

GIP18

The effect of *Helicobacter pylori* infection on the *in vitro* migration behavior of human leukocytes

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Helicobacter pylori is well known for its ability to evade the human immune system and to establish a persistent chronic infection in the human stomach. Various mechanisms were shown to be involved in immune system modulation and evasion. Besides passive defense strategies (e.g. altered LPS- or flagellin structures) that result in avoiding detection by pattern recognition receptors (PRR), *H. pylori* exploits also active approaches. For example the vacuolating cytotoxin (VacA) inhibits IL-2 secretion and proliferation of T-cells at the level of calcineurin and the g-glutamyl transpeptidase (GGT) of *H. pylori* is supposed to interfere with Ras signaling in T-cells. In addition, *H. pylori* seems to makes use of regulatory

T-cells (Tregs) to generate immunological tolerance. In this study we have shown that *H. pylori* influence the migration behavior of cells from the innate or adaptive immune system in different ways. In an initial attempt we used the Boyden-Chamber assay and evaluated isogenic mutants of the cagT4SS of *H. pylori* for the ability to interfere with cell migration. Further experiments employing 2D and 3D *in vitro* migration assays showed that *H. pylori* is able to affect leukocyte migration in a cag Pathogenicity Island (cagPAI) dependent manner in a 2D environment but not in a 3D situation.

GIP19

Helicobacter pylori infection in Nigeria - analyzed by gastroenterology, phenotypic and genotypic characterization of isolates, pathology and statistical correlations

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Introduction: *Helicobacter pylori* is the causative agent for gastritis, peptic ulcer and a risk factor for the development of gastric cancers. In developing countries like Nigeria the infection rate can reach 85% with high antibiotic resistance, a major reason for eradication failure. We established a network of five hospitals in Nigeria, with the coordination centre in Lagos. To study the epidemiological situation biopsies, isolated strains, patient questionnaires and the according diagnosis of the gastroenterologist were collected.
Materials and Methods: 93 isolates were characterized according to their CagA-, VacA- and IL8-status, their ability to colonize in Mongolian Gerbils, as well as by MLST sequencing. Histological grading was performed for 354 biopsies, and resistances were analysed by E-test in 97 strains as well as by FISH in 105 biopsies.
Results: Most patients showed mild pathology, which was always presented as inflammation and approximately 70 % showed a change in MALT. We have not detected any cancer cases. Furthermore the strains were not highly variable. 97 % were CagA- and 90 % were VacA-positive (always s1/m1). 67 % of the strains were able to colonize in the Mongolian Gerbil animal model. Via E-test we have shown high resistance (98 %) to Metronidazol, followed by Amoxicillin (31%), Clarithromycin (17 %) and Tetracycline (4 %). Via FISH we did not detect any resistance to Clarithromycin, but Tetracycline showed a resistance rate of 16%.
Conclusion and Outlook: The controversial results in resistance patterns require further determination of new and not yet described mechanisms. Furthermore the analyses of statistical correlations between patient information, gastroenterology, characterized strains as well as pathology are in progress.

GENOMICS AND METAGENOMICS

GMP01

A Method for Selectively Enriching Microbial DNA from Contaminating Vertebrate Host DNA

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Recent discoveries have implicated the human microbiome as playing a role in certain physical conditions and disease states, and these advances have opened up the potential for development of microbiome-based diagnostic and therapeutic tools. Microbiome research on non-human organisms is also increasing. The majority of microbiome DNA studies to date have employed 16S rRNA analysis, but these provide little information regarding function. In contrast, sequencing of the total DNA of a microbiome sample provides a broader range of information including genes, variants, polymorphisms, and putative functional information. However, many samples, including those derived from vertebrate skin, bodily cavities, and body fluids, contain both host and microbial DNA. Since a single human cell contains approximately 1,000 times more DNA than a single bacterial cell, even low-level human cell contamination can substantially complicate sample analysis. In some cases, as few as 1% of sequencing reads may pertain to the microbes of interest and a large percentage of reads must be discarded, making such experiments impractical.
To address this issue, we developed a magnetic bead-based method to enrich for microbial DNA using methyl-CpG binding domain (MBD) to separate host DNA from microbial DNA based on differences in CpG methylation density. Importantly, microbial diversity and relative abundance is maintained after enrichment. This method was evaluated with multiple challenging samples, and we describe the enrichment of bacterial and protistan DNA from human saliva, human blood, a mock malaria-infected blood sample, human cystic fibrosis sputum and black molly fish, followed by next-generation sequencing. Sequence reads were mapped to reference genomes, and reads aligning to host genomes were reduced approximately 50-fold, while reads corresponding to microbial sequences increased approximately 10-fold. This tool for microbiome sequence analysis holds promise for use with a variety of sample types, enabling enrichment while accurately reflecting the diversity of the original sample.

GMP02

Improved metagenomic analysis using low error amplicon sequencing libraries

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Metagenomic analyses using 16S rRNA or other amplicons are widespread and performed on several next generation sequencing (NGS) platforms. During amplification or library preparation, several errors are inserted by polymerases or can occur during sequencing. Unlike resequencing approaches, metagenomic analyses lack a correcting reference genome, thus such errors cannot be detected or corrected. In this study we assessed and optimized the usability of a low error amplification method for sequencing amplicon libraries in metagenomic analyses. For this purpose we used a mock community with species derived from various habitats (e.g. human associated, soil, aquatic) for generating amplicon libraries. We inserted unique barcodes in a low amount of initial amplification products using a linear PCR step. These templates were then exponentially amplified and sequencing adaptors were inserted for several NGS platforms.
Using these unique barcodes it is feasible to pool all sequences derived from one initial DNA amplicon together and build one consensus sequence. In this way most errors which occur after this linear elongation can be corrected using bioinformatics tools.
The elimination of such amplification errors directly leads to better phylogenetic assignment. This tool also improves the usability of NGS platforms, being able to correct random errors which occur during sequencing. The generation of such low error amplicon reads enables a more precise insight into the analyzed community thus opening new perspectives for metagenomic analyses.

GMP03

Surprising identification of a urease encoding gene cluster in the novel acidophilic iron oxidizer "*Ferrovum*" sp. JA12

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Acidophilic iron oxidizing bacteria related to the novel proposed genus "*Ferrovum*" within the *Betaproteobacteria* [1] have been enriched from a pilot plant for the biological remediation of acid mine drainage [2]. We sequenced the genome of the enrichment culture JA12 using next generation sequencing techniques (454, Illumina) and assembled the complete genome sequence of "*Ferrovum*" sp. JA12. Using comparative genomics we reconstructed potential electron transfer processes required for ferrous iron oxidation and the pathways involved in the uptake and assimilation of the macronutrients C, N, P and S [3]. In this context, we also revealed a gene cluster of 16 open reading frames encoding a putative urea ABC transporter, the urea hydrolyzing enzyme urease and its accessory proteins. Although the

ability to use urea as an additional source of nitrogen or even as carbon source is widely spread among the *Proteobacteria*, no similar gene cluster has so far been identified in related neutrophilic or acidophilic iron oxidizing bacteria. The physiological role and evolutionary origin of the urease gene cluster in "*Ferrovum*" sp. JA12 remain therefore unclear. However, bioinformatic analyses predicted promoter regions similar to those in other urease producing bacteria, indicating that urea is taken up from the environment and cleaved *via* the urease. Furthermore, the comparison of the phylogeny based on the 16S rRNA gene and that of various genes from the urease gene cluster shows that horizontal gene transfer events may have played a role in acquiring the genetic basis for the ability to use urea. Currently, we are working on the establishment of a western blot based detection system to identify the conditions under which "*Ferrovum*" sp. JA12 produces the urease. In particular, we are testing two hypotheses: firstly, the gene cluster represents a means of N acquisition and, secondly, the gene cluster plays a major role in providing a cytoplasmic buffer against acidic pH similar to the urease activity reported in *Helicobacter pylori* [4].

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GMP04

A novel SCC*mec* element in a CC12 MRSA strain

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SCC*mec* elements are potentially mobile genetic elements that include the methicillin/beta-lactam resistance gene *mecA*. Eleven types and several subtypes of SCC*mec* elements are currently known.
An isolate of West Australian MRSA-59 (ST12, Ridom *spa* type t160) was isolated in 2007 and found to carry an un-typable SCC*mec* element. It was characterised by microarray hybridisation and by Illumina next generation sequencing. The SCC*mec* element of this strain comprised *dcs*, *mvaS*-SCC (truncated 3-hydroxy-3-methylglutaryl CoA synthase), Q5HJW6 (a putative protein), *dru* (direct repeat units), *ugpQ* (glycerophosphoryl diester phosphodiesterase), *ydeM* (putative dehydratase), *mecA*, *mecR* (signal transducer protein MecR1), *mecI* (regulatory protein for methicillin-resistance), *copA2* (copper exporting ATPase), *mco* (copper oxidase), A8YZ03 (a putative lipoprotein), *arsC* (arsenate reductase/arsenical pump membrane protein), *arsR* (repressor of arsenic resistance gene cluster), *per2* (plasmidic permease), Q9KX84-M10 (putative protein) and *mcrC* (type IV 5-methylcytosine-specific restriction enzyme subunit C). Any recombinase genes as well as *xylR/mecR2* (homolog of xylose repressor) and PSM-*mec* (SCC-associated phenol soluble modulin) were absent.Staphylococcal isolates belonging to several species were tested by microarray hybridisation in order to find similar SCC*mec* elements. *Mec* complexes lacking *xylR/mecR2* were observed sporadically in *S. aureus* (CC5, CC7, CC398) and in several coagulase-negative staphylococci (S. *epidermidis, S. haemolyticus, S. hominis, S. saprophyticus*). However, these isolates all carried various sets of *ccr* genes. A single *S. lentus* isolate from Germany yielded, with regard to SCC*mec*, the same hybridisation pattern as WA-59.
This suggests, further characterisation pending, a transfer of the novel element between coagulase-negative staphylococci and *S. aureus*.

GMP05

A new role for tight adherence (*tad*)-like genes in natural transformation

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Abstract will not be presented.

GMP06

Microbiological Indoor and Outdoor Air Quality of Two Major Hospitals in Benin City, Nigeria

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Air contains large number of microorganisms including bacteria and fungi and their estimation is important as an index of cleanliness for any particular environment. It becomes imperative to undertake a study of the microbiological air quality of the airborne micro-flora in the environments of two major government hospitals, University of Benin Teaching Hospital (UBTH) and Central Hospital, in Benin City metropolis. Both indoor and outdoor air samples were assessed monthly for the three (3) months in the wet season (June - August, 2010) and dry season (November 2010 - January 2011) using the settled plate methods. The study sites were divided into nine (9) units which include accident and emergency ward, laboratory, male ward, female ward, children ward, labour room, treatment room, theatre and outside the hospital gate. The mean airborne bacterial load in the two hospitals ranges from 8.5cfu/min to 172.5cfu/min and 5.5cfu/min to 64.5cfu/min for UBTH and Central hospital in the wet season. While the mean airborne fungal load in UBTH and Central Hospital in dry season ranges from 2.5cfu/min to 9.5cfu/min and 1.5cfu/min to 19.0cfu/min respectively. The female ward, children ward, accident and emergency ward and outside the hospital gate recorded the highest airborne micro-flora. The result revealed the isolation of ten (10) fungal isolates and six (6) bacterial isolates. These include *Aspergillus niger, Aspergillus flavus*, *Botryodiplodia acerina, Rhizopus stolonifer, Nigospora zimm, Mucor* sp., *Monilla infuscans, Penicillium* sp., *Candida* sp. and *Trichoderma viridis* while the six (6) bacterial isolates include *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus* sp., *Serratia marcescens* and *Micrococcus* sp. The result shows the highest fungal population of 26.5cfu/min (outdoor) in UBTH followed by 24.0cfu/min (outdoor) in Central Hospital. The highest bacterial load of 172.5cfu/min (outdoor) was recorded in UBTH. The fungal isolates *Aspergillus niger* (53.0%) and *Monilla infuscans* (43.9%) were showed to be the most frequently isolated airborne fungal organisms while *Staphylococcus aureus* (91.3%) and *Staphylococcus epidermidis* (85.8%) were the most frequently isolated bacterial isolates. The statistical analysis showed no significant difference between the microbial population obtained during the wet and dry seasons in both hospitals studied. Data generated underline the usefulness of monitoring hospital environments.

GMP07

So much more than a Souvenir - The Relevance of Plasmids in Roseobacters exemplified for *Phaeobacter inhibens* DSM 17395

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Phaeobacter inhibens DSM 17395, an archetype of the marine *Roseobacter* group (Alphaproteobacteria), harbors three plasmids of the RepA-I, RepB-I and DnaA-like replication type with respective sizes of 65-kb, 78-kb and 262-kb. Manual genome annotation indicated that plasmid encoded genes play an important role for a variety of physiological functions as well as the ecological competitiveness of *P. inhibens*. One example is the complete pathway for the production of the antibiotic tropodithietic acid (TDA) that is located on the 262-kb plasmid. Furthermore, about 20 genes for polysaccharide metabolism are located on the 65-kb replicon and the 78-kb plasmid contains genes for the synthesis of siderophores. We used our established plasmid curing approach based on plasmid incompatibility (Petersen at al. 2011) in order to investigate the function of each replicon and the interplay of the plasmids. Accordingly, we generated a complete set of mutants lacking one, two and three of the endogenous plasmids. The comparison of all seven mutants with the wild type via the pulsed field

technology (PFGE) is compatible with the sensitive PCR-assay and documents successful curing. We analyzed biofilm formation, swimming motility and the production of TDA. Moreover, we used the phenotype MicroArray technology (Biolog; PM1, PM2, PM3) to investigate the conversion of nearly 300 different carbon and nitrogen substrates. The characterization of the mutants showed that TDA-biosynthesis is exclusively determined by the 262-kb plasmid. Curing of the 65-kb replicon resulted in the complete loss of attachment and motility, thus justifying the designation as a biofilm plasmid (see also the talk of Jörn Petersen). Finally, the 78-kb plasmid contains several genes for the degradation of L-threonine and the different mutants (Δ78, Δ78+65, Δ262+78, Δ262+78+65) lost the capacity to convert this amino acid. High throughput phenotyping showed apart from the loss of specific plasmid-encoded metabolic capacities a general and cumulative decrease of the metabolic signal hence indicating a largely impaired viability of the bacterium. Taken together, our results clearly demonstrate the important physiological roles of all three plasmids for *P. inhibens* and thus support our former prediction that they are essential *sensu lato*, i.e. indispensable for survival in the natural habitat (Petersen et al. 2013).

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GMP08

Complete Genome Sequence of the acarbose producer *Streptomyces glaucescens* GLA.O (DSMZ 40922) consisting of a linear chromosome and a linear plasmid

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Acarbose is a secondary metabolite of different *Actinobacteria* that inhibits mammalian α-glucosidases and is therefore used for the treatment of type-2 diabetes mellitus. Its medical impact is based on a decreased release of glucose from starch- and sucrose- containing diets in the human intestine, which also leads to reduce blood glucose and serum insulin levels. Besides the sequenced wild type *Actinoplanes* sp. SE50/110 (Schwientek *et al.*, 2012), which is a progenitor of today`s production strains, also *Streptomyces coelicoflavus ZG0656* as well as *Streptomyces glaucescens* GLA.O are known natural producers of acarbose. Whereas a draft genome for *S. coelicoflavus ZG0656* is available only the acarbose biosynthesis cluster sequence is known for *S. glaucescens* GLA.O (Guo *et al.*, 2012; Rockser and Wehmeier, 2009).
Sequencing of the *S. glaucescens* GLA.O genome was performed by combination of mate pair and PCRfree libraries using the MiSeq Sequencer from Illumina (CeBiTec Bielefeld University). The assembled genome comprises a linear chromosome, 7.45 Mbp in size with an average G+C content of 73% and 6,417 protein coding sequences (CDS) and a linear plasmid, 170,574 bp in size, GC content of 69% and 173 CDS.
Based on the genome the acarbose biosynthesis cluster (*gac*-cluster) sequence of *S. glaucescens* GLA.O could be confirmed. In comparison to *Actinoplanes* sp. SE50/110 the *gac*-cluster showed significant organizational differences. In particular, it has been reported that some *gac* genes in *S. glaucescens* GLA.O are unique while other expected genes seem to be missing entirely (Rockser and Wehmeier, 2009). The detailed comparison of the acarbose biosynthesis cluster of *S. glaucescens* GLA.O, *Actinoplanes* sp. SE50/110 and *S. coelicoflavus* ZG0656 will be presented.

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GMP09

Mycobacterium avium subsp. *paratuberculosis* sheep strain JIII-386: Sequencing, Assembling, Annotation, and Genome comparison

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Mycobacterium avium subsp. *paratuberculosis* (MAP) - the etiologic agent of paratuberculosis - affects cattle, sheep and other ruminants worldwide. The deciphering of phenotypic differences observed between cattle and sheep type strains (belonging to Type-II and I/III, respectively) by comparative genome analysis requires data from diverse isolates originating from different regions of the world but is currently hampered by the lack of availability of a fully assembled MAP-Type-I/III strain.
MAP sheep strain JIII-386 from a migrating herd in Germanyand genotyped as MAP-Type-III [1] was sequenced by whole-genome shotgun sequencing (Illumina technique), *de novo* assembled (CLC Genomics Workbench, improved with SSPACE), and annotated by BacProt based on Proteinortho [2]. NcRNAs were annotated by homology search of Rfam families using the GORAP pipeline [3]. Additionally, a new full sequence of cattle isolate JII-1961 from Germany [1], published MAP-Type-II strains K10, MAP4, MAP-Type-III strain S397 (all from U.S.), MAP-Type-I strain CLIJ361 from Australia, and *M. a.* subsp. *hominissuis* strain MAH104 were used for comparison and assembly improvement of JIII-386. These genomes were also fully annotated by BacProt and results compared with NCBI annotation. We hereby present the so far best assembled Type-I/III strain. Using two annotation programs, equal numbers of genes were found, but also 10 % of genes only by either NCBI or BacProt. A new Shine-Dalgarno sequence motif (5'AGCTGG3') differing from the standard (5'AGGAGG3') was extracted for all *M. avium* strains and *M. tuberculosis* strain H37Rv - possibly conserved for all Mycobacteria. Novel mycobacteria-specific proteins were searched with BacProt. For the first time 82(81) ncRNAs and Riboswitches were unveiled for MAP, numbers of which differ between MAP-Type-III and II in three cases (ASpks, G1, ykkC-III) but also between MAP types and MAH104. Some previously described differences (presence or absence of specific ORFs, LSPs) between MAP-Type-I/III and II strains [4] could be partially revised, two new Type I/III specific large regions identified. Results of SNP analysis confirm the strong similarity of MAP-Type-II strains, and show higher diversity between MAP-Type I/III strains.
Enhancement of known strain diversity at genome level as achieved by providing two new MAP sequences and the use of two annotation programs unveiled new insights in MAP-Type specific gene regions and will help to decipher genes responsible for different host association and virulence of Type I, II, and III.

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GMP10

A draft genome of Candidate Division OP3 obtained by physical enrichment of cells

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The bacterial candidate division OP3 was first discovered as sequence in 16S rRNA gene libraries generated from sediment of the Obsidian Pool, a hot spring located in Yellowstone National Park, USA [1]. Since then, OP3 was reported to be present in various anoxic habitats like tidal flats and deep groundwater. OP3 affiliates to the *Planctomycetes*, *Verrucomicrobia* and *Chlamydiae* (PVC) superphylum. Currently, no cultivated representative is available for physiological studies.
16S rRNA genes of candidate division OP3 were recently detected in a limonene degrading methanogenic enrichment culture. CARD FISH studies revealed small cells of OP3 which were alone or attached to larger cells [2]. Based on the limited diversity in the enrichment culture, density gradient

centrifugation was attempted and yielded a fraction with over 80% OP3-cells. The DNA present in this OP3 cells-enriched fraction was isolated and sequenced by next generation techniques. The metagenome is currently assembled and will be analyzed. Recently, four single cell draft genomes of OP3 cells have been published as *candidatus* `Omnitrophica`. These are with sizes of 2037, 768, 518 and 469 kb (150, 60, 54 and 35 contigs) and may be suitable to provide initial insights into the physiology. We expect from the comparison of the draft genomes a first glance at the specific physiology of OP3.

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GMP11
Optimized protocols for Illumina PCR-free whole genome sequencing and evaluation of assembly performance

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Introduction: Illumina Next-Generation Sequencing (NGS) technology has paved the way for rapid and cost-efficient *de novo* sequencing and re-sequencing of genomes. With the introduction of PCR-free library preparation procedures (LPPs) major improvements were made in comparison to the initial PCR containing LPPs, as PCR biases are largely reduced. In the present study, we describe a further optimization of the existing Illumina TruSeq® DNA PCR-free LPP with regard to size selection of DNA fragments and quantification of the resulting library concentration. Furthermore, the impacts of particular parameters on assembly performance such as the read size and the average library insert size were investigated. Finally the relationships between genomic GC content, average library insert size and sequencing quality are highlighted.
Materials and Methods: Five bacterial strains, ranging in genomic GC content from 35.4% to 73.0%, were paired-end sequenced on the Illumina MiSeq sequencing platform. These were *Bacillus cereus* F837/76, *Enterococcus faecalis* OG1RF, *Salmonella* Typhimurium 14028S, *Pseudomonas stutzeri* ATCC 17588 and *Micrococcus luteus* NCTC 2665. Genome sequencing libraries were generated using the Illumina TruSeq® DNA PCR-free LPP and modified versions of it. Sequence assembly was performed using SPAdes. Estimation of assembly performance is mainly based on NGA50/NGA75 and corrNG50 quality metrics, which were calculated using the quality assessment tool QUAST.
Results: In comparison to the standard Illumina TruSeq® DNA PCR-free LPP our modified protocols are suitable for exact calculations of molarity (needed to obtain optimal cluster densities) without the need of qPCR. In addition, the modified LPPs consume only half the reagents per sample doubling the number of preparations possible with the same commercial kit. Furthermore, magnetic bead mediated size selection was optimized for the generation of sequencing libraries with varying average insert size.
Estimating assembly performance with the help of assembly metrics like corrected NG50, NGA50 and NGA75, showed that sequencing libraries with increased average insert sizes can result in substantial assembly improvements. Currently, from a technical point of view, such improvements seem to be limited to genomes within the range of low to medium GC content, since sequencing quality decreases rapidly with increased genomic GC content and average library insert size.
Conclusions: Our modifications to the standard Illumina TruSeq® DNA PCR-free LPP enable the researcher to produce sequencing libraries of varying average insert sizes. In addition, the modifications make the need of labor-intensive and time-consuming qPCR obsolete. Investigations on assembly performance indicate that our modified PCR-free LPPs can create sequencing libraries superior to the standard ones.

GMP12
Gene-expression profiling of peripheral blood mononuclear cells after the confrontation with pathogens of systemic infections

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Introduction: Bloodstream infections are caused by fungi, Gram negative and Gram positive bacteria. For the defense against microbial pathogens, the response of the innate immune system is crucial.
Genome-wide gene expression analysis can help to distinguish the response of peripheral blood mononuclear cells (PBMCs) towards different pathogens and identify pathogen specific patterns.
Materials and Methods: PBMCs of five healthy male donors were isolated via Ficoll density centrifugation and confronted with *A. fumigatus*, *N. meningitidis* or *S. aureus* with a multiplicity of infection of 1. After 3 and 6 hours RNA from PBMCs was isolated and microarray analysis were performed.
Genes which are significantly differentially expressed (FDR < 0.05, Fold Change > 1.5) with regard to an uninfected control were analysed for each pathogen.
Results: The data revealed a pathogen-independent program of gene expression induced by all three pathogens. Within this group we found mainly genes whose products are involved in cytokine-cytokine receptor interactions and the TNF signalling pathway. The strongest upregulation was seen for *IL6*, *TNF* and for genes encoding different chemokines.
A. fumigatus induced a strong specific response which was focused on genes corresponding to the cell cycle and the proteasom.
In contrast, bacterial pathogens shared a large number of differentially expressed genes. In addition *N. meningitidis* induced a specific set of genes encoding cytokine receptors as well as several genes envolved in the Jak-STAT signalling pathway like *IL2* and *IL4* receptor and *IFNα*.
Discussion: These characteristic transcriptional changes can be used as a fundament for future studies to aid the diagnosis of sepsis. Further bioinformatical analysis will adress gene clusters and the structure of the underlying gene regulatory network on the basis of coexpression and additional prior knowledge.

GMP13
MalleiTyper - A bioinformatic tool for MLVA typing of *Burkholderia mallei*

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Introduction: *Burkholderia mallei* is the causative agent of glanders, a zoonotic disease in *Equidae* that can be transmitted to humans. Licensed human vaccines are not available. Several outbreaks of the disease during the last few years had a remarkable economic impact, and the pathogen is within the scope of possible bioterroristic agents (CDC class B). During an outbreak investigation, multiple locus variable number tandem repeat analysis of 23 dedicated loci (MLVA23) is an established method to generate specific molecular fingerprints in order to distinguish among different strains of *B. mallei*. With the development of Next Generation Sequencing (NGS) technology and affordable benchtop platforms, algorithms for *in silico* typing from *de-novo* assembled microbial genomes have been published. However, as a result of high GC content and a multitude of repeat motifs within the genome, *de-novo* assembly is challenging and time-consuming. We therefore developed MalleiTyper, an algorithm that calculates the MLVA23 fingerprint of a sequenced isolate directly from a raw NGS file (FASTQ format) without the necessity of prior assembly using string comparisons and a smart kmer analysis.
Materials and Methods: Published MLVA23 primer sequences and all available finished genomes of *B. mallei* were analyzed and used to generate a MLVA loci library file. Genomes of the agent were downloaded from NCBI reference genome database and randomly shredded into fragments of 75 to 400 bp length to create an artificial FASTQ file to mimic a NGS data set. After optimization of the algorithm, MalleiTyper was challenged with genuine NGS data generated by our Ion Torrent PGM instrument. Finally, the *in silico* results have been compared to data created with conventional MLVA23 assay.
Results: The algorithm was able to detect not only exact matches of MLVA primer and tandem repeats, but also sequences with mismatches that may

occur due to a real single nucleotide polymorphism (SNP) in a particular bacterial strain or to erroneous determination by NGS technology.
Conclusion: MalleiTyper performed well with artificial and real NGS data sets. Written in python, the script can be used either as standalone tool, or the code can be easily integrated in other workflows or even as third party plugin to commercial NGS software packages. Moreover, the program is designed to allow the adaption to other bacterial species just by exchanging the input MLVA loci library file.

GMP14
Differential gene expression of the nosocomial pathogen *Enterococcus faecalis* subjected to metal stress

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Enterococci are nosocomial pathogens capable of causing various infections of the human host such as infections of surgical areas and the blood stream. The most abundant species *Enterococcus faecalis* is able to build thick biofilms on medical devices such as catheters and implants, making it more resistant to antibiotic treatment, phagocytosis and physical and chemical stresses. Together with the huge problem of emerging antibiotic resistance among clinically relevant bacteria this gives rise to a need for novel antimicrobial materials. Here we present the differential gene expression of *E. faecalis* 12030 exposed to AgXX®, a novel antimicrobial surface coating based on micro galvanic elements formed by silver and other precious metals of the platinum group [1].
Using next generation RNA sequencing technology we carried out chrono-transcriptome analysis of *E. faecalis* cells subjected to different metal stress situations to investigate a putative mode of action of this novel antimicrobial surface coating as bioactive contact catalyst.
E. faecalis 12030 was either subjected to metal stress by exposure to stainless steel nets covered with Ag or AgXX® or exposed to uncovered steel nets as control. Different exposition times were chosen to cover both a possible quick and transient metal stress response as well as a possible adaptation reaction. Total RNA was isolated followed by the removal of 5S, 16S, 23S rRNA as well as tRNAs. The remaining enriched mRNA was fragmented enzymatically and used as template for the generation of whole transcriptome cDNA libraries. Strand-specific sequencing was carried out in an Ion Proton™ Sequencer. FPKM values were used to calculate relative gene expression ratios. A gene was considered differentially expressed when the ratio was $\geq |2.0|$. Generally a correlation between exposition time and intensity of gene expression was observed: the longer the exposition time the larger the fold change was. This trend is a strong hint for an adaptation of the bacterium to the specific metal stress situation. In total 286 genes were differentially up- and 156 genes down-regulated in *E. faecalis* cells exposed to steel nets covered with AgXX® for 24 minutes compared to *E. faecalis* cells exposed to uncovered steel nets for the same time. Several gene products of the top up-regulated genes are involved in metal-ion-transport, heat shock response or general stress response. A putative mechanism of action of the novel antimicrobial substance will be presented.

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GMP15
Proteogenomics of *Geobacter metallireducens* exposed to a high and low substrate concentrations of aromatic compounds (toluene, benzoate) and fermentation products (acetate, butyrate, and ethanol)

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Geobacter metallireducens is one of the model organisms for bioremediation of many aromatic compounds such as petroleum-derived pollutants as toluene, phenol, p-cresol and benzene. This strict anaerobe iron-reducing bacterium is capable to completely oxidize organic compounds. The proteogenomic approach was applied to investigate *G. metallireducens* exposed to high and low substrate concentrations of aromatic compounds (toluene, benzoate) and fermentation products (acetate, butyrate, and ethanol). The main aim was to identify possible new and/or modified peptide-coding sequences and increase the number of potentially new proteins relevant for bioremediation.
The measured MS/MS spectra of *Geobacter metallireducens* were compared with all theoretical peptides derived from *in silico* six frame translation of the genome code and with the intergenic open reading frame (ORF). We identified 215 new and/or modified peptide-coding sequences by using the six frame translation database and 15 new peptides by using the intergenic ORF database. Among these, many uncharacterized proteins were identified, but also one modified peptide sequence belonging to the benzoylsuccinyl-CoA thiolase subunit (bbsB), an enzyme that is involved in the catalysis of the anaerobic toluene degradation. The results show that proteomics is a valuable method for validation of genome annotations.

GMP16
Reducing the metagenomic sequence space for analysis of the gut microflora by taxon-specific cell enrichment

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Introduction: The microbiota of the gut consists of a huge number of different microbial species whose composition has been shown to vary greatly between individuals but still remains complex and their whole-scale analysis in individuals is challenging [1].
For certain scientific questions, however, it would suffice to examine in a targeted manner only a taxon-specific sub-metagenome. Examples for study objectives along this line are (i) the in-depth analysis of the metagenomes of selected taxa with respect to variation in individuals including the functional genomic information, (ii) the analysis of changes in the composition and functionality in taxon-specific populations of microbes under different conditions (e.g. in response to diet or inflammation), or (iii) the analysis of changes in the composition and functionality of taxon-specific populations under the influence of e.g. competing or probiotic strains.
Materials and Methods: The method mostly focused on in this project is directed to taxon-specific cell enrichment, by hybridizing polynucleotide probes to intracellular targets of fixed (permeabilized) cells, while parts of the probes remain in the cells periphery presumably by forming inter-probe networks [2].
Cells which react with the polynucleotide probes are enriched by immobilization on (paramagnetic) beads or microplate wells via binding of probe labels to appropriate capture components. The standard polynucleotide probes are comprised of *in vitro* transcripts of 23S rRNA domain III, which can be regarded as roughly genus-specific [3, 4].
Results: Prior to working on real (clinical) samples, immobilization of pure cultures of *Enterococcus* already could be established within this project

period. The results were evaluated by qPCR and 23S rDNA sequencing from these immobilized cells.
Discussion: In this project the focus is on bacteria of relevance in the gut. Furthermore the applicability of the resulting cell enrichments as starting material for submetagenomic studies will be evaluated. With respect to immobilization of intestinal relevant bacteria cells, the application to fecal samples needs to be improved.

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GMP17
Insights into the microbial phosphorus turnover in forest soils using a metagenomic approach

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Phosphorus (P) is an important macronutrient for all biota on earth as it is an essential part of nucleic acids, phospholipids and ATP [1]. Thus, P is a major growth limiting factor affecting plant growth and crop yields [2]. Many soils throughout the world are deficient in free and thus plant available P, since the high reactivity of P leads to precipitation. Besides a large fraction of P is bound to organic compounds that have to be hydrolyzed enzymatically prior to uptake [3]. Nevertheless various microbes are known to be effective in mineralizing organically bound P as well as precipitated forms of inorganic P [4]. With this study we want to deepen our knowledge on the microbial communities involved in P cycling and the regulation processes of their crucial P uptake systems. Therefore, we analyzed samples from two contrasting forest soils containing high or low amounts of P. We assume that in a P lacking soil the recycling of organic bound P is dominant whereas in a P rich soil mineralization processes of inorganic P prevail. We also assume a link between the dynamic of P turnover and the availability of other nutrients like carbon and nitrogen. To compare the microbial communities a tRFLP based on 16S rRNA was applied. Additional insights into community structure were achieved by ERIC based PCR. Whole genome shotgun sequencing was carried out using 454 technology. Data analysis was performed implying MEGAN5. Moreover the microbial phosphorus as well as the microbial carbon and nitrogen content were determined. Taxonomic analysis of sequencing data revealed significant differences in community composition in two forest soils with distinct P content. While *Proteobacteria* form the most abundant phyla on both sites, the amount of annotated reads for *Actinobacteria* and *Acidobacteria* differs significantly. On the family level the soil rich in P is clearly dominated by *Bradyrhizobiaceae* while the microbial community in the P lacking soil tends to be distributed more evenly with a significantly higher percentage of *Acidobacteriaceae*. Sequencing data will further be analyzed on a functional level using KEGG to identify dominant P degrading enzymes as well as enzymes involved in solubilization of mineral phosphate. Additionally sequencing data will be blasted against a selected set of Pfam families to take also in account the considerable amount of not annotated reads.

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GMP18
Next-generation sequencing of bacterial communities in the gut of *Serritermes sp* (Isoptera, Serritermitidae) and *Neocapritotermes sp* (Isoptera, Termitidae): termites from the central savannas

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Introduction: Termites are biomass decomposers that affect on the structure and composition of soil with assistance of their resident gut microbiota. Culture-independent molecular analyses have revealed that the majority of these microorganisms are not identified by traditional methods. In this study we investigate the gut of *Serritermes sp,* an endemic specie, and *Neocapritotermes sp*, not an endemic specie, collected at Campus of University of Brasília, DF, Brazil.
Materials and Methods: Part of the nest of the *Serritermes sp* and *Neocapritotermes* sp were taken to the laboratory, 60 or 40 workers, respectively, were randomly chosen. The gut was immediately dissected and frozen until DNA extraction using a DNA kit (MO-BIO, Brazil) with the aid of a bead beater following the manufacturer's instructions. The extracted DNA was PCR-amplified using 16S rDNA primers for hypervariable regions V3 and V6 and was sequenced by ion torrent next generation technology.
Results: Analyses by Ribosomal Database Project of V3 and V6 amplicon showed the predominance of Bacteroidetes, Proteobacteria, Firmicutes, Spirochaetes in both termites species. Actinobacteria were in equal proportion and a low amount of sequences correspond to Synergisteles Verrumicrobia, Fusobacteria, Elusimicrobia, TM7, Deferribacteres and Tenericutes were observed. Even in comparison, around 9% and 17% of the sequences from V3 region and 16% and 35%, from V6 region were unclassified in *Serritermes sp* and *Neocapritotermes sp* respectivelly. The application of species richness estimators confirmed the highly diverse nature of bacteria in gut samples
Conclusion: The study of the 16S rDNA gene from gut microbiota of two termites inhabiting the same area but with different nest and habits can help the understanding of phylogenetic studies. More accurate analyses are currently being pursued to clarify these microenvironments.

Supported by: CNPq, FAPERJ and CENPES

HOST-MICROBE INTERACTIONS

HMP01
Dual RNA-seq reveals intracellularly expressed Salmonella small RNAs and their impact on the human host response

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Bacterial pathogens encode a plethora of conserved small noncoding RNAs (sRNAs) but their regulatory roles in pathogenesis have been difficult to address owing to a general lack of macroscopic phenotypes in standard virulence assays. Using a novel Dual RNA-seq approach (1) for a single-step RNA expression profiling in both pathogen and host simultaneously, we have discovered molecular phenotypes of sRNAs during the intracellular replication of *Salmonella* and their impact on the host response**.** This approach has revealed candidate STnc440 as a new PhoP-activated sRNA that times the expression of both, invasion-associated effector proteins and the virulence genes required for intracellular survival. Concomitant analysis of the host response shows that this bacterial sRNA impacts the expression of a tenth of all human genes from many diverse pathways, suggesting that it prevents from an imbalanced human cytokine response and a dead-end pathway for both the pathogen and the host. STnc440 also impacts the host genome outside protein-coding regions, inducing infection-specific expression changes in a defined set of long noncoding RNAs (lncRNAs). Our study provides a paradigm for RNA-based analysis of intracellular bacteria without their physical purification from a host and a new discovery route for hidden functions of pathogen genes.

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HMP02
Infection and transfer of chlamydia in human primary macrophages

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Chlamydia are obligate intracellular bacteria, that can either replicate in inclusion compartments or persist inside host cells e.g. macrophages. We hypothesize that depending on the macrophage phenotype or on the cell entry pathway persistence or replication occurs. We suggest the inflammatory (type I) human monocyte-derived macrophages (hMDM) to be the better host for chlamydia. In addition to direct cell entry, usually leading to persistence, our data suggest that chlamydia use apoptotic blebs for their silent uptake by the hMDMs resulting in replication.
We started to characterize chlamydia infections in hMDMs and compared different strains -*Chlamydia pneumoniae* (*Cpn*), *Chlamydia trachomatis* (*Ctr*) and GFP-expressing *Chlamydia trachomatis* (eGFP-*Ctr*). We quantified the infection rate and the chlamydial load in either pro-inflammatory (type I) or anti-inflammatory (type II) hMDMs. With an chlamydia LPS-staining we observed microscopically that independent of the strain, the type II hMDMs are more infected than the type I hMDMs. In contrast, when we assessed chlamydia 16SRNA with qRT-PCR, normalized to housekeeping gene GAPDH, our data suggest that the chlamydia load is higher in type I hMDMs. Correspondingly, we observed more replicative inclusions in type I hMDMs using the chlamydia specific staining. Subsequently, we focused on the chlamydial transfer into hMDM. To investigate this in more detail we use the confocal LSM 7 *Live* system from Zeiss which allows us to perform high speed live cell imaging. Using this system preliminary timelapse data suggest that eGFP-*Chlamydia trachomatis* was able to transfer to hMDMs via apoptotic blebs.
Focusing on primary hMDMs will enable a better understanding of chlamydia infection processes.

HMP03
Comparative analysis of the adaptation of *Staphylococcus aureus* to internalization by different types of human non-professional phagocytic host cells

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Staphylococcus aureus can cause a wide range of diseases ranging from milder skin infections via toxin-mediated diseases to endocarditis, pneumonia, or septicaemia. Although it was formerly regarded to be an extracellular pathogen, it is now established that this Gram positive pathogen can be internalized by host cells and persist and replicate within these cells. In the present study, we compared the survival and physiological adaptation of *S. aureus* HG001 to human bronchial epithelial cell lines (S9), adenocarcinomic human alveolar epithelial cells (A549), and human embryonic kidney cells (HEK 293). During the initial phase of internalization, GFP expressing bacteria were separated from host cell debris by cell sorting and the proteome of *S. aureus* was analyzed by mass spectrometry 2.5 h and 6.5 h post infection. Identification and quantitation of proteins was achieved combining various database searches including spectral comparison and AUC based label-free quantitation. Also the intracellular replication of bacteria was observed by flow cytometry based cell counting and fluorescence microscopy. Additionally, the extra- and intracellular pool of host cell metabolites available at the starting point of infection was determined by ^{1}H-NMR, LC-MS and GC-MS.
Starting with about $2x10^{6}$ bacteria, 1,450 *S. aureus* proteins, comprising roughly 50% of the whole *S. aureus* HG001 proteome, were identified and quantified. The majority of the bacterial adaptation responses was similar for all three cell lines, *e.g.* levels of ribosomal proteins and metabolic enzymes decreased and amounts of terminal oxidases and stress responsive proteins or activity of the sigma factor SigB increased after internalization. However, some pathways like fermentation, threonine degradation, and central carbon metabolism showed different response patterns following internalization. These observations also coincided with altered intracellular growth behavior and cell specific metabolic profiles
With this comparative study we provide a comprehensive overview of the common and specific features of the adaptation of *S. aureus* HG001 to specific host cell lines as starting point for follow-up studies with different strain isolates and regulatory mutants or other host cell models.

HMP04
Occurrence of invasion factor InlB variants among human and animal *Listeria monocytogenes* isolates

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Introduction: *Listeria monocytogenes* is a facultative intracellular pathogen that causes a disease in humans and a broad range of domestic and wild animals. The critical step of infection is an active invasion into normally non-phagocytosing cells. The active invasion is dependent on bacterial invasion factors, InlA and InlB, which directly interact with conservative eukaryotic receptors E-cadherin and Met, respectively. The interspecies variability of E-cadherin and Met causes inability for InlA and InlB of laboratory strains to interact with their corresponding receptors that can be overcome by introduction of site-specific substitutions (Wollert et al., 2007). The aim of the study was to reveal naturally occurring variants of *Listeria monocytogenes* invasion factor InlB and to establish their role in interactions with receptors of different host species.
Materials and Methods: In total, 15 isolates from maternal-fetal cases, 11 isolates obtained from the liver and spleen of wild small rodents and12 isolates from wild marine animals were included in the study. Strains were typed with MLST. *inlB* genes was sequenced for all strains. Virulence was determined on a murine model. Differed alleles of *inlB* were cloned into *L. monocytogenes* strain with the deletion of the chromosomal copy of the *inlB* gene. Invasion efficiency was determined with agentamicin assay.
Results: 5, 4 and 2 STs were revealed among human, rodent and marine animal isolates, respectively. Diverse *inlB* alleles were observed in human and marine animal isolates of the same ST. An identical *inlB* allele was observed for rodent isolates that belonged to independent clones within the phylogenetic lineage. Virulence of isolates that belonged to the same MLST type but distinguished in *inlB* was different in the murine model. Invasion efficiency of recombinant strains was diverse.
Conclusion and Discussion: Invasion into the host cell is a critical step for *L. monocytogenes* infection in humans and other mammals, and invasion factors are extremely important for infection development. Divergence of internalin genes might be related to *L. monocytogenes* fitness to a certain host.

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HMP05
Salmonella uses CMA derived small peptides as a source of nutrition for its intracellular replication within the SCV

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Salmonella enterica serovar Typhimurium (*S*. Typhimurium) is an important human gastrointestinal pathogen with an invasive and facultative intracellular lifestyle. Like other intracellular pathogens, *Salmonella* reside within a membrane-bound compartment called the *Salmonella* Containing Vacuole (SCV) within infected cells. The SCV is commonly considered a nutritionally-deprived environment, but the fact that *Salmonella* replicates

within the SCV indicates the successful adaptation to this intracellular environment. Despite the remarkable increase in understanding of the cellular microbiology of *Salmonella* infections and the molecular functions of virulence factors required for intracellular life, the nutritional basis of life of *Salmonella* within the SCV is still not completely understood. Our study shows that *Salmonella* Typhimurium acquires small peptides by co-opting the host cell chaperone mediated autophagy (CMA)-dependent cytosolic protein turnover pathway. CMA is a selective host cell protein degradation pathway active in all cell types and is involved in the transport of cytosolic proteins into lysosomes for degradation. Here we show for both intracellular *Salmonella* and in purified SCVs that the SCV is associated with the key components and substrates of CMA.
The results of this study highlight a unique phenomenon in host-pathogen interaction wherein an intracellular pathogen monitors the fitness of their infected host cells and are able to couple their own intracellular proliferation rate to the health status of the host.

HMP06
Activation of the alternative sigma factor SigB of *Staphylococcus aureus* following internalization by epithelial cells

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Staphylococcus aureus is a versatile pathogen that can be a commensal but also cause a wide range of different infections. This broad disease spectrum is a reflection of the complex regulation of a large collection of virulence factors that together with metabolic fitness allow adaptation to different niches. The alternative sigma factor SigB is one of the global regulators mediating this adaptation. Our recently established proteomics workflow that combines high efficiency cell sorting with sensitive mass spectrometry allows us to monitor global proteome adaptations with roughly one million bacterial cells. Thus, we can now approach the adaptation of pathogens to the intracellular milieu. In the current study this proteomics workflow was used in conjunction with qRT-PCR and confocal fluorescence microscopy to comparatively analyze the adaptation of the *S. aureus* wild type strain HG001 and its isogenic *sigB* mutant immediately after internalization to the intracellular milieu of human S9 bronchial epithelial cells. The study revealed fast and transient activation of SigB following internalization by human host cells monitored by the exclusively SigB regulated alkaline shock protein 23 (*asp23*). SigB was also required for intracellular growth. Loss of SigB triggered proteome changes reflecting the different growth rates of wild type and *sigB* mutant, the resistance to methicillin, adaptation to oxidative stress and protein quality control mechanisms.
However, SigB is not the only mediator for intracellular adaptation. It is embedded in a regulatory network together with other regulators or regulatory loci, e.g. SarA and *agr*. Therefore, we analyzed the secretome of SigB, SarA and *agr* single, double and triple mutants to understand their ability to provoke host cell reaction and to better understand the interplay of the regulatory network of *S. aureus* to adapt to the intracellular environment.

HMP07
Investigation of the distribution of chemoreceptor genes in *Campylobacter jejuni* strains isolated from different sources

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Chemotaxis plays a central role in *Campylobacter jejuni* host colonization and infection. From different whole genome sequences it is known that *C. jejuni* possesses at least 11 different chemotaxis receptors. However, little is known about their specificity for chemosignals, their coexpression and their functional interplay. The objectives of this study were to investigate the distribution of chemoreceptors in different *C. jejuni* isolates and their association with each other. 291 *C. jejuni* isolates from cattle (46), chicken (70), turkey (25) and humans (150) were MLST-typed and the chemoreceptor genes in each isolate were investigated by PCR. A MLST based UPGMA-dendrogram was constructed and associated each *C. jejuni* subgroup with specific chemoreceptor genes. The genes of TLP 1, 2, 3, 6, 8, 9 and 10 are roughly ubiquitous. In contrast, TLP4 was specifically present in isolates of MLST CC42, CC45, CC48, CC49, and CC 206; TLP5 was specifically detected in isolates of CC21, CC42, CC45, CC48, and CC 206. One notable finding was the association of the non-ubiquitous chemoreceptor TLP11 with the dimeric form of TLP7 in MLST ST21/CC21, ST53/CC21, ST38/CC48 and all STs of CC61 isolates. The TLP7 receptor was shown before to play a crucial role in *C. jejuni* motility, epithelial cell invasion and formic acid sensing. This gave rise for further examination of the role of TLP11in not only chemotaxis but also in motility and invasion. Thus the strain specific expression of TLP4, TLP5, TLP7 and TLP11 contributes obviously to differences in host adaptation and virulence of particular isolates. To determine if TLP 11 plays a role in the pathogenecity of *C. jejuni*, a knockout mutant of the parental strain 84-25 was constructed and chemotaxis, adhesion, invasion and intracellular survival assays on caco-2 cells are currently performed on the wild type, the mutant and the complemented strain.

HMP08
Requirements for intracytosolic replication of *Francisella tularensis*

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Introduction: *Francisella tularensis* is a Gram-negative bacterium and the etiological agent of tularemia (rabbit fever). The Live Vaccine Strain (LVS), which is used as a model organism, is attenuated in humans but remains fully virulent in mice. While the virulence mechanisms are to a great extent unknown, the capability of replicating in the cytosol of macrophages is considered a key mechanism. However, mutants within the *Francisella* pathogenicity island (FPI) have been found to be defective for phagosomal escape, intracellular growth and for activation of the inflammasome (*1, 2*). Since FPI-mutants that are unable to escape from the phagosome also fail to reach the cytosol where replication occurs, it has been difficult to address whether the encoded gene products also play a role during the cytosolic stage of infection. Here, we utilize microinjection to contribute to the understanding of the function of single FPI proteins during cell infection with *F. tularensis*.
Materials and Methods: GFP-labeled *F. tularensis* were injected into murine like macrophages. The replication was followed by live cell microscopy and compared to intracellular growth after phagocytic uptake. *Listeria monocytogenes* served as replication control (3).
Results: L. monocytogenes replicated rapidly upon injection in J774A.1 macrophages. In contrast, some of the tested *F. tularensis* mutants that replicated upon phagocytic uptake showed delayed growth kinetics and, eventually grew to far lower numbers per cell. To our surprise, we also found that strains, unable to escape from the phagosomal compartment, would replicate to a certain extent upon injection, highlighting the importance of the corresponding gene products for the phagosomal escape during infection.
Conclusion: Our data indicate that efficient cytosolic growth appears to be dependent on both host and pathogen factors. Based on our findings, we conclude that the mode of uptake, the location in/and escape from the phagosomal compartment, as well as the intracellular milieu of the host cell cytoplasm controls the bacterial fate.

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HMP09

Identification of new bacterial scent compounds and introduction of the first database of microbial volatiles 'mVOC'

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Microorganisms, similar as other organisms, release distinct scents during their normal metabolism. These scents are microbial volatile organic compounds (mVOCs); they are volatile due to their low molecular weight, high vapor pressure and low water solubility. mVOC are usually fatty acids derivatives (hydrocarbons, alcohols, aldehydes and ketones), terpenoids, aromatic compounds, nitrogen and sulfur containing compounds. Interestingly, often new compounds with unknown structures are detected. *Staphyloccocus schleiferi* DSMZ 4807, which is a normal resident of the human skin, was investigated. In fact,the volatile profile of this bacterium has been analyzed by GC/MS and the results show an impressive VOC profiles with interesting new compounds. The NMR analysis shows that, these compounds are imino alcohols and imino ketone. Are these compounds synthesized by the bacteria with a new biosynthetic partway?
mVOCs play crucial roles in inter- and intra-organismic interactions and are also promising tools for early detection and diagnosis of diseases, we performed a literature search for microbial VOCs and compiled the results in "mVOC", a database of microbial volatiles for public use. It is available without registration at http://bioinformatics.charite.de/mvoc. "mVOC" is the first online database presently composed of 1200 volatile compounds from ca. 350 bacteria and 100 fungal species. It offers different search options (compound names, ID, molecular weight, structure and/or with microorganism names). It is also possible to find information about the biological functions of compounds and the volatile signature of microorganisms. "mVOC" also presents the volatiles which are new to science and therefore cannot be found in any other database, e.g. sodorifen, which is a complete new volatile emitted by *Serratia plymuthica* 4Rx13.

HMP10

Legionella pneumophila GDSL lipolytic enzymes influence bacterial virulence and immune response of the host

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Introduction: *Legionella pneumophila* possesses three GDSL enzymes, PlaA, PlaC, and PlaD, showing phospholipase A (PLA) and acyltransferase activities which may target host lipids and contribute to Legionnaires' disease pathogenesis. PlaC is a PLA releasing fatty acids and the major acyltransferase transferring long chain fatty acids to host sterols. PlaA and PlaD show PLA activity and transfer short chain fatty acids to sterols. PlaA and PlaC are secreted by *L. pneumophila* and activated by the zinc metalloprotease ProA. We here investigated the contribution of the GDSL enzymes to *L. pneumophila* virulence and impact on the host immune response.
Materials and Methods: Single, double and triple GDSL mutants were constructed in *L. pneumophila* and analysed for PLA and acyltransferase activities. Further, impact on host cell infection in amoebae and macrophages was studied in single strain and competition infections. Recombinant proteins were expressed and purified and antibodies were raised to subsequently determine the GDSL enzyme secretion pathway. To analyse the influence of the GDSL enzymes on the modification of the *Legionella* containing vacuole (LCV), LCVs were isolated after infection of *D. discoideum* with wild type or triple mutant strains and proteome analysis was performed. Additionally, impact of the enzymes on hydrolysis of host lipids and generation of eicosanoids was studied.
Results: Western blot analysis confirmed Lsp-dependent type II protein secretion and ProA-dependent processing of PlaA as well as PlaC and showed cell association of PlaD. Competition infection assays of mutants versus wild type revealed that PlaA and PlaC are important for intracellular replication of *L. pneumophila* in *Acanthamoeba castellanii.* LCV Proteomic analysis of LCVs harbouring wild type or GDSL-triple mutant bacteria showed lipid metabolism-related differences. One protein, whose mammalian homologue is involved in eicosanoid generation, was found in wild type but not in mutant LCVs. We determined that *L. pneumophila* wild type triggered arachidonic acid release and eicosanoid generation, which was attenuated in the GDSL triple mutant.
Conclusion: Our results indicate that *L. pneumophila* GDSL enzymes are tightly regulated to prevent hydrolysis of bacterial lipids and contribute to the pathogen-directed modification of host cells and induction of immune responses.

HMP11

Analysis of *in vitro* interactions between human dental folicle stem cells (hDFSC), polymorphonuclear leukocytes (PMN) and oral pathogenic microorganism

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Periodontitis is a chronic opportunistic infection which can result in the loss of teeth. Periodontal pathogens are for example the obligate anaerobic bacteria *F. nucleatum*, *P. gingivalis* and *A. actinomycetemcomitans*. In the periodontal pocket it is possible that local stem cells may interact with these pathogenic microorganisms. During an infection, polymorphonuclear neutrophils (PMNs) are the first cells to be recruited to the site of infection. In our study, it was elucidated whether oral pathogens influence human dental stem cells (hDFSCs) and immune cells in anaerobic conditions.
To monitor the cell count the cells were stained with Trypan Blue staining.The apoptosis of PMNs was quantified by FACS analysis with Annexin V and 7-amino-actinomycin D (7-AAD). The supernatants of the co-culture were analyzed via ELISA (e.g.Interleunkine). HDFSCs were less affected by the lack of oxygen and seemed to be more tolerant for the analyzed bacteria compared to differentiated cells e.g. human gingival fibroblasts. The co-cultivation of stem cells and pathogenic bacteria did not result in massive cytokine responses. The influence of infected hDFSCs on the survival of neutrophils depends on the oral microorganism responsible for the infection. The infection of hDFSCs with *P. gingivalis* prolongs the survival of PMNs in our co-culture system. The results indicate that hDFSCs show fewer interactions with the examined pathogenic bacteria and can prolong the survival of PMNs after an infection.

HMP12

Immune evasion and intracellular survival of phagocytosed Livestock-associated MRSA isolates of clonal complex 398

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of clonal complex 398 (CC398) are on the rise in Europe, and increasingly found in clinical settings. Aiming at the identification of bacterial mechanisms contributing to the epidemiological success of the MRSA CC398 lineage to invade and spread among humans in the community and hospital settings, we investigated two infection relevant processes, the evasion of phagocytosis and the intracellular persistence after phagocytosis. In a first approach, the influence of the staphylococcal immune evasion cluster (IEC) on the ability of *S. aureus* to evade phagocytosis by host immune cells was assayed with a set of IEC harboring vs. lacking CC398 isolates. In a whole blood assay, a marked decrease in phagocytosis by human granulocytes was observed for isolates harboring the IEC, suggesting that this gene cluster affects the ability of CC398 isolates to evade phagocytosis. Comparison of phagocytosis evasion and of intracellular survival after phagocytosis between the most prevalent CC398-associated *spa* types found in Germany (t011, t034, t108) yielded significantly decreased phagocytosis rates in whole blood assays for *spa* type t108. However, when analyzed for intracellular survival, *spa* type t108

isolates were less efficiently re-isolated from human macrophages 24 hours post ingestion than *spa* type t011 and t034 isolates, suggesting that *spa* type t108 isolates albeit less efficiently phagocytosed by leucocytes are more effectively killed by professional phagocytes after ingestion.
Our results underline the heterogeneity within the pool of MRSA CC398 isolates, and demonstrate substantial differences in the immune evasion properties of subpopulations of this clade.

HMP13

Role of a major immunodominant protein GlpQ in *Staphylococcus aureus*. Why does *S. aureus* secrete a glycerophosphodiesterase?

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Staphylococcus aureus is a commensal bacterium and also a major human pathogen. The emergence of Methicillin-Resistant *S. aureus* (MRSA) strains limits the use of existing antibiotics to cure infections and new anti-microbial approaches are needed. In a recent study a group of 11 conserved proteins, that provoked a high immune response in bacteremia patients were identified and thus could constitute new antimicrobial targets. Interestingly, one of the most immunodominant proteins, GlpQ, is a secreted glycerophosphodiesterase predicted protein but its putative role in infection has been underestimated. Importantly, in contrast with many virulent factors, *glpQ* is not redundant as it is widely present in most *S. aureus* genomes. Why does *S. aureus* secrete a glycerophosphodiesterase? Using *in vitro* approaches we were able to identify glycerophosphocholine (GPC) as a specific substrate for GlpQ. GPC derives from phosphatidylcholine, the major phospholipid present in the host cell membranes, after the acyl chains have been cleaved. Furthermore, we show that GPC can be used as a carbon source for *S. aureus* growth in a chemically defined media and GlpQ is important to metabolize this compound. Our findings suggest that GlpQ is able to degrade deacylated phospholipids, glycerophosphodiesters, present in the host cell membrane, for nutrient mobilization and survival inside the host. Conversely, by using deacylated phospholipids, GlpQ might also contribute to pathogenesis. These results support the hypothesis that GlpQ might have an impact in virulence. Therefore, it may represent a promising target for active or passive vaccines.

HMP14

Nucleotide-oligomerization-domain-2 affects commensal gut microbiota composition and intracerebral immunopathology in acute *Toxoplasma gondii* induced murine ileitis

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Introduction: Within one week following peroral high dose infection with *Toxoplasma (T.) gondii*, susceptible mice develop non-selflimiting acute ileitis due to an underlying Th1-type immunopathology. The role of the innate immune receptor nucleotide-oligomerization-domain-2 (NOD2) in mediating potential extra-intestinal inflammatory sequelae including the brain, however, has not been investigated so far.
Materials and Methods: Following peroral infection with 100 cysts of *T. gondii* strain ME49, NOD2$^{-/-}$ mice displayed more severe ileitis and higher small intestinal parasitic loads as compared to wildtype (WT) mice. However, systemic (i.e. splenic) levels of pro-inflammatory cytokines such as TNF-a and IFN-g were lower in NOD2$^{-/-}$ mice versus WT controls at day 7 p.i. Given that the immunopathological outcome might be influenced by the intestinal microbiota composition, which is shaped by NOD2, we performed a quantitative survey of main intestinal bacterial groups by 16SrRNA analysis. Interestingly, Bifidobacteria were virtually absent in NOD2$^{-/-}$ but not WT mice, whereas differences in remaining bacterial species were rather subtle. Interestingly, more distinct intestinal inflammation was accompanied by higher bacterial translocation rates to extra-intestinal tissue sites such as liver, spleen, and kidneys in *T. gondii* infected NOD2$^{-/-}$ mice. Strikingly, intracerebral inflammatory foci could be observed as early as seven days following *T. gondii* infection irrespective of the genotype of animals, whereas NOD2$^{-/-}$ mice exhibited higher intracerebral parasitic loads, higher F4/80 positive macrophage and microglia numbers as well as higher IFN-g mRNA expression levels as compared to WT control animals.
Conclusion and Significance: NOD2 signaling is involved in protection of mice from *T. gondii* induced acute ileitis. The parasite-induced Th1-type immunopathology at intestinal as well as extra-intestinal sites including the brain is modulated in a NOD2-dependent manner.

HMP15

The inhibition of *Helicobacter pylori* adherence to AGS cell line using specific egg yolk immunoglobulin against *Helicobacter pylori* recombinant HP-NAP proteins

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Introduction: Colonization of the gastric mucosa by *Helicobacter pylori* in some people caused chronic gastritis and may develop to gastric cancer. Inhibition of *H. pylori* colonization can decrease the risk of subsequent diseases. Passive immunization by oral administration of antibacterial immunoglobulin may decrease the *H. pylori* colonization. Egg yolk immunoglobulin Y (IgY) is an inexpensive, high yield and non-invasive source of immunoglobulin that has been assessed for preventive-therapeutic means. *H. pylori* neutrophil activating protein (HP-NAP) is one of the most important virulence factor produced by *H. pylori* that can act as an adhesion. In this research, the *H. pylori* adherence to AGS cell line was inhibited by IgY- HP-NAP produced in our lab.
Materials and Methods: Recombinant HP-NAP (rHP-NAP) was expressed and purified. The hens were immunized by rHP-Nap. IgY was Purified, using different concentrations of PEG 6000. The AGS cell line was cultured in 96-well plates and then co-cultured with *H. pylori* which were treated by different concentration of IgY-HP-Nap. Inhibitory effect of IgY-HP-Nap against *H. pylori* in the co-culture was analyzed by ELISA.
Results: The Purified IgY-HP-NAP was verified by ELISA assay, assessed using SDS-PAGE and confirmed by western blot. IgY- HP-NAP significantly inhibited the adherence of *H. pylori* to AGS cells.
Conclusion: IgY against rHP-NAP of *H. pylori* could decrease the adherence of *H. pylori* to AGS cell line and hence can be considered for passive immunization against infections caused by this pathogen.

HMP16

The extracellular adherence protein Eap contributes to binding of *Staphylococcus aureus* to ultra large von Willebrand factor (UVWF) fibers under high-shear-flow conditions

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Staphylococcus aureus is the most frequent cause of infective endocarditis in industrialized countries. In contrast to other pathogenic bacteria causing this type of disease, *S. aureus* is able to bind to intact endothelium. We have recently shown that binding of *S. aureus* to the intact endothelial cell layer is mediated predominantly by luminally secreted ultra large von Willebrand factor (ULVWF) fibers under high-shear-flow conditions, and that bacterial binding to ULVWF is multifactorial, involving wall teichoic acids and cell wall-anchored proteins. Here we show that the secreted and in part cell wall reattached extracellular adherence protein Eap contributes to binding of *S. aureus* to ULVWF at a shear stress of 10 dynes/cm^2, reflecting physiological flow rates found in larger arteries. Inactivation of *eap* in *S. aureus* strain SA113 nearly halved the binding of the mutant to ULVWF under theses flow conditions when compared to the binding rates of the wild type, and preincubation of the mutant with Eap fully reconstituted the binding rates to wild type level. Exponential growth phase cells were found to possess a significantly higher adhesion capacity to ULVWF than stationary phase cells, probably due to an enhanced expression of cell wall-anchored adhesion factors during the early growth stage. However, the lack of Eap decreased binding of *S. aureus* exponential growth phase- and stationary

phase cells to ULVWF to a similar extent, suggesting this Eap effect to be growth stage-independent. Presence of extracellular DNA, which was recently shown to bind to ULVWF under high-shear-flow, enhanced the attachment of *S. aureus* to ULVWF, however, this effect appeared to be independent of Eap.
Our data suggest that Eap is likely to contribute to *S. aureus* ability to cause infective endocarditis by promoting the pathogens capacity to adhere to ULVWF under high-shear-flow conditions.

HMP17
Molecular analysis of centrosome inactivation in vaccinia infection: identification of viral and host pericentriolar material regulators

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Vaccinia is a large DNA poxvirus that replicates in the cytoplasm of the host cell. Vaccinia infection results in remodeling of both the actin and the microtubule cytoskeleton, including the centrosome, of the host cell, to enhance virus spread within and among cells. The centrosome is the microtubule-organizing center of animal cells. It is composed of a pair of centrioles surrounded by pericentriolar material (PCM). The PCM is responsible for the nucleation and organization of the microtubules. As part of the cytoskeleton remodeling, and in common with other intracellular pathogens, vaccinia infection induces centrosome inactivation; i.e. reduction of pericentriolar material limiting the centrosome's microtubule nucleating and microtubule organizing capacity. Centrosome inactivation also takes place (independently of infection) during the cell cycle in the mitosis to G1 transition and upon differentiation. The molecular mechanism underlying centrosome inactivation is unknown. The objective of this study was to identify the viral and host proteins mediating the vaccinia induced centrosome inactivation and their relevance for PCM reduction occuring during cell cycle progression or in differentiation. This was achieved by employing biochemical, proteomics, mutant virus and RNAi approaches.
We will report (a) on the vaccinia proteins that induce centrosome inactivation both upon infection but also when expressed in the non-viral background and (2) on the host proteins, which mediate centrosome inactivation upon vaccinia infection. The latter are also involved in cytoskeleton-mediated signaling in leukemias and lymphomas. Thus, vaccinia can be employed as a tool for probing the mechanisms regulating centrosome integrity but also centrosome dysfunction.

HMP18
EPEC secreted protein B (EspB) induces cytotoxicity in immune but not in epithelial cells

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Enteropathogenic *Escherichia coli* (EPEC) are a leading cause of diarrhoea, especially in young children and in underdeveloped areas. During infection EPEC subvert signaling pathways of targeted host cells by transducing effector proteins into the host cell cytosol using a Type Three Secretion System (T3SS) encoded on the 'locus-of-enterocyte-effacement' pathogenicity island. One of the main T3SS effector molecules is EPEC secreted protein B (EspB). EspB appears to be a multifunctional protein as it forms the T3SS translocator pore in the host cell membrane, is injected into the cytoplasm of host cells, and is also secreted into the supernatant of bacterial cells. Due to its membrane spanning abilities, we speculated that secreted EspB forms complexes in host cell membranes. To answer this question EspB was cloned, expressed, and purified. To investigate a putative cytotoxic effect, the human monocytic cell line THP-1, the colonal cell line T84, and the cervix adenocarcinoma cell line HeLa were incubated with recombinant EspB (rEspB). Release of lactate dehydrogenase (LDH) and uptake of propidium iodide (PI) was measured. THP-1 cells showed an increased efflux of LDH and influx of PI, whereas T84 and HeLa did not show an effect upon rEspB treatment. Preliminary experiments indicated that mitochondria of THP-1 cells were affected by rEspB treatment resulting in the induction of apoptosis. Cell fractionation data showed that rEspB was located in the membrane and the cytosol of monocytes and epithelial cells. We conclude that EspB is not only part of the T3SS arsenal but also as a recombinant protein forms pores in host cell membranes independently of bacterial cells. As the protein is also found in the cytosol rEspB seems to exhibit cell-penetrating properties. It remains to be elucidated why incorporation of EspB leads to cell death in monocytes while epithelial cells appear to be resistant against these cytotoxic effects.

HMP19
Characterization of a multi-functional virulence factor of *Corynebacterium diphtheriae*

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Corynebacterium diphtheriae is a microorganism of global significance for being the etiologic agent of diphtheria and systemic infections. The diphtheria bacillus has been classically described as an extracellular pathogen. However, it was demonstrated that this microorganism is internalized by epithelial cells and presents number of virulence factors that may act independently of diphtheria toxin. Comparative proteomic analysis *in silico* and *in vitro* suggested the importance of the protein DIP0733 and indicated its ability to interact with matrix proteins and cell surfaces, participating in cellular internalization and induction of apoptosis. In this study, we investigate the role of DIP0733 as virulence factor and how it contributes to the process of host cell interaction. A corresponding mutant strain showed an altered adhesion pattern and a drastically reduced ability to adhere and invade Hep-2 and HeLa cells. Interestingly, in *Caenorhabditis elegans* model system, the DIP0733 mutant strain was attenuated in its ability to colonize and kill the host. Based on its binding activity in respect to plasma and extracellular matrix proteins such as fibrinogen, fibronectin and collagen, DIP0733 may play a role to avoid the recognition of *C. diphtheriae* by the immune system. Further investigations are being analysed about this effects to support the idea that DIP0733 is a major effector of *C. diphtheriae* host interaction at molecular level.

HMP20
The Role of Peroxisome Proliferator-Activated Receptors Alpha(Pparalpha) as a Genetic Risk Factor in Visceral Leishmaniasis

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Introduction: The peroxisome proliferator activated receptors (PPARs) comprise a subfamily of nuclear
receptors. These molecules, which act as transcription factors, exhibit different tissue distributions and functions. Until now, it is known that genes regulated by PPARs mainly participate in the regulation of key proteins involved in the metabolism of intracellular and extracellular lipids, fatty acid oxidation and inflammatory processes. The PPARalpha is expressed in many metabolically active tissues, including liver, kidney, heart, skeletal muscle, adipose tissue and macrophages. For the role on regulating lipid levels, PPARalpha is considered a very important target for effective treatment of dyslipidemia by using synthetic drugs that function as agonist activators. Lipid disorders have been reported in human patients and even in domestic dogs with active visceral leishmaniasis (VL) suggesting the role of lipid profile in the onset of infection.
Materials an Methods: This research consists in a population-based case-control study(n=248), seeking to relate the presence of specific mutations (L162V. V227A, intron2 T / C and intron 7 G / C) in the gene encoding PPARalpha with Leishmania infantum infection in patients from the VL endemic area
of Teresina - PI, Brazil. Thepolymorphisms were PCR genotyped by allelic discrimination using TaqMan® probes on a custom Assay-on-Demand basis by Applied Biosystems.
Results: Afler SNP analysis it was observed that the L162V imitation is strongly associated with L.infantum infection. Genotypes containing the mutant allele 162V are significantly more frequent between individuals with LV than among all uninfected individuals ($p = 0.007$) than among neighbors uninfected individuals ($p = 0.048$) than among randomly selected iniinfected

individuals (p = 0.023) than all healthy individuals regardless of their status of infection (p = 0.014) than healthy subjects and neighbors (p = 0.015).
Conclusion and Discussion: It is noteworthy that healthy individuals who have at least one mutant allele 162V, are almost four times more likely to get infected than individuals who do not have the mutation (odds ratio 3.91 ; 95% CI 1.38-11.07).Taken together, this data clearly show the importance of PPARalpha genotypes for VL infection status.

HMP21
Flavonoid-inducible expression of a rhizobial efflux system and of its associated transcriptional regulator

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Efflux pumps enable bacteria to remove toxic chemical compounds from their cell. Rhizobial genome sequences reveal the presence of a number of efflux systems belonging to different families. In the alfalfa symbiont *Sinorhizobium meliloti* strain 1021, 14 efflux systems have been identified (1). In transcriptome analyses, the genes *SMc03167* and *SMc03168* – the deduced proteins are similar to the multi drug resistance proteins EmrB and EmrA of *E. coli*, respectively – were reported to be inducible by luteolin, a plant signal known to induce nodulation genes (2). Using a transcriptional *emrA-gusA* fusion, we demonstrated that the gene is inducible by several flavonoids, strongest by apigenin but also by quercetin, which is not an inducer of nodulation genes. This suggests that the gene is not regulated directly by NodD, which is the activator of nodulation genes. Upstream of *emrA*, a TetR-type regulator (EmrR) is encoded. EmrR is binding to palindrome-like sequences within the *emrA-emrR* intergenic region (3). By creating translational *emrR-lacZ* fusions, we determined the likely translational start site of *emrR*. This revealed that also *emrR* is inducible by apigenin. After integration of the *emrR-lacZ* fusion into an *emrR* mutant background, the fusion was no longer inducible by apigenin, however, the expression level in the non-induced strain was significantly higher than in the wild-type background. This suggests that EmrR acts as a repressor, which regulates the transcription of *emrAB* and of its own gene. Interestingly, a mutation of *emrR* but not of *emrA*, impaired symbiosis with alfalfa (3, 4 and unpublished results). This might indicate that a proper regulation of *emrAB* is essential for the interaction of *S. meliloti* with alfalfa. Further experiments are directed towards a more detailed analysis of the expression and regulation of the efflux system and of *emrR*.

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HMP22
The type VI secretion system 5 mediates intercellular spread of *Burkholderia thailandensis* via host cell fusions in cell lines derived from nine different human organs

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Burkholderia pseudomallei is the causative agent of the potentially fatal disease melioidosis. The disease can present with a diverse range of clinical manifestations due to the ability of the pathogen to infect virtually every organ in the human body. *B. pseudomallei* is a facultative intracellular bacterium and investigating its intracellular life cycle is crucial to understanding the pathogenesis of melioidosis. Previous studies showed that the type VI secretion system 5 (T6SS-5) of *B. pseudomallei* and of its low virulence model organism *Burkholderia thailandensis* enables the bacteria to directly spread from one host cell to another by inducing cell-cell fusions. The target of the T6SS-5 and the mechanism underlying host cell fusions, however, remain unknown. In the present study we aimed to get an insight into the prevalence and cell type specificity of T6SS-5-dependent spread of *B. thailandensis*. To this end, we analyzed the ability of *B. thailandensis* wild type and ΔT6SS-5 mutant to induce host cell fusions in nine different human normal and cancer cell lines representing the digestive (pancreas (PANC-1), duodenum (HuTu-80)), endocrine (adrenal gland (SW-13)), genitouritrary (bladder (5637), prostate (DU-145), ovary (SK-OV-3)) and respiratory (lung (Hel-299)) organ system as well as the sensory (eye (HCEC-12)) and central nervous system (brain (H4)). We found that *B. thailandensis* wild type but not the ΔT6SS-5 mutant induced cell-cell fusions in all cell lines studied. Antibiotic protection assays revealed that both wild type and ΔT6SS-5 mutant were able to invade and replicate in all cell lines included in the study. Altogether, our data suggest that the T6SS-5 facilitates intercellular spread of *B. pseudomallei* in the tissues addressed in this study and potentially in other organs involved in melioidosis. Furthermore, the finding that different types of host cells were fused by the T6SS-5 indicates that it targets a common host cell component or that it can interact with multiple different targets to stimulate the fusion of adjacent host cells.

HMP23
Streptococcus pneumoniae infection of bronchial epithelial cells induces specific changes in microRNA profile

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Streptococcus pneumoniae is a Gram-positive lactobacillales that usually colonizes the human nasopharynx but is also an important pathogen causing pneumonia and other fatal infections. Within the past couple of years it was found that microRNAs (miRNAs), a class of short, non-coding RNAs, play a crucial role as important regulators of gene expression at post-transcriptional level in infectious diseases. They have a key role in the regulation of the inflammatory host response against pathogens. To date, there are no data addressing miRNA expression after pneumococci infection. This study gives a deeper insight in the host-pathogen-interaction and may disclose putative therapeutic targets. For this purpose, we performed a global miRNA expression analysis of human bronchial epithelial cells (Beas-2B) infected with two different multiplicities of infection (MOIs) of *S. pneumoniae* (strain D39) compared to mock-infected and LTA-stimulated cells by Taqman Low Density Arrays. In addition, we tested for cytotoxicity and secretion of interleukin-8 as a marker for inflammation. Out of 759 examined miRNAs we found 495 to be expressed. With regard to the MOIs we identified 22 and 17 significantly deregulated miRNAs after *S. pneumoniae* infection in contrast to mock infection. To identify miRNAs specific for *S. pneumoniae infection* we compared the results with LTA-stimulated cells. There, 12 significantly deregulated miRNAs were determined. Out of the aforementioned 22 and 17 deregulated miRNAs after *S. pneumoniae* infection only one and two miRNAs overlapped in LTA-stimulated cells, respectively. This indicates that the miRNA profile after infection varies between cells exposed to diverse MOIs. Furthermore, the results suggest that the miRNA profile is specifically altered in *S. pneumoniae* infection compared to LTA stimulation. These miRNAs might play an important role in host response to *Streptococcus pneumoniae* infection in human bronchial epithelial cells. Further analysis and greater knowledge of the effect of this deregulated miRNAs within the infection could reveal potential therapeutic targets.
Supported by BMBF-PROGRESS, BMBF e:Med Capsys, and the German Center for Lung Research (DZL).

HMP24
Identification of novel transcripts by dRNA-seq analysis of *Bradyrhizobium japonicum* in liquid culture and in soybean root nodules

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The α-proteobacterium *B. japonicum* is the nitrogen fixing intracellular symbiont in root nodules of soy beans (*glycine max).* Using modern RNA deep sequencing techniques we wanted to take a closer look on its whole primary transcriptome regarding the expression of non-annotated small RNAs under free living and symbiotic conditions. We took advantage of the dRNA-seq method (1) to achieve comprehensive information about non-annotated transcripts which are differentially expressed in in free-living *B. japonicum* cells in liquid culture and during symbiosis (2).Since this approach can distinguish between processed and primary 5'-ends of RNA, it allows the identification of virtually all transcriptional start sites used under the conditions of interest. Total RNA from *B. japonicum* USDA 110 from

liquid cultures and from soybean nodules was subjected to dRNA-seq analysis. Some of the found transcripts expressed in free living cells were verified using Northern blots. Their conservation among different members of the *Rhizobiaceae* was investigated using nBLAST and their putative conserved secondary structure was predicted using LocARNA. The analysis of non-annotated transcripts revealed a plethora of putative regulatory RNAs expressed in free-living cells and/or in symbionts. Our data shows that some of the putative regulatory RNAs are highly conserved within the whole family of *Rhizobiaceae*, while others are restricted to a few strains or are even exclusive for *B. japonicum*.

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HMP25

Host interaction and adaption of *Escherichia coli* in the urinary tract

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Introduction: The main cause of urinary tract infection (UTI) are uropathogenic *E. coli* (UPEC). Besides provoking symptoms, *E. coli* can as well colonize the human bladder asymptomatically. During asymptomatic bacteriuria (ABU), patients carry high bacterial numbers in their urinary bladder without inducing host immune responses or the development of symptoms. For this purpose, commensal and pathogenic bacteria employ sophisticated strategies to interact with their host.
Pathogenic and nonpathogenic *E. coli* strains can be discriminated based on the presence and absence of additional DNA elements contributing to specific virulence traits. Unlike UPEC, which cause symptomatic infections, the clinical isolate *E. coli* ABU 83972 lacks virulence-associated factors and is capable to colonize the bladder for long periods of time in the absence of symptoms. Furthermore, this strain actively suppresses the RNA polymerase II-dependent (Pol II-dependent) host gene expression in inoculated patients and different human cell lines after *in vitro* infection. In contrast, the clinical ABU 83972 re-isolate SN25 is no longer able to actively suppress the Pol II-dependent gene expression.
Materials and Methods: To identify genes that contribute to the abolishment of the active Pol II-dependent gene expression, we investigated the clinical re-isolate SN25 regarding single nucleotide polymorphisms (SNPs). The complete genome of *E. coli* SN25 was sequenced using Illumina sequencing technology. Raw sequence reads of the draft genome were quality trimmed and mapped to the annotated reference genome of ABU strain 83972. To determine a SNP in either a coding or noncoding region, a coverage of 80 sequences and a frequency of 85% divergent nucleotides at a given position was applied.
Results: A set of 6.460.174 sequence reads were quality trimmed. Subsequently, 5.629.200 unique reads could be mapped to the reference genome. With the set of chosen parameter, 43 SNPs were detected. Four of these variants are located within a noncoding, the remaining 39 within a coding region, respectively. In this context, 31 of the 39 variants lead to synonymous amino acids, whereas the remaining 8 nonsynonymous SNPs change the amino acid sequence of the corresponding protein. Additionally, the transcriptome of the clinical re-isolate SN25 and the parental strain ABU 83972 was compared by RNA sequencing.
Discussion: The RNA-seq data provide further insights into differential gene expression in the *E. coli* strain ABU 83972 relative to its re-isolate SN25. In combination with the draft genome sequence, candidate genes can be determined and selected for further in-depth analysis regarding their allocation to regulatory networks. The corresponding gene products can be grouped according to functional categories. Their contribution to bacterial suppression of the Pol II-dependent gene expression in host cells will be investigated.

HMP26

Interaction of *E. coli* O104:H4 German outbreak strain with intestinal epithelial cells from different hosts

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Introduction: In 2011, Germany was struck by the largest outbreak of hemolytic uremic syndrome. The highly virulent *E. coli* outbreak strain LB226692 possesses a blended virulence profile combining genetic patterns of human adapted enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC), a subpopulation of Shiga toxin (Stx)-producing *E. coli* (STEC). STEC are basically adapted to the bovine host but are capable of causing severe diseases in humans, typically following consumption of contaminated food. Intestinal colonization in and bacterial adaption to different hosts depends on specific molecular interactions which are insufficiently unravelled regarding LB226692. Aim of this study was to quantify strain-specific adherence properties of different *E. coli* strains to intestinal epithelial cells of human (CaCo-2, Int407) and bovine (FKD-R 971) origin.
Materials and Methods: Cell lines were incubated for up to 6 hours with equal numbers of the outbreak strain, *E. coli* strains representing different pathovars (classical EHEC, EAEC, bovine STEC isolates) or non-pathogenic *E. coli* strains (commensal). Adhesion assays were conducted to analyze pattern and rate of adhesion (Giemsa) and fluorescent actin staining (FAS) to visualize bacteria-associated actin accumulation. Level of invasion was determined by gentamicin protection assays (cfu count). Amounts of Stx released upon host cell contact were quantified by ELISA.
Results: In general terms adherence properties to epithelial cells varied qualitatively (type, pattern) and quantitatively (extend) between strains and only low numbers of *E. coli* were found intracellularly. Bovine FKD-R 971 and human Int407 cells were strongly colonized by all *E. coli* strains under investigation, while only low bacterial numbers were detected on CaCo-2 cells. Quantitatively, the outbreak strain associated intermediately compared to classical EHEC strains (EDL933, 86-24WT). LB226692 adhered to any of the host-specific cell lines in characteristic stacked-brick adhesion pattern without inducing actin accumulation (negative for FAS-labelling). For classical EHEC strains, the relative amount of Stx correlated well with the level of adhesion. Compared to classical EHEC, strain LB226692 released lower amounts of Stx to the supernatant. However LB226692 released notably higher levels of Stx when attached to bovine FKD-R 971 cells as compared to all human cell lines tested.
Conclusion: Extend of adhesion to and invasion of intestinal epithelial cells varied between strains. The results point to a milieu-dependent control of virulence gene expression by LB226692 which is independent of bacterial attachment to epithelial cells.

HMP27

Icm/Dot-dependent inhibition of phagocyte migration by *Legionella* is antagonized by a translocated Ran GTPase activator

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The opportunistic pathogen *Legionella pneumophila* causes a severe pneumonia termed Legionnaires´ disease. *L. pneumophila* uses a conserved mechanism to replicate within a specific "*Legionella*-containing vacuole" (LCV) in macrophages and protozoa such as the social soil amoeba *Dictyostelium discoideum*. The bacterial Icm/Dot type IV secretion system (T4SS) governs the process of LCV formation and translocates over 300 different effector proteins into host cells. We analyzed the effects of *L. pneumophila* on migration and chemotaxis of amoebae, macrophages and polymorphonuclear neutrophils (PMNs) using under-agarose and scratch assays, as well as single cell tracking. *L. pneumophila* inhibited in a dose- and T4SS-dependent manner the directed cell migration of infected phagocytes. We observed that *L. pneumophila* impairs migration and chemotaxis of murine RAW 264.7 macrophages towards the cytokines CCL5 or tumor necrosis factor α (TNFα), or primary human PMN migrating towards formyl-methionyl-leucyl-phenylalanine peptide (fMLP), respectively. Furthermore, a *L. pneumophila* mutant strain lacking the T4SS-translocated activator of the small eukaryotic GTPase Ran, LegG1, hyper-inhibited the migration of *D. discoideum*, macrophages and neutrophils. Under these conditions microtubule polymerization of infected cells was significantly diminished. The hyper-inhibition phenotype was reverted upon overexpression of *legG1*, and phagocytes infected with *L. pneumophila* Δ*legG1* expressing *legG1* from a plasmid migrated similarly to cells infected

with *L. pneumophila* Δ*icmT* lacking a functional T4SS. Moreover, using scratch assays and RNA interference, we observed that LegG1 promotes random cell migration of macrophages and epithelial cells in a Ran-dependent manner. Single cell tracking analysis of *L. pneumophila*-infected phagocytes revealed that the velocity and directionality were decreased and microtubule polymerization was impaired. Scratch assays with infected macrophages or HeLa cells subjected to "microbial microinjection" confirmed that LegG1 promotes cell migration. Taken together, our findings indicate that *L. pneumophila* inhibits phagocyte migration in an Icm/Dot-dependent manner and the bacterial Ran activator LegG1 antagonizes migration inhibition by promoting microtubule stabilization.

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HMP28

Did you know that laboratory mice are frequently colonized with *Staphylococcus aureus*?

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Staphylococcus aureus (S. aureus) is a facultative pathogen infecting humans as well as a range of livestock animals, such as cattle, sheep and poultry. Mice are the most commonly used infection model for studying *S. aureus* pathogenesis. However, due to a lack of epidemiological data, many *S. aureus* researchers assume that mice are not natural hosts of *S. aureus*. We have recently observed a severe outbreak of *S. aureus* abscesses among male mice (C57BL/6J) shortly after delivery from a commercial vendor. This prompted us to investigate the *S. aureus* colonization rate among mice from commercial vendors and at our local breeding facility. We also elucidated routes of transmission and analyzed whether *S. aureus* colonization induces an antibody response.
All publically available health reports of five commercial mice vendors (n=432) were automatically browsed for positive *S. aureus* tests using the software tools *gedit*, *pdftotext*, *wget, sed* and *gawk* . *S. aureus* colonization was determined in murine stool samples and human nose swabs by plating on mannitol salt and sheep blood agar plates, respectively. *S. aureus* was identified by PCR and clonal relationship was determined by *spa* typing. Antibody responses were analyzed by ELISA and FlexMAP®.
Immune-competent mice in specific pathogen free barriers were commonly colonized with *S. aureus* (Charles River US: 26.1 %; Taconic 10.25 %), whereas specific opportunistic pathogen free (SOPF) mice were *S. aureus*-free. A *S. aureus* screening at our local animal breeding facility revealed that C57BL6/ N mice were colonized with CC1 (t127, t6811), C57BL6/N albino with CC15 (t084, t491) and C57BL6/ J mice with CC88 (t2311, t6728) strains. In contrast, stockmen were colonized with unrelated human *S. aureus* isolates, suggesting that they are not a frequent cause of *S. aureus* transmission. Indeed, we observed a close to 100% transmission of *S. aureus* from colonized breeders to their off-spring, whereas the off-spring of *S. aureus*-free breeders remained negative. Colonization per se induced a serum IgG response in C57BL/6 mice while *S. aureus*-free SOPF-mice remained sero-negative. In conclusion, *S. aureus* colonization of laboratory mice is very common. Conceivably, persistence of *S. aureus* in barriers is maintained by transmission from parental mice to their off-spring. Colonization primes the adaptive immune system and might thus interfere with subsequent infection and vaccination studies.

HMP29

Neisseria meningitidis exploits ERM protein function for pathogen entry

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N. meningitidis interaction with host cells follows a multistep cell adhesion pathway. After initial binding to host cells, meningococci proliferate, form small aggregates, also named microcolonies, at the site of attachment on the cells surface and induce the organization of 'cortical plaque' structures with accumulation of a number of cytoskeletal proteins beneath the bacterial microcolonies.
In this study we have found that ezrin, a member of the ezrin, radixin and moesin (ERM) family that functions as a membrane -cytoskeleton linker, is recruited to the site of bacterial attachment in infected brain endothelial cells (HBMEC). We determined that ezrin is phosphorylated at Thr567 in response to contact with *N. meningitidis,* which involves protein kinase C activation. Moreover, inhibition of ezrin by siRNA ablation significantly reduced meningococcal uptake by HBMEC up to 80%. In addition to ezrin the membrane component CD44, which interacts with ezrin, and actin filaments accumulate underneath microcolonies. By immunoprecipitation we showed a significant increased binding between CD44 and ezrin in *N. meningitidis*-infected cells compared to uninfected cells. Whereas CD44 localizes in rafts, ezrin is found in nonraft compartments. Here, we demonstrated that increased CD44-ezrin coprecipitation and colocalization mechanistically relied on disruption of cholesterol-enriched membrane domains called lipid rafts during meningococcal infection.

HMP30

Differential activation of the acid sphingomyelinase-ceramide system determines invasiveness of *Neisseria meningitidis* into brain endothelial cells

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The interaction with brain endothelial cells is central to the pathogenicity of *Neisseria meningitidis* infections. Recent studies demonstrated that distinct membrane microdomains, named lipid rafts, and ceramide play an important role in infectious biology. Ceramide forms larger ceramide-enriched membrane platforms that are required for segregation of receptors and diverse signal transduction. In this study, we show that *N. meningitidis* causes transient activation of acid sphingomyelinase (ASM) followed by ceramide release in brain endothelial cells. In response to *N. meningitidis* infection, ASM and ceramide are displayed at the outer leaflet of the cell membrane and condense into large membrane platforms wherein ErbB2, an important receptor involved in bacterial uptake, clusters. Mechanistically, *N. meningitidis*-mediated ASM activation relied on binding of the outer membrane protein Opc to heparan sulfate proteoglycans followed by activation of phosphatidylcholine-specific phospholipase C. Pharmacologic or genetic ablation of ASM abrogated meningococcal internalization without affecting bacterial adherence. In accordance, the restricted invasiveness of a defined set of pathogenic isolates of the ST-11/ST-8 clonal complex into brain endothelial cells directly correlated with their restricted ability to induce ASM and ceramide release. In conclusion, ASM activation and ceramide release are essential for internalization of Opc-expressing meningococci into brain endothelial cells, and this segregates with invasiveness of *N. meningitidis* strains.

HMP31

Secretion and processing of *Legionella pneumophila* phospholipases C

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Legionella pneumophila is the causative agent of Legionnaires' disease and replicates in diverse host cells. Bacterial phospholipases are important virulence factors which may modify the intracellular host environment. *L. pneumophila* possesses a variety of phospholipases, including three phospholipases C (PLC) sharing homology with the phosphatidylcholine-specific PLC of *Pseudomonas fluorescens* and hypothetical proteins of

pathogenic fungi. PlcA and PlcB are two formerly defined *L. pneumophila* proteins and PlcA was described as type II secretion system (Lsp)-dependently secreted. Additionally, we found that the Dot/Icm-injected effector CegC1 shows similarity to PlcA and PlcB and therefore is designated here as PlcC. Expressed in *E. coli,* PlcA and PlcB exhibit activatable phosphatidylglycerol (PG)-specific PLC activity, while PlcC hydrolyzed a broad phospholipid spectrum without requiring activation. The addition of Zn^{2+} ions further increased, whereas EDTA inhibited, PLC activity of all three enzymes. A PLC triple mutant, but not single or double mutants, exhibited reduced host killing in a *Galleria mellonella* infection model, highlighting the importance of the three PLCs in pathogenesis. Here we investigated the mode of activation and secretion of the *L. pneumophila* PLCs PlcA and PlcB. For analysis of PLC activity and antibody generation, the PLC proteins were recombinantly expressed and purified. Secreted nature and protein processing was investigated by Western Blotting of culture supernatant of wild type and of secretion and protease mutants. Further, lipid hydrolysis of bacterial products or purified protein was assessed by incubation with phospholipids and by reaction product detection by means of thin layer chromatography.
Upon analysis of secretion system mutants, we found that PlcA and PlcB are secreted via the type II secretion system Lsp. Our analysis revealed further that the *L. pneumophila* zinc-metalloprotease ProA is essential for processing of PlcA and PlcB and activation of PLC activity. Growth kinetics of *L. pneumophila* showed secretion of inactive PlcA from early logarithmic but processing / activation of PlcA from late logarithmic growth phase. Currently, we characterize the specific processing mechanism leading to activation of the PLC proteins by ProA and their localization during host infection.

HMP32

Bacteria-derived bioactivity in the sponge *Haliclona* sp.

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Marine sponges host a variety of symbiotic partners. Up to 30 different bacterial phyla are known for their association with these sessile filter feeders fulfilling numerous functions (Lee et al. 2011). Among others, the production of bioactive secondary metabolites which are regularly used as defense by the host to persist in highly competitive environments. The close association with diverse communities of bacteria makes sponges the promising source for undiscovered bioactive compounds (Webster & Taylor 2012). *Haliclona* sp. is here of special interest because of a specific defense mechanism, causing a severe pain after contact with human skin. To investigate this peculiarity, the bacterial community composition of *Haliclona* sp. was analyzed by denaturing gradient gel electrophoreses fingerprinting of PCR amplified 16S rRNA gene fragments, 16S rRNA gene clone library and cultivation of bacteria including phylogenetic identification. Sponge tissue samples were screened for bioactivity by applying bioassay-coupled high-performance thin-layer chromatography. We tested the extent to which bacteria are responsible for the bioactivity and defense. Antibiotic treatments and changes in dark/light incubations were applied to eliminate selective bacterial symbionts from host tissue. After several weeks of incubation, DGGE pattern clearly indicated changes in community compositions; however, changes in bioactivity were not detected. Preliminary results indicate an association of the sponge *Haliclona* sp. with a diverse bacterial community and bioactivity of unknown origin. Interestingly, representatives of phyla known for producing bioactive secondary metabolites were detected. This study might provide additional insights into the interaction between sponges and bacteria regarding defense mechanisms.

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HMP33

Impact of CodY in *Staphylococcus aureus* on virulence gene expression and host pathogen interactions

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Staphylococcus aureus is a human pathogen of strong clinical significance due to increasing infections with multi-resistant isolates. A better understanding of its infection biology is urgently required to combat this pathogen also in the future. The fitness of this pathogen, which is based on its ability to successfully adapt to host conditions, is crucial for full virulence. Consequently, elucidation of processes involved in the adaptation of the pathogen to the host environments during colonization and infection provides the basis for a more comprehensive picture of the pathogenesis of *S. aureus* and this information may also contribute to new therapeutic strategies to treat infections caused by this pathogen.
In the present study, we focused on the role of CodY in virulence gene expression and host pathogen interactions. CodY is a global regulator that is crucial for adaptation to stationary phase conditions and nutrient limitation. It is activated by GTP and branched chain amino acids and thus couples gene expression to changes in the pool of these metabolites. A loss of CodY resulted in the overexpression of several virulence factors such as lipases, proteases, nucleases and toxins. This is mediated by a modulated activity of RNAIII, SarA, SigB, and SaeRS in the *codY* mutant. The impact of CodY on survival of *S. aureus* phagocytosed by macrophages was analyzed. Proteome analysis of phagocytosed *S. aureus* cells using SILAC metabolic labeling and gel free proteomics in combination with mass spectrometry has been performed to get valuable insights into staphylococcal physiology after phagocytosis. Interestingly, in the *S. aureus* wild type, CodY has been found at elevated levels after phagocytosis. This might be accompanied by a decreased production of extracellular enzymes in the wild type under these conditions and could provide an explanation for the oberservation that *codY* mutants kill macrophages more efficiently than the corresponding wild type strains.

HMP34

Investigation of the cellular envelope phospholipids from *Legionella* species

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Introduction: *Legionella* are Gram-negative bacilli that are ubiquitously found in the environment as intracellular pathogens of protozoa and as components of biofilms. Humans get infected with *Legionella* through inhalation of contaminated aerosols, which allow the bacteria to enter the lung and to replicate inside alveolar macrophages causing a severe form of pneumonia. Our previous investigations showed that in the presence of exogenous choline *L. dumoffii* and *L. micdadei* exhibited a reduced capability of tumour necrosis factor (TNF-α) induction (Palusinska-Szysz *et al.* 2014). The aim of this study is a detailed analysis of phospholipid classes and single species of *L. micdadei* and *L. dumoffii* in order to elicit if changes in the phospholipid composition have an impact on pro-inflammatory response of the host.
Materials and Methods: *L. micdadei* and *L. dumoffii* were cultured on buffered charcoal-yeast extract (BCYE) agar plates for three days at 37 °C in a humid atmosphere and 5% CO_2 or on the same medium enriched with 100 $\mu g \cdot ml^{-1}$of choline-trimethyl-d_9 chloride. Lipids were isolated from

bacterial cells using the Bligh and Dyer method. We applied ^{31}P NMR for the quantitative analysis of phospholipid classes in *Legionella* membranes. GC/MS analysis was performed to investigate changes in the fatty acid pattern and tandem mass spectrometry was employed to identify specific changes in single lipid species.

Results: The organic extracts of *L. micdadei* and *L. dumoffii* were analyzed by ^{31}P NMR spectroscopy. The main phospholipids were phosphatidycholine (PC), and phosphatidylethanolamine (PE). Other minor phospholipids comprised cardiolipin (CL) and phosphatidylglycerol (PG). The fatty acid composition showed the presence of different acids from 14 to 23 carbon atom, including both unbranched saturated and unsaturated fatty acids as well as branched ones (*iso* and *anteiso*). The use of labeled choline enables us to investigate of two PC-synthesis pathways.

Conclusions: The detailed analysis of phospholipid classes and single species of *Legionella* spp allow us to answer if changes in the phospholipid composition have an impact on response of the host.

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This work was supported in part by National Science Centre research grant (N N303822640).

HMP35

The role of listeriolysin O by the interaction of Listeria with vegetable plants

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Foodborne bacterial infection is still a global problem for developing countries and for countries with a high-tech food industry. One such disease is listeriosis, which the who in 1987 related to important foodborne infections. Intercellular interaction of listeriosis pathogens - of Listeria and master explored on plant tissues calli. Biofilm formation stages of *Listeria monocytogenes EGD* and its attenuated mutant *L. monocytogenes Δ hly* lacked listeriolysin O were studied using calluses models of lettuce. By means of light and scanning electron microscopy it was found out that biofilms develop extremely fast on callus surface (18-24 hours). A thicher matrix layer develops during biofilm formation on inorganic surfaces by *Listeria*, at the same time *Listeria* cultures heteromorphism has been marked. Histological studies of infected calluses showed phytopathogenic effects of *L.monocytogenes EGD*, in contrast to *L. monocytogenes Δ hly*. Heat products are prevail in transmission factors of listeriosis but some outbreaks were proved to be caused by vegetables. Interacting with pathogenic *Listeria* plant cells significantly increased in size, at the same time their shape deformed, cell walls became thinner, which resulted in a large number of wall protuberances or retractions inward the host cell. Possibly, at that moment the process of *Listeria* interaction with cells was intensified due to adhesion on their walls, with the sequential penetration of bacterium from the intercellular space by means of wall destruction and their allocation within vacuoles. Many thin sections showed the complete plant cells destruction with a significant accumulation of *Listeria*. It is of interest that individual callus cells formed cytoplasm with electron-dense content in response to bacterial influence, apparently due to synthesis of lipid substances, as well as the formation of phenolic complexes in response to the stress caused by *L. monocytogenes.* Contamination of vegetables with virulent *Listeria* is a potential epidemiological risk.

HMP36

Human contact system activation by *Streptococcus pyogenes* M49 is triggered by streptokinase

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Introduction: S. pyogenes is a Gram positive human pathogen, responsible for mild superficial throat and skin infections on the one hand, but also for severe invasive diseases on the other. Strains are classified according to the M protein, a multifunctional surface-bound virulence factor, involved in streptococcal contact system activation[1].

The human contact system, regulating coagulant and proinflammatory processes, is a plasma proteinase cascade consisting of four proteins: Factor XII (FXII), XI (FXI), prekallikrein (PK) and high molecular weight kininogen (HK). Interaction with single factors has been reported for several bacterial binding proteins and proteases[2].

We investigated the role of secreted and surface-bound streptokinase (Ska) in this context, which binds and activates human plasminogen to plasmin, the key serine protease in fibrinolysis. Moreover, we analyzed whether single contact factors are involved in the regulation of fibrinolysis and thus contributing to the dissemination of the pathogen. We also compared invasive and non-invasive *S. pyogenes* clinical isolates regarding their ability to activate the contact system.

Materials and Methods: PK activation on the surface and by culture supernatants was measured using a specific chromogenic substrate. To investigate the contribution of contact factors to fibrinolysis we determined the ability of an M49 wild type strain to escape from a clot of plasma deficient in PK, HK or FXII compared to normal plasma.

Results: We found that secreted and surface-bound Ska is able to trigger the contact system in normal but not in plasminogen deficient plasma. Comparing PK activation by 52 different strains, there was no correlation obvious between the ability to activate contact factors and an invasive background. However, we found that M12 and M49 serotypes triggered the contact system to a much higher degree than serotypes M1 and M3. Besides, M49 bacteria were significantly impaired in their ability to escape from a clot when PK or HK was absent.

Discussion: Considering that patients suffering from sepsis or septic shock are characterized by a systemic activation of the contact system[3], we hypothesized that *S. pyogenes* strains isolated from invasive or non-invasive infections show different activation patterns. In contrast, we found a correlation with the M protein background. M1 and M3 serotypes, often associated with invasive infections, seemed to be able to evade contact system activation. This could be a survival strategy considering that the contact system is part of the host's immunity. Whether the degree of activation correlates with the Ska secretion or to which extend contact system mediated fibrinolysis contributes to the dissemination of the pathogen remains to be elucidated.

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HMP37

Investigation Of Tandem Lipoproteins In *Staphylococuss Aureus* Usa300 On Host Signaling

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The USA300 strain, a clone of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), was first isolated in 2000 and has successfully epidemic infection in North America, accounting for more than 50% of all disease caused by the entire *S. aureus* species. The previous whole genome sequencing studies revealed that multiple genetic elements on prophages and pathogenic island unique to CA-MRSA strains and are absent in other *S. aureus* strains. It could be a point for these CA-MRSA strains to highly widespread and cause invasive disease.

A tandem lipoprotein cluster, which is located on the *Staphylococcus aureus v*SAα pathogenic island, shows a high biodiversity in all the different *S.aureus* isolates. Among them, USA300 is an invasive clone that exhibits

the longest and most complex cluster. In order to investigate the function of the tandem lipoproteins on host signaling, we constructed and compared the cluster deletion mutant, the complementary with wild-type strain. Interestingly, the tandem lipoprotein mutant (Δlpp) reduced the inflammatory cytokines and antimicrobe peptides production compared to the wild type and complementary in the infection of human MM6 and keratinocyte cell lines. The invasion of mutant cells (Δlpp) into keratinocytes was significantly decreased compared to those of the wild type and complementary. The result suggests that the tandem lipoproteins located on pathogenic island play a role on host signaling.

HMP38

Surface binding properties of *Staphylococcus saprophyticus*

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Introduction: *Staphylococcus saprophyticus* is a gram-positive and coagulase-negative pathogenic staphylococcus causing urinary tract infections in young women. Some of its surface proteins have been characterised in the recent past. These include the uro-adherence factor A (UafA) which shows binding to uro-epithelial cells. We want to find out if *S. saprophyticus* strains 7108 and ATCC 15305 bind additionally to sleek surfaces like glass and plastic.
Materials and Methods: An overnight culture was incubated at 37 °C. The OD_{600} was adjusted to 2.0. For binding to glass bacterial suspension was given onto an object plate and incubated at room temperature for 2 h. The binding to plastic was tested with bacterial suspension incubated for 2 h at 37 °C in plastic dishes. The unbound bacteria were washed off with PBS and bound bacteria were stained using crystal violet. The resulting binding / no binding was visible to the naked eye as illustrated by a violet or clear surface.
Results: The wild type strains of *S. saprophyticus* showed a clear binding to glass and plastic. In contrast, the mutants missing UafA could not bind to the surfaces. This shows that UafA not only mediates binding to uro-epithelial cells but also to clean surfaces.
Discussion and Outlook: The ability to bind to surfaces is usually the first step in biofilm formation. In biofilms bacteria can resist antibiotics and evade the immune response. We want to find out in which way the adhesin UafA acts as a virulence factor in the human host as well as an essential tool to colonise instruments or surfaces, for example, in hospitals.

HMP39

BPI-like proteins induce type 1 fimbriae in different *Enterobacteriaceae*

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Introduction: The Bactericidal/permeability-increasing protein (BPI) and the closely related palate, lung and nasal epithelium clone (PLUNCs) proteins are lipid transfer proteins involved in the innate immune response in epithelia. As antimicrobial proteins (AMPs) they form a first line of defense against bacteria and other pathogens.
BPI has three different antimicrobial functions: 1) neutralization of LPS, 2) direct bactericidal activity against several gram-negative bacteria and 3) opsonization activity. Short PLUNC1, the best studied PLUNC protein, is also capable of binding LPS and is thought to have similar functions as BPI. In addition it was shown that SPLUNC1 has an inhibitory effect on the biofilm formation of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, suggesting that members of the BPI/PLUNC family may have a general function in the defense against bacteria by interfering with bacterial adhesion and biofilm formation.
Adhesion to biotic and abiotic surfaces such as epithelia or catheters plays an important role in the life cycle and pathogenesis of many bacteria and is mediated by a vast number of different adhesive structures. In this study we aimed to analyze the influence of BPI/PLUNC proteins on the adhesion of several bacteria from the family of *Enterobacteriaceae*.
Materials and Methods: Highly purified recombinant short PLUNC1 and long PLUNC1 were generated. BPI is commercially available. The influence of these proteins on bacterial adhesion was analyzed via a quantitative *in vitro* assay and fluorescence microscopy. To identify specific genes involved in the adhesion of the bacteria gene deletions via Lambda Red-recombination were introduced. By plasmid complementation the phenotype of the bacteria was reverted.
Results: While the inhibition of *K. pneumoniae* adhesion by short PLUNC1 was confirmed, other genera of *Enterobacteriaceae*, such as *Salmonella* Typhimurium and *Escherichia coli*, surprisingly showed increased adhesion in the presence of several members of the BPI/PLUNC family of proteins. In *S.* Typhimurium BPI, short and long PLUNC1 induced adhesion in a dose dependent manner when present in the medium, while in *E. coli* this effect was only observed with PLUNC proteins but not BPI.
The observed induction of adhesion could be inhibited by the addition of mannose or methyl α-D-mannopyranoside, known inhibitors of type 1 fimbriae. Moreover adhesion was no longer induced by any of the proteins, if strains deficient for *fimA*, the major structural component of type 1 fimbriae, were used. In accordance the knockout phenotype could be reverted by complementation with a plasmid carrying *fimA*.
Conclusion
Some *Enterobacteriaceae* like *S.* Typhimurium and *E. coli* respond to the presence of AMPs from the BPI/PLUNC family by fimbrial adhesion. This could be a strategy of the pathogens to escape the activity of these AMPs by enforced attachment.

HMP40

Human milk oligosaccharides and their influence on the development of the infant´s intestinal microbiota

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Human milk, as an important nutrient source, plays a crucial role in the development of the infant intestinal microbiota. The bifidogenic effect of human milk oligosaccharides (HMOs) has been shown in numerous *in vitro* studies. These molecules seem to escape digestion by the infant´s intestinal enzymes and reach the colon, providing nutritional sources for the microbiota. However, only few bacteria, in particular *Bifidobacterium* species, are currently known to have the enzymatic equipment for HMO-utilization. It is intriguing to speculate that differences in content and structure of HMOs which are dependent on the secretor status of the lactating woman influence the abundance and activity of specific microorganisms. To investigate the relationship between the composition of the infant gut microbiota and the secretor-dependent HMO-composition, we enumerated bacteria by qPCR with genus- and species-specific primers. Faecal samples of 15 healthy, exclusively breastfed infants (10 secretor-milk-fed, 5 non-secretor-milk-fed) at the age of 1 and 3 months were analyzed. Due to high inter-individual variations, we did not identify a clear secretor- or age-related bacterial distribution pattern. However, the percentage distribution of the studied bacteria showed a higher abundance of *Bifidobacteria* in the faecal samples of secretor-milk-fed infants at the age of 1 month (p=0,037) compared to non-secretor-milk fed infants.
Our preliminary data indicate that the *bifidobacteria* population in the infant gut may be influenced by the secretor-dependent milk-composition of the mother. This relation can be explained by substrate preferences for specific HMOs resulting from the enzymatic equipment, which has been confirmed in several *in vitro* studies.

HMP41

Detection of Quorum Sensing Autoinducers of *P. aeruginosa* via ELISA in different Matrices

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Bacterial quorum sensing signals, called autoinducers (AIs), often have to be analyzed in high sample numbers of different matrices as mucus of human epithels, supernatants of cell cultures or human sputum. Here cost-efficiency and time consumption become critical parameters. In our project, we develop and validate the analysis of AIs from *Pseudomonas aeruginosa*, the acylhomoserine lactones 3-oxoC12-HSL and C4-HSL, via the comparably cheap and fast ELISA method. The ELISA was first evaluated for the detecting of the N-acylhomoserine lactones (HSL) and their degradation products, the homoserines (HS). It is now established, such that it is possible to measure the 3-oxoC12-HSL and C4-HSL in sum and to differentiate

between HSL and HS. To get a better differentiation between the AIs of *P. aeruginosa,* the ELISA was adapted to detect C4-HSL and 3-oxoC12-HSL separately. This competitive assay is performed according to the paper from Cheng et al. (2010)*. During preliminary studies we have tested some native sputum samples and the epithelial growth media on the content of HSL analogoussubstances to get a first overview how the ELISA deals with these different matrices. The assay can handle the different surroundings and it is also possible to make a differentiation between the HSL and the HS. These results indicate that the ELISA works for even complex matrices e.g. mucus or growth media.

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HMP42

Genome wide transcriptome analyses on the population level and single cell reporter analyses identify key parameters of quorum sensing (QS)-dependent gene regulation in *Sinorhizobium fredii* NGR234

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Here we report on the genome wide RNA-seq analysis of newly constructed autoinducer (AI) mutant strains, NGR234-Δ*traI* and NGR234-Δ*ngrI*, on a population level and in combination with the analysis of *traI* and *ngrI* gene expression on a single cell level. The RNA-seq analysis of early stationary phase cultures in the background of NGR234-Δ*traI* suggested that up to 316 genes were differentially expressed in the NGR234-Δ*traI* mutant vs. the parent strain and 466 in the background of NGR234-Δ*ngrI* vs. the parent strain. Accordingly, a common set of 186 genes was regulated by the TraI/R and NgrI/R regulon including flagella biosynthesis genes and genes linked to EPS succinoglycan biosynthesis. Further we also discovered that genes responsible for rhizopine catabolism in NGR234 were strongly repressed in the presence of high levels of *N*-3-oxooctanoyl-L-homoserine lactone. Additional nodulation assays suggested that QS-dependent gene regulation on a population level appears to be of higher relevance during non-symbiotic growth rather than for life within root nodules. While these studies assume a homogenous gene expression within a population, it is likely that this not the natural situation for many of the examined genes. Therefore, we analysed the expression of *traI*, *ngrI* and many other QS-dependent genes on a single cell level. For this we constructed *rfp*-based promoter fusions and monitored their expression on a single cell level over time in laboratory cultures and in plant rhizospheres. Interestingly, the expression of the *traI* and *ngrI* fusions resulted in the observation of high levels of phenotypic heterogeneity in NGR234, furthermore the promoter fusions were strongly affected by addition of micromolar concentrations of *N*-3-oxooctanoyl-L-homoserine lactone or by the absence of corresponding AI synthase gene. Additional studies with plant-root exudates further indicated that gene regulation in plant rhizospheres is strongly affected by plant released compounds and that *traI* and *ngrI* expression is highly homogenous under these conditions. Altogether these findings implied that the AI and plant root exudates trigger levels of gene expression in NGR234 within populations and that populations in laboratory cultures are not necessarily homogenous with respect to AI-dependent gene expression. Our data suggest that population-wide as well as single cell analyses are needed to fully understand the complex regulatory networks of bacterial life in the rhizosphere and during symbiosis.

HMP43

Identification of novel target genes critical for host-microbiota interactions in inflammatory bowel diseases

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Crohn's disease (CD) and ulcerative colitis (UC) represent the two major forms of inflammatory bowel diseases (IBD). Although their exact etiologies have remained unknown, it is widely accepted that dysregulated interactions of the gut microbiota with genetic susceptibility loci play a major role in the onset and perpetuation of both disorders. Thus, we evaluated in this context the association of distinct intestinal microbial colonization patterns with the regulation of defined signaling pathways in mouse models of acute and chronic colitis induced through the application of dextran sulfate sodium (DSS), adoptive T cell transfer or bacterial infection. Utilizing sequencings strategies as well as conventional plating assays, we identified an intriguing regulation of certain enterobacterial species or fungi by Arginase 1, the final enzyme of the urea cycle, CD101, a negative costimulatory molecule [1, 2], and PTPN22, a protein tyrosine phosphatase which is critical for the maintenance of cellular homeostasis. While the redistribution of the intestinal microflora ameliorated the course of disease in Arg1-deficient animals, PTPN22$^{-/-}$ as well as CD101$^{-/-}$ mice exhibited accelerated and prolonged signs of colitis. Based on those promising results, we are currently investigating the interactions of the distinct identified microbial species with the respective Arg1-, PTPN22- and CD101-expressing immune subsets as well as the molecular basis underlying these interactions.

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HMP44

Highly sensitive microarray based identification of antibodies against *Staphylococcus aureus* antigens

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To study the humoral immune response to *Staphylococcus aureus* antigens a protein microarray, containing 64 recombinantly expressed *S. aureus* proteins, was developed using the Alere technology. In addition to a broad repertoire of various known virulence proteins such as enterotoxins and immune inhibitors, 22 secreted proteins with so far unknown function were included. The main advantage of this protein microarray is that a large number of antibody specificities against *S. aureus* antigens can be differentiated in parallel. Moreover different antibody subtypes such as IgM, IgG, IgA and also sub classes of these subtypes can be specifically detected and quantified. The technique is rapid and easy to handle, highly automated and economical, highly sensitive and reproducible, and it needs only small quantities of samples and reagents. Each protein was spotted in up to five different concentrations ranging from 0.01 to 0.5 mg/ml. Blood plasma samples and nasal secretions of healthy human carriers and non-carriers of *S. aureus* were used to find the optimal concentration for each protein and sample dilutions. In this experimental approach, 25 immunogenic antigens have been detected, including four putatively secreted proteins with so far unknown function. Moreover, first experiments were carried out to examine the antibody response in a mouse model during *S. aureus* infection. In future, this protein array will be used for studies analysing the immune response against *S. aureus* antigens during course of an infection in both humans and animals to identify antigens/antibodies that can be used as diagnostic markers for severe *S. aureus* infections. Gaps in the antibody response will be informative with regard to bacterial protein expression regulation and/or manipulation of the host immune response. Moreover, possible *S. aureus* vaccine candidates that have never been taken into consideration by other approaches will be provided. As a final aim the protein chip will be used as diagnostic application giving the opportunity to early identify patients with *S. aureus* infections.

HMP45

Age-dependent infection susceptibility of Salmonella in mice

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Neonates and young infants in developing countries exhibit an enhanced risk to acquire systemic infection with *Salmonella enterica* leading to sepsis and meningitis. The influence of the age-dependent factors of host susceptibility have, however, not been investigated. Here, we comparatively analyzed *Salmonella* infection in adult as well as 1-day-old and 6-day-old neonate mice after oral administration. In contrast to the situation in adult animals, we observed spontaneous colonization, massive invasion of enteroabsorptive cells, intraepithelial proliferation and the formation of large intraepithelial microcolonies. Mucosal translocation in neonate mice was dependent on enterocyte invasion due to the absence of M cells. Epithelial invasion further caused potent innate immune stimulation. Our results identify factors of age-dependent host susceptibility and provide important insight in the early steps of *Salmonella* infection *in vivo*. We also present a new small animal model amenable to genetic manipulation of the host for the analysis of the Salmonella enterocyte interaction *in vivo*.

HMP46

Analysis of the type III-secreted protein NopE1 in nodule extracts and in transiently transformed *Nicotiana benthamiana* leaves

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Bradyrhizobium japonicum is a nitrogen fixing symbiont of soybean. During symbiosis, the bacteria secrete various proteins (Nops, nodulation outer proteins) via the type III secretion system (T3SS). Depending on the host plant, the effector protein NopE1 influences symbiosis either positively or negatively (1). NopE1 contains two metal-ion inducible autocleavage (MIIA) domains (formerly DUF1521), which are cleaved at conserved sites in the presence of calcium (1-3). Cleavage is essential for its biological activity (1). To localize NopE1 in planta, the protein was tagged with a 2xFlag and a polyclonal antibody against the wild-type protein was raised. *Macroptilium atropurpureum* was infected with rhizobia expressing either a cleavable “wild type” protein or a non-cleavable variant, which was created by amino acid replacements (D to A) at the cleavage sites. Within nodule extracts, full-length protein could be detected when the plant was infected with rhizobia expressing the non-cleavable variant. Cleavage products of NopE1 were detected in nodule extracts when rhizobia expressed the “wild-type” protein. To obtain more information about the in vivo function of NopE1 in planta, we are now transiently expressing NopE1 variants in leaves of *Nicotiana benthamiana*. NopE1 was fused to fluorescent proteins in order to elucidate intracellular localization. The expression of NopE1 will be analyzed by Western blot and fluorescence microscopy.

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HMP47

Preclinical infection C57BL/6 mouse model of *Pseudomonas aeruginosa* infection in the immunocompromised host

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The gastrointestinal tract encloses the largest and most complex bacterial community in the human body, the GI microbiota, which protects the host from colonization and infection by pathogenic and opportunistically pathogenic organisms in concert with the host’s immune-system.
Hemato-oncological patients commonly suffer from reduced effectiveness of their immune system, both due to the respective ailments themselves and because of iatrogenic measures. Such patients are constantly threatened by nosocomial infections, including with multi-resistant pathogens such as extensively drug-resistant (XDR) *Pseudomonas aeruginosa*, which can cause particularly severe problems with antibiotic treatment and even lead to fatal outcomes. Colonization with XDR *Pseudomonas aeruginosa* may be favoured by prophylactic broad spectrum antibiotic therapy, disrupting the microbiota-mediated colonization and infection resistance. It is still unclear whether the immunodeficiency, mucositis, the altered microbial pattern, the specific virulence/fitness factors of the pathogens themselves or all these factors together account for the increased susceptibility towards colonization and even bloodstream infection with germs such as *Pseudomonas aeruginosa*. To better understand the mechanisms underlying colonization, translocation and infection of immunosuppressed hosts with multi-resistant Gram-negative pathogens, this project aims at clarifying the role of the immune system as well as the microbiota using a C57BL/6 mouse model for preclinical evaluation of interventions targeting the GI microbiota. Conventional and germfree C57BL/6 mice in particular and other mouse lines of interest are treated with *Pseudomonas aeruginosa* strains, including XDR strains isolated from hemato-oncological patients suffering from septicemia. Immunodeficiency or mucositis may then be induced or an alteration in the microbiota caused through the treatment with antibiotics, the changes in microbial pattern analyzed before and after treatment and/or before and after the colonization attempt and the mice’s susceptibility to colonization and infection evaluated. Differences as well as longitudinal changes in the mice’s microbiota can be analyzed, germfree mice employed directly or colonized with specific germs or another mouse’s microbiota and tested for differences in their colonization and infection resistances to better understand and possibly influence mechanisms shaping and affecting the microbiota.

HMP48

Interaction of human dental follicle stem cells with oral anaerobic bacteria *Prevotella intermedia* and *Tannerella forsythia*

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Periodontitis is a bacterially induced inflammatory disease of the periodontum characterized by progressive irreversible loss of supportive tissue and finally tooth loss. Besides high differential potential human dental follicle stem cells (hDFSC) are considered to be an approach for tissue regeneration.The oral cavity is colonized by over 500 bacterial species forming complex multi-species biofilms. Especially a shift of species to gram-negative anaerobic bacteria like *Prevotella intermedia* or the red complex species *Tannerella forsythia* is associated with progressive periodontitis.Therefore, the interaction between the periodontal pathogens *P. intermedia* and *T. forsythia* with hDFSCs was investigated as well as survival of stem cells under anoxic stress. HDFSCs were isolated from wisdom teeth provided by the Department of Oral and Maxillofacial Plastic Surgery, University of Rostock, and cultivated in DMEM-F12 with 10 % FCS.To assess the influence of anoxic stress on the vitality of the hDFSCs the number of vital cells, metabolic activity and migration activity were analyzed. The strain *T. forsythia* ATCC43037, *P. intermedia* ATCC25611 and *P. intermedia* clinical isolates were co-cultured with the hDFSCs in DMEM.The interactions were analyzed in terms of adherence and internalization, the influence on cellular migration and interleukin secretion.
The hDFSC survived 72 h of anoxia with maintained state of vitality and metabolic activity compared to those under aerobic conditions. *P. intermedia* as well as *T. forsythia* adhere to and internalize into hDFSCs

while co-cultivating. Comparing *P. intermedia* clinical isolates to the ATCC strain no differences were observed in virulence behavior. The internalization of *T. forsythia* was slightly lower compared to all *P. intermedia* strains. Both, anoxic stress and/ or bacteria delayed migration activity of hDFSCs about 50 % even though no additional effect was observed. HDFSCs can cope with lack of oxygen over 72 h without loss of metabolic activity. Therefore, an interaction between hDFSCs and bacteria in anaerobic parts of the periodontal pocket might be possible and both bacteria species interacted with hDFSCs. Interestingly, adherence and internalisation of *P. intermedia* clinical isolates were equal to the ATCC strain.

HMP49

Visualization of Antibiotic Therapy Against *Staphylococcus aureus* by ^{19}F Magnetic Resonance and Bioluminescence Imaging

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The emergence and spreading of antibiotic resistant bacteria such as meticillin-resistant *Staphylococcus aureus* (MRSA) in recent decades has led to a dramatic increase of invasive, life threatening infections. It is therefore of paramount importance to identify new drug targets and to develop new compounds and lead structures to fight bacterial infections. In addition, the development of highly predictive in vivo models and the application of in vivo imaging technologies could help to accelerate the selection process and to capture the whole spectrum of activity of novel compounds in vivo. Our aim was, in this context, to establish an in vivo imaging platform to evaluate the efficacy of new compounds in murine models of *S. aureus* infection. The idea was to combine Bioluminescence Imaging (BLI), a technology that visualizes the luciferase activity of luminescent bacteria, and ^{19}F Magnetic Resonance Imaging (^{19}F MRI), a method that measures the accumulation of ^{19}F at the site of infection after incorporation of perfluorocarbon contrast media (PFC) by phagocytic immune cells in the bloodstream. In principle, the application of both imaging modalities to the murine thigh infection model with *S. aureus* strain Xen29 demonstrated stable BLI signal from day 2/3 to day 7 p.i. as well as strong accumulation of PFCs at the site of infection in the shape of a 'hollow sphere'. We then treated different groups of mice with various clinically relevant (like Vancomycin or Linezolid) or an experimental therapeutic (Lysostaphin) and could see decreased BLI signals and weaker ^{19}F accumulation, both depending on the efficacy of the applied antibiotic. ^{19}F MRI showed furthermore a dramatically decreased abscess area for Linezolid or Lysostaphin treated mice. Overall, both imaging modalities indicated non-invasively the same efficacy pattern than classical colony-forming unit determination and delivered furthermore valuable information about the course of infection in a non-invasive fashion. We think that this combination of imaging modalities delivers a more detailed picture of the state and course of disease/infection than classical methods by visualizing bacterial burden (BLI) and inflammatory immune response (^{19}F MRI) simultaneously and might therefore be a precious platform for the evaluation of promising new drug candidates or to study host pathogen interactions.

HMP50

Deletion of the *Legionella longbeachae* PtdIns(4)*P*-binding effector SidC reveals its role for pathogen vacuole formation and virulence

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Inhalation of aerosols contaminated with *Legionella* spp. may lead to a life-threatening pneumonia termed Legionnaires' disease. *L. pneumophila* and *L. longbeachae* cause the same disease, yet the environmental niches, transmission routes and disease epidemiology of these clinically most relevant species differ greatly. In the accidental host, human alveolar macrophages, *Legionella* avoids lysosomal degradation and replicates within a membrane-bound, endoplasmic reticulum (ER)-derived compartment, called the *Legionella*-containing vacuole (LCV). Crucial for the establishment of the LCV is the *Legionella* Icm/Dot type IV secretion system (T4SS). *L. pneumophila* translocates approximately 300 different "effector" proteins in an Icm/Dot-mediated manner into the host, where they subvert cellular processes. Some of these effectors anchor to the LCV by binding to phosphoinositide (PI) lipids. For *L. longbeachae* ~110 effectors have been predicted, but with one exception have not been mechanistically characterized to date. Notably, more than 50 of these effector proteins are not conserved among other *Legionella* species.
Using PI-bound agarose beads in pulldown assays, we identified in *L. longbeachae* lysates a 111 kDa SidC homologue as the major PtdIns(4)*P*-binding protein. $SidC_{Llo}$ bound with nanomolar affinity to PtdIns(4)*P* via its P4C domain and decorated the LCV upon Icm/Dot-dependent translocation. An *L. longbeachae* Δ*sidC* deletion mutant strain was impaired for the recruitment of the resident ER protein calnexin to LCVs in *Dictyostelium discoideum* amoebae. Calnexin recruitment was restored by $SidC_{Llo,}$ or its orthologues $SidC_{Lpn}$ or $SdcA_{Lpn}$. Therefore, the SidC homologues of *L. longbeachae* and *L. pneumophila*, which are about 40% identical, seem to carry out similar functions. Moreover, an *L. longbeachae* Δ*sidC* deletion mutant strain was outcompeted by wild-type bacteria in *Acanthamoeba castellanii*. Taken together, biochemical, genetic and cell biological data indicate that $SidC_{Llo}$ is an *L. longbeachae* effector, which binds through a P4C domain with high affinity to PtdIns(4)*P* on LCVs, promotes ER recruitment to the LCV and thus plays an important role in pathogen-host interactions.

I. Haneburger and S. Dolinsky contributed equally to the work.

HMP51

dRNA-seq analysis reveals promoters and antisense RNAs in *Bradyrhizobium japonicum*

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Bradyrhizobium japonicum USDA 110 is a soil bacterium which can fix nitrogen in symbiosis with *Glycine max* (soybean). We used the dRNA-seq approach to determine the whole primary transcriptome of free-living *B. japonicum* in liquid culture and in soybean nodules. This approach can distinguish between processed and primary 5'-ends, allowing the genome-wide mapping of transcriptional start sites (TSSs) preceded by promoters, and the detection of novel transcripts like small RNAs (sRNAs) with regulatory functions (1). Using ß-galactosidase activity assays we verified the promoters of two sRNAs corresponding to 3'-UTRs of mRNAs and two antisense RNAs (asRNAs) complementary to 3'-UTRs of mRNAs. For the abundant, trans-encoded sRNA BjrC174 (2) a strong promoter lacking similarity to known promoter motifs was verified. Furthermore, the mapped TSSs were used for *de novo* prediction of promoter motifs in *B. japonicum*. For an asRNA, which is complementary to the mRNA of blr1853 encoding cytochrome P450, a sigma70 promoter with an extended -10 box (GCTATA) was predicted. The importance of the GC extension for blr1853 expression in *B. japonicum* was experimentally confirmed. This asRNA and blr1853 mRNA in exponential and stationary cultures and nodules was analyzed by RT-PCR and strand-specific qRT-PCR. The results confirm the specific expression of blr1853 under symbiosis and suggest a role of the asRNA in the regulation of the transcription of this gene.
References:

1. Sharma CM et al. (2010) Nature. 464: 250-5.
2. Madhugiri et al. (2012) RNA BIol. 9: 47-58.

HMP52

Interaction of *Corynebacterium ulcerans* with host cells

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Corynebacterium ulcerans is a pathogenic species of the genus *Corynebacterium* and closely related to the human pathogen *Corynebacterium diphtheriae* and the animal pathogen *Corynebacterium pseudotuberculosis*. *C. ulcerans* is mainly associated with mastitis in cattle, non-human primates and other animals and is also known as commensal in various domestic and wild animals. However, during the last decade, human infections associated with *C. ulcerans* appear to be increasing in various countries and can most often be ascribed to zoonotic transmission. Besides respiratory diphtheria-like illness, *C. ulcerans* can also cause extrapharyngeal infections in humans, including severe pulmonary infections. To date, only little is known about mechanisms that this emerging pathogen uses for host colonization. Here, interaction with human epithelial cells and human and murine macrophages were examined. For this purpose, *C. ulcerans* wild type strains and corresponding mutants in putative virulence factors were examined. This showed that *C. ulcerans* is highly able to adhere to eukaryotic epithelial cells and to invade into them. Furthermore, infection of phagocytic cells leads to a high amount of intracellular bacteria. *C. ulcerans* is not only able to survive, but also to replicate inside the macrophages, and the assays performed provided indication of host cell death during *C. ulcerans* infection. Further investigations focused on the fate of the host cells. Trypan blue staining exhibited a high number of dead cells after infection. Light microscopy of infected cells and DAPI staining of their nuclei gave hints to induction of apoptosis by *C. ulcerans*. *C. ulcerans* can, like *C. diphtheriae*, produce diphtheria toxin upon lysogenization by *tox*-carrying corynephages. However, the strains examined here lack the genomic information for this toxin, suggesting other factors besides the diphtheria toxin to be crucial for *C. ulcerans* virulence. The ability of *C. ulcerans* to survive and replicate within phagocytic cells and to kill them, makes this organisms of greater interest with respect to systemic infections.

HMP53

A *Verticillium longisporum* resistant rapeseed line accumulates specific bacterial communities in the rhizosphere and the endophytic compartment

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Agricultural plants harbor a high diversity of microbes colonizing roots, among those several plant growth promoting and antagonistic bacteria affecting plant growth, health and yield. Specific exudation pattern and other so far unknown plant-derived factors can affect root colonization. We investigated root-associated microbial communities of two contrasting oilseed rape double haploid lines from a cross of a susceptible and a resistant parent exhibiting resistance to *Verticillium longisporum*. Seedlings were germinated and grown for ten days in sand or soil, dip inoculated with a *V. longisporum* spores solution and grown in a sand/soil mix or soil for further four weeks. A clear disease pattern was developed after infection of the susceptible, but not of the resistant cultivar. Prior, two and four weeks after infection, roots from infected and non-infected control plants were harvested and microbial communities of the rhizosphere and the endophytic root compartment were compared by denaturing gradient gel electrophoreses (DGGE) using universal and actinobacterial and *Pseudomonas*-specific 16S rRNA gene-targeting primers. Preliminary data clearly indicated cultivar specific rhizosphere and endophytic microbial communities affected by pre-growth in sand/soil mix or soil. Infection with *V. longisporum* furthermore affected the compositions of the microbial communities. The data indicate that the ability to accumulate specific rhizosphere and endophytic microbial communities by rapeseed may play a role in the resistance to *V. longisporum* infections. Thus, bacterial strains phylogenetically identified as *Pseudomonas*, *Lentzea*, *Duganella* and *Burkholderia* spp. which we isolated from the resistant oilseed rape line are promising candidates to find antagonistic effects against *V. longisporum* infections.

HMP54

Novel *Staphylococcus aureus* phagosomal escape factors identified by automated microscopy

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Staphylococcus aureus escapes from phagosomes of professional and non-professional phagocytes. Whereas production of phenol-soluble modulins (PSM) is required for escape, PSMs are not sufficient. We hence used the JE2 transposon mutant library [Bose JL, Fey PD, Bayles KW (2013) *Appl Environ Microbiol* 79: 2218-2224] to screen for further candidates in medium-throughput microscopy on an Operetta platform. Using a fluorescent phagosomal escape recruitment marker assay [Giese B, Glowinski F, Paprotka K, Dittmann S, Steiner T, Sinha B, Fraunholz MJ (2011). *Cell. Microbiol.* 13: 316-329] we investigated a wide range of JE2 single gene mutants and assessed the impact of the mutation on the ability of *S. aureus* to escape from the phagosomes of its host cells. We found that several mutants demonstrated significant changes in their capability to escape from the phagosome. Here we discuss the identified candidates.

HMP55

Effect of the extracellular adherence protein (Eap) of *Staphylococcus aureus* on migration and proliferation of human keratinocytes

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Staphyloccocus aureus is a major human pathogen, and a common cause for superficial and deep seated wound infections. The pathogen is equipped with a large arsenal of virulence factors allowing it to attach to various host cell structures, and to modulate the immune response of the host. One of these factors is the extracellular adherence protein Eap, a member of the "secretable expanded repertoire adhesive molecules" (SERAM) that possess adhesive and immune modulatory properties. Eap was previously shown to impair wound healing by interfering with host defense and repair mechanisms. However, its impact on cell proliferation and migration of keratinocytes, two major steps in the re-epithelialization process of wounds, has not been determined yet. Here we analyzed the effect of Eap on keratinocyte proliferation and migration using a culture inserts-based migration assay, and an EdU (5-ethynyl-2'-deoxyuridine)-based proliferation assay. Additionally, thexCELLigenceRTCA (real-time cell analyzer) system was utilized,which measures changes in electrical impedance as cells attach and spread in a culture dish covered with a gold microelectrode array. By challenging HaCaT cells with Eap, we consistently observed an inhibition of cell migration in both the culture insert- and the RTCA-based migration assay. In the EdU-based proliferation assay, a significant decrease in proliferation rates was found in response to Eap treatment. Interestingly, in the RTCA proliferation assay, a distinctive increase in the impedance was observed in presence of Eap, indicating either an enhanced proliferation rate or changes in cell morphology.Atomic force microscopy based topology studies confirmed profound morphological changes of HaCaT cells upon Eap challenge, which markedly widened and flattened the keratinocytes. Our data suggest that Eap represses both migration and proliferation of epithelial cells by inducing morphological changes that may alter the cytoskeletal structure of the keratinocyte.

HMP56
A lipid zipper triggers bacterial invasion
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Glycosphingolipids are important structural constituents of cellular membranes. They are involved in the formation of nanodomains ('lipid rafts'), which serve as important signaling platforms. Invasive bacterial pathogens exploit these signaling domains to trigger actin polymerization for the bending of the plasma membrane and the engulfment of the bacterium - a key process in bacterial uptake. However, it is unknown whether glycosphingolipids directly take part in the membrane invagination process. Here, we demonstrate that a 'lipid zipper', which is formed by the interaction between the bacterial surface lectin LecA and its cellular receptor, the glycosphingolipid Gb3, triggers plasma membrane bending during host cell invasion of the bacterium *Pseudomonas aeruginosa. In vitro* experiments with Gb3-containing giant unilamellar vesicles revealed that LecA/Gb3-mediated lipid zippering was sufficient to achieve complete membrane engulfment of the bacterium. In addition, theoretical modelling elucidated that the adhesion energy of the LecA-Gb3 interaction is adequate to drive the engulfment process. *In cellulo* experiments demonstrated that inhibition of the LecA/Gb3-lipid zipper by either LecA knockout, Gb3 depletion or application of soluble sugars that interfere with LecA binding to Gb3 significantly lowered *P. aeruginosa* uptake by host cells. Of note, membrane engulfment of *P. aeruginosa* occurred independently of actin polymerization, thus corroborating that lipid zippering alone is sufficient for this crucial first step of bacterial host cell entry. Our study sheds new light on the impact of glycosphingolipids in the cellular invasion of bacterial pathogens and provides a novel mechanistic explication of the initial uptake processes.

HMP57
The role of peptidoglycan in the development of food allergies
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The human immune system responds to a variety of exogenic signals present in our environment. Microbial associated molecular patterns (MAMPs) from the human microflora, such as the peptidoglycan (PGN), are activating cells of the innate immune system.
This pattern recognition by intra- or extracellular Toll-like receptors (TLRs) of dendritic cells (DCs) directs the adaptive immune response towards distinct T helper (Th) cell responses, mostly T_h1 (Inflammation), T_h2 (allergy) or T_h17 (regulatory). A putative role in the development of food allergies is still under debate and the major goal of this study. The elucidation of the exact role of PGN and its recognition in the context of food allergies could lead to the development of strategies to prevent food allergies, especially in young children, where the incidence is increasing.
To answer this question, several ovalbumin-expressing staphylococcal species have been cloned with the aim to generate a helpful tool, bringing together a model allergen and PGN to enabling us to characterize a possible allergic reaction in a spatial and temporal manner. A synthetic ovalbumin gene (SERPINB14) from chicken (*Gallus gallus*) was cloned with or without a signal peptide (SP) and a propetide (PP) from *Staphylococcus (S.) hyicus* into an inducible plasmid, named pTX30. The SP led to a secretion of the ovalbumin into the culture supernatant, whereas the PP (putative chaperone) was essential for expression. Sequence and western blot analysis could verify successful protein expression and secretion.
In parallel, innate immune signalling capabilities of PGN were carried out with several murine cell lines and cytokines as readout. We could show, that PGN from a lipoprotein (Lpp)-deficient
S. aureus mutant *(*SA113Δ*lgt)* did not induce any cytokine (IL-6, IL-12p70, TNF-α) in any cell type, we used (Mono-Mac-6, DCs, J774). This was not the case, when a co-stimulatory TLR signal (e.g. from Lpps) was present. This indicates that PGN itself is no TLR2 ligand and that the observed activation of immune cells was due to Lpp contaminations of the used PGN preparations.

HMP58
Anaerobic processes in gut contents of the methane-emitting earthworm *Eudrilus eugeniae*
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The earthworm gut represents a mobile, saccharide rich, anoxic microzone in aerated soils. Earthworms emit H_2 and the greenhouse gas N_2O, and it has been recently discovered that the earthworm *Eudrilus eugeniae* from Brazil also emits methane. The objective of this study was to examine anaerobic processes that might be important to the methanogenic foodweb in gut contents of *E. eugeniae*.
A methanogenic enrichment was obtained from gut contents, and was utilized to examine activities and competitive potentials of methanogens and acetogens. RNA-based stable isotope probing of bacterial 16S rRNA and *mcrA* of methanogens was performed with [^{13}C]-glucose as a representative saccharide in gut contents. H_2-CO_2 and formate but not acetate or methanol stimulated the production of methane in methanogenic enrichment cultures. Four different species-level *mcrA* phylotypes affiliated with *Methanobacteriaceae* were detected in enrichment cultures. Acetogens outcompeted methanogens for H_2-CO_2 and formate, indicating that acetogenesis might be ongoing in the gut. Various concomitant as well as successive fermentations were augmented by the rapid consumption of glucose, and potential methanogenic substrates were produced and consumed. Facultative aerobes and obligate anaerobes were associated with the diverse H_2-forming fermentations, and hydrogenotrophic methanogens affiliated with *Methanobacteriaceae* and *Methanoregulaceae* were labeled by [^{13}C]-glucose. These findings provide insights on the occurrence of various microbes that participate in the methanogenic food web in the alimentary canal of methane-emitting *E. eugeniae.*

HMP59
The KEKE motif of the *C. burnetii* T4SS effector protein CaeA is important to maintain the anti-apoptotic effect
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Coxiella burnetii is the causative agent of Query fever (Q fever), a zoonotic disease. In animals, such as cattle, sheep and goats it provokes abortions and thus leads to significant deficits in agriculture. In humans, Q fever either manifests as acute or chronic disease.Acute Q fever presents as a mild, flu-like illness but can develop into pneumonia and hepatitis. Chronic Q fever often results in endocarditis and cases often have a fatal outcome.
C. burnetii is highly infectious. Upon inhalation and uptake by human alveolar monocytic phagocytes the pathogen is able to reside and to replicate in a phagolysosomal compartment. After infection of its host cell *C. burnetii* translocates a variety of effector proteins into the host cell cytosol by use of a type IV secretion system (T4SS). To date, over 100 *C. burnetii* effector proteins are identified, yet their function remains largely unknown. Only few *C. burnetii* effector proteins have been further analyzed so far and one of them is CaeA (CBU1524). CaeA localizes to the host cell nucleus and has been shown to inhibit host cell apoptosis after UV treatment when exogenously expressed in HEK293 cells. Its mode of action, however, is unknown. We isolated genomic DNA of different *C. burnetii* animal isolates and analyzed the sequence polymorphism of *caeA* in these strains. Interestingly, we found that CaeA contains a KEKE motif that varies in the number of KEKE repetitions in the analyzed strains. KEKE motifs have been proposed to facilitate protein-protein interactions. Although they are present in a large number of proteins there is not much known about their function. We analyzed the role of the CaeA KEKE motif and could show that a deletion of the motif abrogates the anti-apoptotic effect of the protein. Introduction of additional KEKE repetitions as it could be found in some of the isolates, however, restores the anti-apoptotic function of CaeA. Thus, we demonstrate that the CaeA KEKE motif is important for the anti-apoptotic effect of the protein. Interestingly, all *C. burnetii* strains isolated from cattle which we have analyzed showed an increased number of KEKE motifs. Thus, we show that CaeA may represent a strain and host specific virulence factor of *C. burnetii*.

General and Hospital Hygiene

HYP01
Do Carbapenemase-producing strains make a difference in nosocomial outbreaks caused by *Klebsiella pneumoniae*? - Results of a systematic review

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Introduction: Nosocomial outbreaks (NO) caused by carbapenemase-producing *Klebsiella pneumoniae* represent an emerging threat for health care systems worldwide but few are know about characteristics of such NO. We therefore conducted a systematic review on this topic and compared infection control measures in outbreaks of carbapenemase positive *Klebsiella pneumoniae* (NO-CPK) strains with measures in outbreaks caused by carbapenemase negative *Klebsiella pneumoniae* (NO-CNK) strains.
Materials and Methods: The analysis is based on the Outbreak Database, PubMed and the reference lists of relevant articles. Search terms for NO-CPK were ["CARBAPENEMASE" AND "KLEBSIELLA" AND "NOSOCOMIAL" AND ("OUTBREAK" OR "EPIDEMIC")]. Data on NO-CNK was retrieved directly from the Outbreak Database. Only primary outbreak reports in English, German, French or Spanish got included.
Results: A total of 40 NO-CPK (starting in the year 2004) with at least 830 affected patients (median: 13) got included, thereof at least 388 infected patients (median: 7) and 161 fatal cases (median: 4). The average duration of these outbreaks was 8 months (median: 6). 46.6% of the affected patients presented with a nosocomial infection. The most frequent infections were bloodstream infections (166), pneumoniae (93), urinary tract infections (80) and wound infections (54). The mortality in NO-CPK was 43.7%. Data on the geographical distribution of outbreaks, type of medical department and type of carbapenemase (3 most often mentioned items only) is shown in FIGURE 1. 38 of the 40 NO-CPK were published within the last 5 years.
A total 123 NO-CNK (starting in the year 1972) with at least 3,364 affected patients (mean: 15) got included for the comparison of infection control measures. The proportion of infected patients in this group was significantly lower compared to NO-CPK (13.8%; p<0.01). Most infection control measures were significantly more often used in NO-CPK (TABLE 1).
Conclusion and Discussion: The rather large proportion of infected patients may be a consequence of the empiric antibiotic therapy regimen which covers "standard" *Klebsiella pneumoniae* but doesn't include carbapenemase-producing strains. Furthermore, a mortality rate of nearly 50% clearly underlines the clinical importance of this multidrug resistant pathogen.

Figure1

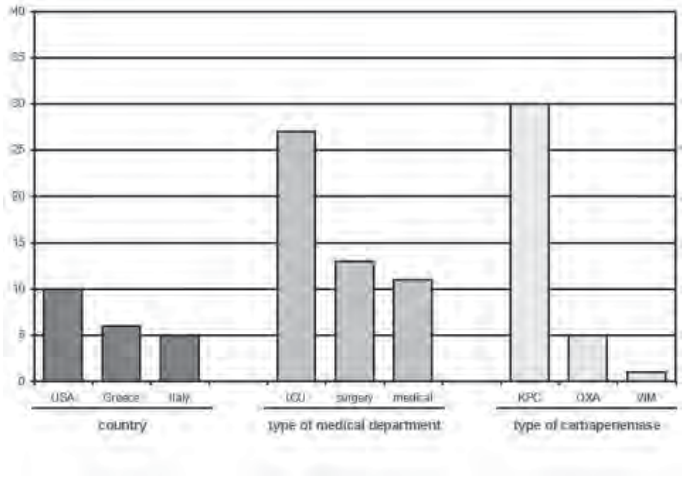

Figure 2

	Outbreaks caused by *Klebsiella pneumoniae*		
	Carbapenemase-producing strains (n=40)	non Carbapenemase-producing strains (n=123)	p-value
isolation/cohorting of patients	27 (67,5%)	42 (34,1%)	<0,01
enforcing hand hygiene	24 (60,0%)	65 (52,8%)	0,43
disinfection/sterilization	24 (60,0%)	45 (36,6%)	<0,01
changes in antibiotic stewardship	23 (57,5%)	48 (39,0%)	0,04
screening of patients	22 (55,0%)	64 (52,0%)	0,74
use of protective clothing	21 (52,5%)	30 (24,4%)	<0,01
education of staff	20 (50,0%)	29 (23,6%)	<0,01
environmental screening	16 (40,0%)	23 (18,7%)	<0,01
changes in the use of medical devices	12 (30,0%)	35 (28,5%)	0,85
improvement of patient-to-staff ratio	12 (30,0%)	10 (8,1%)	<0,01
total closure of the ward/unit	3 (7,5%)	10 (8,1%)	0,90
screening of personnel	2 (5,0%)	30 (24,4%)	<0,01

HYP02
Comparability of two different bio-medical atmospheric pressure plasma sources related to their antimicrobial activity

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Introduction: Physical plasmas are excited ionized gases which are generated by energy supply to a neutral gas. The medical application of physical plasma is an innovative field known as plasma medicine. A field of plasma medicine is focused on the inactivation of microorganisms for example in the treatment of chronic wounds or plasma-based treatment of medical materials or devices [1, 2]. A broad spectrum of technically different cold atmospheric pressure plasma sources for biomedical applications is available [2]. In despite of the technical differences, standardized parameters for the uniform identification of biological performances are necessary. Therefore, conventional microbiological test procedures have to be adapted to get standardized procedures to compare different bio-medical plasma sources related to their antimicrobial effectiveness.
Materials and Methods: We compared two technically different atmospheric-pressure plasma sources with regard to their antimicrobial effectiveness: a plane surface dielectric barrier discharge and the spot-like atmospheric-pressure plasma jet kINPen MED [3] which is already licensed as a medical product. This study describes the test of antimicrobial effects of plasma treatment using three different microorganisms by agar spread plate technique. Different methods of examination are compared to get a most comprehensive plasma source characterization.
Results: Agar spread plate method is in general a useful technique to characterize antimicrobial efficacy of cold atmospheric pressure plasma sources. To compare the biological performances of spot-like and plane plasma sources, a normalization of treatment area is necessary. Moreover, using a modified version of zone of inhibition test characterization of area effects of different plasma sources is possible.
Discussion: This study illustrates that a uniform standardized protocol for the biological characterization of technically different plasma sources is necessary. Some defined basic criteria for performance characterization *in vitro* should be helpful to enhance the comparability of characterization results of different bio-medical plasma sources.

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Acknowledgement: The financial support of the German *Federal Ministry of Economics and Technology* (grant no. 01FS12001) is gratefully acknowledged.

HYP03

Messung der Keimbelastung im OP - Das neue mediclean® CPM. Reinluftkontrolle in Echtzeit.

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Abstract has not been submitted.

HYP04

Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in healthy carriers in Antananarivo, Madagascar

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Introduction: *Staphylococcus aureus* (*S. aureus*) can be transmitted through asymptomatic carriers in the community or in health care facilities[1]. Data on the prevalence of *S. aureus* and methicillin resistant *S. aureus* (MRSA) in Africa remains scarce[2].

Aim: A cross-sectional study was conducted to describe the prevalence and local clonal epidemiology of *S. aureus* and MRSA among health care workers (HCWs) and non-medical University students in Antananarivo, Madagascar.

Materials and Methods: We screened nasal swabs from students and HCWs for *S. aureus* and MRSA by agar culture. Multiplex PCR was performed to identify *S. aureus* specific (*nuc*), MRSA specific (both *mecA* and *mecC*) and Panton-Valentine leukocidin (PVL) toxin specific genes (lukF-PV) of all methicillin sensitive *S. aureus* (MSSA*)* and MRSA isolates. *Spa* typing was performed for all PCR confirmed MRSA isolates[3]. Associated Multi Locus Sequence Types (MLST) were allocated with Ridom SpaServer[3]. Frequency distribution of *S. aureus* and MRSA nasal carriage in HCWs and non-medical students was determined using Pearson's χ^2 test.

Results: We detected 180 (11.6%) *S. aureus* and 28 (1.8%) MRSA from 1.550 nasal swabs (**Table 1**). MSSA nasal carriage was significantly associated with female gender (13.4%, p= .003). The prevalence of MRSA in HCWs was 2.8% and 1.0% in non-medical students. 21 *S. aureus* isolates were PVL positive of which one was a MRSA (**Table 2**). The *mecC* gene was not present in any isolate. The *spa* type t186 with the associated MLST-88 was the predominant MRSA clone (21/28) in the study population.

Conclusion: Our data showed a low rate for MSSA and MRSA nasal carriage in non-medical students and HCWs from Antananarivo. The predominant MRSA clone t186 ST-88 has been described in hospital patients before but not in the community[4]. Implementation and improvement of existing preventive measures against MRSA in malagazy hospitals are needed.

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Figure 1

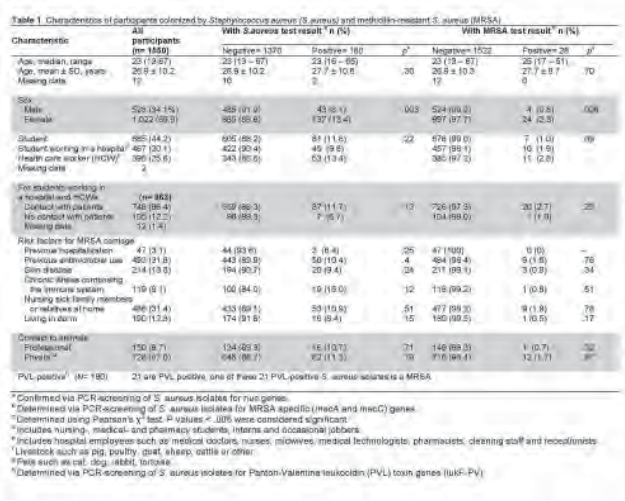

Table 1. Characteristics of participants colonized by *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA)

Characteristic	All participants (n= 1550)	With *S.aureus* test result [a] n (%)			With MRSA test result [a] n (%)		
		Negative= 1370	Positive= 180	p [c]	Negative= 1522	Positive= 28	p [c]
Age, median, range	23 (19-67)	23 (19 – 67)	23 (16 – 65)		23 (19 – 67)	25 (17 – 51)	
Age, mean ± SD, years	26.9 ± 10.2	26.9 ± 10.2	27.7 ± 10.6	.36	26.8 ± 10.3	27.7 ± 9.7	.70
Missing data	12	10	2		12	0	
Sex							
Male	528 (34.1%)	485 (91.9)	43 (8.1)	.003	524 (99.2)	4 (0.8)	.006
Female	1.022 (65.9)	885 (86.6)	137 (13.4)		997 (97.7)	24 (2.3)	
Student	685 (44.2)	605 (88.2)	81 (11.8)	.22	676 (99.0)	7 (1.0)	.69
Student working in a hospital	467 (30.1)	422 (90.4)	45 (9.6)		457 (99.1)	10 (1.9)	
Health care worker (HCW)	395 (25.6)	343 (86.8)	53 (13.4)		385 (97.3)	11 (2.8)	
Missing data	2						
For students working in a hospital and HCWs	(n= 862)						
Contact with patients	748 (86.4)	662 (88.3)	87 (11.7)	.13	726 (97.3)	20 (2.7)	.28
No contact with patients	105 (12.2)	98 (93.3)	7 (6.7)		104 (99.0)	1 (1.0)	
Missing data	12 (1.4)						
Risk factors for MRSA carriage							
Previous hospitalization	41 (3.1)	44 (93.6)	3 (6.4)	.25	47 (100)	0 (0)	~
Previous antimicrobial use	453 (31.8)	443 (90.9)	58 (10.4)	.4	494 (99.4)	9 (1.8)	.76
Skin disease	214 (13.8)	194 (90.7)	20 (9.4)	.24	211 (99.1)	3 (0.9)	.34
Chronic illness compromising the immune system	119 (8.1)	100 (84.0)	19 (16.0)	.12	118 (99.2)	1 (0.8)	.51
Nursing sick family members or relatives at home	486 (31.4)	433 (89.1)	53 (10.9)	.51	477 (98.1)	9 (1.9)	.78
Living in slums	180 (12.3)	174 (91.8)	16 (8.4)	.15	180 (99.5)	1 (0.5)	.17
Contact to animals							
Professional	150 (9.7)	134 (89.3)	16 (10.7)	.71	149 (99.3)	1 (0.7)	.52
Pets [b]	726 (47.0)	645 (88.7)	82 (11.3)	.19	714 (98.4)	12 (1.7)	.07
PVL-positive [d] (N= 180)	21 are PVL positive, one of these 21 PVL-positive *S. aureus* isolates is a MRSA						

[a] Confirmed via PCR-screening of *S. aureus* isolates for nuc genes.
[b] Determined via PCR-screening of *S. aureus* isolates for MRSA specific (*mecA* and *mecC*) genes.
[c] Determined using Pearson's χ^2 test. P values < .005 were considered significant.
[d] Includes nursing-, medical- and pharmacy students, interns and occasional jobbers.
[e] Includes hospital employees such as medical doctors, nurses, midwives, medical technologists, pharmacists, cleaning staff and receptionists.
[f] Livestock such as pig, poultry, goat, sheep, cattle or other.
[g] Pets such as cat, dog-, rabbit, tortoise.
[h] Determined via PCR-screening of *S. aureus* isolates for Panton-Valentine leukocidin (PVL) toxin genes (lukF-PV).

Figure 2

Table 2. Molecular characteristics of methicillin resistant *Staphylococcus aureus* (MRSA) in students and healthcare workers in Antananarivo, Madagascar

n = 28 [a]	*spa* types [b]	MLST [c]	PVL+ [d], (n)
21	t186	ST-88	0
1	t852	unknown	1
1	t2393	unknown	0
1	t5562	unknown	0
2	t5772	unknown	0
1	t11285	unknown	0
1	t13653	unknown	0

[a] Confirmed via PCR-screening of *Staphyloccus aureus* (*S. aureus*) isolates for MRSA specific (*mecA* and *mecC*) genes.
[b] Staphylococcal protein A (*spa)* sequence typing was performed on MRSA isolates with the Ridom Staph standard protocol and the Ridom *Spa*Server (http://spa.server.ridom.de).
[c] Associated Multi Locus Sequence Types were allocated with Ridom *Spa*Server, if possible.
[d] Determined via PCR-screening of *S. aureus* isolates for Panton-Valentine leukocidin toxin genes (lukF-PV).

HYP05

Form Surveillance to Awareness: Current developments in electronic infection control support systems

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Introduction: Surveillance and timely reaction on changes in the epidemiology of nosocomial pathogens, healthcare acquired infections and antibiotic resistance as well as monitoring of antibiotic usage are key elements in infection prevention and control. However, conventional, paper-based Surveillance is time consuming and can severely impair the time to reaction e.g. in the case of nosocomial outbreaks. Electronic systems could offer an easier and more effective way, but need a careful planning and a sensible validation of reported Results:

Materials and Methods: We will show chances, challenges, opportunities and applications for electronic support systems and their advantages and drawbacks in contrast to conventional, paper based methods. The technical environment of such systems, data sources and ways of data management and presentation will be discussed.

Results: Electronic systems could have the potential to greatly increase the efficacy and speed of surveillance and reaction, given that they are connected to clinical and laboratory data sources and are able to collect and manage all relevant data and could be a real support for the infection control staff. Electronic systems could therefore guide the way from retrospective surveillance to proactive awareness approaches in infection control.

HYP06
The AWARENESS-Study

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Contact precautions are a cornerstone against multidrug resistant organisms (MDROs). As with all hygienic measures, the impact of contact precautions strongly depends on the compliance of health care workers (HCWs) with these measures, the more, as contact precautions have been associated with less HCW contact and adverse clinical outcomes in the past. Still, little is known about the perceptions, attitudes and beliefs of HCWs with measures to control MRDOs. To assess the perceptions of HCWs in Mecklenburg - Westpommerania, the AWARENESS (Auswirkungen von MRE auf die Versorgung von Betroffenen in der Gesundheitsregion Ostseeküste)-study was conducted as part of the HICARE - Gesundheitsregion Ostseeküste project. We will present the results of this just finished study that was based on standardized questionnaires that were sent to 39 healthcare facilities.

HYP07
Development of a molecular method for quantification of *Escherichia coli* in raw and drinking water

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Introduction: Fecal pollution of the water cycle can be assessed via detection of indicator bacteria as waterborne pathogens. Conventional approaches apply culture based methods; hence they are time-consuming and laboratory intensive. A faster microbial contaminant detection method will be molecular approaches as the quantitative real-time PCR (qPCR), although the state of development of similar methods described in literature is still insufficient for the routine monitoring of drinking water [1; 2]. The aim of the study described here was to especially develop a technique for water analysis in terms of fast discrimination of live, dead and viable but non-culturable (VBNC) organisms.
Quantitative *real-time* PCR (qPCR) Water samples spiked with a distinct number of *E. coli* cells were filtered and cells were pretreated with propidium monoazide (PMA). PMA binds to DNA of dead bacteria and bound DNA will subsequently not be amplified by a PCR reaction. The derived genome counts of viable bacteria were quantified using SYBR Green-based qPCR with *E.coli*-specific primers.
Conclusion and future prospects: First results of our studies suggest that the developed technique can complement culture-based methods by discrimination of live and dead bacteria which is of great importance when bacteria occur in the VBNC-state after disinfection procedures or usage of copper in tap systems. To evaluate the new technique, it will now be applied to raw- and drinking water samples in parallel with accredited techniques of routine analysis.

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HYP08
Intensive care physicians' and nurses' attitude towards the preventive effect of hand hygiene in terms of reducing pathogen transmissions: Are there associations with psychological predictors of compliance to hygiene standards?

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Infection prevention is a necessity in all medical facilities and should meet the highest requirements in order to protect health care providers and patients. At the same time, data from Hanover Medical School, Germany, has shown a decreasing hand disinfection compliance in intensive care units in recent years [1]. In order to improve hand hygiene behaviour and to reduce health care associated infections (HAIs), current hand hygiene training approaches need to be advanced. The present study focuses on the question whether there are associations between the belief that hand disinfection prevents transmission of pathogens related to HAIs and psychological predictors of compliance to hygiene standards, including motivation. In the research project PSYGIENE (PSYchologically optimised hand hyGIENE promotion), 307 intensive care physicians (P) and 348 nurses (N) at Hanover Medical School completed a hygiene-related questionnaire. This assessed pathogen transmission-related risk perceptions; perceived overall preventive effectiveness of hand disinfection (outcome expectancy); motivation defined as the intention to disinfect one's hands compliantly; self-efficacy, i.e. the belief in one's capabilities to organize and execute compliant behaviour; and guideline awareness. Logistic regression analysis was utilized to assess the impact of the belief that one's hand disinfection behaviour prevents pathogen transmissions. The results show that the health care providers generally believe that their own hand hygiene prevents the transmission of pathogens. However, physicians are less convinced that their own hand hygiene is effective in this sense (N: 67% vs. P: 48%, $p < .001$). While overall, 75% of physicians and 78% of the nurses reported highest motivation to implement hand hygiene, this was most frequent when reduction in transmission risk was perceived as high (P: 96% vs. 71% in the group without perceived risk reduction, p=.029; N: 91% vs. 67%, p=.007). Similar patterns were obtained for the belief that one's hand hygiene eventually contributed to infection prevention (P: 89% vs. 66%; p=.039 and N: 92% vs. 67%; p=.004), and for the belief to be able to consistently maintain hand hygiene (self-efficacy; P: 70% vs. 44 %, p= .029 and N: 61% vs. 20%, p=.001). Awareness of guidelines was generally more infrequent, and not significantly associated with transmission risk-related beliefs (P: 37% vs. 19%, p= .085; N: 44% vs. 33%, p= .310). Results suggest that while promoting the belief that hand disinfection prevents pathogen transmission may increase a motivational mindset, particularly for physicians, guideline awareness is not associated with this risk perception, and thus may require experientially oriented approaches such as skills training.

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INFECTION IMMNUOLOGY

IIP01
Hypoxia promotes *Mycobacterium tuberculosis* specific upregulation of Granulysin in human T-cells.

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Introduction: Oxygen tension affects local immune responses in inflammation and infection. In tuberculosis mycobacteria avoid hypoxic areas in the lung as they persist and reactivate in the apex of the lung. On the other side hypoxia is beneficial for the host, as oxygen restriction activates antimicrobial effector mechanisms in macrophages and restricts growth of intracellular *Mycobacterium tuberculosis* (*M.tb*).
Materials and Methods: Cell culture. Preparation and viability of T-cells and antigen presenting cells (APC). Hypoxia chamber. Measurement of cytokines. Quantitative LightCycler PCR. Flow cytometry.
Results: To investigate the influence of hypoxia on adaptive immunity in tuberculosis, we measured the expression of the antimicrobial effector molecule granulysin which is expressed in cytolytic lymphocyte subsets. We found a 2.4-fold up regulation of granulysin mRNA-levels after stimulation of *M.tb*-specific T-cells with secreted mycobacterial antigens (PPD) at 1% O_2 as compared to physiological oxygen levels (20% O_2). Flow cytometry analysis showed that granulysin was initially secreted, followed by an up regulation 48 h after stimulation with PPD and heat inactivated *M.tb*. The cellular composition of granulysin positive cells was independent from oxygen tension.
Discussion: We suggest that hypoxia supports the efficacy of the adaptive immune response in human *M.tb* infection by up regulation of the antimicrobial effector protein granulysin.

IIP02

Burkholderia pseudomallei-mediated modulation of iron homeostasis promotes bacterial growth in macrophages

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Introduction: Iron is essential for both human and bacterial life, and infection initiates a competition for this nutrient between host and invading pathogen. Iron homeostasis is tightly regulated by the peptide hormone hepcidin, primarily produced in hepatocytes. Hepcidin triggers degradation of the iron export protein ferroportin, thereby decreasing the delivery of iron into the circulation. A few studies indicate that iron contributes to enhanced growth of *B. pseudomallei* in soil as well as biofilm formation, but how the pathogen competes with the pathways of iron export and storage of their host cell is unknown. Thus, we investigated whether *B. pseudomallei*, the causative agent of melioidosis, alters iron homeostasis and how exogenous modulation of host cell iron content affects intracellular bacterial growth.
Results: Infection of murine bone marrow-derived macrophages with *B. pseudomallei* caused an upregulation of hepcidin and downregulation of ferroportin expression. Treatment with hepcidin as well as iron supplementation by ferric ammonium citrate (FAC) enhanced intracellular growth of *B. pseudomallei*, whereas limitation of macrophage iron by desferrioxamine (DFO) reduced bacterial survival. Following intranasal infection with *B. pseudomallei*, mice displayed decreased ferroportin expression and elevated iron concentration in the liver, but higher systemic hepcidin and lower systemic iron levels (hypoferremia). Intraperitoneal administration of FAC promoted *B. pseudomallei* growth at the primary site of infection accompanied by increased dissemination to distant organs, higher proinflammatory response and serum hepcidin levels. Conversely, iron chelation by DFO diminished bacterial loads in lung, liver, and spleen, attenuated both serum cytokine and hepcidin levels and improved survival of mice.
Conclusion: Our data provide evidence that modulation of the iron balance by hepcidin and ferroportin might be a strategy used by *B. pseudomallei* to increase cellular iron and promote their own intracellular growth. Thus, defects in iron homeostasis can have an important role in susceptibility to melioidosis.

IIP03

Construction of multi-functional *Salmonella* vaccine strains using mini-Tn7 transposons for targeted chromosomal integration of multiple heterologous expression cassettes

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Stable and targeted introduction of expression modules for heterologous antigens or other factors into bacterial chromosomes is one important aspect for the development of bacterial vector vaccines. mini-Tn7 transposons have been used for the insertion of such modules into a single chromosomal site of Gram-negative bacteria, so called *attTn7* . However, chromosomal integration of multiple copies of an expression cassette might be more suitable for the expression of appropriate amounts of antigen. In addition, other expression modules for factors mediating antigen delivery or potentiating vaccine efficacy should also be stably introduced. Here we demonstrate that mini-Tn7 integrates simultaneously at multiple locations in the chromosome of the aroA-attenuated *Salmonella enterica* serovar Typhimurium vaccine strain SL7207 when an artificial *attTn7* site was introduced at positions of choice by targeted homologous recombination. To us, this was surprising in the light of previously reported Tn7 target immunity. We accomplished simultaneous integration of up to four mini-Tn7 transposons. Integrations were verified by PCR and whole genome sequence analysis. In addition, alternating chromosomal introduction of artificial *attTn7* sites followed by transposition allowed the consecutive integration of two different mini-Tn7 transposons harbouring expression cassettes for ß-galactosidase and a bacterial luciferase. When applied to Balb/c mice these bacteria were colonization proficient and stably retained both expression cassettes. Thus, we suggest the use of mini-Tn7 for targeted and stable multi-copy integration of expression cassettes into the genome of *Salmonella* vaccine strains, which should facilitate the equipment of such vaccines with multiple functions.

IIP04

Identification of infectious microalgae *Prototheca spp.* specific immunogenic proteins using proteomic *analysis.*

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Introduction: Microalgae of the genus *Prototheca (P.)* are non-green unicellular algae closely related to *Chlorella spp*. *Prototheca* sp. are associated with rare but severe infections in vertebrates, often referred to as Protothecosis. Among cattle, it is associated with a resistant form of bovine mastitis and hence is of economic importance. The main infectious agent appears to be *P. zopfii* genotype 2 (GT 2), whereas genotype 1 is classified as non-virulent. In addition, *P. blaschkeae* is also isolated from cattle affected by bovine mastitis. It is of interest to investigate specific antigenic proteins present in GT2 that might be responsible for its infectivity.
Materials and Methods: Whole cell proteins extracts from *P. zopfii* GT1, GT2 and *P. blaschkeae* were separated using two-dimensional gel electrophoresis (2D-PAGE) followed by Western Blotting using serum from rabbits experimentally challenged with GT2. 89 spots (23 of *P. zopfii* GT2, *33* of GT1, and 35 of *P. blaschkeae)* were excised, digested with trypsin and identified using MALDI-TOF mass spectrometry.
Results: In the absence of *Prototheca* genome information, about 68% (61 proteins) of investigated proteins were identified. They were mainly involved in energy metabolism, transcription, cellular transport, and cell signaling. Three proteins (transcription elongation factor 1-alpha, malate dehydrogenase, and heat shock protein 70) appear to be common antigens among *P. zopfii* GT1, GT2 and *P. blaschkeae*.
Conclusion: Enhanced metabolism might be attributed to the survival of the algae in the macrophages during the early stage of infection. Further investigations using serum from naturally infected hosts such as cows or dogs might provide additional clues on the mechanism of this algal infection.

IIP05

Dense Granula proteins derived from *Toxoplasma gondii* as target structures for B- and T- cell responses

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Protection against the parasite *Toxoplasma gondii* in infected individuals is realized by humoral and cellular immune responses. We here analyze the capacity of Toxoplasma-Lysat-Antigen (TLA) and of a range of different recombinant Toxoplasma proteins (GRA1, GRA2, GRA7, GRA9, MIC5, BAG1, SAG1) as targets for cellular and humoral immunity in infected and noninfected individuals of three different species.
Humoral response was evaluated by Western Blot analysis of human, murine and porcine sera, revealing the presence of specific antibodies against different recombinant Toxoplasma proteins. Cellular immunity was investigated by determining T- cell proliferation to TLA or different recombinant antigens in spleen cell cultures or isolated PBL cultures. Analyzing sera of seropositive human, swine and mice, antibodies against GRA1 and GRA2 could be found most frequently (100%), followed by GRA7 (90-100%) and GRA9 (60-91%). Cells from all infected individuals proliferated to TLA whereas no response was observed in cultures of the uninfected control group. Response to the recombinant antigens was variable between individuals in all species and none of the analyzed antigens elicited proliferation in all cultures from seropositive individuals. Therefore we come to the conclusion that infection with *T. gondii* can be detected by T-cell proliferation in response to TLA or recombinant antigens. In addition the tested recombinant antigens can be used diagnostically to detect the presence of specific antibodies against the parasite.

IIP06
Global and compartment-specific kinase activation in response to TDM via Mincle

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The glycolipid Trehalose-6,6-dimycolate (TDM), also known as cord factor, is a major constituent of the mycobacterial cell wall and may play a dual role in infection. On the one hand TDM is a virulence factor of pathogenic mycobacteria, which inhibits phagosome maturation and by association the destruction of the bacteria. On the other hand TDM is also recognized as a pathogen-associated molecular pattern (PAMP) that triggers the innate immune system. Our group and others have identified the C-type lectin receptor Mincle as the Pattern recognition receptor for TDM and also for its synthetic analogue Trehalose-6,6-dibehenate (TDB). After TDM recognition, downstream signaling is effected by means of the Syk-Card9-Bcl10-Malt1 pathway. In this work the effector functions and molecular mechanisms, triggered by the recognition of TDM via Mincle, shall be investigated more precisely to obtain insight into the reprogramming of macrophage signal transduction by the cord factor. The main interests are the global and compartment-specific kinase activation in response to TDM via Mincle. The compartment-specific examinations bear on the recruitment and the phosphorylation of signaling proteins to the cell membrane and the phagosome by TDM. For this we isolate the phagosomes of murine bone marrow-derived macrophages by magnetic beads and aim to identify kinases, adapter molecules and other signaling proteins recruited to the phagosome.

IIP07
Role of Nrf2-dependent regulation of glutathione synthesizing enzymes in *Burkholderia pseudomallei* infection

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Introduction: Nuclear erythroid-related factor 2 (Nrf2) is a redox-sensitive transcription factor protecting cells from oxidative stress by regulating cytoprotective genes, including the antioxidant glutathione (GSH) pathway. GSH homeostasis is regulated by *de novo* synthesis dependent on glutamate-cysteine ligase (GCL) and glutathione synthase (GSS) as well as GSH redox state mediated by glutathione reductase (GSR). The present study was designed to investigate the role of Nrf2 in the regulation of GCL and GSR during *in vitro* and *in vivo* infection with *B. pseudomallei*, the causative agent of melioidosis, and to elucidate their function in bacterial host defence.
Results: Infection of bone marrow-derived macrophages from C57BL/6 or Nrf2 wild-type ($Nrf2^{+/+}$) mice with *B. pseudomallei* resulted in nuclear accumulation of Nrf2 and increased transcription of catalytic and modifier subunit of GCL and GSR. Induction of GCL enzymes was completely dependent on Nrf2 activation, whereas GSR mRNA expression was regulated by Nrf2-independent pathways as well. Deficiency of Nrf2 ($Nrf2^{-/-}$) significantly promoted clearance of *B. pseudomallei* in macrophages and was associated with reduced release of lactate dehydrogenase. Furthermore, inhibition of GSH synthesis by the GCL inhibitor buthionine sulfoximine (BSO) decreased intracellular growth of the pathogen. C57BL/6 mice intranasally infected with *B. pseudomallei* displayed significantly higher mRNA levels of Nrf2, GCL and GSR in spleen compared to non-infected mice, but lower hepatic expression of respective genes 48 hours after infection. In accordance with the observed *in vitro* effects, intraperitoneal administration of BSO to mice diminished bacterial loads in lung, liver and spleen compared to vehicle treated mice.
Conclusion: Our data suggest that increased Nrf2-dependent expression of glutamate-cysteine ligase impairs host defence against *B. pseudomallei*, which might be due to enhanced activation of cytoprotective antioxidant responses. Further investigations are needed to elucidate the function of GSH as well as glutathione reductase during *B. pseudomallei* infection.

IIP08
Lipobiotin: A synthetic triacyl lipopeptide facilitates noncovalent labelling of microorganisms for magnetic phagosome isolation

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Here we describe the synthesis and the properties of Lipobiotin, a triacyl lipopeptide, which can be used to functionalize hydrophobic surfaces.
Lipobiotin is the synthetic triacyl lipopeptide PHC-KKKKK(biotin-Aca-Aca). It has been previously used to label hydrophobic surfaces with biotin [1]. It is synthesized by solid phase peptide synthesis using Fmoc/tBu chemistry followed by N-terminal modification to obtain the triacyl membrane anchor N-Palmitoyl-S-(1,2-bishexadecyloxy-carbonyl) ethyl-[R]-cysteine (PHC). Biotin is attached to the membrane anchoring lipid part via spacer molecules at the side chain of the C-terminal lysine. Due to the hydrophilic peptide moiety, Lipobiotin can be administered from aqueous solutions. The interaction with avidin or streptavidin makes it a versatile reagent which can be used for detection, labelling or immobilisation in many research applications. Lipobiotin has been recently successfully employed to label different microorganisms and has been the key step for subsequent magnetic isolation and characterization of pathogen-containing phagosomes. The approach facilitates the purification of intact bacteria-containing phagosomes for their comprehensive biochemical characterization from as few as $2x10^7$ primary cells [2]. In addition the new labelling approach was also used to isolate apoptotic vesicles from human polymorphonuclear cells [3]. Lipobiotin is thus a useful tool to noncovalently label microorganisms and subsequently isolate and characterize microbe-containing compartments, in order to identify microbial and host cell targets for novel anti-infective strategies.

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IIP09
The Evaluation of Th17 cell effector cytokines Levels in *Helicobacter Pylori*-infected peptic Ulcer and Gastritis Patients

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Introduction: *Helicobacter pylori*(*H.pylori)*is causally associated with peptic ulcer andgastritis. This microorganism induces cellular immune response. Among different lymphocytes, Th17 plays an important role in defense against *H.pylori* and causes inflammatory response. *H.Pylori* may cause gastritis and peptic ulcer is because of increased activation of Th17 and changes of cytokines such as IL-17A, IL-21, IL-22, IL-23 and TGF-β.
Aim: To find the relationship between Th17 and IL-17A, IL-21, IL-22, IL-23, TGF-β in the patients suffering from *H. pylori* infection with signs peptic ulcer, and gastritis.
Materials and Methods: Thirty-sixsamples of patients with gastrointestinal problems were collected.The percentage of Th17 was measured by Flowcytometry. The levels of IL-17A, IL-21, IL-22, IL-23, and TGF-β cytokines were measured by ELISA.
Results: Patients were divided into three group's Peptic ulcer, gastritis, and normal (15 male, 21 female)with the mean age of 4116 years. The percentage of Th17 in the patients with peptic ulcer and gastritis was significantlymore than the normal counterparts ($p \leq 0.001$). The serum levels of IL-17A,IL-23 andTGF-β in peptic ulcer and gastritis groups were significantlyhigher compared to the levels in the normal population ($p \leq 0.01$). Changes in levels of IL-21 and IL-22 in peptic ulcer and gastritis groups were low.Increased levels of IL-17A, IL-22, IL-23, and Th17 were significantly observedin gastritis compared to normal population ($p \leq 0.001$).

Conclusion: We can conclude that Th17 and the associated cytokines such as IL-21, IL-23, IL-22, IL-17A and TGF- β can play a critical role in peptic ulcer and gastritis in the individuals infected with *H. pylori*.

IIP10

Association of IL-21 and TGF-β with Th17 in peptic ulcer and gastritis inducer by *Helicobacter pylori*

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Introduction: *Helicobacter pylori* (*H. pylori*) is a spiral shape bacterium, it is important human pathogens, responsible for most peptic ulcer diseases and gastritis. Infection by *H. pylori* can caused the immune response in mucosal immunity.IL-21 and concentration of TGF-β induce differentiation of Th17 cells.Th17 cells are associated with inflammatory diseases such as gastritis. Accordingly, we aim to investigate the relationship of TGF-β, IL-21 and Th17 cells, with peptic ulcer and gastritis in-patient's infected with *H. pylori*.
Materials and Methods: Twenty-four *H. pylori*-infected patients were enrolled into the study, consist of: 12 peptic ulcers, 12 gastritis (Moderate and Mild) and 12 individual negative for *H. pylori*. PBMCs isolated from the patients were stimulated with *H. pylori* antigens and then the levels of TGF-β and IL-21 in the culture supernatants and Th17 frequency was measured using ELISA and Flow cytometer, respectively.
Results: In patients with peptic ulcer and gastritis Th17 frequency were significantly higher than the normal counterparts ($p \leq 0.001$).Significantly increased levels of TGF-β with IL-21 were observed in peptic ulcer and gastritis, compared to that in normal group ($P \leq 0.0001$).
Conclusion: A significant association ofIL-21 and TGF-β with Th17cells was observed in gastritis patients, compared with control group.

IIP11

Infection course of an attenuated *Salmonella enterica* serovar Typhimurium vaccine strain with deficiencies for synthesis of aromatic amino acids, diaminopimelic acid and the virulence factor *sifA* in HeLa cells

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Live attenuated *Salmonella* vaccines have a proven track record in human and veterinary medicine. Engineered variants of such strains constitute promising carriers for heterologous antigens and DNA vaccines. Prerequisites for the delivery of heterologous cargo from the bacteria into the cytoplasm of the infected mammalian host cell are the escape from the endosomal compartment and release of bacterial content within the host cell. Here, we used a GFP-tagged derivative of the *aroA*-attenuated *Salmonella enterica* serovar Typhimurium strain SL7207 lacking in addition genes *asd* and *sifA*. *asd* mutant bacteria are auxotroph for diaminopimelic acid (DAP), an important component of peptidoglycan cross-links. Upon removal of the complementing DAP from the culture, such bacteria quickly lyse and release intracellular content into the supernatant. The *sifA* deficiency has been shown to increase the number of bacteria escaping from the endosomal compartment into the host cell cytoplasm. In order to find optimal conditions for *Salmonella* mediated delivery of protein or DNA into the cytoplasm of HeLa cells, we studied the course of bacterial infection under varying conditions of DAP supplementation of the cell culture medium with respect to DAP concentration and time of DAP withdrawal. Intracellular green fluorescent bacteria were tracked microscopically and quantified by plating. Under such conditions our data revealed the time frame of bacterial lysis and HeLa cell toxicity depending on the bacterial load. Thus, this work helps us to further optimize the protocol for *Salmonella* mediated delivery of heterologous cargo into mammalian cells.

IIP12

Antibody detection against *Plasmodium vivax* apical membrane antigen 1 using ELISA method among residents in Siahoo, southeast of Iran

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Introduction: Although *Plasmodium vivax* infection basically considered benign, it causes heavy burden of disease in endemic areas. Successful control of malaria needs numerous factors such as detection the level of antibodies in individuals resident in the endemic areas. This study aimed to detection antibody response against *Plasmodium vivax* apical membrane antigen 1 among residents in Siahoo, Iran.
Materials and Methods*:* A total of 487 sera were collected from individuals residing in Siahoo, a district in Hormozgan province located in southeast of Iran where no malaria transmission has been occurred for recent three years. Sera were separated according to the age groups to determine whether there was a correlation between the frequency of antibodies and age. For this purpose, samples divided into three groups including: Group I: 15-19 years old, Group II: 20-29 years old, Group III: ≥ 30 years old. Antibody detection was assessed by indirect ELISA using recombinant *PvAMA-1*.
Results: The results showed that 14% of the total subjects in Siahoo district presented totalIgG antibodies to AMA-1 recombinant protein. But frequency of serum samples containing specific antibodies in groupIwas significantly lower than the other groups, while there was not statistically different between groups (II)& (III).
Discussion:It is important to notethat thesubjects were significantly differed in years of exposed to malaria in Siahoo, this matter reflected most likely exposure to malaria parasite and possibly maturation of the immune system over time, moreoverpersisted ofthe detected antibodies against PvAMA-1 correlated with the long term of exposure to malaria in Siahoo.
Conclusion: There was correlation between the frequency of antibody response to PvAMA-1 and the age, also humoral immunity to *P. vivax* can be maintained even in the absence of continuous re-exposure to the parasite.

IIP13

In vivo interactions of *Pseudomonas aeruginosa* with the innate and adaptive immune system

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The Gram negative bacterium *Pseudomonas aeruginosa* is an ubiquitous opportunistic pathogen. Major targets are patients with cystic fibrosis, an autosomal-recessive inherited disease of secretory glands. Favored by the inflammation inside such patient's lung, *P. aeruginosa* rapidly converts from its motile, planktonic form to the persistent biofilm growth state. Besides its intrinsic resistance to many antibiotics, formation of biofilms additionally protects the bacteria from exogenous assaults like antibiotics as well as the immune system of the host. Thus, the understanding of the molecular mechanisms that lead to formation of biofilms is most important for the design of new intervention strategies.
Our recently established model of solid murine tumors colonized by *P. aeruginosa* was used to investigate biofilm formation *in vivo*. BALB/c wild type or syngeneic lymphopenic Rag1-/- mice were subcutaneously injected with CT26 colon carcinoma cells and intravenously infected with the highly virulent *P. aeruginosa* wild type strain PA14 (UCBPP-PA14). After 24h, the bacteria colonized the tumors of all mice in high numbers. In addition they formed biofilms. This was first assessed by an antibiotic resistance assay where biofilm-competent PA14 appeared to be more resistant. In addition

electron microscopy was performed. PA14 formed microcolonies. Within such clusters, the individual bacteria were embedded in nests of extracellular matrix material i.e. biofilms. When tumors were analyzed by immunohistochemistry, co-localization of *P. aeruginosa* and neutrophils (PMNs) was detected. Whether such cells are involved in the development of biofilms as observed for other bacteria could not be tested. After depletion of PMNs by anti-Gr1 and subsequent infection, the mice were not able to combat the infection anymore. During these experiments we noticed that the tumors of BALB/c mice shrank after colonization by *P. aeruginosa*. Some mice even cleared the tumors. Reconstitution of lymphopenic Rag1-/- animals with T cells from such mice revealed the presence of tumor specific T cells. Interestingly, when CD4+ T cells as well as CD8+ T cells of uninfected mice bearing CT26 tumors were transferred, a similar anti-tumor activity could be demonstrated. Obviously, the infection of tumor bearing mice activates T cells that are already present in such mice but are not able to reject the CT26 tumors. Which T cell subsets are responsible for the opposed tumor development will be studied now.
Thereby, our solid tumor model enables future investigations concerning the interaction of immune cells and *P. aeruginosa in vivo*. Signaling pathways which lead to the recruitment of neutrophils inside the colonized tumor as well as screenings for intervention strategies for the development of bacterial biofilms are issues which might be addressed.

IIP14

Protective role of coagulation factor XIII in *Streptococcus pyogenes* skin infections

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The intrinsic pathway of coagulation (contact system) represents a double-edged sword in immunity and infection. Systemic contact activation during invasive *Streptococcus pyogenes* infections contributes to the pathogenesis of sepsis and severe diseases. However, today it is generally accepted that coagulation is also part of the early innate immune response against *S. pyogenes*. In this process, surface proteins, such as the streptococcal M1 protein are targeted by coagulation factor XIII (FXIII), leading to the entrapment of *S. pyogenes* within the fibrin clot.
In this study the FXIII mediated immobilization of *S. pyogenes* within the fibrin network was investigated by plating experiments and scanning electron microscopy (SEM). The role of FXIII *in vivo* was analyzed by using a murine skin infection model.
Our results suggest that the M1 serotype of *S. pyogenes* adopt a special role in comparison to other serotypes, because the entrapment of this serotype within the fibrin clot is influenced by FXIII. Reduced numbers of bacteria were found associated with the fibrin network when either FXIII-deficient plasma or *S. pyogenes* mutant strains, lacking the M1-protein were used. Nevertheless, entrapment of the M1-deficient mutant strains was not completely abolished, suggesting that also other surface structures are targeted for bacterial entrap-ment. To analyze if FXIII plays also a role during *S. pyogenes* infection in vivo, a murine skin infection model was used. To this end, CBA wildtype and FXIII-knockout (FXIII-KO) mice were infected subcutaneously with *S. pyogenes* strain 5448 of serotype M1T1. The survival of FXIII-KO mice was dramatically reduced compared to wildtype animals but reconstitution with a human FXIII concentrate (Fibrogammin®P, CSL Behring) could significantly improve the survival of FXIIIKO mice. The measurement of the activated partial thromboplastin time (aPTT) in the plasma of infected animals showed normal clotting times 72h after infection in wildtype mice, whereas the aPTT of FXIII-KO mice was highly prolonged, suggesting a systemic contact system activation in these animals. In reconstituted FXIII-KO mice the clotting times after 72h of infection remained normal.
Taken together, our results implicate that immobilization of *S. pyogenes* into the fibrin network is influenced by FXIII and the streptococcal M1 protein. This pathogen entrapment represents an early host defense mechanism to prevent bacterial spreading and systemic complications.

IIP15

Delivery of functional DNA and messenger RNA to murine and human antigen-presenting cells by recombinant fission yeast

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The import of functional nucleic acids like DNA and messenger RNA into mammalian cells has proven to be a powerful tool in cell biology and several delivery systems have been described. However, as targeting of particular cell types is a major challenge and DNA/mRNA vaccination represents a promising means for the induction of cellular immune responses, there is a need for novel delivery systems that permit the introduction of functional nucleic acids to immune cells. Here, we describe a delivery system based on the yeast *Schizosaccharomyces pombe* that allows the delivery of functional DNA and mRNA to mammalian antigen-presenting cells such as human dendritic cells. Further, we demonstrate that *Sz. pombe* cells effectively induce the maturation of human dendritic cells, an important step for T-cell activation. In conclusion, this new yeast-based system suggests itself as a promising approach for the development of a novel type of live vaccines.

IIP16

Comparative proteomic analysis of caspase-1/11 deficient and wild-type macrophages infected with *Burkholderia pseudomallei*

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Introduction: *B. pseudomallei*, the causative agent of melioidosis, is able to invade and survive within the cytosol of professional phagocytes. Its type III secretion system 3 plays a major role in the early NOD-like receptor C4-mediated activation of the caspase-1 inflammasome. Subsequent events involve the processing of caspase-9, -7 and PARP, the release of IL-1β and activation of pyroptotic cell death, which serves as a host defence mechanism to restrict intracellular bacterial replication. Deficiency of caspase-1/11 in murine melioidosis is associated with reduced survival and increased *B. pseudomallei* growth. Caspase-1/11 knockout macrophages exhibit induction of apoptotic cell death, dependent on processing of apoptosis-related caspases, at a later stage of infection. However, the underlying molecular mechanisms are still unknown.
Materials and Methods: We carried out a proteomic analysis of bone marrow-derived macrophages from caspase-1/11 knockout and C57BL/6 wild-type mice with and without infection in order to study the host cell responses to *B. pseudomallei* and absent caspase-1/11-mediated defence mechanisms. Proteins were analysed at 1.5 hours after infection and peptide identification was carried out gel-free by HPLC/ESI-MS/MS. Altered functions and pathways were mapped using semi automated pathway analysis.
Results: Using a cutoff of 2-fold we identified 274 proteins differently regulated between infected and non-infected C57BL/6 macrophages. With a number of 226 the majority of these proteins were decreased in abundance, whereas 48 proteins were higher expressed in response to *B. pseudomallei*. Altered proteins were found to be involved in molecular transport, mitochondrial dysfunction, oxidative stress and inflammatory response. On the contrary, comparison of infected caspase-1/11-deficient and wild-type macrophages revealed 99 out of 153 changed proteins to be upregulated in macrophages lacking caspase-1/11. Pathway analysis suggests that differentially abundant proteins are related to both cytokine and apoptosis signalling as the primary pathways.
Conclusion: Our results indicate that already at early stages of infection, *B. pseudomallei* can cause substantial changes in the proteome of macrophages. Furthermore, this study provides valuable information concerning cellular processes affected by caspase-1/11.

IIP17
Evaluation of a latex agglutination test based on the A2 antigen derived amastigote forms of *Leishmania infantum* for rapid detection of human and canine visceral leishmaniasis

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The diagnosis of visceral leishmaniasis (VL) in humans and animal reservoir hosts is difficult, particularly in rural areas where the disease is endemic and laboratory facilities are limited. This study aimed to develop a latex agglutination test (LAT) for the rapid detection of anti-*Leishmania* antibodies against the A2 antigen derived from the amastigote form as well as those against crude antigens derived from the promastigote form of an Iranian strain of *Leishmania* (*Leishmania*) *infantum*. The A2 antigen (42-100 kDa) was prepared from the amastigote form of *L. infantum*, purified with electroelution and compared with the crude antigen from the promastigote form of *L. infantum.* Both antigens showed appropriate intensity reactions, were selected using dot blotting of positive and negative pooled sera and used to sensitize 0.9-µm latex beads. The tests were carried out on sera from 43 symptomatic, human patients with VL confirmed by parasitological examination and direct agglutination test (DAT), 30 healthy controls and 32 patients with other infections but without VL. Canine sera were collected from 63 domestic dogs with VL confirmed using parasitological examination and DAT and 31 healthy dogs from areas non-endemic for VL. Compared with the controls, human sera from DAT-confirmed patients yielded a sensitivity of 88.4% (95% CI, 82.1%-94.5%) and specificity of 93.5% (95% CI, 87.0%-99.7%) on A2-LAT (amastigote) when 1:3200 was used as the cut-off titre. Kappa analysis revealed a good degree of agreement between A2-LAT and DAT (0.914). LAT required 3-5 min to complete, versus the 12-18 h needed for DAT. Compared with the controls, A2-LAT of canine sera from DAT-confirmed cases yielded a sensitivity of 95.2% (95% CI, 95.0%-95.4%) and specificity of 100% (95% CI 100%) when 1:320 was used as the cut-off titre. Kappa analysis revealed a good degree of agreement between A2-LAT and DAT (0.968). Similarly, the sensitivity and specificity of Pro.-LAT (promastigote) was calculated to be 88.4% and 91.9% respectively for human sera and 96.8% and 90.3% respectively for canine sera. No statistically significant differences were observed between A2-LAT and Pro.-LAT for the detection of human and canine *L. infantum* infections. In conclusion, A2-LAT and Pro.-LAT could be used in parallel to screen for *L. infantum* infections in humans and dogs in areas endemic for VL in Iran.

CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

KMP01
Clinical, epidemiological and molecular investigation of a natural pneumonic plague outbreak caused by *Yersinia pestis*, Madagascar, 2011

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Yersinia (Y.) pestis, the causative agent of plague is endemic in Madagascar, particularly in the central highlands. Although plague has never been reported from the very North of the country, an unexpected pneumonic plague outbreak occurred in Ambilobe district, an extremely remote area. During only 27 days the index case infected 21 other people, and 15 of them died. According to WHO diagnostic guidelines 17 suspected, two presumptive and three confirmed human plague cases were identified. Subsequent epidemiological investigation revealed 39 contact persons. Trapping of animals was carried out in the surrounding natural focus. Molecular typing of *Y. pestis* from two human samples and five animal samples (*R. rattus)* revealed Malagasy specific 1.ORI3-k-SNP-genotype and four different CRISPR patterns.

From this highly progressive pneumonic plague outbreak with a fatality rate of 100% among non-treated patients, we conclude and discuss that 1.ORI3-k-*Y.-pestis* genotypes do have potential to cause larger epidemics. The occurrence of multi-resistant strains and the persistence of the pathogen in natural foci close to human settlements pose severe risks to the population in endemic regions and force the need of outbreak frontline response strategies.

KMP02
Rapid and multiplexed detection of *Legionella pneumophila* serogroups in urine samples by antibody microarrays on the analysis platform MCR3

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Introduction: Legionellae are ubiquitous waterborne bacteria that can infect susceptible people. The most common species *L. pneumophila* has at least 15 serogroups (Sg) and several monoclonal subgroups. Based on the frequent occurrence of diverse *Legionella* ssp. in water or air, a direct rapid serotyping would be promising. Furthermore, for epidemiological purposes a direct typing in patient's urine samples might be a valuable tool.
Materials and Methods: For the measurements of *L. pneumophila* Sgs on the MCR3, a flow-based chemiluminescence sandwich microarray immunoassay (CL-MIA) was developed. Single-useable antibody microarrays were produced. The sample was pumped through the chip after injection. Lipopolysaccharide structures were bound selectively on each microarray spot where the specific capture antibody was immobilized. A sandwich assay was developed by using biotin-labeled detection antibodies. After flow-based incubation with streptavidin-labeled horseradish peroxidase, subsequent washing and incubation with luminol-based substrate, the microarray was imaged by a CCD camera. The assay was performed within 36 min.
Results: First antibodies have been screened and calibrated on the MCR3. Using heat-inactivated *L. pneumophila* Sg1 subtype Bellingham cells, the limits of detection were determined to 6×10^3 CFU/mL for a commercially available polyclonal antibody, and 2.8×10^3 CFU/mL for a monoclonal antibody, respectively. Furthermore, in a proof-of-principle study, urine samples from patients were analyzed. Positive and negative urine samples, tested with the Standard method ELISA showed similar results in comparison with the new array. A screening of other monoclonal antibodies against Sg 1 - 15 is planned in the future. With the MCR3, a faster outbreak management could be possible.

KMP03
Comparison of three NAT assays for the detection of cytomegalovirus infection

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Introduction: CMV infection in immunocompromised patients represents a significant risk for a serious morbidity. Indeed, those patients belong to the major groups of transfusion recipients and were considered as high risk patients for transfusion-transmitted (TT)-CMV infection. The introduction of leukodepletion of blood products and the provision of CMV-negative blood products reduced the incidence of TT-CMV infections in risk populations. However, CMV break-through infections persist with 1-3 % of transfused high risk patients, not least possibly due to the window-phase donations during acute primary CMV-infection. In the present study, the sensitivity and performance of different amplification systems were evaluated for (a) individuals with acute or chronic infections and (b) blood donor pool screening.
Materials and Methods: The analytical sensitivity and the precision of the three commercial assays RealStar CMV PCR Kit, Sentosa SA CMV quantitative PCR-Kit (Vela Diagnostics), and the CMV R-gene PCR-Kit (bioMèrieux (Argene)) was compared using a twofold dilution series of plasma inoculated with the first WHO international Standard for CMV. DNA was extracted with the Nuclisens easyMAG for ID-NAT and a high volume extraction protocol (4.8 ml, chemagic Viral 5K) for blood donor pool screening. Plasma sample of a total of 54,451 blood donations from 18,405 individual donors were screened for CMV DNA using the RealStar CMV PCR assay (Altona Diagnostic Technologies). Furthermore, the

presence of anti-CMV IgA, IgM and IgG antibodies in CMV DNA positive individuals was determined using different immunological assays.
Results: All assays provided a good analytical sensitivity ranging from 37.66 to 57.94 IU/ml IU/ml for the ID-NAT. The analytical sensitivities observed using the high volume extraction method ranged from 10.23 to 11.14 IU/ml. All assays demonstrate intra- and interassay variabilities with variation coefficients <5%. In total, five CMV DNA positive donors (0.03%) were identified by routine CMV screening, with DNA concentrations ranging from 435 to 4.30E+03 IU/ml. Four donors already showed reactive IgA, IgM and/or IgG antibody titers (IgA+/IgM+/IgG-, IgA+/IgM+/IgG+), whereas one donor showed no presence of anti-CMV specific antibodies.
Conclusion: The occurrence of transfusion-associated CMV infection still requires further investigations and the evaluated methods present powerful basic tools providing sensitive possibilities for viral testing to diminish CMV break-through infections. CMV PCR screening facilitated the identification of one donor with a window-phase donation during acute primary CMV-infection.

KMP04
Development and usage of protein microarrays for the quantitative measurement of Panton-Valentine leukocidin

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Staphylococcus aureus (*S. aureus*) is a human pathogen that may harbor several exotoxins. One of these clinically important toxins is the Panton Valentine leukocidin (PVL). It is associated with skin- and soft tissue infections and necrotizing pneumonia. Different PVL-positive strains express different levels of PVL concentrations. Protein microarrays allow the quantitative measurement of the toxin expression as well as the influence of co-expressed proteins, e.g., TSST and alpha toxin or of external factors such as antibiotics.
Different PVL antibodies were generated and in different dilutions spotted on microarrays. In detail, the assay, including hybridization and precipitation staining, was described previously by Stieber et al. (2013). In this assay, 226 clinical isolates (MRSA and MSSA) were tested for their PVL expression and analyzed relating to their clonal complexes (CC), *agr* types and severity of disease. Additionally, the effect of various concentrations of oxacillin on PVL expression was tested with selected strains. The toxin concentration was normalized on colony forming units (CFU) counts.
In general, PVL levels correlated with clonal complex affiliations with, e.g., CC8 and ST93 isolates being high level or CC5 and CC80 isolates being low level producers. In some few CCs the amount of expressed PVL varied widely (e.g., CC 398, sequence type 291/813). In general, agr group I and IV strains produced more PVL than strains belonging to agr groups II and III. Isolates that were positive for the *tst1* gene yielded conspicuously low PVL levels or no PVL at all. First experiments showed increased PVL levels per CFU under influence of oxacillin.
A protocol for quantitative measurements of bacterial exotoxins by protein microarrays was established that can readily be applied also to other toxins and even to several toxins simultaneously. It facilitates fast data collection and a wide range of data analysis. In future experiments, the assay could be expanded using further antibiotics or other substances that might influence the toxin release by bacteria.

KMP05
Shigatoxin quantitative measurement using antibody microarrays

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Shigatoxin (Stx) producing Escherichia coli (STEC) cause human diseases such as diarrhea and hemolytic-uremic syndrome (HUS). The therapy of a STEC infection aims mainly on treating symptoms; and antibiotics are considered contraindicated assuming an increased toxin release when bacterial cells are destroyed. Stx protein microarrays allow the quantitative measurement of toxin expression. This allows measuring the effect of antibiotics on stx expression in order to identify antibiotics that can be used without causing an additional toxin release. Anti-stx antibodies against stx subtypes 1 and 2 were generated by phage display and spotted on microarray. Resulting arrays were used to discriminate the stx subtypes as defined by Scheutz et al. (2012). STEC strains were characterized by this antibody array; and in parallel also by DNA microarray in order to confirm stx subtyping. Furthermore, stx concentrations in culture supernatants after overnight incubation in Gram Negative (GN) broth were measured based on a method previously described for a staphylococcal toxin, PVL, by Stieber et al. (2013). Measured stx levels were normalized on CFU counts.
The stx antibody arrays were used to distinguish between stx subtypes using known isolates. In first experiments, the assay was able to detected and discriminate the variants stx 1, stx 2a, stx 2b, stx 2c/2d and stx 2e/2f. Two strains (O157:H7 and O104:H4) with common and clinically relevant subtypes stx1a and stx2a, respectively, were selected for further study. Cultures of these strains were incubated with several antibiotics in different dilutions. This included compounds blocking the bacterial cell wall synthesis (Cefotaxime, Imipenem, Fosfomycin), and antibiotics blocking bacterial protein / DNA synthesis (Gentamicin, Azithromycin, Cotrimoxazol). When testing without antibiotics, the two strains yielded different amounts of *in vitro* expressed stx. The O157:H7-positive isolate (stx1a) yielded an average stx concentration of 33 ng/ml; the O104:H4 isolate (stx2a) expressed 3 ng/ml. Cefotaxime decreased the stx levels in both isolates and the combination of cefotaxime and azithromycin caused an even more distinct decrease of stx expression. Contrarily, levofloxacin increased the stx expression in both strains. All other tested antibiotics showed only an insignificant influence on toxin expression.
This assay offers a new application for antibody protein arrays for subtyping and quantitative measurement of shiga toxins. In this case, their response on antibiotic treatment is available. First experiments indicate usability of cefotaxime, possibly combined with macrolides, while it should be cautioned against a use of fluoroquinolones in stx-related conditions. Tests with more EHEC strains are underway.

KMP06
Use of cotrimoxazole is a risk factor for colonization with PVL-positive *Staphylococcus aureus* in Gabon: Implications for cotrimoxazole prophylaxis in HIV patients

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Cotrimoxazole is recommended as a prophylaxis of opportunistic infections in HIV-patients to reduce the burden of *Pneumocystis jirovecii*, malaria and bacterial infections. However, as cotrimoxazole is also a broad-spectrum antimicrobial agent, its use might have impact on colonization patterns and resistance rates of the normal bacterial flora. The objective of this study was to compare colonization, antimicrobial resistance and virulence factors of *Staphylococcus aureu*s between HIV-patients and healthy controls.
HIV-patients (n=141) and healthy controls (n=206) frequency-matched for age were included in a cross sectional study in Gabon (3-9/2013). Nasal and pharyngeal swabs were screened for *S. aureus*. Isolates were subjected to susceptibility testing (Vitek 2), genotyping (*spa* typing, multilocus sequence typing, MLST) and PCR-based detection of virulence factors. Risk factors were assessed using a standardized questionnaire. Categorical data were compared using logistic regression and chi^2-test.
Ergebnisse: HIV-patients were more frequently colonized with *S. aureus* (36.9 vs. 31.6%, p=0.043). Isolates from HIV-patients were more frequently positive for the Panton-Valentine leukocidin (PVL, 42.1 vs. 23.2%, p=0.025). This was in line with a higher proportion of classical PVL-positive lineages (ST15 and ST152) in HIV-patients (51.8 vs. 34.8%, p=0.06). Use of cotrimoxazole was a risk factor for colonization with PVL-positive isolates (OR=2.8, p=0.036) and cotrimoxazole resistance (OR=3.0, p=0.048). PVL-positive isolates were associated with cotrimoxazole

resistance (OR=25.1, p<0.001) and skin and soft tissue infection (SSTI, OR=2.7, p=0.012).
Cotrimoxazole prophylaxis could select for PVL-positive and cotrimoxazole-resistant isolates. The higher proportion of PVL was a risk for SSTI. If patients are under cotrimoxazole-prophylaxis, physicians might consider a higher risk of SSTI in regions where cotrimoxazole-resistance is associated with PVL.

KMP07
Molecular detection and genotyping of porcine reproductive and respiratory syndrome virus (PRRSV) from first outbreak of PRRS in India

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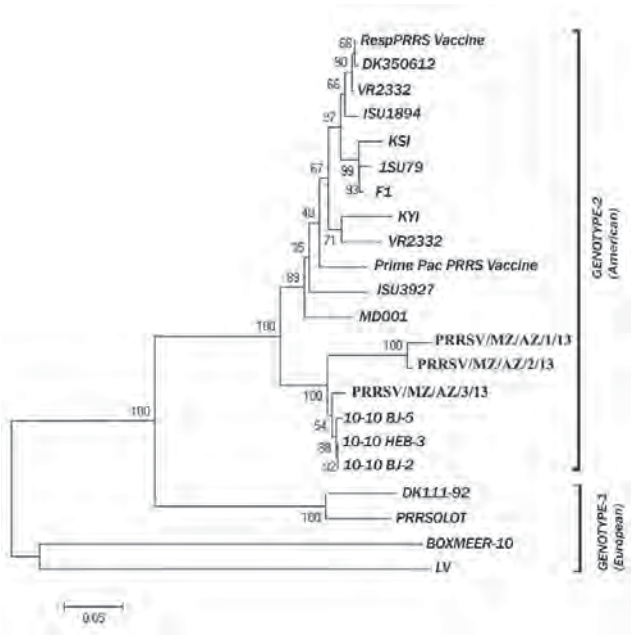
Introduction: PRRS is an economically important viral disease for the pig industry worldwide. In recent years, the disease has causedhuge losses to the Chinese pig production, particularly following the emergence of highly pathogenic PRRSV. India has reported its first outbreak of PRRS in pig populations of Mizoram, India, to OIE on 27th June, 2013.
Materials and Methods:A total of 880 pigs of different age groups from Mizoram, India were examined clinically at farm premises during the outbreak period from March to June, 2013. Serum samples from affected pigs were tested for PRRSV specific antibodies by ELISA. Detailed necropsy examination was carried out in dead animals and representative tissue samples from lungs, spleen, tonsil and mesenteric lymph nodes were collected for histopathological examination and molecular diagnosis by gel based RT-PCR.The PCR amplified 300bp fragment of ORF7 and 548bp fragment of ORF4 of PRRSV positive samples were cloned and the recombinant plasmids containing gene fragments were subjected to DNA sequencing for phylogenetic and molecular evolutionary analyses.
Results: Epidemiological studies on pigs of different age groups revealed 65% morbidity and 63.11% case fatality with highest mortality in pre-weaned piglets (81.06%).Enzyme immunoassay in the clinically suspected pigs revealed 73.65% sero-positivity for PRRSV-specific antibodies. Gross & histopathological examinations revealed severe interstitial pneumonia, congestion and hemorrhages with lymphoid depletion in lymph nodes and spleen. RT–PCR assay on tissue samples targeting the ORF7&ORF4 genes of PRRSV have confirmed the first PRRS outbreak in India. The nucleotide sequence identity and phylogenetic analysis of the gene sequences from ORF–7 (Acc.No. KF976728, KF208423) and ORF–4 (Acc. No.KF208420, KF208421, KF208422) revealed that the strain of PRRSV detected in India belonged to the North American type (genotype II) and had close link with the highly pathogenic PRRSV of genotype II, circulating in China.
Conclusion: Detection of PRRSV of genotype II in Mizoram, which caused high mortality in pigs and showed close link with the highly pathogenic PRRSV isolates of China, is a matter of great concern for the entire pig populations of India. A thorough and systematic study on surveillance, epidemiology and diagnosis is essential to know the magnitude of the disease and also for designing strategy to control and containment of PRRS in India.

Figure 1



KMP08
Prevalence of intestinal parasitic infection among school children in Nepal

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Intestinal parasitosis is endemic in least developed and developing countries including Nepal and is responsible for different types of morbidity and mortality. The objective of the study is to find out the prevalence of intestinal parasitic infection among school children in different districts of Nepal. We carried out a cross- sectional descriptive study of intestinal parasitosis in school children of four districts of Nepal in 2012. A total of 359 school children (boys: 166 and girls: 193) aged 5-16 of Gorkha (Aruchanaute and Aruarbang VDCs) (n=97), Gulmi (Turang and Balithum VDCs) (n=90), Nuwakot (Halde-Kalika VDC) (n=97) and Rupandehi (Padsari VDC) (n=75) were included. Stool samples collected in clean, dry, screw capped plastic container were mixed with 10% formal saline and transported to SICOST laboratory and examined by formal-ether concentration technique. A total of 33.4% (120/359) children were positive for at least one type parasitic infection (boys: 33.1%; girls: 33.6%). Marginally high prevalence was observed in Gorkha (40.2%) followed by Gulmi (33.3%) and others. Altogether 9 types of parasites were detected. Protozoan infection was more common (19.8%) followed by helminth infection (16.4%) and mixed infection (2.8%). Among the helminthes, Trichuris trichiura (49.1%) was more common followed by Ascaris lumricoids (28.8%) and other whereas among the protozoa Giardia lamblia (26.8%) was commonest followed by Entamoeba coli (15.5%) and others. Present findings indicated that intestinal parasitosis in Nepal still remains a challenge despite nationwide deworming program together with vitamin A and suggests an effective implementation of sanitation and safe drinking water programs together with basic hygienic practice among school children in these areas.

KMP09

Bacterial nutrient-sharing: Haemophilus influenzae stores and distributes hemin by using Protein E

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Introduction: The human pathogen *Haemophilus influenzae* causes mainly respiratory tract infections such as acute otitis media in children and exacerbations in patients with chronic obstructive pulmonary disease. We recently revealed the crystal structure of *H. influenzeae* protein E (PE), a multifunctional adhesin that is involved in direct interactions with lung epithelial cells and host proteins. Here we report that *H. influenzae* contains a PE-dependent storage pool of hemin, and this may result in a hemin supply to the *H. influenzae* populationwhen there, for example,is a paucity of hemin in the environment.
Materials and Methods: Hypothetical model of the PE-hemin complex was illustrated by putative docking of a hemin molecule in the binding pocket of the PE-dimer. Enhanced chemiluminescence (ECL) showed the difference in hemin binding to the surface of *H. influenzae.* An interaction between hemin and recombinant soluble PE was also demonstrated by native-PAGE and UV-visible spectrophotometry. Affinity for the hemin-PE interaction was calculated by Surface plasmon resonance. Hemin loaded NTHi 3655 or NTHi 3655 Δ*hpe* were co-cultured with hemin starved NTHi 3655*lux* to analyze transfer of hemin between the strains.
Results: *H. influenzae* mutant devoid of PE bound significantly less hemin in comparison to the PE-expressing wild type counterpart. Accordingly, *E. coli* expressing PE resulted in a heme-binding phenotype. An affinity (K_d) of 1.6×10^{-6} M for the hemin-PE interaction was revealed. Importantly, hemin that was bound to PE at the *H. influenzae* surface, was donated to co-cultured luciferase-expressing *H. influenzae* that were starved of hemin.
Conclusion: PE is hemin binding outer membrane protein of *H. influenzae.* When hemin is bound to PE it thus may serve as a storage pool for *H. influenzae*. To our knowledge this is the first report showing that *H. influenzae* can share hemin via a surface-located outer membrane protein.

KMP10

Effects of Helicobacter pyloriosis treatment with platelet-rich plasma (PRP) and antibiotics on liver in the guinea pig

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Introduction: Several studies in different countries showed that more than 50% of gastric world's adult population is infected with Helicobacter pylori. Secretion of growth factors from platelets of different practices can play in the healing process. This study is effect of Helicobacter pyloriosis treatment with platelet-rich plasma (PRP) and antibiotics on liver in the guinea pig.
Materials and Methods: Guinea pigs transferred to the Urmia, infected with Helicobacter pylori. Treatment methods with given a bacterium was started and after giving effect of platelet rich plasma and antibiotic was observed. Platelet-rich in the two groups are autologous platelet-rich plasma and PRP of humans and other groups of treatment, treatment with antibiotics.
Results: From total of 8 guinea pigs treated with either PRP therapy and antibiotics, the following results were obtained. **Treatment with human PRP:** Even the portal vessels were congested but also hepatocytes showed no pathological signs. **Treatment with autologous PRP:** Pathological changes in the liver was observed as Hindi nutmeg liver, the central artery that all without exception were congested. **Treatment with antibiotics:**Hyperemia of the hepatic artery was strictly observed. Hyperemia widely but focus on different parts of the liver were observed. Liver cells or hepatocytes in normal tissue and were unchanged.
Discussion: Much research has been done on platelet-rich plasma and H.pylori, but so far the research on the effect of platelet-rich plasma on the healing of lesions caused by H. pylori not has been made.

Keywords: platelet-rich plasma, liver, Guinea pig, Helicobacter pylori

KMP11

Blood stream infections with Enterobacteriaceae resistant to third-generation cephalosporins - change of resistance mechanisms over a 10-year period

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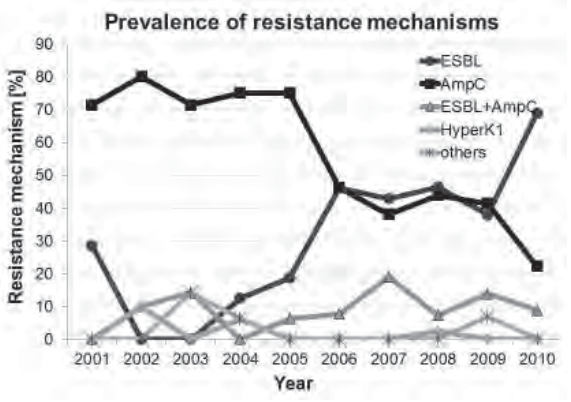
Objectives: to assess the changes in resistance to 3rd generation cephalosporins (Ceph3G) in Enterobacteriaceae from blood stream infections (BSI) over a 10 year period and to analyse the underlying resistance mechanisms.
Materials and Methods: Retrospective analysis of all BSI by Enterobacteriaceae from the University Hospital Cologne (1/2001-12/2010), inclusion criteria: Enterobacteriaceae with cefotaxime resistance. The CLSI combination disk test was performed for the phenotypic analysis of resistance mechanisms. Genotypic analysis of relevant ESBL and AmpC genes was done by a microarray based method (Checkpoints CT101, Wageningen, Netherlands) and PCR and subsequent sequencing of $bla_{\text{CTX-M}}$ genes.
Results: 1919 BSI episodes by Enterobacteriaceae were identified during the study period, with 223 fulfilling the inclusion criteria; of these, 205 isolates were available for analysis. The total number of BC isolates with Ceph 3G resistance increased from 7 in 2001 to 45 in 2010, corresponding to 5.1% and 15.1% of all Enterobacteriaceae BC isolates. Over the whole study period, *E. cloacae* was the most frequently isolated species (35.5%) from BC with resistance to Ceph 3G, followed by *E. coli* (32.2%). In 2006-2010, *E. coli* was the dominant species. Parallel to the change in species distribution, the underlying resistance mechanisms changed over time. In 2001-2005, resistance to Ceph 3G was mostly mediated by AmpC, but was outnumbered by ESBL in 2006-2010 (fig. 1). CTX-M1 group was the most frequent ESBL group detected (N=69), followed by CTX-M9 group (N=18), SHV-type ESBLs (N=17), CTX-M2 (N=3) and TEM-type ESBLs (N=2). CTX-M-15 was the most frequent ESBL-type within CTX-M1 group. Resistance mediated by the hyperproduction of chromosomal AmpC was observed in 54 isolates, whereas in 43 isolates a plasmid encoded AmpC was identified (ACT/MIR, CMY2 or DHA). A combination of ESBL and AmpC was detected in 19 isolates (9.3%).
Conclusion: The incidence of bacteraemia with Enterobacteriaceae resistant to Ceph 3G has markedly increased from 2001-2010. This is the result of 1) the total increase of bacteraemia with gram-negative pathogens and 2) the higher percentage of ESBLs, mainly in E. coli. ESBLs have replaced AmpC as the dominant resistance mechanism during the study period, with CTX-M15 now dominating over other ESBL-types.

Figure 1



KMP12
Rapid and Economic Identification of Carbapenemase Genes in Gram-Negative Bacteria with a Multiplex Oligonucleotide Microarray-Based Assay

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Rapid molecular identification of carbapenemase genes in Gram-negative bacteria is crucial for infection control and prevention, surveillance and for epidemiological purposes. Furthermore, it may have a significant impact on selection of an appropriate initial treatment and great benefit for critically ill patients. A novel multiplex oligonucleotide microarray-based assay was developed to simultaneously detect genes encoding clinically important carbapenemase genes as well as selected extended (ESBL) and narrow spectrum (NSBL) beta-lactamase genes directly from clonal culture material within a short time. Additionally, a panel of species specific markers was included to identify *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii/braakii*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* during the test. The assay was validated using a panel of 117 isolates collected from urinary, blood and stool samples and subsequent cultures. For these isolates, phenotypic identification and susceptibility tests were available as well as an independent detection of carbapenemase, ESBL and NSBL genes. In direct comparison, the microarray based assay correctly identified 98.2% of the covered carbapenemase genes. This included *bla*VIM (n=13), *bla*GIM (n=2), *bla*KPC (n=27), *bla*NDM (n=5), *bla*IMP-2/4/7/8/13/14/15/16/31 (n=10), *bla*OXA-23 (n=13), *bla*OXA-40-group (n=7), *bla*OXA-48-group (n=33), *bla*OXA-51 (n=1) and *bla*OXA-58 (n=1). Furthermore, the additional beta-lactamase genes [*bla*OXA-1 (n=16), *bla*OXA-2 (n=4), *bla*OXA-9 (n=33), OXA-10 (n=3), *bla*OXA-51 (n=25), *bla*OXA-58 (n=2), CTX-M1/M15 (n=17) and *bla*VIM (n=1)] were correctly identified. In the analyzed collection, 114 of 117 (97.4%) isolates, including *Acinetobacter baumannii* (n=28), *Enterobacter spec.* (n=5), *Escherichia coli* (n=4), *Klebsiella pneumoniae* (n=62), *Pseudomonas aeruginosa* (n=12), *Citrobacter freundii* (n=1) and *Citrobacter braakii* (n=2), were correctly identified with the panel of 15 species specific probes. This assay can be easily extended, adapted and transferred to point of care platforms enabling fast surveillance, rapid detection and appropriate early treatment of infections caused by multiresistant Gram-negative bacteria.

KMP13
Intestinal colonisation by ESBL-producing *Enterobacteriaceae* in patients suffering from *Clostridium difficile* infection

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Objectives: Patients infected by *C. difficile* are often exposed to many conditions that may predispose to acquisition of other health-care associated pathogens, e.g. multidrug resistant Gram-negative bacteria. This study was performed to assess the frequency of intestinal colonisation by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* in patients with clinically apparent *C. difficile* infection (CDI).
Materials and Methods: We conducted a prospective survey to determine the prevalence of colonisation by ESBL-producing Enterobacteriaceae in patients with laboratory confirmed CDI, who were treated in different healthcare facilities (32 hospitals, 3 nursing homes) inHesse, a federal state in southwestGermany. From February 2011 to November2013, a single faecal sample from each patient was screened for the presence of ESBL-producing gram-negative bacteria using a chromogenic agar. The isolated organisms were subjected to biochemical identification and susceptibility testing with an automated system. Presence of common ESBL genes was assessed by PCR. Presence of risk factors for antibiotic-resistant organisms was evaluated using a questionnaire.
Results: Of 236 patients with CDI who were treated in 35 healthcare facilities in Hesse, 38 (16.1%) were colonised by ESBL-producing Enterobacteriaceae. Among these, 32 (84.2%) patients carried ESBL-producing *E. coli* and 6 (15.8%) ESBL-producing *K. pneumoniae*. The median age of the patients was 78 years and 208 (88.1%) patients were ≥65 years old. According to the information provided by the questionnaire, 40.2% of the patients had been previously hospitalized within the past 3 months, 16.1% had one or more previous episodes of CDI within the past 3 months (recurrent CDI), and 9.7% were residents of nursing homes. Among the risk factors evaluated, recurrent CDI was significantly associated with colonisation by ESBL-producing Enterobacteriaceae (p=0.019).
Conclusion: We found a high prevalence of colonisation by ESBL-producing *E. coli* and *K. pneumoniae* in diarrhoeal patients with CDI. Our data suggest that patients with CDI may contribute to the spread of multi-resistant gram-negative bacteria in healthcare facilities. They underline the importance of good adherence to infection control measures in the management of patients with CDI and the need for dedicated antibiotic stewardship programs to combat the spread of multi-resistant pathogens.

KMP14
Frequency of *Toxoplasma gondii* Infection in HIV Positive Patients from West of Iran

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Introduction: *Toxoplasma gondii* is one the most prevalent parasite that infects a broad range of warm-blooded animals as well as humans. In immunocompromised individuals such as AIDS patients the infection could be life threatening**.**
Materials and Methods: 100 serum samples from HIV positive patients were collected from west of Iran. *Toxoplasma gondii* antibody was determined by IgG ELISA. *T. gondii* antigen was detected by capture-ELISA. PCR was performed on samples with *T. gondii* antigenemia.
Results: From 100 serum samples, 18 had IgG against *Toxoplasma*. 6 samples showed *T.gondii* antigenemia in which 1 sample had positive result in PCR.
Conclusion: The result showed that, capture-ELISA and PCR could confirm the *T.gondii* acute infection in HIV positive patients.
Key words: *Toxoplasma gondii*, IgG ELISA, capture- ELISA, PCR, Iran

KMP15
Hand hygiene compliance on two ICUs at Hanover Medical School: indication-specific analysis of compliance rates per bedside

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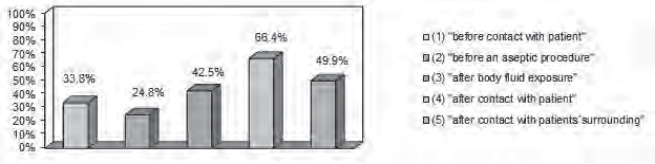
Introduction: Health care-associated infections (HAIs) are still a major problem on intensive care units (ICUs) and are known to have serious impact on patients' mortality, length of hospital stay, and costs for the health care system. Hand hygiene (HH) is considered to be the most important tool to prevent HAIs particularly in situations of great impact on patients' safety, such as aseptic procedures (AP). However, compliance with HH still remains low among health care workers (HCWs). The aim of this study was to generate indication-specific HH compliance focussing the AP.
Materials and Methods: For a period of two weeks direct bedside observation was performed on a surgical ICU (SICU) and a medical ICU (MICU) in accordance to the World Healthcare Organization guideline "my five moments for hand hygiene": (1) "before contact with patients", (2) before an aseptic procedure", (3) "after body fluid exposure risk", (4) "after contact with patients", and (5) "after contact with patients´ surroundings". Bedside observations of HCW were performed for 12 hour periods (from 7:00am to 7:00pm) three days a week by a single observer. HH opportunities were documented on a standardized observation record. AP were stratified into manipulation of ventilation devices, intravascular catheters, urinary catheters, dressing, and other aseptic procedures.
Results: During the 144 hour observation period, a total of 1,896 opportunities for HH were observed for the two ICUs included in this study. The frequency of observed HH indications is illustrated in Fig. 1. Thus, indication (2) "before an aseptic procedure" was the most commonly observed indication (28.3%; n=537).
The overall HH compliance rate (CR) was 42.6%. As Fig. 2 shows, the highest CR was evaluated for indication (4) "after contact with patients" (66.4%). In contrast, the lowest CR was calculated for indication (2) "before an aseptic procedure" (24.8%).
Stratifying the AP into the different device manipulations described below revealed that "manipulation of intravascular catheters" was the most

frequently observed AP but only reached low CR (24.2%; n=293). The highest CR was evaluated for "manipulations of urinary catheters" (42.9%; n=14). In contrast lowest compliance was observed with "manipulation of ventilation devices" (18.7%; n=134). CR and frequencies of investigated AP are given in table 1.

Conclusion: The overall compliance rate per bedside was poor in this observational investigation in particular with indication (2) "before an aseptic procedure" although AP are well known to increase the risk of HAIs. Thus, future interventions to improve HCWs adherence to hand hygiene and therefore patients´ safety should focus on AP.

Figure 1

Figure 2



KMP16
Phenotype-genotype correlation studies on a clonal *Pseudomonas aeruginosa* outbreak

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Introduction: By RNA-sequencing of a large collection of *P. aeruginosa* clinical isolates, a clonal outbreak consisting of 15 isolates exhibiting a small-colony variant (SCV) and multidrug-resistance phenotype was identified. Despite the high genetic similarity among the outbreak isolates, we discovered differences in phenotypic traits in respect to virulence, biofilm growth pattern and motility, indicating the occurrence of adaptational processes within the host. To uncover the underlying mechanisms we correlated the gene expression profiles and other phenotypic characteristics to genetic alterations within this set of isolates.

Materials and Methods: Qualitative whole-transcriptome sequencing was applied to investigate both gene expression profiles and sequence alterations. The phenotypic characterization of the isolates included a wax moth larvae (*Galleria mellonella*) virulence assay, biofilm growth pattern investigation by confocal laser scanning microscopy and motility agar plate assays to provide information about swimming and swarming ability.

Results: Detailed investigation of the gene expression profiles revealed differences in several gene clusters among the clonal *P. aeruginosa* outbreak isolates, e.g. genes encoding the two-component system PmrAB and downstream genes were differentially expressed. We furthermore found isolates that were significantly more virulent towards *G. mellonella* and more motile in terms of swimming ability. In addition, the isolates could be clearly grouped into two distinct biofilm forming clusters, plus some exhibiting a unique pattern.

The analysis of genetic alterations (of nine of the isolates) revealed less than 50 non-synonymous mutations that differed among the isolates. One candidate gene, responsible for a specific biofilm phenotype, was the sigma factor encoding gene *algU*, where mutations (in the coding sequence or 5´-upstream) were identified in the respective isolates. Furthermore, *pmrB* was found to be up-regulated in some of the isolates, in line with the finding of an insertion within this gene in the other part of the isolates.

Analysis of the phylogenetic background based on sequence similarity provided a likely route of transmission between the isolates.

Discussion: The investigated set of very closely related isolates with only few genetic alterations but different phenotypic characteristics provides an optimal possibility to directly correlate genetic changes, that occurred within host-adaptational processes, to a certain phenotype. The introduction of the identified mutations into a mutation negative strain background will be essential to prove the effect of single mutations on specific bacterial phenotypes.

KMP17
Biobanking platform of the German Center for Infection Research (DZIF)

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A central goal of the biobanking infrastructure inside BMBF-funded German Centre of Infection Research (DZIF) is to provide fast and standardized access to biomaterials which is nowadays mandatory for multi-site and translational research.So far, there is a lack of existing harmonized biobanking structures for infectious diseases in Germany and of biobanking structures and expertise at several DZIF partner sites.To build up a biobanking platform, pre-existing biomaterials as well as respective expertise and technologies at different partner sites will be integrated. During implementation, structures like quality and project management, IT infrastructure and ELSI aspects will be harmonized within DZIF biobanking wherever possible. The DZIF biobank structure will be governed by a board consisting of the three columns tissue (Heidelberg), liquid (Munich) and pathogens & microbial producers (Braunschweig) Central coordination (Heidelberg) of the platform is essential for further development, harmonization, and embedding in national and international research activities of DZIF biobanking. After the initial phase all positions at the main sites are filled and structures are implemented. Furthermore, there is a regular and recorded exchange of information between the biobanking representatives of all DZIF sites. A DZIF Biobanking homepage (www.dzif-biobanken.de) and catalogue has been developed and up to now 29 biobanks are registered. Future work includes extending the catalogue and networking within the national (DZGs, AG BMB TMF, GBN) and international (e.g. ESBB) biobanking community. Moreover, harmonized DZIF standard operating procedures (SOPs) are of central importance to establish biomaterial banking as a common DZIF resource with a consistent high standard.

KMP18
MRSA risk factors associated with predominant *spa*-types in hospitals - experience from a statewide MRSA admission screening (MRSAarNet)

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Introduction: Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are a major health concern. The Saarland regional network for prevention and control of MRSA (MRSAarNet) has set out to determine the burden of MRSA (and other multidrug-resistant organisms) throughout the Saarland health care institutions, and the acute care hospital admission prevalence study has recently been concluded (1). The goal of this additional analysis was now a detailed association of the risk factors ascertained and the *spa*-types of the MRSA cases identified in this admission prevalence study.

Materials and Methods: 378 MRSA-isolates from 24 different Saarland hospitals were genotypically characterized by *spa*-typing. Patient characteristics and MRSA risk factors were analyzed using patient questionnaires as previously described (1, 2). Distribution of *spa*-types and association of the predominant spa-types with risk groups was analyzed.

Results: A total number of 42 different *spa*-types as well as one unknown *spa*-type could be found. Although MRSA isolates were obtained at hospital entry, the four predominant *spa*-types corresponded to genotypes typically considered as hospital associated and were found to be as follows: t008 (n=10), t002 (n=14), t504 (n=63) and t003 (n=225). The analysis of the *spa*-type distribution in the different risk factor groups revealed no age specific *spa*-type association (Figure 1), i.e. the four predominant *spa*-types could be detected with similar rates in all age groups. Interestingly, in the age group of patients younger than 30 years, a larger portion of *spa*-types not included

in the four predominant *spa* groups could be found; in contrast, isolates of *spa*-type t002 was missing in the younger age group. Risk factors for MRSA were detected with similar rates in groups infected with the various *spa*-types.
Conclusion: Our analysis failed to identify a clear association between the *spa*-type distribution and the presence of certain risk factors in MRSA positive admission patients. This leads to the conclusion that in our hospital admission prevalence cohort, the MRSA isolate clades as defined by *spa*-typing were homogenously distributed independently of risk factors for MRSA acquisition, yet with a trend for increasing predominance of the prevalent *spa*-types t002, t003, t008 and t504 in older age groups.

[1] Herrmann et al., PlosOne, 2013, 8(9)
[2] Ruffing et al., PlosOne, 2012, 7(12)

Figure 1

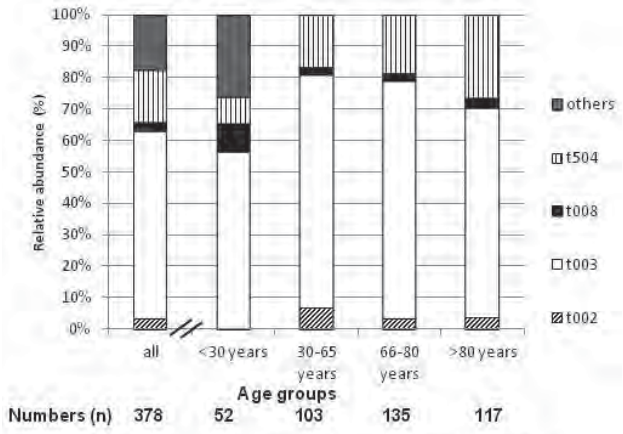

Fig. 1: Age dependent *spa*-type distribution analysis in four predefined age groups and in the whole study group.
Results are the relative numbers of the different *spa*-types in the single groups.

KMP19
Evaluation of the eazyplex SuperBug CRE assay for the rapid detection of CTX-M genes as resistance marker in positive blood cultures

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Sepsis is one of the most important causes of death in intensive care units. Fast microbiological diagnosis including information on antibiotic resistance is highly important to initiate a targeted therapy as early as possible. In a pilot study we evaluated the eazyplex Superbug CRE assay (AmplexDiagnostics, Gars-Bahnhof, Germany) for the identification of beta-lactamases of the CTX-M-1 and CTX-M-9 families directly from positive blood culture (BC) flasks. The eazyplex system is a rapid molecular test based on isothermal amplification with a minimum of hands-on time. The study retrospectively included BCs that were tested positive for *Klebsiella pneumoniae* and *Escherichia coli*. A volume of 25 ml was taken from the BC flask. Besides a short lysis and centrifugation step no further preparation of the sample was performed. All *K. pneumoniae* and *E. coli* ESBLs were correctly identified by the detection of CTX-M genes. Non-ESBLs were also correctly identified by a negative CTX-M result. The mean time to result of amplification was 16 min for the internal control and 11 min for CTX-M targets. The results of this study show for the first time that eazyplex SuperBug CRE can be used as a simple rapid test for the identification of ESBL resistance directly from positive blood cultures. For the application in routine diagnostics the combination of eazyplex SuperBug CRE with a rapid species identification assay is needed.

KMP20
Blood culture for pathogen detection in patients with spondylodiscitis

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Objectives: Spondylodiscitis is a rare but severe disease that contains a heterogeneous group of infections affecting the vertebral column, intervertebral disc and/or epidural abscess. Due to unspecific symptoms diagnosis is often delayed. Detection of the causative pathogen should be sought for optimal choice of antibiotic therapy, especially since a several week course of antibiotics is necessary. We evaluated the use of blood cultures as a non-invasive method for pathogen detection in patients with spondylodiscitis.
Materials and Methods: We conducted a retrospective analysis of patients treated for spondylodiscitis at the department for neurosurgery and in the department for orthopaedic surgery of a university hospital from 2004 to 2010. Diagnosis of spondylodiscitis was based on clinical presentation, laboratory values and radiologic findings. Pathogen detection was sought by blood culture or by CT-guided or open biopsy in patients that were treated by surgery.
Results: 227 patients with spondylodiscitis (18 cervical, 75 thoracal, 112 lumbocacral, 22 multisegmental) were included. 23 patients (10%) were treated conservatively with immobilisation and 204 patients (90%) were treated with surgery. All patients received additional antibiotic therapy. A causative pathogen could be detected in 144 patients (63%). *Staphylococcus aureus* was the most commonly detected pathogen (n=55, 38%), with 18% (n=10) being MRSA. CoNS were found in 23 patients (16%), streptococci in 18 patients (13%) and gram-negative rods in 14 patients (10%). 12 patients (8%) had spondylodiscitis due to *Mycobacterium tuberculosis* and one patient from each *Pasteurella multocida* and *Brucella sp*and 20 patients suffered from polymicrobial infections. Blood cultures were taken from 75 patients (33%). One pair of blood culture were taken from 36 patients, two or more pairs of blood culture were taken from 39 patients. The rate of pathogen detection was 33% (13 out of 36 patients) in patients with one blood culture and 72% (28 out of 39 patients) in patients where two or more blood cultures were taken ($p<0.01$).
Conclusion: When two or more pairs of blood cultures are taken, blood cultures are a sensitive and non-invasive method for pathogen detection in patients with spondylodiscitis. Especially patients treated conservatively could be spared from invasive diagnostic procedures.

KMP21
A Universal Protocol for the Extraction of Nucleic Acids from Clinical Specimens Adapted to Different Laboratory Automation Platforms

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Robust , fast and reliable purification of nucleic acids from diverse and complex clinical samples remains a challenging task for diagnostic applications. Many diagnostic laboratories are faced with the task to extract different nucleic acids from a variety of clinical samples during a typical working shift. To collect batches of similar specimens or to sort by nucleic acid type is not always feasible or desirable. Consequently, different protocols or kits are used for the various applications. A simplification of this practice towards the use of only one unified protocol would reduce the average cost, labor and sources of error. We have developed a reagent set with universal protocols for the parallel extraction of nucleic acids (DNA and RNA) from clinical samples (e.g. EDTA-blood, plasma, urine, sputum). The system is based on either magnetic bead or 96-well filtration plate technologies. The protocols have been adapted to various laboratory automation platforms including walk-away applications on the STARlet (Hamilton) and the InviGenius® (Stratec Molecular) or semiautomated extraction using the KingFisher™ (Thermo) and the protocol is also amenable to manual Spin-Filter use. The data presented in this poster demonstrate the equivalence of the universal protocol in terms of sensitivity of extracted nucleic acids from clinical samples compared to specific manual methods for the extraction of genomic DNA, viral RNA, and DNA, as well as bacterial DNA. It is furthermore demonstrated that the extracted nucleic

acid can be used in different downstream applications for detection and analysis. The kits will be CE-IVD compliant.

KMP22

Identification of human pathogenic fungi via DNA-microarray analysis for clinical applications

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Patients with a weak immune system like people receiving immunosuppressive treatment for cancer and organ transplantation or patients who suffer from AIDS or cystic fibrosis are representing a high-risk group for secondary infections with human pathogenic fungi. Those invasive fungal infections show a high morbidity and mortality rate between thirty to eighty percent. Deciding reasons may be inadequate medication due to inaccurate and time consuming classification of moulds and yeasts in clinical laboratories. For an increased life expectancy an effective and early medication is necessary.Conventional molecular biological methods to identify human pathogenic species like PCR, qRT-PCR or sequencing preclude parallel detection resulting in material and sample intensity. To overcome these limitations we are developing a Fungal-Yeast-Identification-Chip as a fast and reliable device for the accurate identification of 55 human pathogenic moulds and yeasts. To this end we take advantage of DNA sequences of specific target genes which are representing evolutionary conserved sequences variable enough for discrimination of the relevant species. Sequence databases of ribosomal RNA genes as well as functional target genes are established to design probes with high discrimination power and primer pairs for the amplification of diagnostic target regions. To evaluate the DNA-Microarray in a first step individual PCRs with DNA of reference species were established. Up to now evaluation of the microarray occurred with 55 reference species. After hybridization of labeled amplicons we obtained highly specific signals. Compared with specific probe signals background fluorescence is very low and could be discounted. To increase specific signals of designed probes a probe-redesign took place. Furthermore, the results will be validated with patient samples like broncho-alveolar lavage or tracheal secretion provided by our clinical partner.For clinical application the Fungal-Yeast-Identification-Chip will be embedded in a Lab-on-a-chip-system. All required steps like cell lysis of human and pathogenic cells, amplification of target genes via PCR and the identification of 55 fungal pathogens via microarray are integrated in a disposable cartridge. Reliable and fast identification should be possible within a few hours and provides rapid therapeutic intervention in an early state of infection. This provides rapid therapeutic interventions in an early state of infection and will increasing patients life expectancy.

KMP23

Virulence factors and antibiotic susceptibility patterns of *Staphylococcus* sp. strains isolated from skin swabs in patients with inflammatory skin disorders

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Introduction: *Staphylococcus aureus* has a peculiar ability to colonize the skin of patients with inflammatory skin disorders (ISD) (eczema, psoriasis, acne, atopic dermatitis) and is consistently found in eczematous skin lesions in these patients, being considered an important aggravating factor for skin lesions, especially when resistant to antibiotics. The aim of the study was the characterization of virulence markers and antibiotic susceptibility profiles of skin *Staphylococcus* sp. in patients with ISD and healthy controls and the assessment of structural variants of the *mec* element in methicillin resistance strains.
Materials and Methods: Swab-scrubbed skin samples of ISD patients and controls were cultured on Blood agar and manitol salt agar plates and incubated at 37°C for 24 hrs. Characteristic *Staphylococcus* sp. colonies were identified by standard bacteriological procedures and API Staph kits and analyzed for soluble virulence factors expression, i.e. pore forming toxins, proteases and DN-se by cultivation on specific media (sheep blood, Tween 80, yolk, milk, starch, gelatin, blue toluidine). Drug resistance patterns were assessed by disk diffusion method (CLSI, 2013). Structural variants of the *mec* element in methicilin resistance strains were determined by multiplex PCR.
Results: *S. aureus*, *S. capitis*, *S. hominis* and *S. warneri* were isolated only from the ISD patients, while *S. xylosus*, *S. lugdunensis* and *S. sciuri* from healthy controls. The strains isolated from ISD exhibited with higher prevalence pore forming toxins (lecithinase) and proteases (caseinase, gelatinase), with potential roles in pathogenesis by degrading host proteins causing irritation and jeopardizing the physiological skin condition. The *Staphylococcus* sp. isolates exhibited a similar antibiotic resistance pattern (skin lesion isolates/controls) with high resistance to penicillin (53.85%/50%), erythromycin (46.15%/50%) and cefoxitin (23%/25%). Clindamicin (3 strains) and linezolid (1 strain) resistance was obtained in strains from ISD patients. A high resistance rate was noted for trimethroprim-sulfamethoxazole (50%) in case of *Staphylococcus* sp. isolated in healthy controls. The molecular investigations showed that all *S. aureus* strains were harboring *mec*A gene and the *ccr* gene complex.
Conclusion: Increasing incidences of community-acquired methicillin-resistance (CA-MRSA) in *S. aureus* skin and soft tissue infection raise concerns that ISD skin would be a favorable reservoir for CA-MRSA.

KMP24

Development and Comparison of Different Diagnostic Assays to Analyze Hantavirus Infections

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Hantaviruses are 80-120-nm enveloped viruses in the Bunyavirus family having a negative sense single-stranded and three-segmented RNA. The viral RNA segments - small (S), medium (M) and large (L) - are complexed with a nucleocapsid (N) protein. There are over 20 characterized species globally (six so far in Europe), differing in their virulence and clinical manifestation. Old world Hantavirus (e.g. Hantaan, Dobrava and Puumala) are causing Haemorrhagic fever with renal syndrome (HFRS) or Nephropathia epidemica (NE). New world Hantavirus (e.g. Sin Nombre and Andes) are causing cardiopulmonary syndrome (HCPS). The severity of the disease depends on the infecting virotype with case fatality rates from 0.1% - 10% for HFRS and 25% - 35% for HCPS.
Hantaviruses are rodent-borne viruses. Hantaviruses are transmitted to man by inhalation of aerosols of contaminated rodent excreta like urin, droppings or saliva. The diagnosis of hantavirus disease mainly relies on the detection of IgG and/or IgM antibodies, through immuno-fluorescent assays (IFA), Enzyme Immuno Assays (EIA) or Blots.
Here we show the performance characteristic of the newly developed Dobrava IgG/IgM , Puumala IgG/IgM ELISA, Hantavirus Screening IgG/IgM ELISA (DOBV, PUUV, HTNV, SOUV), multiplex Hantavirus IgG/IgM Lineblot (DOBV, PUUV, HTNV, SOUV, SNV, ANDV) including Leptospira antigens for the differentiation between Hantavirus and Leptospira infections and several species specific lateral flow rapid tests. Samples for evaluation were obtained from the German, reference center for Hantavirus, the German Army, the Royal Tropical Institute (KIT) in Amsterdam and commercial sources.
Due to the improved antigen design, expression method and test setup a superior assay performance was achieved compared to other test methods.
All developed assays will help to detect two of the most underdiagnosed diseases and help to administer patient to proper treatment as early as possible.

KMP25

A proteomics approach to decipher molecular interactions in polymicrobial biofilms during catheter-associated urinary tract infections

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Catheter-associated urinary tract infections (CAUTIs) account for up to 40% of all nosocomial infections and are thus the most prevalent source of hospital-acquired infective diseases. The risk that CAUTIs become symptomatic increases dramatically with the time of the catheterization due to the formation of mostly mixed-species bacterial biofilms in and on the catheters. Most prominent pathogens isolated from long-term catheterized patients are *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*. To elucidate the impact of synergistic or competitive interactions between different species within the biofilm on the physiology of two important uro-pathogens, the proteome profiles of *P. aeruginosa* and *P. mirabilis* grown in mono- and mixed cultures and employing iron-depleted artificial urine media were analyzed by a semi-quantitative shotgun proteomics approach (GeLC-MS/MS). Our proteomic analysis demonstrated that the presence of *P. mirabilis* leads to a decrease of *P. aeruginosa* proteins involved in the up-take of *P. aeruginosa*-specific iron-binding siderophores and heme, whilst proteins involved in the uptake of heterologous siderophores were found to be highly expressed. This strongly indicates, that *P. aeruginosa* is capable of acquiring iron bound to siderophores produced by other species. Notably, *P. mirabilis* proteins involved in iron uptake were generally more abundant in the co-cultures. Moreover, we observed an adaptation of *P. aeruginosa* to alkaline pH due to *P. mirabilis* urease activity in the co-culture, which was mirrored by an increased expression of proteins involved in the glycerate pathway, of the outer membrane protein OmpH1 and of xxx. Interestingly, OmpH1, which was found to be highly abundant during co-culturing is also involved in antibiotic and cationic antimicrobial peptide resistance. We are currently validating the importance of these proteins for *P. aeruginosa* fitness in multi-species biofilms by comparing the growth behavior of the corresponding GFP-tagged transposon mutants and the wild-type strain in mixed biofilms with *P. mirabilis*. In conclusion, our study will help to elucidate the complex molecular interactions occurring in polymicrobial uro-pathogenic biofilms.

KMP26

Whole genome based molecular epidemiology of tuberculosis in Hamburg: a population based study in a low incidence setting

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Introduction: Classic molecular genotyping of clinical *Mycobacterium tuberculosis* complex isolates (MTBC, causative agents of tuberculosis [TB]) has been successfully employed to analyze transmission dynamics and guide TB control measures. In recent years, multidrug and extensively resistant MTBC strains have emerged, further underlining the urgent need for tools enabling us to understand local and global population structure and dispersion of MTBC strains. A number of studies have looked into the suitability of whole genome sequencing (WGS) based molecular typing using next generation sequencing technologies (NGS) for genotyping MTBC isolates, and consistently reported a higher resolution of isolates compared to classical typing techniques such as 24-loci MIRU-VNTR (mycobacterial interspersed repetitive units - variable number of tandem repeats) typing and spoligotyping (interspaced palindromic repeats; CRISPRs). However, in order to employ WGS typing in MTBC outbreak analysis, parameters for genome based cluster definitions and its performance in longitudinal molecular epidemiological studies has to be investigated.
Materials and Methods: In total, 947 MTBC isolates were obtained from patients living in the city state of Hamburg in the years 2008 - 2012, representing more than 90% of all cases reported in that time period. Extracted DNA was used for NGS library construction and sequenced on Illumina instruments. Spoligotyping and 24-loci MIRU-VNTR typing were performed in parallel.
Results: Groups defined from WGS typing correlated well with phylogenetic strain classification and cluster analysis based on classical typing. Strains belonging to previously defined major lineages were attributed to respective branches in the tree drawn from the concatenated list of 31,566 single nucleotide polymorphism (SNP) positions found by NGS. These lineages include *M. tuberculosis* (e.g. Beijing, East African Indian, Delhi-CAS, Haarlem, LAM, X-type, S-type), *M. africanum* (West African 1 and 2) as well as *M. bovis* strains.
In line with published reports, WGS typing offered a much higher resolution, with additional subdivisions suggested especially for the Euro American Lineage. With the added resolution, several clusters defined by classical typing techniques were split, enabling a significantly improved cluster analysis. Detailed investigations are currently ongoing.
Discussion: Applying WGS typing in a population based study confirmed its great potential for longitudinal molecular epidemiological studies. Clinical isolates could be reliably attributed to phylogenetic groups and seperated for cluster analysis with greatly improved discriminatory power compared to classical typing techniques.

KMP27

Biofilm production and virulence factors in staphylococcal isolates from orthopaedic vs. non-orthopaedic deep tissue specimens

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Objectives: *Staphylococcus aureus* (SA) and *Staphylococcus epidermidis* (SE) are the most common pathogens of bone, joint and prosthetic infections associated with increased morbidity, mortality and an enormous economy burden to healthcare systems. Orthopaedic infections are frequently connected with biofilm formation, which is difficult to eradicate. Therefore, understanding of difference in biofilm forming capacity between isolates causing orthopaedic and non-orthopaedic infections is of particular interest.
Materials and Methods: The main adhesion and biofilm characteristics of 57 SA (12 orthopaedic and 45 non-orthopaedic) and 58 SE (31 orthopaedic and 27 non-orthopaedic) isolates from deep tissue specimens collected in a German university hospital during 2012 were studied. Only monomicrobial infections were considered. The biofilm- or adhesion-associated genes *embp*, *aap*, *icaA*, *bhp* for SE and *sasG*, *sasC*, *icaA*, *fnbA*, *bap* and various toxin (*sea-see*, *seg-sej*, *eta, etb*, *tst*, *edina-edinc*, *etd*, *pvl*) and regulatory (*agr1-agr4*) genes for SA were screened by PCR. The phenotypic biofilm-forming capacity was investigated by a quantitative assay.
Results: The phenotypic biofilm production was much higher in SA than in SE (71.9 vs. 24.1%; p<0.005). However, 94.7% of SA isolates were *icaA*-positive, compared to only 41.4% of SE (p<0,005). All *icaA*-negative SE isolates possessed *embp* and/or *aap* genes. In total, 86.2% and 69.0% of SE were *embp*- and *aap*-positive, respectively. The prevalence of adhesion factors in SA was also high: *sasG* 47.4%, *sasC* 50.9% and *fnbA* 96.5%. The *bap* gene was not detected and the *bhp* gene only in 27.6% of SE isolates. SE from orthopaedic samples contained more frequently *icaA* (51.6%) and *aap* (74.2%) genes compared to non-orthopaedic (29.6% and 63.0%). *embp* was less common among orthopaedic samples (80.6 vs. 92.6%). The phenotypical ability to form a biofilm in SE isolates was equal (12.9 vs. 11.1 %; p<0.477). Biofilm formation was higher in orthopaedic SA isolates compared to non-orthopaedic (83.3 % vs. 68.9 %) despite similar distribution of the virulence factors (*icaA*, *sasG*, *sasC* and *fnbA*). Certain toxin genes were detected with orthopaedic or non-orthopaedic SA isolates, e.g. *seg* and *sei* (75.0 vs. 48.9%), *sec* (33.3 vs. 15.6%) or *sea*, *sed* and *sej* (0 vs. 17.8%). One of the non-orthopaedic SA isolates was tested positive for the PVL-encoding genes and four (8.9%) for the toxic shock syndrome toxin 1 gene *tst* (none and two (16.7%) of orthopaedic isolates, respectively).
Conclusions: While nearly every clinical isolate of deep tissue infections had the molecular equipment to produce biofilms, not all isolates displayed the biofilm formation *in vitro*. Orthopaedic isolates revealed a higher capability for biofilm production.

KMP28
Carbonmonoxid releasing nanofibers as a potential anti-biofilm treatment

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In the clinic day routine, biofilms are formed on various surfaces like tubes and artificial devices, but also on epithelia (bladder, lung, mucosa), or on open wounds. According to the National Institutes of Health, biofilms coincide with more than 80 % of all nosocomial bacterial infections. Accrued biofilms are almost impossible to eradicate by common drug treatments since microbes embedded in a biofilm benefit from the protective biofilm environment exhibiting elevated tolerance to antibiotics and increased protection against the immune system. Additionally, since most antibiotics target metabolic processes they are inefficient in killing persister cells in deeper biofilm layers. Therefore, alternative therapeutic strategies that interfere with biofilm integrity would offer great benefits for clinical treatment. *Staphylococcus aureus* present one the most abundant biofilm forming pathogen, often related with various implant-associated infections and skin wound infections. We aimed to eradicate *S. aureus* biofilms by controlled photo-stimulated (405 nm and 480 nm) release of toxic CO that have been incorporated in nanofibers. The water insoluble and photoactive CO releasing molecule dimanganese decacarbonyl (CORM-1) has been non-covalently embedded into poly(L-lactide-*co*-D/L-lactide) nanofibers via electro-spinning to enable bioavailability and water accessibility of CORM-1. Anti-biofilm tests were performed in chamber slides and analyzed by confocal laser scanning microscopy (CLSM) as well as evaluated using the in-house software that phenotypically and statistically determine the living and dead cells. The results showed that the controlled release of CO effectively eliminate the bacteria of existing biofilms. Since the toxic effect of CO base on the impaired electron transport chain, emergence of resistance mechanisms is unlikely. Moreover, CO might also impact persister cells. Thus CO-nanofibers might be an interesting possibility for the antimicrobial treatment, e.g. of wound infection.

KMP29
Evaluation of the Rida®Gene Norovirus I & Ii and Rotavirus/Adenovirus Duplex Real-Time Rt-Pcr Assay for the Laboratory Diagnosis of Gastrointestinal Infections

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Objectives: Diarrheal disease is a major health care problem worldwide. Whereas Noroviruses cause by far the most cases of non-bacterial gastroenteritis outbreaks, rotavirus is the main cause of diarrhoea in children aged under five. Adenovirus is the third main virus causing gastroenteritis. Apart from gastroenteritis, adenoviruses can cause viremia and life-threatening conditions such as sepsis in immunocompromized patients. This study evaluated the performance of the RIDA®GENE real-time RT-PCR assay compared to the immunological RIDASCREEN® assay for the detection of norovirus, rotavirus and adenovirus in hospitalized patients including immunocomprimized individuals
Materials and Methods: For real-time RT-PCR analysis, 200 stool specimens from neonates/infants and oncological patients with acute diarrhea as well as 100 control samples from small children were isolated with the EZ1 Advanced automated extraction platform (Qiagen). RNA was analyzed with the RIDA®GENE Norovirus I & II multiplex real-time RT-PCR and the RIDA®GENE Rotavirus/Adenovirus Duplex real-time RT-PCR on the ABI®7500 (Life Technologies). PCR analysis was compared to RIDASCREEN® ELISA assays for norovirus, rotavirus and adenovirus.
Results: 263 samples were included in the analysis. 24 (9.1%) norovirus-positive samples were identified by the RIDA®GENE Norovirus I & II multiplex real-time RT-PCR. 22 positive samples were identified by the RIDASCREEN® Norovirus ELISA. Overall, 25 (9.5%) samples showed an infection with rotavirus as detected by the RIDA®GENE Rotavirus/Adenovirus Duplex assay. Here, 10 stool specimens of the RIDASCREEN® Rotavirus ELISA-test were in concordance with the real-time RT-PCR. The RIDA®GENE Rotavirus/Adenovirus Duplex assay identified 28 adenovirus-positive samples (10.6%). The RIDASCREEN® Adenovirus ELISA detected 2.3% positive samples only. Those samples showed 100% concordance compared to the RIDA®GENE Rotavirus/Adenovirus Duplex assay.
Conclusion: The RIDA®GENE real-time RT-PCR for norovirus, rotavirus and adenovirus are highly sensitive and rapid multiplex real-time PCR assays for the diagnosis of viral gastroenteritis. The high detection rate of adenovirus-positive stool specimens with the real-time RT-PCR technology could be due to additional detection of other adenovirus types than usually found in stool samples. In addition to the enteric adenoviruses (types 40/41) many other adenovirus types have been shown to cause high viral load in immunosuppressed patients after stem cell transplantation with potential viremia followed by life threatening organ manifestation. Real-time RT-PCR assays such as the RIDA®GENE Rotavirus/Adenovirus Duplex real-time RT-PCR assay present a major advantage over other detection platforms for routine diagnostic testing in hospitalized and immunocompromized patients.

KMP30
Attributable costs of infections with multi-drug resistant Gram-negative pathogens (ESBL) at a German University hospital

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Objectives: Multidrug-resistant Gram-negative pathogens have obtained a worrying increase in their clinical significance during the last years. So far, the economic impact of infections with these pathogens has been little studied. The aim of our study is to identify the main cost drivers with reference to the German DRG-System.
Materials and Methods: A retrospective analysis cost analysis was conducted over a 1-year period in 2012 from the hospital perspective. The analysis determined empirically the cost of hygienic measures, laboratory costs and opportunity costs due to isolation time and extended length of stay.
Results: 173 patients were included in the analysis. Mean length of hospital stay was 16.21 days and mean time in isolation was 8.86 days, respectively. Opportunity costs due to prolonged length of stay making up the largest share with € 4,615.58 per case on average.
Conclusion: Our study provides real data for the burden of multidrug-resistant Gram-negative pathogens in the hospital. Based on this, further studies are feasible to assess the cost effectiveness of targeted preventive measures in the infection control.

KMP31
Fast and cost-effective pathogen diagnosis on an automated DNA/protein diagnostics platform based on cylindrical microarrays (hybcells)

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Objectives: Polymerase Chain Reaction is commonly used to detect microbial nucleic acids in clinical samples. Current microbiology practice takes days to find pathogens in clinical samples, which is sometimes too slow to provide prompt and specific therapy for the patient especially concerning fungal pathogens. On the other hand these pathogens are sometimes weakened due to antimicrobial therapy so that their growth is even decelerated or suspended. A PCR based method would also solve this issue.
Materials and Methods: Samples from clinical sensitive material were parted into thirds. The first part was used for bacteriological culture, the second part for manual DNA extraction with SeptiFast Prep Kit® and Lys Kit® (Roche) and testing with SeptiFast® (Roche). The third part was used for automated extraction with Select NA Blood Pathogen Kit® on Select NA® (Molzym) and further processed with Hybcell Pathogen DNA plexA® (Anagnostics) according to the instruction manual from the Hyborg System® (Anagnostics). This System is an automated Microarray based technology, a highly sensitive, multi-target detection platform. The method starts with a PCR amplifying present bacterial 16S rDNA accompanied by fluorescence labeling. Amplified DNA is used as template for hybcell Solid Phase Primer Extension. In case of matching strain-specific primers, these primers are extended whereas unmatched primers are not. A washing step removes all non-extended primers. Fluorescence signals of all primers (array) are measured and results are derived automatically. The method

delivers results for a panel of bacteria and fungi. Only potent pathogens are considered, multiple pathogens are presented.
Results: Thirty-three clinical sensitive direct Probes were included in this study. Clinically important material like EDTA blood, lung swabs (posthumous), liquor, mitral valve, orthopedic material and paraffin embedded tissue was included. The detection rate of PCR was higher with both methods than of culture. Results were similar in both methods (SeptiFast 12/33, Anagnostics 11/33). Both methods take about six hours turnaround time. Due to the automated extraction method, hands on time is two hours shorter with automated extraction process with Select NA (Molzym).
Conclusion: With its simple workflow and user-friendly format, Anagnostics/Molzym has developed a high sensitivity high specificity detection method for human pathogens. This technology is an essential step toward routine diagnostics and is ideally suited to address this issue and provides fast and cost effective workflow.

KMP32
Viral respiratory tract infectious agents circulating in Bavaria during the 2013/2014 influenza season

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Introduction: During the influenza season 2012/2013 influenza viruses were detected in only 32% of cases with acute respiratory tract infections. To identify and further characterize which additional viruses may be involved in influenza-negative respiratory tract infections, the EMBIS-study (ErregerMonitoring im Bayern Influenza Sentinel) was established to investigate the circulation of respiratory tract viruses in the Bavarian population during 2013/2014 season.
Materials and Methods: Between calendar week (CW) 40/2013 to CW 17/2014, two swabs per week of patients with acute respiratory tract infection (according to WHO definition, May 2011) were provided by selected General Practitioners. These samples were tested for influenza viruses at the Bavarian Health and Food Safety Authority (LGL) using real-time RT-PCR. When positive rates exceeded 20% (KW6) influenza-negative samples were examined for 13 additional viruses using Luminex xTag RVP (RSV, 4 parainfluenza-, 4 corona-, entero/rhino-, adeno-, human metapneumo- and human bocavirus). Results were published via the LGL website.
Results: In total, 1,838 samples were investigated for influenza viruses. 298 (16 %) proved to be positive and were typed as follows: Influenza A Virus: 207 H3N2, 61 H1N1, 13 non typable; Influenza B Virus: 17. Of the 1,540 influenza-negative samples 609 were further characterized by EMBIS. For 49 % of these samples, no data were obtained. 313 positive samples were identified as entero/rhinovirus (17 %), human metapneumovirus (14 %), adenovirus (9 %), RSV (10 %). All remaining viruses were detected in <5 % of samples. Whether there are age-related differences is still being investigated.
Conclusions: Results obtained by EMBIS show that various viral agents circulate during the influenza season. Disease symptoms alone do not permit classification of the agent(s). Reliable classification of the causative agents relies on proper application of diagnostic tools. Apart from viral agents, bacterial infections or allergic reactions may also be cause for respiratory symptoms.

KMP33
Evolution and interactions of co-colonizing *Staphylococcus aureus* and *Pseudomonas aeruginosa* cystic fibrosis isolates

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Cystic fibrosis (CF) is the most common inherited lethal disease among Caucasians leading to a defect CF transmembrane conductance regulator causing an impaired epithelial chloride ion transport. This, in turn, leads to the production of a sticky mucus in in the airways of the lungs and the colonization of the lung epithelium by opportunistic bacterial pathogens. Early in life, CF patients are usually colonized by *Staphylococcus aureus* and non-capsulated *Haemophilus influenzae*, followed by mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*, which in most cases will establish chronic infections. Whilst the pathogenicity and physiology of individual CF-pathogens has been investigated in detail, only little is known about interspecies interaction of polymicrobial CF-infections.
In order to unravel evolution and molecular interactions of early and late *S. aureus* and *P. aeruginosa* isolates, of one CF-patient, the strains were phenotypically characterized and potential molecular interactions during co-infection were elucidated by proteomics. Determination of virulence factors such as protease, motility, hemolysis and biofilm-forming capacity revealed that late *P. aeruginosa* isolates were less virulent and motile compared to the early isolates; in contrast, only minor differences could be observed between the early and late *S. aureus* isolates.. Co-culturing of *S. aureus* and *P. aeruginosa* isolates followed by gel-free semi-quantitative proteomics indicated that the presence of *P. aeruginosa* leads to an induction of *S. aureus* proteins involved in anaerobiosis and to a decreased expression of proteins involved in ROS detoxification. In *P. aeruginosa* potential virulence factors such as the alkaline protease AprA seem to be inducing during co-culture with *S. aureus*.
Moreover, fluorescenctly-tagged isolates were grown as mono- and mixed-species biofilms in artificial flow-chambers and inspected by confocal laser scanning microscopy to investigate the synergistic or competitive interactions during biofilm formation. The elucidation of molecular interactions during polymicrobial infections in the CF airway will significantly contribute to a better understanding of niche adaptation and is thus an essential prerequisite for personalized therapies.

KMP34
Development of a freeze-dried qPCR assay for the detection of Monkeypox virus under field conditions

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In the past decades, quantitative real-time-PCR (qPCR) has brought numerous new opportunities for the rapid, sensitive, and specific detection of novel and emerging disease agents. However, the widespread use of qPCR, especially in tropical countries with the greatest burden of emerging disease is limited as most qPCR reagents have to be transported and stored under cooled or frozen conditions. Currently only a few freeze-dried qPCR assays are commercially available for certain economically interesting diseases. Most of these assays are based upon proprietary technologies and are not openly accessible to other researchers. In an effort to improve this situation for the diagnosis of human Monkeypox virus infections we developed a novel freeze-dried qPCR assay for the detection of this neglected viral disease. Monkeypox is a viral zoonosis with symptoms in humans similar to those seen in the past in smallpox patients. Monkeypox still occurs sporadically primarily in remote villages in Central and West Africa, near tropical rainforests. The virus is transmitted to people from a variety of wild animals and it spreads in human population through human-to-human transmission. We developed a 5'-exonuclease dual-probe qPCR assay for simultaneous detection of Monkeypox virus and other Orthopox viruses (including smallpox virus). Based on the analysis of more than 130 Orthopox virus genomes we selected a suitable target region within the crmb gene encoding a soluble TNFa receptor molecule. In parallel, a novel qPCR chemistry was developed and optimized for freeze-drying. Real-time PCR was performed on a SmartCycler II system, which is frequently used by field teams in outbreak investigations. Analytical sensitivity was determined by probit analysis with serial dilutions of quantified Orthopox virus DNA. Analytical specificity was determined using a collection of non-Monkeypox viruses and other pathogenic viruses and bacteria. Clinical testing will be performed with spiked positive samples and negative clinical samples. The stability of the freeze-dried qPCR reagents was determined by accelerated aging testing. In summary, our novel freeze-dried Monkeypox virus qPCR allows sensitive and specific detection of human Monkeypox infections without the need of a cold chain. It can be sustainably produced in-house and provides a suitable tool for further investigations in field laboratories within limited resource settings in the tropics.

FOOD MICROBIOLOGY AND FOOD HYGIENE

LMP01
In vitro investigation of anti-fungal activity of two essential oil of herbal plant (THYMUS VULGARIS. L, MENTHA PULEGIUM. L) on some food spoilage molds.

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Introduction: Due to the harmful effects of chemical preservatives, natural preservatives, such as essential oils have been used recently. Molds are the important agents of food spoilage and production of toxic compounds called mycotoxins during storage. In this survey, the effects of Thymus vulgaris and pennyroyal (Mentha pulegium) essential oils on mycelium growth of Aspergillus flavus PTCC 5004 and Penicillium chrysogenum PTCC 5033 was investigated.
Materials and Methods: At first, concentrations of 0.25, 0.5, 1, and 2% (w/v) of the essential oils were prepared in molten potato dextrose agar (PDA) with an approximate temperature of 45-43°C. Then, mycelium of 5 to7 day's culture of molds were transferred on PDA agar containing different concentrations of the essential oils using with sterile cork borers (Ø=5mm). After that, diameter of the molds growth were measured on days 3, 5, 7, and 10 of incubation at 25°C; and inhibitory percent of different concentration of essential oils was calculated by comparing the diameter of the molds growth in test groups with control group.
Results: The results showed that the essential oil of thyme in all tested concentrations inhibited the growth of Aspergillus flavus and Penicillium chrysogenum by 100 percent. Also, pennyroyal essential oils at all concentrations inhibited the growth of Penicillium chrysogenum by 100 percent, while this oli inhibited the growth of Aspergillus flavus in concentrations of 0.5, 1, and 2% by 100 percent.
Discussion: From the result, it can be concluded that among the tested essential oils, essential oils of thyme had the most antifungal effects, while essential oil of pennyroyal at 0.25% had the least antifungal effects on tested molds. Therefore, it is recommended that thyme essential oil as alternatives to chemical preservatives used throughout the food industry.

LMP02
Growth potential of *Listeria monocytogenes* and *Escherichia coli* in a food model and sliced ham under argon modified atmosphere packaging.

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The past decades have seen a significant societal and demographic change which fuelled the enormous demand for ready-to-eat (RTE) foods on the market today. In this context, modified atmosphere packaging (MAP) evolved to one of the most useful techniques to maintain the sensory quality attributes of foods of various origin as well as to ensure microbial safety.
While oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) played a mayor role in MAP so far, the inert, odourless and tasteless noble gas argon (Ar) is under discussion as a possible alternative to N_2 that could be capable of enhancing the protective effect of commonly used CO_2-N_2 MAP applied for RTE meat products. Since there is a lack of information on the effectiveness of Ar containing MAP on the growth or survival of relevant pathogenic bacteria relevant in RTE foods, the purpose of the present study was to assess the influence of Ar MAP on the growth of *L. monocytogenes* and *E. coli* strains inoculated on a gelatine-agar matrix as well as on cured, pasteurised and sliced ham. Therefore, a N_2-CO_2 MAP was tested against a CO_2-N_2-Ar and CO_2-Ar MAP via bacterial growth potential determination. Additionally, a shelf life study was conducted to evaluate the effect of the respective MAP on the background flora of ham. The findings suggest that the two Ar containing MAP gas mixtures negatively influenced the growth of *L. monocytogenes* and therefore could be used to enhance the safety of RTE products. However, this effect could not be confirmed for *E. coli*, since all tested gas mixtures reduced the microbial counts equally. Further, a shelf life prolongation could not be observed for tested products.

LMP03
Transcription analysis of toxin genes *stx*, *cdtV* and *subAB* in Shiga toxin-producing *E. coli* O113:H21 strain TS18/08

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are among the most important food-borne pathogens and are frequently transmitted via contaminated beef [1]. During former studies *E. coli* serotype O113:H21 strain TS18/08 was isolated from ground meat, characterized by molecular methods and found out to harbor three different genes for cytotoxins namely *stx2*, *cdtV* and *subAB* [2]. Shiga toxin (Stx) is the main virulence factor of STEC and responsible for enzymatic cleavage of an adenine group at the ribosomal 28S RNA leading to inhibition in protein biosynthesis and further to death of the eukaryotic cell [3]. Cytolethal distending toxins (CDTs) cause irreversible cell cycle arrest leading to target cell death [4]. The subtilase cytotoxin (SubAB) works as serine protease cleaving a Hsp70 family chaperone called BiP/GRP78 of the endoplasmic reticulum leading further on to apoptosis [5]. The aim of this study was to answer how far all three toxin genes are transcribed during growth and to gain further insight in pathogenic potential and molecular mechanisms of this strain.
Materials and Methods: The analyzed *E. coli* serotype O113:H21 strain TS18/08 was cultured in Luria-Bertani broth at 37°C and 180 rpm shaking. The cells were harvested at six time points during growth (t=1 h, 2 h, 3 h, 4 h, 6 h, 8 h), RNA was isolated, transcribed to cDNA by reverse transcriptase and amplified during real-time PCR with specific oligonucleotides in a SYBRgreen assay. Analysis of real-time data was done by the absolute standard curve method based on the copy number of the target with normalization to a non-regulated housekeeping gene. Due to this, differences in time of transcription of the toxin genes and in relative amount of transcripts can be analyzed.
Results and Discussion: During growth of strain TS18/08 samples at different time points were analyzed. Transcription of all three toxin genes was detected. Most transcripts of *subAB* and *cdtV* were found 3 h after incubation while *stx* had its maximum 6 h after start of the experiment. Comparing the normalized RNA levels of the three toxin genes *cdtV* level was highest followed by *subAB1* and *stx* with a mean proportion of 104:27:1. As a conclusion, STEC strain TS18/08 has to be considered as a potential pathogen in terms of food safety. The consequent sequel is now to determine how far the corresponding proteins are expressed in the wild type strain and how big is their cytotoxic potential.

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LMP04
Selective action of silver nanoparticles against bacteria and fungi

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Introduction: Silver nanoparticles (SNP) colloidal solutions have an antimicrobial action against bacteria, molds, and yeasts. The efficiency of this action depends on a number of factors such as the size of the particles, the stabilizing additives included into the colloidal solution compound, and concentration of nanoparticles in the substrate. Also SNP are not equally effective against various species of bacteria and fungi, and minimal inhibitory concentration (MIC) of nanoparticles in a medium is different for each species. Due to significant differences in cell wall structure fungi are able to sustain higher SNP concentrations as compared with bacteria. Therefore it may be possible to select a range of nanoparticles concentrations inhibiting the growth of bacterial microorganisms while not affecting fungi microorganisms. Thus, it will enable the usage of SNP

colloidal solutions as bactericidal agents during fungal producers of antibiotics and enzymes cultivation.

Materials and Methods: SNP colloidal solutions produced using the method of chemical reduction of a water-soluble silver salt in the water medium by ascorbate or sodium citrate with consequent addition of food stabilizers (Gum arabic or chitosan). Estimated size of nanoparticles was 10-15 nm.

The research was effected using axenic cultures of microorganisms of gram-negative bacteria *Escherichia coli, Erwinia herbicola, Pseudomonas fluorescens* and gram-positive bacteria *Sarcina flava*, and *Bacillus subtilis*.

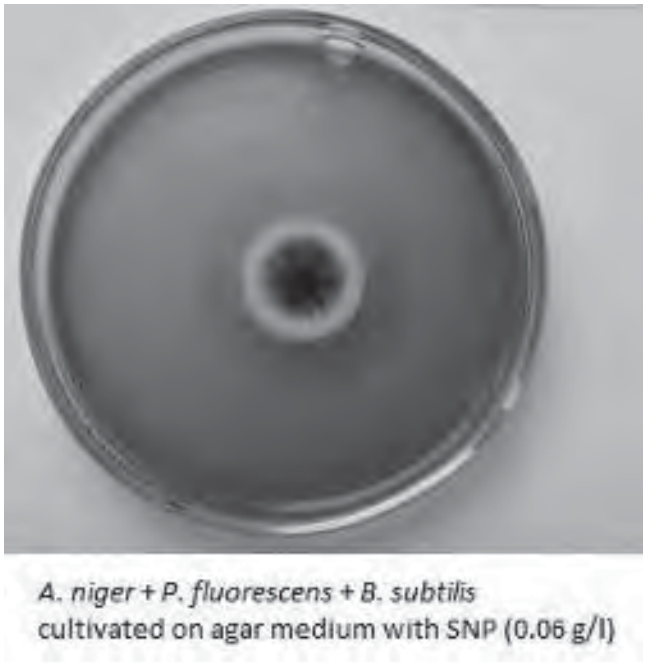
Following fungi species were tested: *Aspergillus niger*, *Penicillium candidum, Rhizopus oryzae, Saccharomyces cerevisiae.*

In order to determine MICs for various microorganisms fungi and bacteria were cultivated simultaneously on agar plates containing different SNP concentrations.

Results: SNP colloidal solutions inhibit the growth of tested bacterial species within the range of concentrations of 0.03-0.06 g/l. MICs for tested fungi microorganisms vary from 0.09 to 0.14 g/l. Concentrations within the limits of 0.06-0.09 g/l inhibited the growth of bacteria while not affecting the growth of fungi (Figure 1 and Figure 2).

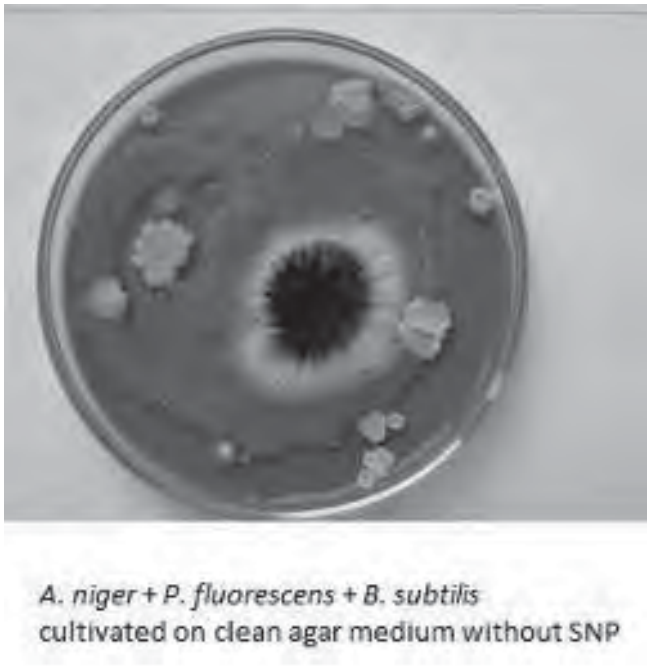
Conclusion and Discussion: Tested fungi microorganisms can be cultivated on substrates containing from 0.06 to 0.09 g/l of SNP. This will reduce the risks of bacterial contamination.

Figure 1



A. niger + P. fluorescens + B. subtilis
cultivated on agar medium with SNP (0.06 g/l)

Figure 2



A. niger + P. fluorescens + B. subtilis
cultivated on clean agar medium without SNP

LMP05

A comprehensive toolbox for tracing emetic *Bacillus cereus* and its toxin in food production and foods

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Introduction: The incidence of foodborne intoxications caused by bacterial toxins has been steadily increasing in Europe throughout the last years [1]. Especially the toxin cereulide, produced by a specific class of *Bacillus cereus,* is increasingly recognized as a serious threat. The production of this emesis-inducing toxin is known to be dependent on intrinsic and extrinsic factors [2; 3]. Nevertheless, once present in food, cereulide will be unlikely destroyed or inactivated throughout further processing. Investigating the prevalence of emetic *B. cereus* strains and assessing boundaries of emetic toxin production could pave the way for novel intervention and prevention measures to control this pathogen.

Materials and Methods: The "toolbox for toxigenic *Bacillus cereus*" includes various novel molecular, immunological and bioanalytical methods, and was designed to cope with the requirements of future *B. cereus* diagnostics [4]. By combining the methods/techniques differently, (i) the prevalence of emetic *B. cereus* strains among 822 food matrices was detected and determined, (ii) the toxin production potential of the isolates was investigated and (iii) food samples were categorized according to their potential in supporting the formation of cereulide.

Results and Discussion: Our results showed that emetic *B. cereus* strains occur more frequently and in a much broader diversity of foods than noticed so far. Categorizing food samples into high, medium and low risk foods helped to understand that some compositional and physicochemical parameters affected cereulide production to a greater extent than others.

In summary, the toolbox presented not only allows to effectively trace the pathogen and its toxin but also to specifically analyse and systematically determine key parameters affecting cereulide formation boundaries in food production and processing. It is expected that the knowledge gained will open new avenues for control and prevention of cereulide formation in food production.

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LMP06
Hazard and Risk Mapping of water and food borne diseases in Iran: A 20 year trend

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Introduction: Water and food borne diseases are still of great importance in Iran. There is a few evidence about the vulnerability, hazard and risk of these diseases in our country and their awareness can be an essential issue. The aim of this survey is the assessment of the 20 year trend and determination of the hazard and risk rate of four food and water borne diseases in Iran.
Materials and Methods: Data was obtained from Iranian Center for Disease Control and Prevention (CDC). Data regarding to cases of Cholera, Typhoid, Shigellosis and Schistosomiasis reported to CDC, classified according to provinces and the year of occurrence. The linear trend of these four diseases were plotted in the EXCEL (2007) software. The hazard and risk maps were drawn using ARC GIS software.
Results: Our analysis showed that during 20 years of study average cases of typhoid, cholera and shistosomiasis were 133027 , 20323 and 101 respectively. The data of shigellosis cases was not accessible for the period of 1991 to 2001 , therefore analysis was done for the last 10 years.
Typhoid has a decreasing trend with an exception that belonged to an outbreak in 2007. In the case of cholera analysis showed an every 2 year raising cases during 1990 to 1996 and a great outbreak in 1998. During the first decade of study schistosomiasis showed to be an endemic disease in Khuzestan province (south western of Iran) , but then after there was no cases reported from 2002 resembling that the disease is going to be eliminated in this region. The total cases of shigellosis in 10 years was 71400 that is high for this disease although it's trend was decreasing. For all 4 diseases the risk rate was high in Sistan va Baluchestan, Kohgiluyeh and Buyer Ahmad , Ardebil, Kordestan, Hmadan, Lorestan and west Azerbaijan provinces. But the hazard rate was the highest in Sistan va Baluchestan, Ghom, Qazvin and Zanjan for cholera. In the cases of shigellosis hazard rate was very high in Sistan va Baluchestan, south Khorasan, Kohgiluyeh va Buyer Ahmad and Bushehr and the risk was high in south Khorasan, Kohgiluyeh va Buyer Ahmad and Ardebil provinces.
Conclusion: Although report of water food borne diseases are mandatory by local health centers of Iran and their trends show decreasing pattern, but it seems it needs more inspection especially in provinces with low socioeconomic situation that have risk and hazard rate for these kind of diseases.

LMP07
Detection, characterization and comparison of *Campylobacter* spp. isolates from poultry, pork liver, raw milk and diarrheal patients in the Czech Republic

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Objectives: The most common etiological agent of human gastrointestinal tract infections in Europe are *Campylobacter* spp. isolates. There are several known reservoirs of this pathogen, especially poultry meat, pork meat and liver, and raw cow's milk. The objectives of this study were to evaluate the prevalence and species distribution of *Campylobacter* spp. in these foods at retail sale as well as to characterize their antibiotic resistance and genetic diversity with respect to identification of sources of the infections in the Czech Republic.
Materials and Methods: Between May 2013 and May 2014 a set of samples were collected in Czech supermarkets of fresh and frozen poultry, fresh pork liver and raw cow's milk from vending machines. These foods were examined for *Campylobacter* spp. presence. The isolates were identified with MALDI-TOF MS. The antimicrobial susceptibilities were tested with the microdilution method. Beta-lactamase OXA-61 was detected by polymerase chain reaction. Selected human and poultry isolates, were subtyped by pulsed-field gel electrophoresis (PFGE).
Results: The prevalence of *Campylobacter* spp. isolates was the highest in fresh poultry, the contamination of frozen poultry and fresh pork liver was lower. *Campylobacter* spp. were not detected in raw milk. *C. jejuni* prevailed over *C. coli*; both species were isolated from fresh poultry samples. High prevalence of resistance to ciprofloxacin, nalidixic acid and ampicilin was detected among poultry isolates. Pork liver isolates were resistant to quinolones (ciprofloxacin, nalidixic acid) and the majority of pork isolates were resistant to tetracycline and streptomycin, too. Human isolates showed resistance to quinolones. High prevalence of $bla_{\text{OXA-61}}$ gene was detected. PFGE analysis revealed that only several human isolates clustered with poultry isolates.
Conclusion: Our results revealed high prevalence of *Campylobacter* spp. at retail sale in the Czech Republic. The isolates are mainly resistant to quinolones, pork liver isolates also to streptomycin and tetracycline. Only several of human campylobacter isolates were genetically related to genotypes found in poultry.

Supported by the grant IGA MZCR NT/14392.

LMP08
Identification of a potential bacteriocin gene of an enterohemorrhagic *Escherichia coli* O104:H4 strain

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In 2011, the largest outbreak of enterohemorrhagic *E. coli* (EHEC) occurred in Germany with more than 3800 infections including nearly 3000 cases of EHEC gastroenteritis and over 800 cases of hemolytic uremic syndrome (HUS). The causative agent was a member of a rare *E. coli* serotype O104:H4 and has been classified as an enteroaggregative *E. coli* that had acquired Shiga toxin gene and other genetic elements.Interestingly, this strain is negative in production of a classical colicin. This differentiates the strain from other O104:H4 strains and raises the question of how this strain is able to outcompete other bacterial strains in the human gut.
First evidence of a potential bactericidal activity was assessed by a competition assay [1], where strain *E. coli* O104:H4 (E965) was incubated together with different *Enterobacteriaceae* strains (*E. coli, Salmonella, Citrobacter, Klebsiella* and *Hafnia* species). Growth inhibition of all *E. coli* strains (11 applied) was detected, while no inhibition was observed with strains of other genera.
I*n silico* analysis of the published genome of *E. coli* O104:H4 str. 2011C-3493 [2] with the BAGEL automated bacteriocin mining tool (http://bagel2.molgenrug.nl/) indicated the existence of potential bacteriocin genes. In order to identify the gene or gene cluster conferring potential bactericidal activity a genome library of *E. coli* O104:H4 (E965) was constructed. 860 cosmid clones were screened using two *E. coli* indicator strains (E162 and E164). Four bactericidal activity conferring cosmid clones were identified. Sequence analyses revealed a single locus in the genome of the *E. coli* O104:H4 (E965), which could confer bactericidal activity against other *E. coli* strains.
Further investigations will focus on detection of the activity conferring open reading frame or operon using transposon mutagenesis.

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LMP09
Meat-associated pseudomonads: molecular characterization and identification

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Pseudomonads belong to the main spoilage microbiota of cold stored meats. Currently recognized dominating species are represented by *P. fluorescens*, *Ps. lundensis* and *P. fragi*. However, the identification and characterization of the Gram-negative microbiota of meats is still far from exhaustive. This is especially true for the pseudomonads, a genus with high heterogeneity and biodiversity on species and subspecies level. Many pseudomonads still lack a clear taxonomic classification. Therefore, conventional phenotypical methods often deliver dissatisfying Results: On the other hand, molecular identification and subtyping methods, including rapid methods such as whole-cell protein fingerprinting by MALDI-TOF MS have become powerful diagnostic tools and are expected to considerably improve our knowledge of microbial species composition in this area.

In an attempt to characterize and identify *Pseudomonas* isolates from pork and beef using classical microbiological methods, published genus- and species-specific PCR and MALDI-TOF mass spectrometry (MALDI-Biotyper Microflex, Bruker Daltonics GmbH) we were able to assign all isolates to two major groups, (1) the highly diverse *P. fluorescens* group comprising more than 20 validly described species, and (2) the *P. chlororaphis* group including *Ps. fragi* and *P. lundensis* among others (Anzai et al., 2000). While most strains identified by PCR as *P. fragi* were confirmed by MALDI-TOF MS (log score > 2.3), this was not the case for all of the other isolates. By additional sequencing of the rpoB gene the species ID's of a number of isolates were confirmed and the respective strains could be allocated to *Ps. fragi* , *P. lundensis* and a distinct separate cluster which was also suggested by MALDI-TOF MS. A considerable number of unclear isolates, all belonging to the *Ps. fluorescens* group, clustered close to *P. synxantha* and/ or *P. libanensis* and *P. gessardii*.
Further comparison, including more type strains, is necessary in order to obtain a more precise picture of meat associated pseudomonads.

LMP10
Release of bioactive peptides from milk protein by proteolytic enzymes from lactic acid bacteria; Cloning and expression of two cell-envelope associated proteinases

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Lactic acid bacteria (LAB) are extensively used as starter strains in the manufacture of various fermented products. They are increasingly marketed as health-promoting bacteria. Certain strains have been shown to produce from milk protein bioactive peptides, which are released through proteolysis from casein by the cell-bound proteinases [1]. Subsequently, released peptides are predominantly translocated into the cytoplasm. Bioactivity, such like ACE-inhibition, could be found in the peptides remaining in the medium [2].In our study, we screened for proteolytically active lactobacilli and lactococci with the FITC assay [3]. Highest proteolytic activity was found in strain 92202, followed by strain 92059 [2]. For species identification, we performed 16S rDNA sequencing and used bioinformatical tools (ARB) for species-tree construction. Nucleotide sequence analyses together with physiological sugar fermentation tests suggested that strain 92059 is a *Lactobacillus delbrueckii* subsp. *bulgaricus* and 92202 is a *Lactobacillus delbrueckii* subsp. *lactis* strain, respectively. Presence of proteinase genes in both strains was confirmed by Southern blot analysis. Proteinase genes from both strains were amplified via PCR with primers designed on the basis of published sequences of *Lactobacillus delbrueckii* and cloned into pSMART vector for sequencing. The modified genes coding for all domains essential for activity were expressed in *E. coli* using vector pBad. In *Lactococcus lactis*, over expression is being optimized with the nisin controlled expression system (NICE). The purified enzymes will be used to degrade milk protein for release of bioactive peptides.

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METABOLISM AND METABOLIC NETWORKS/ METABOLOMICS

MMP01
Microfluidic Single-Cell Cultivation Systems: Versatile Tools for Microbiology

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Single-cell studies are of interest in many disciplines in microbiology. Traditionally, flow cytometry and agarose pad based image analysis are used to study single-cell behaviour. Microfluidics provides novel technologies, especially for single-cell analysis at well controllable environmental conditions and high spatiotemporal resolution. Thus far, microfluidic single-cell growth studies were not pursued systematically, since many systems operated only on the level of proof of principle. This has drastically changed during the last years and especially disposable microfluidic PDMS-glass systems are of increased popularity [1].

In this contribution we present the versatility of our microfluidic single-cell analysis platform, which was continuously improved over the last three years [1,2]. We will demonstrate, how tailored designs allow for the analysis of specific biological questions on single-cell level. Combined with automated time-lapse microscopy, microbial processes can be investigated with high temporal resolution or in a high throughput manner.
As a model, the growth pattern of *Corynebacterium glutamicum* WT was investigated in detail under various environmental conditions. Remarkably, individual cells within isogenic microcolonies showed significant differences within growth and morphology. The occurrence of these cells was investigated further by analyzing strains with genetically encoded reporter systems optically visualizing SOS response. High-throughput screening experiments revealed spontaneous SOS induction in 0.07-0.5% of the total population which is comparable to results obtained from large-scale cultivation in combination with flow cytometry [3].
Our results prove that microfluidic single-cell cultivation systems are powerful tools to further understand microbial processes and can be used for both, the investigation of fast cellular processes as well as to quantify rare cellular events. Nevertheless, these systems are still in an early phase of development, making a critical discussion of advantages/disadvantages and interdisciplinary collaborations between engineers and biologists necessary.

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MMP02
The crystal structure of 3-sulfinopropionyl-coenzyme A (3SP-CoA) desulfinase from *Advenella mimigardefordensis* DPN7^T: a novel desulfinase with an acyl-CoA dehydrogenase fold

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A novel 3-sulfinopropionyl-coenzyme A (3SP-CoA) desulfinase from the betaproteobacterium *Advenella mimigardefordensis* strain DPN7^T (Acd_{DPN7}) was identified during investigation of the 3,3'-dithiodipropionate (DTDP) catabolic pathway [1]. DTDP is an organic disulfide and a precursor for the synthesis of polythioesters (PTEs) in bacteria. Acd_{DPN7} catalyzes the sulfur abstraction from 3SP-CoA, a key step during the catabolism of 3,3'-dithiodipropionic acid. Additionally, three other 3SP-CoA desulfinases, which were previously annotated as acyl-CoA dehydrogenases, were later identified and biochemically characterized [2]. However, the actual structure of 3SP-CoA desulfinases and the distinct differences to acyl-CoA dehydrogenases were unclear. Hence, the availability of a crystal structure and the comparison with the well investigated acyl-CoA dehydrogenases was highly desired. Here we present the crystal structure of the Acd_{DPN7} apo enzyme and its complex with the substrate analog succinyl-CoA with a resolution of 1.9 Å and 2.46 Å, respectively. Acd_{DPN7} belongs to the acyl-CoA dehydrogenase superfamily fold and that it contains one non-covalently bound FAD per monomer (Fig. 1). Each FAD interacts with amino acid residues of two subunits, indicating a structural role of this cofactor. The enzyme does not show any dehydrogenase activity while the desulfination reaction does not require oxygen or another electron acceptor. The latter provides further evidence that the FAD cofactor is not used for the desulfinase activity. The positioning of the succinyl-CoA group in the crystal complex confirms Arg84 as a key residue in the desulfination reaction, as indicated by R84K, C122S and Q246E mutant activity assays. Acd_{DPN7} is the first desulfinase with an acyl-CoA dehydrogenase fold to be reported, which points at the versatility of this enzyme scaffold to adapt other activities than dehydrogenation.

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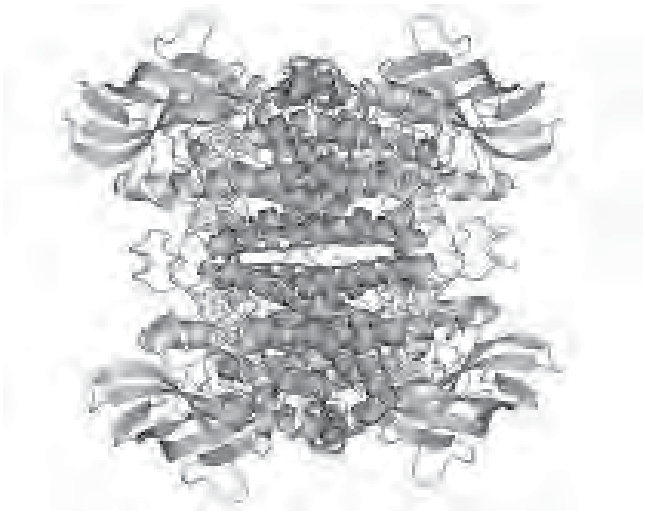
Figure 1



MMP03

^{13}C Metabolic Flux Analysis of *S. cerevisiae* Using Gas Chromatography-Tandem Mass Spectrometry

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Knowledge of metabolic fluxes in living cells is important for both fundamental research on (microbial) metabolic network operation and for guiding rational strain engineering in industrial biotechnology. ^{13}C-tracer based metabolic flux analysis (^{13}C-MFA) is a powerful tool for the quantification of intracellular fluxes. Cells are fed with ^{13}C-labeled substrate and in dependence of metabolic network operation the ^{13}C-carbon isotopes are distributed within the cellular constituents. The resulting labeling patterns are determined and used for computational analysis of the flux distribution. The common way of measuring labeling patterns of, e.g., amino acids is gas chromatographic (GC) separation combined with mass spectrometric (MS) detection. However, the information content obtained from the analysis of single fragmented amino acids is limited and often insufficient to unambiguously determine all fluxes within the central carbon metabolism. [1-3] Here, to increase the quality of ^{13}C-based MFA, we applied tandem mass spectrometry (MS/MS) using a triple quadrupole MS for the measurements of ^{13}C labeling patterns of proteinogenic amino acids. The applicability of this approach to obtain high-quality flux distributions is indicated by revisiting central carbon metabolism of *Saccharomyces cerevisiae*. Within this study, the amino acids fragments resulting from electron impact ionization were separated in the first quadrupole and underwent a second fragmentation step in the collision chamber. This fragmentation step was optimized for distinct amino acid fragments that carry the information required for high quality flux analysis. The additional fragmentation increases the number of fragments significantly and hence the information about the distribution of mass isotope isomers (isotopomers) of the analytes. Importantly, it is straight forward to gather the information of the MS and MS/MS experiments in a single run and the combined isotopomer data can be analyzed in one go. The advantage of combined MS - MS/MS-experiments for high-quality MFA is discussed. The ease of the combination and the significant increase in information content argue for coupled MS- and MS/MS-experiments and is therefore the method of choice for future applications.

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MMP04

Mycobacterium tuberculosis has a natural ornithine aminotransferase (*rocD*) mutation discontinuing a highly expressed pathway for arginine utilization

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Introduction: During bacterial infection arginine is the central metabolite in the pathogen-host interaction as it's the substrate for nitric oxide synthase (iNOS). Utilization of host arginine by other intracellular pathogens supports bacterial survival during infection. We hypothesize that arginine provides a nitrogen source for *Mycobacterium tuberculosis* (*Mtb*) within the nutrient limited microenvironment during infection.
Methods and Results: Using a genetic and biochemical approach involving gene expression analysis, generation of knock-out mutants in virulent *Mtb* as well as ^{13}C- and ^{15}N- based fluxome analysis we show on the one hand that *Mtb*, growing under arginine conditions, highly expresses a gene cluster encoding a putative arginine transporter and homologues for the arginase pathway for example *rocD* (ornithine aminotransferase). On the other hand *rocD* in *Mtb* is non-functional due to a frameshift mutation. Our results further suggest that in *Mtb* utilization of arginine occurs via an altered pathway leading to formation of proline.
Conclusion: We conclude during evolution *rocD* in *Mtb* has been inactivated, which might be associated with the intracellular life style. Arginine, which is present in the nutrient limited environment of the host, provides an efficient substrate for proline synthesis.

MMP05

Genome-scale reconstruction of the *Streptococcus pyogenes* metabolic network reveals growth requirements and indicates potential drug targets

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Introduction: *S. pyogenes* (group A streptococcus, GAS) belongs to the group of lactic acid bacteria (LAB) which are characterised by their capability to ferment glucose to lactic acid [1]. LAB colonize multiple biotopes, including foods, plants or the human body. Some LAB play essential roles in the fermented food and beverage industry, while others, such as *S. pyogenes* possess pathogenic features [2]. Metabolic networks comprise associations between genes and metabolic reactions and facilitate the analysis of the microbial metabolism. We reconstructed the metabolic network of *Streptococcus pyogenes* using the genome sequence of the NZ131 serotype M49 strain of *S. pyogenes*, together with already existing and curated networks of other species, and validated the model experimentally.
Materials and Methods: The AUTOGRAPH method [3] was applied to the Genbank NCBI annotation file of *S. pyogenes* M49 together with four manually curated metabolic networks from *B. subtilis* subsp. subtilis str. 168 [4], *E. coli* K12 [5], *L. plantarum* WCFS1 [6] and *L. lactis* IL1403 [4]. The output file was further curated manually. The model was simulated and analysed using PySCeS-CBM which is a platform for constraint-based modelling. Model validation included amino acid auxotrophy measurements and carbon flux analysis of wild type and mutant strains in chemostat cultures.
Results: The developed network comprises 577 reactions and 558 metabolites, uncovering specific metabolic features of *S. pyogenes*. E.g., initial inconsistencies between model predictions and published data on amino acid auxotrophies were solved experimentally. As predicted by the model and in contrast to published data, *S. pyogenes* is not auxotrophic for serine, proline, glycine and cysteine. For further model validation, the measured steady state concentrations of amino acids and endproducts of *S. pyogenes* wild type and corresponding *arcA* (arginine deiminase)and *glnA* (glutamine synthetase) deletion strains grown in a bioreactor at two dilution

rates and two pH values, were transformed into fluxes of utilization. These experimentally measured values were used to restrict the exchange and transport reactions in the model.
Conclusions: Finally, the established genome-scale model can be used to understand the growth requirements of the human pathogen *S. pyogenes* and define optimal and suboptimal conditions, but also to describe differences and similarities between *S. pyogenes* and related lactic acid bacteria such as *L. lactis* in order to find strategies to reduce the growth of the pathogen and propose drug targets.

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MMP06
Bacterial Metabolites for a New Cost-Effective Method of Diagnosing Urinary Tract Infections

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Urinary tract infection (UTI) is the second most common infection in humans. It is mainly caused by bacteria and is often classified as simple (uncomplicated) or complicated, both treated with antibiotics. Recurrent UTIs and bacterial resistance to antibiotics represent a challenge in the therapy. For a precise treatment, fast and cost-effective method for the identification of strain dependent UTIs is needed. Here, we are searching for metabolic signatures of bacteria causing UTI as potential biomarkers by using high-resolution QTOF/MS as an analytical platform. For the determenation of optimal metabolite composition, growth rates in artificial urine medium were detected for selected typical bacteria found in patients with urinary tract infections.

MMP07
An in silico metabolic model of the organohalide-respiring Sulfurospirillum multivorans and DehaloCyc: A database for reductively dehalogenating bacteria

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Organohalide respiration is an ecologically important and physiologically not well understood process in certain bacteria. Much of the information about this process currently known is derived from genomic data. We present here a genome-wide metabolic model of the organohalide-respiring epsilonproteobacteriumSulfurospirillum multivorans. The model contains approximately 1300 reactions and 1000 metabolites. With this model we can predict i) fluxes through all reactions in mmol/h, ii) growth rates of the organism, iii) substrate uptake and product formation, iv) pathway usage and v) gene knockout effects. Results of computational analyses are compared to experimental data.
Furthermore we present a database called DehaloCyc (*) for organohalide-respiring bacteria. The focus of DehaloCyc will be (i) a genome grounded collection of organism specific informations, (ii) to make these informations publicly available and (iii) to permit comparative analysis between different dehalogenating organisms. Curation of the database can be set up with the help of the scientific community. Created with Pathway Tools software (Stanford Research Institute International), DehaloCyc offers the same functional capabilities as the well-known BioCyc database collection. This allows insertion of additional informations about e.g. regulation. Currently there is data of 23 bacterial species and strains available. Among them, S. multivorans has received extensive manual curation.

MMP08
Diversity of Bacterial Steroid Degradation: Degradation of Cholate by *Novosphingobium* sp. strain Chol11

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Bacterial transformation of steroid compounds is an essential part of the production of steroid hormones in the pharmaceutical industry. Furthermore, bacterial degradation of steroid hormones became of major interest in ecotoxicology because some of the synthetic steroid hormones are only poorly degraded in sewage treatment plants and agricultural settings and are believed to act as endocrine disruptors. Despite this relevance of bacterial steroid degradation the knowledge about the metabolic pathways is still small.
The best-described pathway for bacterial degradation of steroid compounds proceeds via oxidation of the A-ring and stepwise removal of the side chain leading to the formation of $\Delta^{1,4}$-diene-3,17-diones (ADDs). We investigate this pathway using *Pseudomonas* sp. strain Chol1 as model organism and the bile salt cholate as a model substance. There, DHADD, a dihydroxy-derivative of ADD is formed as an intermediate. Recently, we have isolated a cholate-degrading actinobacterium, *Dietzia* sp. strain Chol2, which does not form DHADD or other intermediates with 3-keto-$\Delta^{1,4}$-diene structure. Instead, an intermediate with a triene structure, 3,12-dioxo-4,6-choldienoic acid (DOCDA), was formed [1]. To investigate whether also other bacteria degrade cholate via this intermediate we set up enrichment cultures with DOCDA as substrate and could easily isolate bacteria that degraded cholate via DOCDA. In culture supernatants of one of these strains, *Novosphingobium* sp. strain Chol11, a further unknown intermediate was detected and identified as 12β-hydroxy-androsta-1,4,6-triene-3,17-dione (HATD). In cell extracts of strain Chol11 cholate and 3-ketocholate were converted into DOCDA via Δ^4-3-ketocholate. In the presence of phenazine methosulfate (PMS) a further double bond was added at Δ^1-position in the A-ring of DOCDA. Interestingly, the central $\Delta^{1,4}$-diene intermediate of the formely known pathway, DHADD, was also converted to the triene HATD.
The formation of 3-keto-$\Delta^{1,4,6}$ -triene-intermediates with whole cells and cell-free extracts indicated that at least the initiating reactions of the degradation sequence for cholate in strain Chol11 is different from the known pathway via 3-keto-$\Delta^{1,4}$-diene intermediates. We are currently applying transposon mutagenesis further investigating this unexplored pathway for cholate degradation in strain Chol11.
[1] Holert, Yücel *et al.* (2014) Evidence of distinct pathways for bacterial degradation of the steroid compound cholate suggests the potential for metabolic interactions by interspecies cross-feeding. *Environ Microbiol* 16(5):1424-1440

MMP09
Carbohydrate catabolism in *Phaeobacter inhibens* DSM 17395, member of the marine *Roseobacter* clade

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Transport, catabolism and substrate-specific regulation for *N*-acetylglucosamine, mannitol, sucrose, glucose and xylose in *Phaeobacter inhibens* DSM 17395 was investigated by proteomics and metabolomics, as genome analysis had not allowed unambiguous reconstruction. These carbohydrates can pass the outer membrane *via* porins identified in the outer membrane protein-enriched fraction. Subsequently, carbohydrate-specifically formed ABC transport systems channel the carbohydrates through the cytoplasmic membrane. Their coding genes mostly co-localize with the respective "catabolic" and "regulatory" genes. *N*-acetylglucosamine is catabolized *via* *N*-acetylglucosamine-6-phosphate and glucosamine-6-phosphate directly to fructose-6-phosphate. Notably, two of the three involved enzymes were newly predicted and identified. Mannitol is degraded *via* fructose, sucrose *via* fructose and glucose, glucose *via* glucose-6-phosphate and xylose *via* xylulose-5-phosphate. In total 30 proteins are predicted to be involved in carbohydrate uptake, regulation and degradation. 28 out of these were identified by proteomics and 19 were assigned to the respective function for the first time. The peripheral degradation pathways feed into the Entner-Doudoroff pathway, which is connected to the lower branch of the Embden-Meyerhoff-Parnas pathway. The enzymes involved in these pathways are more abundant in carbohydrate-grown *P. inhibens* DSM 17395 compared to succinate-grown

cells. Oppositely, proteins needed for the gluconeogenesis were present when *P. inhibens* DSM 17395 was grown with succinate. Abundances of proteins involved in the TCA cycle did almost not change. Conversely, the abundance profiles of TCA cycle metabolites reflected the differing growth rates achieved with the tested substrates. Homologs of the 74 genes involved in the reconstructed catabolic pathways and central metabolism are present in various *Roseobacter* clade members.

MMP10

Insights into the metabolome of *Streptococcus pneumoniae* under nutrient-limiting conditions

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is a human pathogen transmigrating from the upper nasopharyngeal cavity to the lungs thereby causing severe diseases such as pneumonia and bacteremia. Pneumococci are members of the clade of lactic acid bacteria lacking the tricarboxylic acid cycle and electron transport chain for aerobic or anaerobic respiration. We aimed for a protocol to gain metabolome samples to get deeper insight into basic metabolism under nutrient limiting conditions as they may appear inside the host.

Methods: We present here a detailed protocol for the reproducible and reliable generation of metabolome data for pneumococci. *S. pneumoniae* was cultivated in a chemically-defined medium (CDM) established on the basis on the eukaryotic cell culture medium (RPMI 1640). Extracellular metabolites were collected by filtrating the culture supernatant and analyzed by ^{1}H-NMR spectroscopy. To gain access to the intracellular metabolites the bacteria were rapidly separated from the growth medium and the metabolism was stopped on ice cold ethanol followed by liquid nitrogen freezing. The extracted metabolites were analyzed by LC-MS.

Results: The exometabolome of dynamically cultured non-encapsulated *S. p.* D39D*cps* and isogenic glucose-6-phosphate dehydrogenase deficient mutant D39D*cps*Δ*zwf* was analyzed under microaerophilic conditions and a surplus of glucose. Glucose was primarily catabolized via glycolysis to pyruvate and the most prominent extracellular metabolite due to homolactic fermentation was lactate. Remarkably fumarate, an intermediate of the tricarboxylic acid cycle (TCA), was also detected extracellularly, although pneumococci lack the TCA and the electron transport chain for aerobe and anaerobe respiration. When galactose was used as the sole carbon source in CDM, pneumococci execute a mixed-acid fermentation resulting in lactate, formate, acetate, and ethanol as end-products. First results of the intracellular metabolites showed that pneumococci accumulate high amounts of fructose-1,6-bisphosphate, an intermediate of the glycolysis and UDP-GlcNAc a precursor metabolite in cell wall metabolism.

Conclusion: With the established protocol in hand, we are able to analyze the exo- and endometabolome of pneumococci under diverse conditions, which allows deciphering the physiology of this human pathogen.

MMP11

Temperature- and nitrogen source-dependent growth of *Listeria monocytogenes* implicates an important role of 2-oxoglutarate as internal signal during adaptation to changing environments

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The ubiquitous pathogen *Listeria monocytogenes* lives either saprophytically in the environment or within cells in the vertebrate host. Thus it has to adapt its lifestyle to its ecological niche and the availability of nutrients. The aim of this study was to investigate the impact of different nitrogen sources and temperatures on growth of *L. monocytogenes*.

Growth analyses were conducted in a chemically defined minimal medium at 24 °C or 37 °C with either glutamine (Gln) or ammonium (NH_4^+) as nitrogen source. A preference for ammonium over glutamine was observed. This might be ascribed to the interrupted tricarboxylic acid (TCA) cycle in *L. monocytogenes* which is due to the lack of 2-oxoglutarate dehydrogenase. To allow a permanent glucose metabolism by continuous channeling of pyruvate in the TCA cycle, removal of the end product of the oxidative branch, 2-oxoglutarate, is important. 2-oxoglutarate is also the main building block for the *de novo* biosynthesis of glutamate. During growth on ammonium twice the amount of 2-oxoglutarate is removed from the TCA cycle compared to growth on glutamine to build up glutamate, suggesting an increased flux trough the oxidative branch of the citric acid cycle when grown on ammonium. Indeed, a slightly induced transcription of the TCA cycle genes *lmo1566* (isocitrate dehydrogenase CitC), *lmo1641* (aconitase CitB) and *lmo1072* (pyruvate carboxylase PycA) was observed via qPCR during growth with ammonium compared to growth with glutamine.

Interestingly, reduced growth on glutamine was more obvious at 24 °C than at 37 °C. When grown on the same nitrogen source but at different temperatures the removal of 2-oxoglutarate is mainly determined by the amount of glutamate used for the *de novo* biosynthesis of amino acids which is expected to be elevated at the optimal growth temperature of 37 °C. Combining temperature and nitrogen source leads to a model with highest intracellular 2-oxoglutarate concentration at 24 °C/Gln, a medium level at 37 °C/Gln and 24 °C/NH_4^+ and lowest levels at 37 °C/NH_4^+.

This model is further strengthened by qPCR studies in the wild type and a Δ*gltC* mutant. The transcription regulator GltC, which regulates transcription of *gltAB* (glutamate synthase) depending on the 2-oxoglutarate/glutamate ratio in *B. subtilis* (1), activates transcription of *gltB* in *L. monocytogenes* grown at 24°C/Gln and to a lesser extent in cells grown at 37 °C/Gln and 24 °C/NH_4^+ and represses *gltB* transcription at 37 °C/NH_4^+. Therefore, it is assumed that in *L. monocytogenes* the intracellular 2-oxoglutarate concentration might serve as internal signal that allows optimal adaptation of *L. monocytogenes* to changing environments by combining adaptations to various parameters, e.g. temperature and nitrogen source, at the same time.

(1) Picossi et al., 2014. Molecular mechanism of the regulation of *Bacillus subtilis gltAB* expression by GltC. *J Mol Biol.* Feb 2;365(5):1298-313.

MMP12

Beta beware: Microbial degradation of aromatic beta-amino acids

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The metabolism of canonical amino acids (i.e. the proteinogenic L-alpha amino acids) is well investigated with respect to uptake, enzymatic conversion reactions and degradation as well as production and excretion. By contrast, little is known about the fate of non-canonical amino acids (ncaa) bearing the amino group in beta or gamma position or in D-configuration or which are substituted with non-proteinogenic residues. These ncaa are often included in peptide-based natural compounds, presumably not least due to the high protease resistance of such peptides.

So profound knowledge of the biodegradation mechanisms of these definitely not “unnatural” amino acids is crucial in terms of

- defense mechanisms of microorganisms affected with these natural compounds - pharmacokinetics of these natural compounds when used as a drug (e.g. cytostatics containing aromatic beta-amino acids) - environmental aspects referring to the persistence of ncaa in soil and water. We established a fermentation process for a model organism *Burkholderia* sp. which we showed previously to degrade beta-phenylalanine, beta-tyrosine and para-chloro-beta-phenylalanine but not beta-homophenylalanine (Tab. 1, [1]). Using beta-phenylalanine as model substrate, we were able to calculate growth parameters, substrate usage and product formation, thus giving a first quantitative insight in the biodegradation of this ncaa.

[1] J. Rudat, J. Maur, S. Dold, C. Syldatk (2013), Proceedings of the Annual Conference of the VAAM, PHYP054 (ISNN 0947-0867)

Figure 1

β-amino acid	growth	enantiopreference
β-phenylalanine	++	(S)
β-homophenylalanine	-	(-)
β-tyrosine	+++	(S)/(R)
p-chloro-β-phenylalanine	+	(S) > (R)

MMP13

Glucose uptake in *Streptococcus pyogenes* M49

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Introduction: *S. pyogenes* (Group A *Streptococcus,* GAS) is a human pathogen responsible for a wide variety of different diseases, ranging from uncomplicated non-invasive infections of skin and mucous membranes to life-threatening infectious diseases [1]. While the virulence factors of GAS have been intensively studied, very little is known about the metabolism and its regulation, although it has been shown that the transcription of virulence genes is related to an altered expression of genes of carbon metabolism [2]. Belonging to the group of lactic acid bacteria, GAS metabolize a number of carbohydrates to lactate with glucose as preferred substrate [3]. In contrast to closely related bacteria, the major uptake system for glucose in *S. pyogenes* is still unknown. In *Lactococcus lactis*, glucose is mainly transported via the non-PTS permease GlcU, but also cellobiose- and mannose-PTS are involved. Here we discuss the role of several PTS and non-PTS permeases in glucose uptake in *S. pyogenes* serotype M49 [4].
Materials and Methods: We deleted genes encoding for four different PTS (PTS^{Man}, PTS^{Cel1}, PTS^{Cel2}, PTS^{Glu}) and one putative non-PTS permease potentially involved in glucose uptake in *S. pyogenes* M49 strain 591. BIOLOG phenotype microarrays were applied to analyze the substrate utilization of the deletion strains in comparison to the cognate wild type. Furthermore, we assessed growth in complex nutrient rich and chemical defined medium supplemented with glucose. Glucose consumption and generation of fermentation products were measured.
Results:: While none of the mutants had any growth deficiencies in complex THY medium, we noticed a significantly decreased biomass formation of mutants lacking GlcU, PTS^{Man}, PTS^{Cel2}, and PTS^{Glu}, the latter showing the most pronounced effect. Anyway, none of the mutants completely lost its ability to grow on glucose. The substrate utilization investigations revealed that the analyzed systems do not only play a role in glucose transport but are also involved in uptake of other sugars.
Conclusion: The differences in growth behavior in chemically defined medium indicate, that PTS^{Glu} apparently possess a significant function in glucose uptake in *S. pyogenes* M49 591, although the corresponding protein in *S. pyogenes* M1 MGAS5005 has been shown to transport maltose and maltotriose instead of glucose [5]. The potential GlcU permease is apparently less important than the respective protein in *L. lactis*. Our data indicate that three PTS and a putative GlcU are redundant systems involved in glucose uptake.

References

[1] Cunningham (2000), Clin. Microbiol. Rev. 13: 470-511, [2] Shelburne et al. (2008), PNAS 105: 1698-1703, [3] Fiedler et al. (2011), Appl. Environmet. Microbiol. 77: 612-17, [4] Castro et al. (2009), Mol. Microbiol. 71: 795-806, [5] Shelburne et al. (2008), Mol. Microbiol. 69: 436-52

MMP14

Substrate specificity of the dipeptide ABC transporter DppBCDF and its periplasmic substrate-binding proteins DppA1-A5 from *Pseudomonas aeruginosa*

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The ABC transporter DppBCDF of *Pseudomonas aeruginosa* PA14 is responsible for the uptake of di- and tripeptides. The substrate specificity of ABC transporters is determined by its associated substrate-binding proteins (SBPs). SBPs are located in the periplasm of Gram-negative bacteria. They bind their ligands with high affinity and deliver them to the associated ABC transporter. Five putative dipeptide-binding proteins (DppA1-A5) are present in *P. aeruginosa* PA14. We used Biolog phenotype microarrays to determine the substrate specificity of the DppBCDF transporter and its SBPs. The substrate spectrum of the SBPs was elucidated by complementation of a penta mutant deficient of all five SBPs with plasmids carrying individual SBPs. We found that 84 out of 162 dipeptides are recognized and delivered by the SBPs to the permease. DppA2 was able to recognize more di- and tripeptides than any other SBP. Furtermore, we observed that DppA2 and DppA4 are more efficient in tripeptide recognition than the other SBPs. DppA5 was not able to complement the penta mutant suggesting that this SBP is not involved in uptake of di- and tripeptides. Phaseolotoxin, a toxic tripeptide inhibiting the enzyme ornithine carbamoyltransferase, is also transported into *P. aeruginosa* via the DppBCDF transporter. The SBPs DppA1 and DppA3 are responsible for delivering phaseolotoxin to the permease.

MMP15

Calculation of the Coupled System "*Lactobacillus-Candida*" of Balanced Multispecies Knot of Biotope Network Depending on Biofilm Forming

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The pool system "*Candida+Lactobacillus*" is important for keeping healthy balance in urogenital biotope and highly sensitive for monitoring interstrains interactionsin the reaction of biofilm forming (BFF). Earlier we proposed multiknot coupled net microbiocenosis conception of biotope. The aim was to calculate completed prognostic/ diagnostic 2-directional coupled pool interspecies relationships balanced biotope knot "*Lactobacillus-Candida*" formula.
Clinical strains *Lactobacillus acidophilus* (I: 106,124,183a), *L.brevis* (II: 104,109,143), *L.casei* (III: 124b,183), *Candida albicans* (IVa: 23,147,320, IVb: 3,26,116), *C.krusei* (V: 5,60,125,135,185,309) and *C.tropicalis* (VI: 97,144,162,438,897) and were isolated from patient urogenital biotope. Mono- and mixed cultures in MRS were grown in micropanels (48 h, 37ºC). Stain of BF formed was extracted and measured at 620 nm. Strains BFF were calculated, ranged and species blocks within sets obtained were compared. The knot interspecies relationships were characterized as formula's immediate constituents.
Biotope knot coupled ordered formula included the following constituents (left: pool of influence, right: resulting preferential species BFF decreasing): 1) Directions *Lactobacillus—Candida*: I+II+III—IVa>VI>IVb. *Lactobacillus* pool increased ordering strains within functionally similar *Candida* group (IV and VI) succession of prolonged blocks: [23,320,147], [144,135,97,5,438,897] and [116,3,26]. Blocks IVa and IVb are not differed in antimycotic type resistance (IVa as more sensitive to antimycotics). Non-capability to utilize Sac (IVb,VI), Lac and Raf (IVb) was observed. Blastopore germ tubes were early registered (IVa>IVb). Population IVa reveals relatively higher pathogenic potential. 2) Directions *Candida—Lactobacillus*: IVa—I; IVb—II; VI—III; IVa+IVb—II>I>III; IV+V+VI—III>II. Summary cooperation of IVa and IVb resulted in synergistic appearance of *C.albicans* pool influence III similarly to *C.tropicalis* pool action.
Results indicate strong potential of calculated biotope populations' network formulas for prognostic and diagnostic evaluation of biotope intramicrobiocenosis coupled relationships.

MMP16

D-serine inhibits L-serine metabolism of *Staphylococcus saprophyticus*

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Introduction: The amino acid D-serine is present in relatively high concentrations in human urine and bacteriostatic or toxic to several non-uropathogenic bacteria. *Staphylococcus saprophyticus* is the only species of the staphylococci that is typically uropathogenic and the genome is the only of all sequenced staphylococci that possesses a D-serine-deaminase. This enzyme converts D-serine to pyruvate and ammonia. Therefore, *S. saprophyticus* is able to use D-serine as sole carbon and energy source as we could show previously. Here we report that D-serine is not only an additional nutrient for *S. saprophyticus* but also an inhibitor of growth. We show that this inhibition somehow affects the L-serine metabolism.
Materials and Methods: *S. saprophyticus*, a *dsd*A-knock-out mutant, the complemented *dsd*A-knock-out mutant and a D-serine-transporter mutant were grown in our chemically defined medium supplemented with glucose in presence of D-serine, L-serine or in presence with both enantiomers. Growth was monitored by determining OD_{600}.
Results: In presence of D-serine, wild-type *S. saprophyticus* and the *dsd*A-knock-out mutant exhibited a pro-longed lag phase. Interestingly, this effect

was more distinct in presence of both enantiomers. Neither the complemented *dsd*A-knock-out mutant nor the D-serine transporter mutant had a pro-longed lag phase under these conditions. In presence of L-serine all strains started to grow immediately.
Discussion: For several bacteria it was shown that D-serine inhibits pantothenic acid biosynthesis at different points. As *S. saprophyticus* requires pantothenic acid for growth and the chemically defined medium contains pantothenic acid, we can exclude that the observed growth inhibition is caused by this classical mechanism. We hypothesize that D-serine somehow inhibits the L-serine metabolism. The wild-type grew immediately in presence of L-serine and obviously utilized it as nutrient. In presence of D- and L-serine, *S. saprophyticus* had a pro-longed lag phase and was not able to utilize L-serine as long as D-serine was present. The D-serine deaminase converted D-serine to pyruvate, therefore the strain started to grow later. The lag phase of the *dsd*A-knock-out mutant is longer compared to the wild-type because it has no D-serine deaminase to detoxify D-serine and L-serine utilization is inhibited all the time. The complemented mutant did not show inhibitory effects as D-serine is converted faster due to the gene dosage effect. In the transporter mutant, the D-serine uptake is low and thereby the intracellular D-serine concentration. D-serine is converted directly by the D-serine deaminase and L-serine metabolism is not inhibited. Further experiments to analyze if D-serine inhibits the L-serine deaminases themselves or their expression are in progress.

MMP17
Evolutionary stability of heterogeneous nutrient consumption strategies in populations exposed to continuous and fluctuating environments

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Multiple nutrients, which can substitute each other with respect to the bacterial nutrition, are known to be consumed using different strategies. The best known are catabolite repression , i.e. substrate B is only consumed if substrate A is used up, and parallel consumption of substrates A and B by generalists. The theory, which strategy runs best under which conditions, is quite well understood. However, there is increasing knowledge that bacterial populations do not necessarily act uniformly, but that physiologically distinct subpopulations develop even in isogenic populations. Here, we analyze by a mathematical modelling approach, under which conditions a third strategy may be dominant, in which cells can switch between two subpopulations in a bet hedging-like way, where each subpopulation is only able to degrade one nutrient. We distinguish between two pathways: a degradation of both substrates a) by using (partly) the same enzymes (substrate competition) and b) via independent pathways. Our analysis showed that in the latter case, the generalist is always dominant, and bet-hedging never pays off. In substrate competition case however, bet-hedging can become optimal compared to strict catabolite repression, if environmental conditions fluctuate in a way that the future nutritional conditions cannot be predicted by the actual state.

MMP18
Role of three (p)ppGpp synthases (RSH, RelP and RelQ) in the stringent response of *S. aureus*

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Introduction: The stringent response is a global regulatory mechanism accountable for the synthesis of the bacterial alarmone (p)ppGpp upon nutrient limitations. Stringent response in *Staphylococcus aureus* is characterised by a decrease of the translational apparatus, lowering of the GTP pool, de-repression of the CodY regulon and activation of phenol-soluble modulins [1,2]. In Gram-positive bacteria, such as *S. aureus,* three (p)ppGpp synthases are detectable: the bifunctional RSH protein (RelA/SpoT homologue), consisting of a (p)ppGpp synthase domain and a (p)ppGpp hydrolase domain, and two small (p)ppGpp synthases designated as RelP and RelQ. In *S. aureus* the gene coding for the bifunctional enzyme RSH is essential.
Materials and Methods: Deletion mutant (*rsh, rsh^{syn}, relP, relQ* and *CodY*) were created and transformed with different *rsh* construct. The activity of this constructs was monitored by Northern Blot, growth curves and HPLC.
Results: We could show that the essentiality of RSH is attributed to the (p)ppGpp hydrolase activity of the enzyme, which is indispensable to prevent a toxic accumulation of (p)ppGpp due to RelP and RelQ activity. This is based on the observation that, in contrast to an *rsh* mutant, *rsh/relP* or *rsh/relQ* double mutants and a strain mutated only in the synthase domain of RSH (*rsh$_{Syn}$)* are all viable with no or little growth defects under nutrient-rich growth conditions. *In vitro* assays, using recombinant expressed purified proteins, revealed a slightly higher (p)ppGpp synthase activity of RelP compared to RelQ. Both enzymes use GTP and GDP as pyrophosphate acceptors to generate pppGpp and ppGpp, respectively. Over-expressing experiments with different *rsh* constructs indicated that N-terminal (p)ppGpp synthase activity requires the enzymatic activation through the C-terminal sensing domain. In contrast the hydrolase activity results independent by the same domain.
Discussion: The stringent response of *S. aureus* seems to be achieved by the interplay of three proteins, each of one responding to different environmental signals. Since *S. aureus* responds very sensitive to intracellular (p)ppGpp accumulations, the fine tuning of synthesis and degradation of these molecules seems to be very essential for an optimal growth and survival of this major human pathogen.

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MMP19
Metabolic footprint comparison of *Burkholderia pseudomallei* morphotypes

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Introduction: As an environmental saprophyte and facultative human pathogen *Burkholderia pseudomallei* is able to survive and grow in habitats with various biochemical compositions. It has to adapt to nutrient limiting environments in the soil but also to at least in certain components nutrient rich environments in the mammalian host. A common feature of *B. pseudomallei* is the expression of different colony morphotypes of single strains. These morphotypes can differ in virulence, biofilm formation and survival in host cells and might therefore reflect an adaptation process to different niches. We therefore hypothesized, that morphotypes differ in their carbon and nitrogen source utilization as well as in the secretion of metabolites.
Materials and Methods: By using ^{1}H-NMR we are able to monitor and quantify the uptake of carbohydrates and amino acids and simultaneously the secretion of metabolites (metabolic footprints) of two different *B. pseudomallei* strains K96243 (6 morphotypes) and E8 (8 morphotypes). To accomplish this we sampled the supernatant of growing cultures over 60 hours. We monitored more than 60 signals of which at least 30 were identified and quantified.
Results: A key finding of our analysis is the extracellular appearance of gluconate simultaneously to the decrease of extracellular glucose concentration which indicates extracellular conversion of glucose to gluconate. The extracellular oxidation of glucose prior to uptake has been shown previously for *Pseudomonas* species and *Burkholderia cenocepacia*. Furthermore we could differentiate between amino acids, that were taken up in a similar rate between all morphotypes like glutamine and aspartate and amino acids whose uptake varied between the morphotypes like the branched chain amino acids. Beside signals for known metabolites, we detected the appearance of yet unidentified compounds with a broad diversity among the morphotypes.
Conclusion: In conclusion our results indicate that different morphotypes of *Burkholderia pseudomallei* share common metabolic features like the conversion of glucose into gluconate prior to uptake, but also generate metabolic footprints by which they might be recognized and differentiated. Yet unknown metabolites secreted by different morphotypes might serve as an interesting recognition feature once identified.

MMP20

Sulfoquinovose degradation in the typical soil bacterium *Pseudomonas putida:* First evidence for a proposed Entner-Doudoroff-type pathway

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Sulfoquinovose (SQ) is the polar headgroup of the plant sulfolipid sulfoquinovosyl-diacylglycerol (SQDG), which is present in all higher plants, mosses, ferns, algae and most photosynthetic bacteria. With an estimated annual production of SQ of about 10 billion tons, it comprises a major proportion of the organosulfur in nature, where SQ is degraded by bacteria. Very recently, the first degradation pathway for SQ has been discovered in the Enterobacterium *Escherichia coli* K-12 *via* a sulfoglycolytic pathway. However, in contrast to the intestinal habitat of Enterobacteria, the bacteria and pathways involved in SQ mineralization in terrestrial habitats, e.g. in soils, or in limnic habitats for a recycling of the organosulfur as sulfate, remained undefined. We isolated a typical soil bacterium, *Pseudomonas putida SQ1*, which is able to utilize SQ for growth. Our aim is to elucidate its degradation pathway for SQ. The genome of *P. putida* SQ1 was sequenced using Illumina Hiseq2000 and annotated in the IMG pipeline. In contrast to *E. coli*, *P. putida* lacks the glycolytic key enzyme phosphofructokinase and, thus, no sulfoglycolytic pathway for SQ degradation is possible. Subsequent proteomic, transcriptional and bioinformatic analyses suggested five inducible genes for SQ degradation, which are located in one gene cluster. The proposed SQ pathway would proceed in analogy to the Entner-Doudoroff pathway for glucose, where intracellular SQ would be oxidized to 6-sulfo-gluconolactone *via* an NAD-dependent dehydrogenase, dehydrated to 6-sulfogluconate *via* a lactonase, and further dehydrated to 2-keto-3-deoxy-6-sulfogluconate *via* a dehydratase. The latter would be cleaved into pyruvate and sulfolactaldehyde by an aldolase. Pyruvate can enter the central metabolism to sustain growth of *P. putida*, and sulfolactaldehyde would be oxidized *via* a dehydrogenase to sulfolactate.
Hence, we suggest SQ degradation in *P. putida via* an Entner-Doudoroff-type pathway to sulfolactate, which is excreted. Other bacteria utilize the sulfolactate completely *via* a defined pathway, and release the sulfonate-group as sulfate. Thus, the sulfur cycle for SQ is closed within a bacterial community. Due to the high abundance of this compound in plants, it is a major component of the organosulfur in nature, and therefore its mineralization is likely to play an essential role in sustaining the sulfur cycle in soils. Now, through the availability of candidate genes for SQ degradation, the significance of these pathways, e.g. in soils, can be tested by molecular methods.

MMP21

Development of a genetically encoded trehalose sensor and application for trehalose detection *in vitro* and *in vivo*.

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The disaccharide trehalose is widely known for its function as compatible solute under conditions of hyperosmotic stress and to protect proteins and membranes during desiccation or heat stress [1]. Under these conditions, trehalose detection is simplified by its accumulation to high concentrations. In contrast, trehalose is only found in trace amounts in plants, where it serves as signaling molecule [1]. In mycobacteria and related bacteria trehalose is essential as a precursor for cell wall glycolipids but its detection is hampered by the intracellular lifestyle of some of these pathogenic bacteria [2]. Genetically encoded nanosensors can be used for the visualization of the intracellular concentrations of ions, amino acids, sugars, and other metabolites in living cells [3]. These sensors are composed of a binding protein terminally fused to two GFP variants like CFP and YFP. They translate metabolite binding into an altered Förster-Resonance-Energy-Transfer efficiency and thus allow the online determination of metabolite concentrations in a non-disruptive manner [3].
We here show the construction of a genetically encoded trehalose sensor by linking the periplasmic trehalose binding protein from *Corynebacterium glutamicum* to variants of CFP and YFP. The sensor response to the addition of trehalose was optimized by truncation of the peptide linkers and the adjacent protein domains. *In vitro*, the resulting sensor specifically binds trehalose with a dissociation constant of 0.24 µM, enabling the sensitive and specific detection of trehalose. Using site-directed mutagenesis, variants with increased dissociation constants for trehalose were also constructed. To further test the functionality of the sensor, culture supernatants of *C. glutamicum*, which is known to accumulate trehalose in the supernatant, were analyzed both enzymatically and using the trehalose sensor. The obtained results are in good agreement. To show its function *in vivo*, the sensor was expressed in *Escherichia coli* BL21. When subjected to an osmotic upshift during growth in minimal medium, the sensor displayed the immediate intracellular accumulation of trehalose as an osmoprotectant in this bacterium. The trehalose sensor presented here adds to a toolbox of genetically encoded nanosensors for different metabolites. Its high affinity and specificity and its successful application *in vitro* and *in vivo* shown here demonstrate its potential for further applications.

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MMP22

Methylaspartate cycle in *Haloarcula hispanica*

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Microbial life is feasible in all ranges of salt concentration, even saturated, and one of the most important microbial habitants of these environments is haloarchaea of *Halobacteriaceae* family. In spite of their common hypersaline environment, they are different in their nutritional demands and metabolic pathways. Many organic compounds are routinely metabolized via acetyl-CoA. Assimilation of acetyl-CoA into cellular building blocks requires dedicated anaplerotic pathway(s). Although some haloarchaea use the glyoxylate cycle for growth on acetate, many species possess an alternative pathway, the methylaspartate cycle, which was recently discovered in *Haloarcula marismortui* (*1*). The discovery was based mainly on the studies of *H. marismortui* cell extracts, and most of the characteristic enzymes of the cycle (with the exception of malyl-CoA/β-methylmalyl-CoA lyase) have not been characterized, although the corresponding genes were putatively identified. Here we chose another species, *Haloarcula hispanica*, as a model organism for further research on the methylaspartate cycle because it exhibits unusually low restriction activity in the genome and is therefore one of the best tractable models for haloarchaeal studies (*2*). All enzyme activities required for the functioning of the methylaspartate cycle were detected in cell extracts of acetate-grown *H. hispanica*. The activities of the key enzymes of the cycle were strongly upregulated during growth on acetate, indicating their involvement in acetate assimilation. Knockout mutants of the genes encoding key enzymes of the methylaspartate cycle (glutamate mutase, methylaspartate ammonia-lyase, putative succinyl-CoA:mesaconate CoA-transferase and putative mesaconyl-CoA hydratase) were unable to grow in the presence of acetate, although the growth on pyruvate (or acetate plus pyruvate) was not impaired. Furthermore, with these mutants we could confirm the identification of HAH_1336 and HAH_1340 as succinyl-CoA:mesaconate CoA-transferase and mesaconyl-CoA hydratase, respectively. Incubation of the mutants in the presence of acetate led to the accumulation of the corresponding intermediates of the methylaspartate cycle, further confirming its functioning in haloarchaea.

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MMP23

1,4-dehydrogenating Acyl-CoA dehydrogenase: a key enzyme in a novel anaerobic cyclohexane carboxylic acid degradation pathway

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Cyclohexane carboxylic acid (CHC) mainly occurs as functional group of ubiquitous biological compounds, e.g. naphthenic acids in crude oil or polyketide antibiotics. The aerobic degradation of CHC proceeds via oxidative aromatization to 4-hydroxybenzoate involving mono- and dioxygenases dependent oxidation and the sunbsequent degradation in the beta-ketoadipate pathway (1). The anaerobic CHC degradation has so far only been studied in the phototrophic *Rhodopseudmonas palustris* and involves the activation to the corresponding CoA ester followed by the 1,2-dehydrogenation to cyclohex-1-enoyl-CoA. The latter is then degraded by

specific enzymes of benzoyl-CoA degradation pathway of aromatic compounds. However, the degradation of aromatic compounds via cyclohex-1-enoyl-CoA is has so far uniquely been described for *R. palustris*; in all other aromatic compound degrading anaerobes the degradation pathway proceeds via cyclohexa-1,5-dienoyl-CoA. For this reason, it was unknown how cyclohexane carboxylic acid can be degraded in anaerobic organisms other than *R. palustris* (2). We present a novel pathway found in obligately anaerobic Deltaproteobacteria involving a succinyl-CoA:CHC CoA-transferase and two FAD-containing acyl-CoA dehydrogenases yielding cyclohexa-1,5-dienoyl-CoA as a common intermediate for aromatic compound and CHC degradation (3). Formation of the latter from the cyclohex-1-enoyl-CoA intermediate is achieved by a 1,4-dehydrogenation instead of the typical 1,2-dehydrogenation reaction catalyzed by standard acyl-CoA dehydrogenases. Sequence alignments and 3D-structure modeling of the corresponding cyclohex-1-ene-1-carboxyl-CoA dehydrogenases from *Geobacter metallireducens* and *Syntrophus aciditrophicus* revealed that the proton-accepting glutamate, present in all known acyl-CoA dehydrogenases, is lacking. Instead, an aspartate is located at the opposing side of the substrate binding pocket as potential proton acceptor for the proposed 1,4-dehydrogenation mechanism. Site-directed mutagenesis and heterologous expression of the underlying gene in *Escherichia coli* were in accordance with the proposed mechanism. The 1,4-dehydrogenating acyl-CoA dehydrogenase from *G. metallireducens* is the first one described and may be useful for future biotechnological syntheses.

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MICROBIAL PATHOGENESIS

MPP01
Role of *hpyAVIBM* gene (HP0051) of *Helicobacter pylori* in virulence

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Helicobacter pylori is a common human pathogen that colonizes in human stomach and thereby causes gastritis, peptic ulcer, and gastric cancer. *H. pylori* uses different mechanisms to modulate its interaction with host in order to evade host immune response. The *H. pylori* genome consists of numerous restriction-modification genes but the relevance of having such huge number of these genes is still not clear. It is now established that N^6-adenine methylation plays a crucial role in bacterial gene regulation and virulence but not much is known about the effect of C^5-cytosine methylation on these aspects. In this study we have examined the influence of an orphan cytosine methyltransferase, *hpyAVIBM* on gastric infection in mice and cultured cells. Histopathological staining showed that deletion of this *hpyAVIBM* gene in *H. pylori* strain (SS1) has more damaging haemorrhagic effects on mice stomach. The gelatin-zymography result demonstrated that the mice infected with mutant *H. pylori* strain (SS1Δ*hpyAVIBM*) have significant up-regulation of pro-MMP-9 than the mice infected with wild type bacteria. In addition ELISA results of pro-inflammatory cytokines (IL-6 and IL-1β) also proved that mutant strain has more inflammatory effect on mouse stomach than its wild type counterpart. The immunohistochemistry data also added strong evidence on the fact that mutant strain is causing more epithelial cell damage. In agreement, cell culture studies revealed that this SS1Δ*hpyAVIBM* strain caused more apoptotic death in AGS cells compared to the SS1 strain. Knockout strain induced strong immune response as monitored by higher pro-inflammatory cytokines induction, probably because of the up-regulation of various virulence factors. Our data indicates that DNA methylation by this methylase could be playing a critical role in modulating virulence and its interaction with the host.

MPP02
Development of a new human *ex vivo* organ infection model for analyzing the adherence of *Bartonella henselae* using human umbilical cord veins

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Bartonella henselae is an endotheliotropic human pathogen causing, e.g., bacillary angiomatosis. The ability to adhere to endothelial cells (ECs) is crucial for establishing a successful infection. The trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) is responsible for binding to extracellular matrices and ECs. Previously, *in vitro* infection models (static and dynamic) have been developed; however, still no appropriate animal infection model is available for *B. henselae*.
To better understand the natural infection processes, we refined the dynamic *in vitro* experimental approaches to an *ex vivo* infection model using fresh human umbilical cord veins. *B. henselae* wild type (WT) and BadA deficient *B. henselae* (BadA$^-$) strains were used to elucidate the BadA-mediated host cell adherence. To determine the exact amount of bacteria associated with ECs, we established two methods using (a) direct fluorescence measurements and (b) absolute quantification via qRT-PCR. For fluorescence measurements, live bacteria were labelled with CFSE (carboxyfluorescein succinimidyl ester) before infection and the human umbilical cord derived ECs were stained with an EC-specific antibody (CD31; Alexa®647) post infection. Adherent bacteria and ECs were quantified by measuring the relative fluorescence units (RFU). For absolute quantification via qRT-PCR, total genomic DNA of infected ECs was isolated and used as a target for primers amplifying the *B. henselae* gene *glyA* (serine hydroxymethyltransferase) and the human gene *hmbs* (hydroxymethylbilane synthase). Results revealed that *B. henselae* WT bacteria adhere more efficient to ECs under static and dynamic *in vitro* conditions compared to the *ex vivo* model. In static *in vitro* infections, BadA-mediated adherence was ~ 3-fold (fluorescence) or ~ 6-fold (qRT-PCR) higher compared to BadA-deficient bacteria and in dynamic *in vitro* infections, BadA-mediated adherence was ~ 2-fold (fluorescence) or ~ 11-fold (qRT-PCR) higher. These data were additionally confirmed by fluorescence microscopy (CFSE-labelled bacteria, TRITC-phalloidin-stained actin, DAPI stained nuclei).
Our newly described *ex vivo* model mimics physiologically occurring pathogen-EC interactions more realistically since viable *in situ* endothelium is infected. It represents a new tissue infection model which also might be useful for analyzing the infection biology of other vasculotropic pathogens and thereby helps to avoid unnecessary animal infection experiments.

MPP03
Proteolytic activity of the IgM-degrading enzyme of *Streptococcus suis* (Ide_{Ssuis}) represents a novel complement evasion mechanism

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Streptococcus (*S.*) *suis* is an important invasive, extracellular pathogen in pigs, which causes meningitis, arthritis, serositis and other diseases. Furthermore, it is also an emerging zoonotic agent. Recently, we identified a highly specific IgM protease, Ide_{Ssuis}, in *S. suis* [1]. The objective of the current study was to investigate the function of Ide_{Ssuis} in interaction with complement and the adaptive immune system *ex vivo*.
N-terminal Edman-sequencing revealed that Ide_{Ssuis} cleaves the heavy chain of porcine IgM. Analysis of complement deposition and activation showed a function of Ide_{Ssuis} in complement evasion. Opsonophagocytosis assays including a virulent *S. suis* wild type strain and its isogenic ide_{Ssuis} deletion mutant (10Δide_{Ssuis}), porcine serum as well as purified porcine neutrophils confirmed a complement-dependent phenotype of 10Δide_{Ssuis}. Furthermore,

bactericidal assays in porcine blood indicated that the antibody-dependent adaptive immune response had a strong impact on the phenotype of the 10Δ*ide*$_{Ssuis}$ mutant.
In conclusion, this study demonstrates that Ide$_{Ssuis}$ is involved in a novel complement evasion mechanism, which is important for bacterial survival during the early adaptive immune response.
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MPP04
Identification and modification of *Helicobacter pylori* HtrA signature sites in the tumor suppressor E-Cadherin

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Introduction: *Helicobacter pylori* (*Hp*) is a bacterial class I carcinogen that colonizes the human gastric epithelium where it induces inflammatory disorders like chronic gastritis or neoplastic diseases like gastric cancer. Loss of function of the adhesion-junction and tumor suppressor protein E-Cadherin has been frequently observed during gastric carcinogenesis. Recently, the secreted serine protease HtrA of *Hp* was discovered, which directly targets E-Cadherin.
Materials and Methods: Based on initial analyses putative E-Cadherin recognition and cleavage sites were mutated as a first attempt to generate an HtrA-resistant recombinant E-Cadherin for *in vitro* cleavage assays, as well as constructs for transfection into gastric epithelial cells deficient in E-Cadherin expression. The transfected epithelial cells were infected with *Hp* and the amount of cleaved E-Cadherin in the supernatant as well as full-length E-Cadherin in the lysate was determined by Western blotting. Apart from the effect of HtrA we also investigated the effect of E-Cadherin cleaving host proteases like MMP3/7 or ADAM10.
Results: Here, we present a detailed analysis of HtrA signature sites in human E-Cadherin, which represent putative cleavage sites. A wide range of different point mutations has been inserted in the E-Cadherin gene. Functional analyses indicated that E-Cadherin mutants were either still cleavable or showed a defect in subcellular localization. However, E-Cadherin deletion experiments suggested that the putative recognition/cleavage sites represent functional motifs for *Hp* HtrA. We found differences in the amount of soluble E-Cadherin fragments originated from the different mutated versions. In parallel, we also tested several substrate-derived peptides that effectively inhibited E-Cadherin cleavage by HtrA.
Discussion: By disrupting adherens-junctions HtrA might contribute to the *Hp*-induced loss of epithelial integrity and to the promotion of carcinogenic changes in epithelial cells. Therefore, understanding the complex role of E-Cadherin in *Hp* pathogenicity and its efficient inhibition might contribute to the prevention of gastric carcinogenesis.

MPP05
Bacteria-derived Cell-penetrating Effector Proteins of the LPX Subtype

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During infection many pathogenic bacteria use a type III secretion system (T3SS) to inject effector proteins into the host cell cytoplasm. As several of these effectors interfere with host defence mechanisms and suppress immune signalling, they might be exploited as biological therapeutics for inflammatory diseases. This possibility has become even more interesting as the LPX effector protein YopM of *Yersinia enterocolitica* was identified as a novel cell-penetrating protein (CPP) that is able to translocate into host cells in a T3SS-independent manner. Moreover, YopM exerts anti-inflammatory properties upon autonomous internalization (Rüter *et al.*, 2010). Recently, also the LPX protein SspH1 of *Salmonella typhimurium* was shown to have cell-penetrating abilities (Lubos, Master's thesis, 2011). Both YopM and SspH1 belong to bacterial effectors of the LPX subtype of leucine-rich repeat (LRR) proteins which further comprises different IpaH proteins of *Shigella* as well as the *Salmonella* proteins SspH2 and SlrP. Since these effectors share significant homology in sequence and structure, the hypothesis of a general concept for T3SS-independent uptake was raised. Hence, the ability of several recombinantly expressed LPX effector proteins of *Shigella flexneri* and *Salmonella typhimurium* to autonomously translocate into eukaryotic cells was investigated using different approaches including cell fractionation, immunofluorescence microscopy and FACS analyses. Functionality of the recombinant proteins was assessed e.g. by *in vitro* ubiquitination assays. Indeed, intracellular localization was confirmed in the course of this study. Moreover, our results indicate the contribution of endocytosis in T3SS-independent cellular uptake. The enzymatic activity of these proteins as ubiquitin E3-ligases was confirmed *in vitro*. Taken together, further evidence for a general concept of T3SS-independent translocation of bacterial LPX effectors is provided. Along with their capacity to modulate and suppress host immune signalling pathways, for instance by ubiquitination of cellular targets, these bacterial effectors might serve as potential 'self-delivering' biological therapeutics in the future.

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MPP06
Several "*en bloc*" transfers led to different evolutionary tracks of the High Pathogenicity Island and its neighboring backbone genome in *Escherichia coli*

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are among the most prevalent and important human pathogens causing infections such as septicemia, urinary tract infections and meningitis. Horizontal gene transfer is a hallmark in the evolution of pathogens. In ExPECs the presence and acquisition of pathogenicity islands (PAIs), which encode several virulence factors, are of particular importance. In this study, we investigated the principle mechanisms of PAI transfer in ExPECs and *E. coli* in general. For this, we focused on three non-self-transmissible PAIs, which are widely spread among ExPECs - the HPI, the *pks*-island and the *serU*-island.
We applied a whole genome sequencing approach to an *E. coli* reference strain collection (ECOR), selected clinical *E. coli* isolates as well as certain ExPEC archetypal strains. Using the CLCbio Genomics Workbench we analyzed the relation and sequence composition of the three PAIs as well as the respective neighboring backbone genome. To confirm our hypothesis of a clustered and large scale DNA transfer experimentally, we used an F'-plasmid transmission to transfer different PAIs simultaneously. For this, we introduced the F'-plasmid from *E. coli* XL1-Blue MRF' in ExPEC strain NU14*Cm*, the HPI of which was tagged with an antibiotic cassette. This strain served as donor in mating experiments with *E. coli* K12 strain MG1655 as recipient. Transconjugants were analyzed by PCR and whole genome sequencing. The phylogenetic trees revealed (i) a distribution pattern of the three PAIs (HPI, *serU*- and *pks*-island), which reflects both vertical and horizontal transmission, (ii) a clear association of the distribution of the two PAIs with the adjacent HPI. Interestingly, also the phylogenetic relation of the respective neighboring backbone genome matched with the respective PAIs. This indicates that not only the respective PAIs, but also larger DNA regions were transferred "*en-bloc*". We could proof that the HPI and the *pks*-island are transmissible in a single event by an F'-plasmid transfer between two *E. coli* strains. The further spread occurred during several events "*en bloc*" as the distribution of the PAIs correlate with identical subtypes of these PAIs in different clonal groups. This study provides strong evidence for F-plasmid transfer being an outstanding mechanism for intra-species horizontal transfer of PAIs within extraintestinal pathogenic *E. coli*.

MPP07
Evaluation of *Acinetobacter baumannii* Trimeric Autotransporter Adhesin as an Adhesion and Virulence factor

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Introduction: The gram negative non-motile gamma-proteobacterium *Acinetobacter baumannii* is an emerging pathogen causing severe nosocomial infections. With increasing frequency, multidrug and pan-drug resistant isolates are reported. However, little is known about its virulence

mechanisms. Trimeric autotransporter adhesins (TAA) have been hypothesized to be important virulence factors and these TAA are modularly constructed consisting of an N-terminal head domain, neck/ stalk domains and a C-terminal membrane anchor. Our aim was to investigate the TAA of *Acinetobacter baumannii* (Ata) and its impact on host adhesion.
Materials and Methods : *A. baumannii* ATCC 17978, two isogenic *ata* deficient mutants, ATCC 19606 and five multidrug resistant clinical isolates were analysed. AtaA expression was confirmed by qRT-PCR and *ata* gene mutations were analysed by Sanger sequencing. The daTAA Bioinformatic toolkit was used to evaluate TAA domain structures. Adhesion experiments were performed to evaluate adherence to collagen-1, fibronectin, laminin or VCAM-1.
Results: Expression of *ata* was detected in ATCC 17978, ATCC 19606 and clinical isolates but not in the *ata* deficient mutants. Up to 7-fold Ata expression rates were noted in clinical isolates and in ATCC 19606 in relation to ATCC 17978. *ata* mutations (range: from 4-12% nucleotide and 4-8% amino acid sequences) are present in ATCC 19606 and clinical isolates compared to ATCC 17978 with yet unknown biological consequences. Mutations in Ata domain structure were noted with differences occurring in the head and neck domains. All clinical isolates and the type strains *A. baumannii* ATCC 19606 and ATCC 17978 showed higher adherence rates to fibronectin (up to ~24-fold), to collagen-1 and laminin (up to ~12-fold) and to vascular cell adhesion molecule (VCAM)-1 (up to ~9-fold) compared to Ata deficient mutants.
Conclusion: Clinical isolates and *A. baumannii* type strains differ in their Ata expression rates. Genetically, mutations are present in the head and stalk region *ata*. Phenotypically, Ata mediates adhesion to several abiotic surfaces. Currently, we are investigating the adhesion of *A. baumannii* on biotic surfaces under static and shear stress conditions. In addition, effects of Ata virulence are being analysed by the use of the *Galleria mellonella* infection model.

MPP08
*Aureo*Wiki - the repository of the *Staphylococcus aureus* research and annotation community

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Because of the increasing amount of knowledge and experimental data, in particular from functional genomics studies, integrated repositories of genomic, functional and gene expression data become increasingly important. The Gram-positive opportunistic pathogen *Staphylococcus aureus* is a frequent cause of clinically important infections ranging from superficial skin infections to serious diseases such as pneumonia, osteomyelitis and septicemia. In order to make the wealth of information on *S. aureus* available to the scientific community, the wiki-type database *Aureo*Wiki (http://www.protecs.uni-greifswald.de/aureowiki/Main_Page) was established. *Aureo*Wiki centers on the genes and proteins of clinically and experimentally relevant *S. aureus* strains. All essential information related to a given gene/ gene product is compiled on a strain-specific gene page. The *Aureo*Wiki gene pages contain genome-based information complemented by functional, regulatory, gene expression and other data. All the data is provided together with links to the external sources such as various databases and published literature. Importantly, orthologous genes - based on the analysis of 32 *S. aureus* genomes - are presented side by side using strain-specific tabs. Orthologous genes are linked by a so called pan-genome gene identifier and a unified pan-genome gene name which can be found on the respective pan-genome gene page. The pan-genome gene pages contain the multiple alignment coordinates of 32 *S. aureus* strains, the gene occurrence frequency, a multiple-strain genome viewer as well as other comparative information. The data included in *Aureo*Wiki is also accessible through various download options in order to meet the specific requirements of bioinformatic applications.
*Aureo*Wiki is the first tool supporting annotation of biological entities of *S. aureus* in a wiki-based environment. Wiki technology was chosen to allow for collating data and information in a concerted effort where all users can add and update the existing content. Successful examples of scientific wikis devoted to bacterial organisms already exist, to name in particular *Subti*Wiki and EcoliWiki. Taken together, the long-term aim of *Aureo*Wiki is combining the collective knowledge of the *S. aureus* community in order to provide researchers with comprehensive, up-to-date information on this important pathogen.

MPP09
Staphylococcus aureus induces clotting of plasma as an immune evasion mechanism

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Recent work has shown that coagulation and innate immunity are tightly interwoven host responses that help eradicate invading pathogens. Activation of the coagulation cascade in response to *Streptococcus pyogenes* infection leads to an immobilization of bacteria within a formed clot which is followed by their subsequent killing. In contrast, clinical isolates of *Staphylococcus aureus* secrete pro-coagulant factors that in turn can modulate these immune reactions. Such mechanisms may not only protect the microorganism from a lethal attack, but also promote proliferation and establishment of the infection. In the present study we investigated the molecular mechanisms *S. aureus* bacteria employ to promote their survival in and dissemination from a formed fibrin clot.
The morphology of fibrin networks induced by coagulase positive *S. aureus* bacteria was analysed by electron microscopy, bacterial survival and uptake into the clot were monitored by measuring colony forming units. A murine skin infection model was established to study the fate of *S. aureus* bacteria at the infected site. Our data show that *S. aureus* induces clotting of plasma by the secretion of coagulase to promote their own uptake into the fibrin network. No fibrin network formation was observed when a coagulase-deficient mutant strain of *S. aureus* was used. Further *in vitro* studies show that this ability constitutes a virulence mechanism that supports the aggregation, survival, and persistence of the microorganism within the clot. These findings were also confirmed when bacterial agglutination and persistence at the local focus of infection were studied in a murine model of subcutaneous *S. aureus* infection. The coagulase-positive wildtype strain of *S. aureus* was found in large defined aggregates at the local focus of infection, whereas the mutant strain lacking coagulase failed to aggregate and persist in vivo. Our data suggest that *S. aureus* bacteria have evolved mechanisms to utilize the coagulation system for their own benefit. The coagulase-dependent induction of clotting represents a bacterial virulence and immune evasion mechanism. *S. aureus* survives and persists within the self-induced fibrin network where it is protected from the host's innate immune response. Thus, *S. aureus* is able to take advantage of a host defense mechanism to undergo the immune system and promote proliferation to establish host infections.

MPP10

Staphylococcus aureus adapts to the airways of cystic fibrosis patients by higher abundance of SodM and iron transporters

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Due to mutations in the gene encoding the *cystic fibrosis transmembrane conductance regulator*, the lungs of cystic fibrosis (CF) patients exhibit dehydration of airway mucus leading to decreased mucociliary clearance, chronic bacterial infections, lung insufficiency and early death(1). In many patients a predominant *S. aureus* clone can be isolated from the airways for many years(2). In this project we studied adaptational mechanisms of *S. aureus* during persistent infection to the hostile niche of CF airways.
From a unique longitudinal collection of *S. aureus* strains, which were isolated from the airways of CF patients, strain pairs consisting of clonal early and late isolates (determined by multilocus sequence typing, *spa*-typing and pulsed-field gel electrophoresis) from individual patients were chosen for further analysis. The cytoplasmic proteome of one strain pair with a persistence of more than 13 years was investigated using 2D-gel electrophoresis, the cell-surface associated proteins in a gel-free approach. Five additional strain pairs were chosen for verifying proteomic data by qRT-PCR and functional assays.
The data revealed multiple changes for the cytoplasmic and cell-surface associated proteome. Interestingly, SodM, one of the superoxide dismutases of *S. aureus*, was higher abundant in the late isolate. qRT-PCR revealed up-regulation of *sodM* expression in 5 out of 6 strain pairs. In addition, three proteins involved in iron-acquisition (IsdA, IsdE, SirA) were more abundant after long-term persistence.
Since CF airways are characterized by neutrophil-dominated inflammation, bacteria are highly exposed to oxidative stress(4). Therefore, it seems to be beneficial for the bacteria to up-regulate *sodM* as shown for long-term persisting *S. aureus* isolates in order to counteract neutrophil attack. The fact that several iron transporters were more abundant in the late isolate indicates that *S. aureus* is facing iron-restricted conditions in CF airways and that a higher abundance of iron transporters facilitate persistence in this location. Taken together, our data reveal insights into the *in vivo* adaption of *S. aureus* to the hostile environment of the CF lung.

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MPP11

Overexpression of the functional amyloid Fap in *Pseudomonas aeruginosa* strongly affects physiology and biofilm formation

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The curli functional amyloid system of *E. coli* is of great importance for adhesion and virulence[1]. Recently, we described a novel functional amyloid operon (*fapA-F*) within the *Pseudomonas* genus[2]. The biological function of this amyloid system is generally unknown, but overexpression of the *fap* operon leads to enhanced aggregation and biofilm formation. It is however not known whether only the functional amyloid is expressed or other physiological changes of importance for the biofilm formation or virulence take place as well. To answer this, the facultative pathogen *P. aeruginosa* PAO1 wild type (PAO1wt) and a Fap overexpression mutant (PAO1pFap) were investigated at distinct growth stages to study potential differences in aggregation properties and physiology. Electron and confocal microscopy of the cultures showed the deployment of the Fap fibrils on the PAO1pFap cell surface. Furthermore, clear macro- and microscopic cell aggregation could be observed. Label-free protein quantification unveiled significant changes in their physiology as more than 500 proteins significant changed in abundance between PAO1wt and PAO1pFap (p-value < 0.05, twofold change, ~2800 proteins quantified). The data indicated lowered abundance of classical virulence factors, such as elastase B, secretion of alkaline protease A, and increased abundance of the alginate and pyoverdine synthesis machinery. The modulators Vfr and AmrZ as well as cAMP-concentrations thereby seem to be central to the underlying regulatory network. In contrast to the general assumption that *P. aeruginosa* PAO1 is non-mucoid[3], our results show that the Fap overexpression significantly alters PAO1 towards an immobile and mucoid phenotype, often assumed to be less acute virulent. However, this combination is generally associated with poor health prognosis in chronic infections of cystic fibrosis patients. Although the functional amyloids itself likely do not have any regulatory function, their overexpression leads to drastic proteome changes. Many of those changes involve pathology and biofilm associated factors, which suggests the possible importance of these amyloid structures in chronic infections of pseudomonades.

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MPP12

Emergence of pan drug-resistant *Serratia marcescens* harbouring the carbapenemase NDM-1

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Introduction: Pan drug-resistant *Enterobacteriaceae* represent an emerging public health threat: untreatable infections. We report and characterize a pan drug-resistant *Serratia marcescens* isolate recovered from a patient admitted to the University hospital in Frankfurt.
Materials and Methods: Antibiotic susceptibilities were determined by the E-test method. Bacterial isolates were screened by PCR for the presence of acquired resistance genes. Transferability of bla_{NDM-1} was tested by transformation and *in vitro* transconjugation using sodium azide-resistant laboratory strain *E. coli* J53 as recipient. Pathogenicity was investigated using the *Galleria mellonella* infection model.
Results: A carbapenem-resistant *S. marcescens* isolate was repeatedly recovered in significant numbers from urine samples of one patient. Antibiotic susceptibility testing revealed resistance towards all beta-lactam antibiotics, fluorchinolones, aminoglycosides, folate pathway inhibitors, polymyxins, fosfomycin and chloramphenicol. The isolate was therefore classified as pan drug-resistant. Due to the lack of treatment options, only symptomatic care was administered to the patient suffering from a nosocomial urinary tract infection. The carbapenemase NDM-1 was identified, next to the resistance genes SHV-12, CMY-6, TEM-1 and *rmtC*. The NDM-1 harbouring plasmid was identified as IncA/C type with a size of approximately 140 kb. Conjugation experiments using the NDM-1 carrying *S. marcescens* and the *E. coli* J53 resulted in NDM-1 harbouring *E. coli* transconjugants. Donor and acceptor conjugation frequency was determined to be 0.5 (CI 95%: 0,24 - 0,75; SD: 0,443). *In vivo* experiments in *Galleria mellonella*, were performed to assess the pathogenicity of the clinical isolates. Analysis revealed an LD_{50} dose of $1x10^7$ cfu within 24 h after injection.
Discussion: To our knowledge this work presents the first report of a *S. marcescens* strain harbouring NDM-1. Further accumulation of SHV-12, CMY-6, TEM-1 and *rmtC* resistance genes caused the pan drug-resistant phenotype of the clinical isolate. The NDM-1 harbouring IncA/C plasmid was found to be similar to pKP1, a NDM-1 positive plasmid recently described in *K. pneumoniae* in Australia. Our work represents an elucidating example of the concurrent worldwide spread of carbapenemase genes in association with pan drug-resistance and points towards a post antibiotic era, in which screening, disinfection and isolation are the only option to handle pan drug-resistant bacteria.

MPP13

Mechanism of colistin resistance in *Klebsiella pneumoniae* and impact on fitness and virulence

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Introduction: *Klebsiella pneumoniae*, a Gram-negative pathogen, causes severe infections in both, humans and animals. As multidrug resistance in these bacteria rapidly evolves, development of resistance towards lastline antibiotics such as colistin (polymyxin E) is of great concern. So far, knowledge of the underlying mechanism of colistin (CT) resistance in *K. pneumoniae* is scarce. Our objectives were to investigate the resistance mechanism of clinical CT-resistant *K. pneumoniae* isolates and the impact on bacterial fitness and virulence.
Materials and Methods: Antibiotic susceptibility was evaluated using Vitek 2, E-test and broth microdilution. Genetic relatedness was investigated by the semi-automated REP-PCR (DiversiLab, BioMérieux). PCR analyses and DNA sequencing of several genes involved in lipid A metabolism (e.g., *lpxA-D*, *phoP, phoQ*, *pmrAB*, *mgrB*) were carried out to screen for possible CT resistance mechanisms. Transmission electron microscopy was performed to evaluate the cell surface morphology. Competitive growth experiments and the *Galleria mellonella* infection model were used to analyse fitness and pathogenicity respectively.
Results: Six *K. pneumoniae* isolates were recovered from colonisation and infection sites of a patient who had never been treated with polymyxins before. Whilst the first recovered isolate presented a CT MIC of 0.5 mg/L, subsequent isolates were CT resistant, exhibiting a MIC of 4-8 mg/L. All isolates showed a genetic similarity of >98% suggesting clonality. They featured an ESBL phenotype, possessed the beta-lactamase genes for CTX-M-15, SHV-1, OXA-1 and TEM-1 and belonged to the sequence type ST48. Using *in vitro* competitive growth experiments and the *in vivo Galleria mellonella* infection model, neither a significant difference in fitness nor in virulence of CT-S versus CT-R isolates could be detected. Both isolates were highly virulent (LD_{50} <100 CFU) in contrast to ATCC reference strains of *K. pneumoniae*, *E. coli* and *A. baumannii*.
Sequence analyses of 20 genes involved in the biosynthesis of the CT target lipid A did not unveil any disparity between CT-R isolates and the CT-S isolate. However, evaluation of CT resistance on cell surface morphology by electron microscopy revealed fine fibers, covering the outer surface of the CT-S isolate, which were absent in the isogenic CT-R isolate.
Conclusion: We demonstrate the development of CT resistance in a clinical *K. pneumoniae* isolate, independently of CT selection pressure. This was not associated with a loss of fitness or virulence. To identify the molecular mechanisms of CT resistance in this isolate, analysis of whole genome sequencing data is currently being performed. The development of CT resistance in the absence of antibiotic selection pressure in highly virulent clinical *K. pneumoniae* isolates is of great concern, especially if the gain of resistance is not associated with a loss of fitness.

MPP14

Role of HIF-1 in Infections with *Streptococcus pneumoniae* at the Blood-Brain Barrier

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The blood-brain barrier (BBB) is a physiological barrier formed by endothelial cells (ECs), supported by astrocytes and pericytes of the brain microvasculature, which protects the brain from circulating toxins and pathogens yet transporting essential nutrients such as glucose and iron. In brain infections such as meningitis circulating pathogens gain access to the central nervous system by breaching the BBB and infecting the brain parenchyma. Mechanisms underlying the transfer of meningeal pathogens across the BBB are still poorly understood. Based on our previous reports showing a general activation of hypoxia inducible factor (HIF)-1 in bacterial infections, we hypothesized that the activation of HIF-1 leading to secretion of vascular endothelial growth factor (VEGF; initially described as vascular permeability factor VPF), is involved in the invasion process of pathogens across the BBB. To test our hypothesis, we performed pneumococcal infections of brain ECs *in vitro* and obtained permeability of the endothelial monolayers to dextrans of various molecular sizes and to pathogens. We further performed confocal immunofluorescence analysis to visualize the localization of the *S. pneumoniae* on ECs. Expression of several components of EC cell junctions (claudins, occludins) was analyzed by quantitative (q) RT-PCR analysis. Our results demonstrate an increase in paracellular permeability of endothelial monolayers to various sized dextrans. Correspondingly, bacterial counts confirmed migration of bacteria across such monolayers. Immunofluorescence analysis showed localization of pneumococci predominantly at the cell-cell junctions, which was supported by qRT-PCR analysis that showed a downregulation of claudin-5, a critical member of endothelial tight junctions, upregulation of HIF-1α and VEGF. These preliminary results suggest paracellular route for pneumococcal transmigration potentially involving HIF-1 activation in ECs. Our results from oxygen consumption kinetics analysis showed that *S. pneumoniae* infection leads to rapid reduction of oxygen concentration in ECs in cell culture. Furthermore, *in vivo* analysis of brain tissue samples also showed upregulation of HIF-1α in the brain vessels in different meningeal infections indicating HIF-1α as a mediator of vessel permeability. Protein level expression for cell-junction-associated proteins and monitoring of the endothelial electrical resistance using CellZscope is currently underway in addition to permeability studies in HIF-1 knock down ECs under normoxic and hypoxic conditions.

MPP15

A sticky issue: Acquisition of complement regulators by *Acinetobacter baumannii* biofilms

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Introduction: *Acinetobacter baumannii* plays an important role in hospital acquired infections such as pneumonia, sepsis, wound- and urinary tract infections. This pathogen is increasingly resistant to a large number of antibiotics, is able to produce biofilms on biotic and abiotic surfaces, and therefore often involved in device-associated infections. Biofilms of *A. baumannii* contribute to antibiotic resistance and could help to protect the bacteria from innate immunity. How *A. baumannii* can overcome innate immunity remains largely unknown. In this study, the interactions of complement factors with the biofilms of several *A. baumannii* isolates have been investigated, in order to elucidate one potential mechanism by which this particular pathogen escapes complement-mediated killing.
Materials and Methods: Several *A. baumannii* strains were screened for their potential to produce biofilms at different temperatures using a crystal violet staining assay. Binding of complement factors such as complement factor H (CFH), C1-inhibitor (C1-Inh) and C4b-binding protein (C4BP) to biofilms of *A. baumannii* was investigated by ELISA. Additionally, binding of CFH and C4BP was visualized by confocal microscopy. Furthermore, we analyzed whether complement factor CFH and C4BP bound to biofilm surfaces retain their functional activity.
Results: All strains (n=8) of *A. baumannii* produce biofilm under the tested conditions, and growth of biofilm is temperature dependent. Most *A. baumannii* strains grown at room temperature showed higher amounts of biofilm production, compared to strains grown at 37°C. Concerning binding of complement regulators CFH, C1-Inh and C4BP was investigated by ELISA. Out of eight strains, two strains bound all three regulators. For CFH, binding could be observed for four strains, while C4BP and C1-Inh were only bound by two strains. In contrast, planctonically grown cells did not bind complement regulators at all. Furthermore, interaction with complement regulators was visualized by confocal microscopy. Binding of CFH and C4BP could be observed for four *A. baumannii* strains. Cofactor assays showed that CFH and C4BP attached to the biofilms of *A. baumannii* strains remained functionally active and could degrade C3b and C4b.
Discussion: Virulence of *A. baumannii* is known to be influenced by its ability to build up persistent biofilms. The mechanism(s) how biofilm of *A. baumannii* protect the pathogens from attack by innate immunity are not well understood. In this study, we demonstrate for the first time that only biofilm-producers can bind complement regulators CFH, C4BP and C1-Inh.

Furthermore, we could show that biofilm-bound CFH and C4BP can degrade activated complement components C3b and C4b. Serum resistance of *A. baumannii* is thus at least in part influenced by the generation of biofilms, and the subsequent binding of complement regulators to the biofilm surface, limiting complement activation and thereby protecting *A. baumannii* from its detrimental effects.

MPP16

A novel small non-coding RNA affects virulence in *Streptococcus pyogenes* M49

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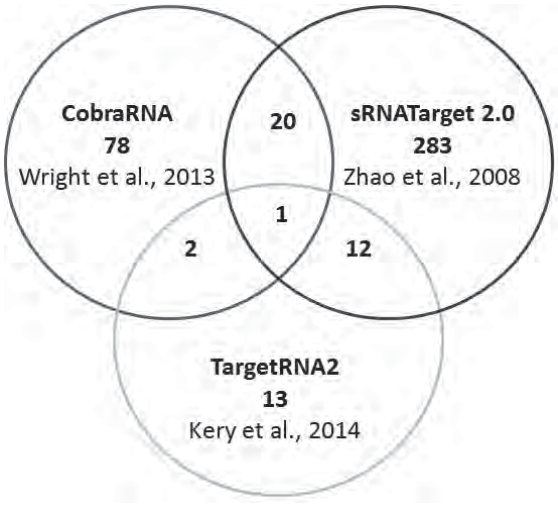
Introduction: *Strepcotoccus pyogenes* (Group A Streptococcus, GAS) is an important Gram-positive human pathogen that causes diseases ranging from infections of the skin and mucous membranes of the naso-pharynx, to severe toxic, like toxic shock syndrome, and invasive diseases. A new class of regulators of gene expression are small non-coding RNAs (sRNAs). The regulatory mechanisms include the stabilization and destabilization of target transcripts. In 2012 our group detected 55 putative sRNA candidates in *S. pyogenes* with bioinformatics analysis and tiling array probe sequences.
The aim of this project was the characterisation of one small non-coding RNA, sRNASpy491738 (Candidate 56),inthecontextofvirulence by construction of sRNA expression mutants as well as by bioinformatics analysis.
Materials and Methods: Construction of sRNA deletion-, complementation- and over-expression strains in *S. pyogenes* M49. Characterization of the mutant strains: investigation of growth behavior in complex media, bacterial survival in human blood, and adherence to and internalisation into human keratinocytes (HaCaT-Cells). To investigate putative targets of the sRNA, thesecondarystructure was calculated using RNAfold and subsequently analyses with the online tools CobraRNA, sRNATarget 2.0 and TargetRNA2 were performed.
Results: Different algorithms resulted in 35 overlapping putative sRNA targets, one of which was predicted in all online tools (fig. 1).In growth experiments in complex media, the different strains showed similar growth-rates. Consequently, all strains could be compared in following experiments, because general fitness was not affected by the genetic modifications.The bacterial survival of the deletion mutant in human blood did not differ from the survival of the wildtype. In contrast, thecomplementation- and over-expression strains showed a decreased ability of growth in blood.Adherence to HaCaT-Cells was comparable in all strains tested. However, the deletion mutant showed a decreased internalisation into HaCaT-Cells compared to the wildtype, whereas internalisation of the over-expression mutant was increased.
Conclusions and Discussion: The sRNA Candidate 56 showed no effect on general fitness. Our results suggest that this sRNA takes part in virulence control of *S. pyogenes* M49. The immediate goal, is to identify the sRNA targets and affected pathways to elucidate the role of Candidate 56 on virulence control in *S. pyogenes* M49.

Figure 1



MPP17

The mutltifunctional adhesins PavB and PspC are major human thrombospondin-1-binding proteins of *Streptococcus pneumoniae*

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Introduction: A prerequisite of bacterial invasive infections is the adhesion of the bacteria to host cells. Adherence of pathogenic Gram-positive bacteria to their host cells is facilitated by surface-exposed structures, which especially target components of the extracellular matrix. The matricellular glycoprotein human thrombospondin-1 (hTSP-1) is secreted mainly by activated thrombocytes and interacts with a wide range of other matrix molecules. Thereby it is involved in angiogenesis, proliferation and apoptosis of cells. Furthermore hTSP-1 can acts as molecular bridge between host cells and Gram-positive bacteria, thus facilitating adherence to and invasion into human epithelial and endothelial cells (Rennemeier et al., 2007). In this study, we identified the adhesins PavB and PspC of *Streptococcus pneumoniae* as hTSP-1 binding proteins.
Materials and Methods: To investigate the contribution of PavB and PspC to TSP-1-binding *S. pneumoniae* D39Δ*cps* and serotype 35A mutant strains deficient for PavB, PspC or both proteins were analyzed for their ability to bind hTSP-1 by flow cytometry. Binding experiments using surface plasmon resonance and enzyme-linked immunosorbent assays were carried out with heterologously expressed protein domains of PavB and PspC. Differences in adherence of *S. pneumoniae* D39Δ*cps* and the PavB and PspC deficient mutants to human epithelial cells pretreated with hTSP-1 was examined using fluorescence microscopy.
Results: Binding and adherence studies identified PavB and PspC as major hTSP-1 adhesins on the pneumococcal surface. Moreover, deletion mutants deficient in PavB, PspC or both of different pneumococcal strains demonstrated a significant reduction in binding of soluble hTSP-1 compared to the isogenic wild-type. Remarkably, the loss of PavB and PspC had an additive effect. Finally, a decreased adherence of the mutants to human epithelial cells after hTSP-1 incubation was determined by fluorescence microscopy.
Conclusions: Taken together, we present here for the first time pneumococcal adhesins interacting directly with hTSP-1 and in addition, we demonstrate the influence of these pneumococcal hTSP-1-binding proteins on hTSP-1 mediated pneumococcal adherence to human epithelial cells.

MPP18

Molecular analysis of the role of LscR in levansucrase expression and bacterial motility in different *Pseudomonas* species

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Virulence of the bacterial blight pathogen of soybean, *Pseudomonas syringae* pv. g*lycinea* PG4180, is favored by the temperature dependent production of levansucrase, an enzyme needed for production of the exopolymer levan and encoded by *lscB*. Previous results showed that expression of *lscB* in the heterologous host, *Pseudomonas putida* KT2440, required a PG4180-borne gene designated *lscR. In planta* growth of PG4180 and its *lscR* mutant on soybeans plants was performed to investigate whether *lscR* gene plays a role in the virulence of PG4180. Soybean plants were inoculated with PG4180 wild type and *lscR* mutant with approximately 10^7 CFU/ml. All the period after inoculation, plants were grown in a greenhouse (19-21°C) with a 12-hr light period. But no difference was observed in the virulence between PG4180 wild type and its *lscR* mutant. Moreover, different plasmid constructs were transformed into *P. putida* KT2440 wild type and then grown on mannitol-glutamate plates containing 0.25% agar and appropriate anitibiotics. Plates were incubated at 18°C and left for 8 days. An interesting phenotype was observed were constructs carrying only a 1,350-bps region containing *lscR* with its neighboring PSPPH 0652 gene showed the highest swimming radius. However, the construct harboring a phage borne ~25-kb region contained in a cosmid and flanking *lscR* showed less swimming radius. Because of the exciting phenotypic differences, current focus is on the analysis of the proteome of *P. putida* KT2440 carrying *lscR* or the prophage DNA, respectively by two-dimensional protein analysis and MALDI-TOF.

MPP19

Type Three Secretion Export Apparatus: The Structural Makeup of the Membrane Proteins

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Many Gram-negative pathogens express type III secretion systems to deliver substrates into the host cells. These common virulence factors consist of a well-defined needle complex that spans both bacterial membranes and connects the bacterial and host cytoplasm, and a less well-studied export apparatus at the inner membrane center of the needle complex. The export apparatus plays an important role in substrate recognition, specificity switching and translocation across the inner membrane. To determine the structure of the export apparatus, we used Salmonella enterica serovar Typhimurium as a model organism. We assessed the transmembrane topology of the export apparatus components using the substituted cysteine accessibility method (SCAM), and their stoichiometry using a mass-spectrometry based peptide-concatenated standard strategy. The export apparatus consist of five transmembrane proteins, SpaPQRS and InvA. Preliminary results show that the switch protein SpaS and the smallest protein SpaQ are present in equal amounts, albeit not necessarily connected. The SpaPR subcomplex is made up mainly of SpaP with a maximum of two copies of SpaR. The largest protein InvA forms a nonameric ring according to the crystal structure of a *Shigella* homolog and, unlike the SpaPR subcomplex, the topology of InvA concurs thus far with the existing predictions. The structural makeup of the export apparatus components promotes the functional understanding of the apparatus and its role in the type III secretion.

MPP20

Small colony variants of *Staphylococcus aureus* - impact on biological fitness in a *Galleria mellonella* infection model

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Introduction: Small colony variants (SCVs) of *Staphylococcus aureus* have been implicated in persistent, relapsing, and treatment-resistant infections. *S. aureus* SCVs are frequently isolated from the chronically infected airways of patients suffering from cystic fibrosis and primarily exhibit thymidine-auxothrophism. It is argued that virulence of SCVs is affected due to reduced expression of virulence factors and impaired fitness. We analyzed the virulence of a defined thymidine-auxotrophic SCV isolate and its isogenic normal-colony counterpart in a *Galleria mellonella* infection model in order to further elucidate the impact of SCVs on pathogenesis in vivo.
Materials and Methods: We constructed a defined and stabile thymidylate synthase (*thyA*) knock-out mutant of a wild-type, normal colony variant (NCV) *S. aureus* strain SH1000. For this purpose, the *thyA* gene of *S. aureus* RN4220 was interrupted by inserting an *ermB* cassette. We generated a *thyA* mutant, due to homologous recombination, by growth at a nonpermissive temperature and selection for erythromycin resistance. The interrupted *thyA* gene was finally transferred by phage transduction from *S. aureus* RN4220 to *S. aureus* SH1000 using phage *Φ*80 resulting in a stabile thymidine-auxotrophic *S. aureus* SCV (SH1000Δ*thyA*).
S. aureus strains SH1000 (NCV) and SH1000Δ*thyA* (SCV) were grown in BHI at 37°C for 2 h and the optical densities at 600 nm (OD600) were measured. The bacterial cells were then collected and washed twice before being diluted with PBS so that 10 µL of bacterial suspensions A, B and C of both strains contained approx. 10^6, 10^5 and 10^4 cfu, respectively. Colony counts were performed to confirm consistency of inoculum. One group of 16 larvae per suspension (A, B, and C) and per strain (SCV and NCV) were inoculated in the last left proleg. Two negative control groups were used, i.e. one group underwent no manipulation, while the other group was injected with PBS only. Test and control larvae were stored in Petri dishes in the dark at 37°C for 5 d and survival was monitored.
Results: The inactivation of the chromosomal *thyA* gene in *S. aureus* SH1000 resulted in the formation of a thymidine-auxotrophic SCV phenotype. SH1000Δ*thyA* showed all typical features described for clinical SCVs such as formation of tiny colonies on solid agar, decreased pigment formation, reduced haemolytic activity, auxotrophism for thymidine, as well as resistance to cotrimoxazol.
The effect of *S. aureus* SCV and NCV on the survival of the larvae appeared to be dose dependent, i.e. survival was reduced with increasing inoculum of *S. aureus*. The survival rate of larvae infected with *S. aureus* SCV, however, was demonstrated to be significant higher than for larvae infected with *S. aureus* NCV.
Discussion: We present conclusive evidence that virulence of *S. aureus* SCV compared to *S. aureus* NCV is significantly diminished in vivo.

MPP21

Efficient genetic manipulation of *Yersinia enterocolitica* and characterization of *Ye* single gene knockouts involved in OMP biogenesis

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The trimeric autotransporter adhesin Yersinia adhesin A (YadA) and the type Ve autotransporter Invasin (Inv) are important pathogenicity factors of the human pathogen *Yersinia enterocolitica* (*Ye*). During infection YadA and Inv are mediating the binding to host cells which is then followed by the translocation of Yersinia outer proteins (Yops) via a type III secretion system. The biogenesis of the outer membrane proteins (OMP) YadA and Inv depends on the β-barrel assembly machinery (BAM) (1,2). The BAM-complex consists of the essential proteins BamA and BamD and the non-essential proteins BamB, BamC and BamE. The unfolded OMP are guided through the periplasm to the BAM-complex with the help of the periplasmic chaperones DegP and SurA. The BAM-complex then inserts the OMP into the lipid bilayer (3).
The aim of this study is to find out, how factors involved in OMP biogenesis contribute to *Ye* virulence. We will therefore investigate if the deletion of the non-essential proteins BamB, BamC, BamE and of the periplasmic chaperones DegP and SurA affect *Ye* virulence, outer membrane integrity and composition, adhesion and invasion to host cells and growth behaviour.

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MPP22

Platelet aggregation induced by *S. gallolyticus* subsp. *gallolyticus*, an emerging pathogen in infective endocarditis

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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (*S. gallolyticus*) is an emerging pathogen in infective endocarditis (IE). After adhesion to endocardial cells, the persistence and establishment of vegetation are crucial factors for IE development. Throughout the establishment of endocardial vegetation, induction of platelet aggregation by bacterial structures could have strong impact on IE development. However, the pathomechanisms underlying the *S. gallolyticus* IE are still not enlightened. This study demonstrates the induction of platelet aggregation by *S. gallolyticus*.
Materials and Methods: Platelet rich plasma of 4 healthy individuals was used for characterization of platelet aggregation by 9 different *S. gallolyticus* isolates. *S. gallolyticus* was grown overnight. The bacterial titer was determined by BactiFlow and bacterial suspensions were diluted up to approximately 4×10^9 cfu/mL. Platelet rich plasma (200×10^3 platelets/µL) was inoculated with *S. gallolyticus* isolates and aggregation was recorded using light-transmission aggregometry.
Results: *S. gallolyticus* was able to induce platelet aggregation. Furthermore, an individual platelet host response to *S. gallolyticus* was shown. Isolate AC 6827 was not able to induce platelet aggregation of the platelets of probands 1 and 4, whereas isolate AC 1181 was not able to induce platelet aggregation of platelets from probands 2 and 4. It is particularly interesting that aggregation of platelets of proband 4 was induced only by 4 of the 9 isolates. The platelet aggregations of other probands demonstrate that only individual isolates of *S. gallolyticus* are not able to induce platelet aggregation and most of them were an appropriate agonist for platelet aggregation.

Conclusion: The study shows individual platelet aggregation throughout the contact of platelets with *S. gallolyticus*. Furthermore, the results demonstrate host-dependent reaction and also strain dependent divergences. In course of persistence and establishment of vegetation on the endocardium, platelet aggregation could be an intriguing virulence factor. Further studies will focus on platelet adhesion of *S. gallolyticus* as well as on the characterization of the induction mechanisms for platelet aggregation.

MPP23
„Impact of hypoxia on *Salmonella* Pathogenicity Island 2 (SPI-2) activity"

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Introduction: Levels of available oxygen differ significantly in distinct niches of the mammalian body. For successful colonization *Salmonella* is challenged to adapt to these conditions during its pathogenesis. *S.* Typhimurium contains a variety of molecules to sense and to react upon changes in pO_2. This includes not only adaptation of metabolism but also the modulation of virulence-related genes. Here we analyzed the impact of hypoxia on *Salmonella* Pathogenicity Island 2 (SPI-2) encoding a type III secretion system required for intracellular survival.
Materials and Methods: Intracellular survival under normoxic and hypoxic conditions was compared using gentamicin protection assays. To characterize transcriptional regulation of SPI-2 genes under different oxygen levels we applied flow cytometry with fluorescent SPI-2 reporter constructs and RT-qPCR to quantify *ssaG* mRNA levels. In all experiments an isogenic *ssrB* mutant was used as a negative control. SsrB is the response regulator of the SsrAB two-component signal transduction system which is essential for SPI-2 expression.
Results: Under hypoxic conditions (pO_2 = 0.5%) an increase in intracellular survival and replication was observed in different host cells. This effect could be in part attributed to an increased expression of SPI-2 under hypoxia. A subpopulation of *Salmonella* isolated from infected host cells or grown in inducing media under limited oxygen showed higher induction of a SPI-2 dependent reporter using flow cytometry. For RT-qPCR analyses *Salmonella* were grown for 4, 6 and 8h under normoxia or hypoxia in SPI-2 inducing medium. Hypoxia-treated samples revealed a slight peak in *ssaG* transcription levels at 6h, whereas under normoxia an almost linear increase was observed.
Discussion: RT-qPCR data revealed differences in regulation of mRNA transcription of the whole population. Flow cytometry data indicated a faster and stronger activation of SPI-2 in a subpopulation under hypoxic conditions compared to normoxia underscoring the relevance of oxygen availability for SPI-2 activity.

MPP24
Cell-surface abundance of pneumococcal lipoproteins and impact of specific anti-lipoprotein antibodies on opsonophagocytosis

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Introduction: The cell surface of *Streptococcus pneumoniae* is decorated with several clusters of proteins. A major proportion is represented by lipoproteins fulfilling important functions in cell signaling, protein export, substrate transport and protein folding. Lipoproteins are highly conserved among pneumococcal serotypes and may therefore represent potential candidates for a serotype independent subunit vaccine. In this study the immunogenicity of pneumococcal lipoproteins was assessed and the efficiency of specific anti-lipoproteins antibodies to opsonize pneumococci and to enhance phagocytosis by macrophages was tested by flow cytometry.
Materials and Methods. The relative antibody titers of specific mouse anti-lipoprotein IgGs were determined by an ELISA. The abundance of selected lipoproteins in *S. pneumoniae* D39 was investigated by flow cytometry, and similar the opsonophagocytosis was determined by flow cytometric analysis. For this purpose the murine macrophage cell line J774A.1 was infected with constructed GFP-producing pneumococci, which were pre-incubated with the specific mouse anti-Lipoprotein IgGs.
Results: The lipoprotein abundance on the pneumococcal cell surface differs among the selected candidates. The most abundant lipoproteins were PpmA, a foldase and SlrA, a peptidyl-prolyl cis/trans isomerase, both directly contributing to virulence of pneumococci [1]. Furthermore, DacB, an L,D-carboxypeptidase and PsaA, which is part of a metal-ion ABC transporter, showed a high abundance, while e.g. the extracellular thioredoxin Etrx2 [2] is represented at a lower level on the pneumococcal cell surface. Currently the role of specific anti-lipoprotein antibodies in pneumococcal opsonophagocytosis is under investigation. The results so far demonstrate an enhanced phagocytosis by macrophages *in vitro* after pre-incubation of pneumococci with specific anti-lipoprotein IgGs. Differences between the anti-lipoprotein IgGs will be shown.
Conclusions. Pneumococcal lipoproteins are highly conserved and are immunogenic pneumococcal surface components. Thus, anti-lipoprotein antibodies promote pneumococcal opsonophagocytosis. Therefore, lipoproteins represent potential candidates for a conjugate or a subunit vaccine, which are urgently needed to combat pneumococcal infections.

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MPP25
Studies on Structural Virulence Factors in *Aeromonas* Spp. Bacteria Isolated from Cultured Fish

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Introduction: *Aeromonas* spp. are inhabitants of a wide range of aquatic ecosystems. These Gram-negative, opportunistic bacteria have been associated with human infections such as gastroenteritis caused by contaminated water or food of animal origin. Common carp and rainbow trout are popular food fish with annual production in Poland estimated at 33,000 tons and thus it should be considered as a potential source of *Aeromonas* infection.
Aim: We asked whether the diversity of structural virulence factors, i.e. LPS and the S-layer, correlates with pathogenicity of *Aeromonas* spp. strains isolated from farmed fish.
Materials and Methods: Bacteria isolated from cultured fish (carp, trout) were identified to a species level by restriction analysis of 16S rDNA. Heterogeneity studies of LPS were performed by SDS-PAGE of proteinase K-digested cells after visualization by silver staining. For compositional analysis of degraded polysaccharide, the gas chromatography-mass spectrometry technique (GC-MS) was employed. Coomassie Brilliant Blue agar was used to quantify the frequency of the S-layer phenotype in different isolates.
Results: SDS-PAGE analysis of LPS showed that a majority of the isolates synthesized O-antigen containing S-LPS. Moreover, the rough LPS (R-LPS) was independent of the cultivation temperature. GC-MS analysis of degraded polysaccharide obtained from LPS after hydrolysis showed riburonic acid, 6-deoxyhexoses, hexoses, aminohexoses, D-glycero-D-manno-heptose, L-glycero-D-manno-heptose, and Kdo. In addition, LPS samples revealed 3,6-dideoxy-3-aminohexose (strains: K49, K132, K223, Pt59 and P1S), 2,6-dideoxy-2-aminohexose (K49 and P1S), and 4,6-dideoxy-4-aminohexose (strain K223). Three strains contained 3,6-dideoksyhexosamine N-acylated with 3-hydroxybutyric acid. Growth on CBB agar showed that most isolates had an S+ phenotype and synthesized a protein layer. The latter conclusion was confirmed by the SDS-PAGE profile, which revealed a ~50 kDa protein subunit.
Conclusion: The structural heterogeneity of lipopolysaccharide revealed by SDS-PAGE and GC-MS analyses appeared to be a strategy, which increases competitiveness of *Aeromonas* sp. bacteria in the aquatic environment. When they harbor structural attributes such as O-antigen LPS and the S-layer together with extracellularly secreted toxins, they can be considered potential foodborne pathogens.
This work was supported by National Science Centre research grant (DEC-2011/03/B/NZ1/01203).

MPP26

Studies on the Regulation of Type VI Secretion Systems in *Escherichia coli*

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Introduction: Type VI secretion systems (T6SS) are the most recently identified secretion machineries and widespread among pathogenic Gram-negative bacteria. Initially, this type of secretion system was believed to serve as a secretion system targeting eukaryotic cells. However, it has become evident that they often target other bacteria. Hence, T6SSs might be involved in both, bacterial competition within microbial populations or bacterial interaction with host cells. Accordingly, they may contribute directly or indirectly to pathogenesis.
Genome sequencing and analysis of the uropathogenic *Escherichia coli* (UPEC) strain 536 revealed the presence of two genomic islands encoding putative T6SSs. As both genomic islands are located at distinct positions in the UPEC 536 genome and differ in their genetic organization, they were most likely acquired in two independent events from different sources. Therefore one could speculate that they differ regarding their regulation of gene expression and their function.
Materials and Methods: To elucidate the transcriptional regulation of the T6SSs in UPEC strain 536, chromosomal and plasmid-based promoter-reporter gene fusions to core genes of both genomic islands have been constructed. The promoter activity has been tested in different *E. coli* K-12 deletion backgrounds as well as in various wild type *E. coli* strains.
Results and Discussion: The screening using the promoter-reporter gene fusion demonstrated that the availability of nucleoid-associated proteins and the growth phase, i.e. the stationary phase, affect transcription of genes coding for core elements of T6SSs.
The expression of T6SSs seems often to be tightly regulated and therefore mostly inactive under standard laboratory conditions. The screening for potential regulators directing gene expression of T6SSs is a first step to get a better understanding under which conditions these secretion systems may be active and contribute to the fitness and/or pathogenicity of *E. coli*.

MPP27

The phospholipases of *Acinetobacter baumannii* - role in interbacterial competition and pathogenicity

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Acinetobacter baumannii is an emerging threat in hospital environments due to its increasing resistance to antibiotics. During infection of the human host *A. baumannii* encounters many stress factors such as iron- and phosphate-limitation, low pH and oxidative stress. But knowledge with respect virulence mechanisms and survival of *A. baumannii* in the human host is scarce. Phospholipases are known to contribute to interbacterial competition and have been identified as virulence factors in different pathogenic bacteria, such as *Pseudomonas aeruginosa, Listeria monocytogenes* and *Clostridium perfringens*. Phospholipases are implicated in the specific destruction of endosomal or phagosomal membranes, which releases the bacterium into the nutrient rich host cell cytoplasm. This together with the identification of potential phospholipase genes in the genome *of A. baumannii* led to the suggestion that phospholipases might contribute to the survival and metabolic adaptation of *A. baumannii* to the human host.
To analyze the role of phospholipases in metabolic adaptation and virulence of *A. baumannii* we developed a marker-less mutagenesis system. A set of different phospholipase mutants was generated and analyzed in *E. coli* killing assays and in a *Galleria mellonella* infection model. These studies unraveled distinct roles of the phospholipases in virulence of *A. baumannii*.

MPP28

The novel 18.4 kb genomic island in monophasic *Salmonella* Typhimurium - a phage remnant encoding a secreted effector?

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The 4,[5]:i:- monophasic variant of *Salmonella* Typhimurium has become one of the predominant agents causing foodborne infections in humans in Germany and other European countries. In 2013 the monophasic variant represented 65.5% of all human *S.* Typhimurium isolates sent to the RKI (Quelle: RKI SalmoDB) and was associated with several outbreaks. In spite of minor variations in phage type and resistance pattern within the emerging monophasic strains one type characterised by phage type DT193 and tetra-drugresistance towards antibiotics including ampicillin, streptomycin, sulfamerazine and tetracycline (ASSuT) clearly dominates in Germany. Previously we described a novel 18.4 kb genomic island adjacent to the *thrW* tRNA locus that is characteristic for this prevalent strain type [1]. On nucleotide level, homologous sequences for parts of the island have been found in *E.* coli and *Shigella* but not in so far published *Salmonella* genomes. Protein BLAST analyses for the 27 potential open reading frames revealed i. a. a CPS-53-like prophage integrase and several more phage-related but mainly uncharacterised gene products. Structural phage components like head and tail proteins are not encoded within the island and therefore it is not a functional prophage but more likely a phage remnant. Nevertheless, it had been shown that the island is able to form a circular intermediate and conjugational transfer to an appropriate recipient strain had been proven. *In vitro* and *in vivo* infection experiments showed no differences between the wildtype and the island-deleted mutant strain regarding the invasion or intracellular replication capacities, respectively. However, the deletion of the island as well as its transduction into other *S.* Typhimurium recipients resulted in an altered phage type, indicating that the island encodes proteins involved in phage immunity. ORF 10 was predicted to encode a type III-effector and the secretion of the gene product was successfully demonstrated. Although the function of that protein to which no homologues have been described so far is still unknown, the gene might represent a so-called moron. The moron-encoded functions are not necessary for the (remnant) phage itself but increase the fitness of the host and thereby enhance the chance of prophage propagation [2]. Prophages and phage remnants might also encode secreted virulence factors as had been shown e.g. for the *Salmonella* phage SopEΦ [3].

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MPP29

Impact of Mat fimbriae on fimbrial gene regulation of *E. coli*

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Introduction: Bacterial adherence to host cells via fimbriae represents a crucial step for the establishment of an infection. Besides promoting receptor-mediated contact to host tissues, fimbrial adhesins may also support microbial colonization of mucosal surfaces by contributing to biofilm formation. Regulatory cross-talk between fimbrial operons has been described for different fimbrial determinants of uropathogenic *Escherichia coli* (UPEC).
Mat (Meningitis associated and temperature regulated) fimbriae mediate biofilm formation of newborn meningitis-associated *E. coli* (NMEC) and UPEC isolates as well as adherence to HEp-2 cells by enteroaggregative *E. coli* strain 042. Mat fimbriae were initially identified in NMEC isolate IHE3034 and show temperature-dependent expression, preferentially at 20 °C. Mat fimbriae expression is positively regulated by the LuxR-type regulator MatA, which additionally represses expression of the main flagellar master regulator FlhDC. *E. coli* strain MG1655 encodes for several chaperone-usher type fimbriae including Sfm fimbriae that are only poorly expressed *in vitro*. A putative regulatory interaction of MatA and the *sfm* operon is part of this study.
Materials and Methods: To analyse a regulatory effect of MatA on the promoter activity of several chaperone-usher type fimbriae of *E. coli* K-12 strain MG1655, constitutively *matA*-expressing reporter strains carrying chromosomal replacements of these fimbrial operons with a promoterless *lacZ* gene were monitored regarding ß-galactosidase activity. Additionally, quantification of *sfm* transcript levels in *E. coli* MG1655 was performed by quantitative real-time PCR.
Expression of Mat fimbriae in selected atypical UPEC isolates and the impact of MatA on Sfm fimbriae expression in *E. coli* MG1655 will be analysed by immunoblotting.

We studied a putative regulatory interaction of MatA with the *sfm* operon by gel shift assays. The role of Mat- and Sfm fimbriae coexpression regarding biofilm formation was analysed *in vitro*.
Results: Expression of Mat fimbriae was not detected in atypical UPEC isolates cultivated under *in vitro* conditions.
We identified a MatA-dependent increase of *sfm* gene expression, which was more pronounced in cultures grown at 25 °C than at 37 °C. We discuss the results of our study against the background of a putative regulatory cross-talk between Mat and Sfm fimbriae and the role of Mat fimbriae as putative virulence- or fitness factors contributing to host colonization.

MPP30

Adaptation of an anti-sense screening approach for the identification of genes involved in the formation of the *Staphylococcus aureus* small-colony variant phenotype

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Introduction: *Staphylococcus aureus* small-colony variants (SCVs) represent a naturally occurring subpopulation of *S. aureus,* which has been associated with chronic, persistent and relapsing courses of infection. *S. aureus* SCVs are characterized by slow growth, reduced pigmentation, altered expression of virulence factors and frequently by auxotrophism for hemin, menadione and/or thymidine. Although mutations have been described for certain menadione biosynthesis genes (*menB*, *menC*, *menE* or *menF*) and the thymidylate synthase gene (*thyA*), the genetic basis of a large part of clinical SCV phenotypes is still unknown. Aim of the project was to establish an episomal screening system for the rapid identification and investigation of putative SCV phenotype-associated genes in different genetic *S. aureus* backgrounds via anti-sense gene silencing.
Materials and Methods. A tetracycline-dependent gene regulation system consisting of the repressor protein TetR and the promotor P(*xyl/tet*) was used. To investigate the impact of anti-sense silencing of the target genes on the *S. aureus* phenotype, growth curves were performed in liquid media with and without 0.4 µM anhydrotetracycline (ATc).
Results: The tetracycline-dependent gene regulation system was modified by separating the repressor *tetR* from the promotor P(*xyl/tet*). *TetR* was cut from the plasmid pRAB11 and integrated into the low-copy-number plasmid pCN33 resulting in plasmid pCN33-TetR. Fragments of genes of interest including *thyA* (thymidylate synthase) and *fabI* (enoyl-acyl carrier protein reductase) were amplified and cloned in anti-sense orientation downstream of the P(*xyl/tet*) promotor in pRAB11ΔTetR. *S. aureus* SA113 was transformed with both plasmids pRAB11ΔTetR-AS´gene´ and pCN33-TetR. *S. aureus* SA113 harboring both the empty pRAB11ΔTetR and pCN33-TetR was used as control. Induction with ATc resulted in significant growth retardation for both the *thyA* and the *fabI* harboring strain, while the non-induced strains as well as the induced empty vector strain grew unaffected.
Conclusion. The optimized episomal anti-sense regulation system allows a rapid identification and functional analysis of genes potentially involved in the SCV formation in different genetic backgrounds. Here, *thyA* and *fabI* genes were used as a proof of concept. Further studies will include a broader range of genes to identify novel targets involved in the SCV formation. Furthermore, the system will allow a conditional and adjustable induction of the SCV phenotype *in vitro* including cell culture systems and, as a long-term goal, in animal models.

MPP31

Regulation of colibactin biosynthesis in *Escherichia coli*

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Introduction: Colibactin, a polyketide cytotoxin, is synthezised by a variety of extraintestinal pathogenic *E. coli* (ExPEC) and commensal strains of the phylogenetic lineage B2. Upon infection with colibactin-producing (Clb^+) *E. coli*, the cell cycle of mammalian cells is blocked due to the induction of double-strand breaks, thus leading to megalocytosis [1]. Synthesis of colibactin is mediated by multiple enzymes encoded on the 54-kb colibactin (*clb*) island. Understanding the expression regulation of these enzymes is a prerequisite to study the function of colibactin and to prevent the cytopathic effect on mammalian cells caused by Clb^+ *E. coli.*
Methods and Results: We identified a gene within the *clb* island, *clbR*, coding for a LuxR-type transcriptional factor. Deletion of *clbR* in *E. coli* abolishes megalocytosis of mammalian cells. Our luciferase-based reporter gene and electric mobility shift assay studies suggest that ClbR acts as an activator of *clb* gene expression. Further results provide evidence, that the two-component system BarA/UvrY is involved in the regulation of *clb* gene expression. BarA/UvrY regulates the synthesis of the small non-coding RNAs CsrB and CsrC, which antagonize the function of the carbon storage regulator CsrA, a global RNA-binding protein [for review: see 2]. A putative CsrA binding motif has been identified within the 5´UTR of the gene *clbQ*. In order to investigate the effect of different CsrA levels on *clbQ* expression reporter constructs using post-transcriptional as well as translational fusions are being analysed.
Discussion: Polyketides represent compounds of clinical and pharmaceutical relevance. Furthermore, colibactin may function as a virulence factor of ExPEC. Thus improved understanding of regulation of colibactin expression is critical to elucidate the role of colibactin during ExPEC pathogenesis as well as to allow the purification and structural analysis of this polykektide.

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MPP32

In vitro reconstitution of capsule biosynthesis in *Staphylococcus aureus* - role of the CapAB kinase complex in pathway regulation

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Staphylococcus aureus is an opportunistic bacterial pathogen responsible for a diverse spectrum of human diseases, like wound infections, sepsis and other invasive diseases. Most microorganisms that cause invasive diseases produce extracellular capsular polysaccharides, which protect the pathogen from opsonophagocytosis and thereby enhance virulence.[1] Despite its importance for pathogenicity, staphylococcal capsule biosynthesis is not fully understood on the molecular level; especially the membrane-associated biosynthetic reactions and the posttranslational regulation of CP production, mediated by the tyrosine kinase complex CapAB, remain largely ambiguous.[2]
In vitro synthesis of the lipid-bound capsule precursors lipid Icap and/or lipid IIcap was only achieved in the presence of the tyrosine kinase complex CapAB.Phosphorylation assays identified several proteins involved in capsule biosynthesis as protein substrates of the tyrosine kinase CapB. The impact of tyrosine phosphorylation on the individual enzymatic reactions has been analysed and specific phosphorylation sites have been determined.

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MPP33

A neonatal CNS infection model following mucosal challenge of *Listeria monocytogenes*

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Introduction: Infection of the central nervous system (CNS) represents an important cause of morbidity and mortality in neonates. Mechanisms of host susceptibility, the route of infection and tissue tropism as well as the underlying mechanisms of inflammation and tissue damage in the CNS, however, are ill-defined. Here, we established a model of neonatal CNS infection with *L. monocytogenes* following mucosal challenge in order to investigate the cellular and molecular mechanisms.
Methods and Results: Neonatal C57BL/6 mice were infected intranasally with 10^4 CFU *L. monocytogenes* strain EGDe. Five days after infection, pups were killed and bacterial dissemination was evaluated by replica plating. Interestingly, in addition to spleen and liver, *Listeria* was detected in brain tissues in large amounts. This strong CNS tropism prompted us to

investigate the distribution of *L. monocytogenes* within the parenchyma in more detail. Hence, entire brains of infected animals were prepared aseptically and divided according to its anatomical structures. Hereby, *Listeria* was preferentially reisolated from the olfactory bulb and the cerebrum. In contrast, meninges, cerebellum, and brain stem exhibited only low amounts of bacteria. Subsequent staining of the infected areas with antibodies directed against *Listeria* and various immune cells, showed an intralesional accumulation of bacteria concomitantly with an increase of $CD3^+$ T cells, $Mac3^+$ microglia/macrophages and $GFAP^+$ astrocytes. To study the interaction between *Listeria* and professional phagocytes of the brain in greater detail, we performed *in vitro* infection assays using purified primary microglia cells. A significant increase and expression of TNFα, Ccl2, and Cxcl2 was shown by qRT-PCR and ELISA and, thus, confirmed the results obtained in *in vivo* studies. Interestingly, an up-regulation of pro-inflammatory cytokines was diminished in *L. monocytogenes* strains lacking the cholesterol-dependent pore forming toxin Listeriolysin O (LLO) suggesting an important role in establishing neonatal *Listeria* infections.
Discussion: The possibility to study neonatal CNS infection by *L. monocytogenes* following the natural route is essential for entirely understanding onset and progression of disease. A standardized *in vivo* model reflecting the pathogenesis of neonatal *Listeria* infection may, thus, be essential for discovering new therapeutical approaches.

MPP34
Defects in the TCA cycle result in increased phagocytic uptake of *Salmonella enterica*

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Bacterial invasion of, and phagocytosis by mammalian cells have been demonstrated to be dependent on the motility of pathogens (Lovewell et al. 2011). The gastrointestinal pathogen *Salmonella enterica* achieves coordinated movement by clockwise (CW) or counter clockwise (CCW) rotation of peritrichous flagella. Alternation of direction of flagella rotation enables the bacterium to move in two different modes, called tumbling and running, in response to relative concentrations of chemotactic stimuli.
We investigated the role of primary metabolic pathways of *S. enterica* in host pathogen-interactions. Interestingly, a *S. enterica* Δ*fumABC* mutant strain showed an increased phagocytic uptake during infection of macrophages. Microscopic analysis revealed less switching events from CCW to CW rotation in the mutant strain, leading to an increased running movement. Furthermore, the enhanced motility results in increased events of contact to host cells and higher degree of uptake by macrophages. Mutant strains with defects in the chemotaxis regulator CheY phenocopy this phenomenon. Internal fumarate accumulation during growth of the Δ*fumABC* mutant strain accompanied this observation. Whereas earlier investigations showed that fumarate accumulation in *Escherichia coli* leads to increased CW flagella rotation (Prasad et al. 1998), our data suggest a different mechanism for *Salmonella*. Electron microscopy of the *S. enterica* Δ*fumABC* strain showed the presence of inclusion bodies in the polar regions of the bacterium. Further experiments reveal a high glycogen accumulation in the Δ*fumABC* strain, which might bias the signal transduction of methyl-accepting chemotaxis proteins at the cell poles. To elucidate how deletion of the fumarases leads to increased CCW flagella rotation and running movement and its link to glycogen metabolism, proteome analyses and metabolome profiling were performed. We present a model how the primary metabolism affects the interaction of *Salmonella* with host cells.

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MPP35
YadA-dependent interaction of *Yersinia enterocolitica* with vitronectin

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One of the major virulence determinants of *Yersinia enterocolitica* is the Yersinia adhesin A (YadA). YadA is the prototype of trimeric autotransporter adhesins (TAA) and has multiple functions such as mediating adhesion to host cells, autoagglutination and serum resistance. Thus YadA is a decisive determinant for host-pathogen interactions. It is known that YadA mediates serum resistance by direct interaction with the complement regulatory factors (CRFs) factor H, C4bp und C3 [1-3].
Recently, the glycoprote*in vitro*nectin (VN) has been recognized as CRF that is exploited by several pathogens to evade the complement system. VN inhibits the assembly of the terminal complement complex (TCC) that induces cell lysis by binding the C5b-7 complex and C9 [4, 5]. It has been shown by other groups that e.g. *Moraxella catarrhalis* binds to VN via the TAA UspA2 and that this binding plays a key role for adhesion to host cells and in the control of the terminal pathway of complement activation [6].
The aims of our study are to find out:

- Does *Yersinia* bind vitronectin?
- Does the binding modulate the interaction of *Ye* with the complement system?
- Does the binding influence Yop translocation via the type III secretion system?

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MPP36
Role of SpaS autocleavage in *Salmonella* type III secretion systems

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Introduction: The type III secretion system (T3SS) is a specialized molecular transport apparatus used by many pathogenic bacteria to translocate virulence proteins (effectors) into the host cell.
The T3SS is also known as 'injectisome' or 'needle complex' and it is organized in three main structures: a multi-ring basal body embedded in the bacterial membranes that houses the export apparatus; a extracellular needle, and a translocon in the eukaryotic host cell membrane which allow effectors injection.
To assemble a functional T3SS, specific substrates must be targeted to the secretion apparatus in the correct order. The substrate specificity switch from early substrates (rod and needle subunits) to intermediate substrates (translocators) occurs once the needle has reached its full length.
The *Salmonella* T3SS protein SpaS is part of the export apparatus. SpaS is a multifunctional protein involved in the substrate specificity switch. It consists of a transmembrane region and a C-terminal cytoplasmic domain that undergoes autocleavage at a conserved NPTH motif. Autocleavage is required for the substrate specificity switch.
Materials and Methods: We test the expression and secretion levels of SpaS-FLAG and other T3 proteins by immunoblotting. The use of blue native gel electrophoresis is a suitable way to study the composition of the assembled needle complex. Also our data are complemented by injectisome purification and its visualization by electron microscopy.
RESULTS: We show the time course analysis of SpaS autocleavage in different genetic backgrounds and its incorporation into the T3SS. We

mutate the residues on the NPTH motif or introduced an exogenous cleavage site and analysed its phenotype in terms of cleavage, secretion and needle assembly.
Conclusion: Our results demonstrate that the kinetics of SpaS autocleavage is not regulated by injectisome state of assembly or state of secretion and that the autocleavage mechanism is independent from the needle length control. Rather, autocleavage is a process occurring before the incorporation of SpaS into the injectisome base. We also found that the NPTH motif is not the prerequisite for the substrate specificity switch but seems to facilitate a flexible state that must be achieved.

MPP37
Analysis of the virulence factor ProA from *Legionella pneumophila*

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Legionella pneumophila is a Gram-negative, rod-shaped bacterium and the main causative agent of Legionnaire´s disease, a severe form of an atypical pneumonia. It replicates intracellularly in protozoa but also in human alveolar macrophages. The zinc-metalloprotease ProA is a major virulence factor and the most secreted protein of *L. pneumophila*. The 38 kDa protein is secreted via the type-II secretion system and homologue to the elastase of *Pseudomonas aeruginosa* and thermolysin of *Bacillus proteolyticus*. It plays a role in the pathogenesis of *L. pneumophila,* because it shows cytotoxic effects against eukaryotic cells, tissue necrosis after intradermal injection and acute pulmonary damage after inoculation. In an *ex-vivo* human lung tissue model we could confirm that ProA contributes to the observed pathology of tissue destruction. In order to further understand the molecular action of ProA and to be able to exploit it as an alternative anti-infective target we aimed at solving its structure. For this purpose we produced ProA recombinantly in different *E. coli* expression strains and under various conditions. First crystals were received by using sitting drop method. Further experiments are planned for optimizing production and crystallization of ProA in order to identify possible substrates and promising inhibitors.

MPP38
Stable isotope labeling by amino acids in cell culture (SILAC) technique for global protein quantification identifies the tRNA modifying enzyme GidA as potential global translational regulator in *Pseudomonas aeruginosa*.

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The opportunistic bacterium *Pseudomonas aeruginosa* exhibits a huge repertoire of complex adaption strategies. Besides transcriptional control also translational regulation plays an important role for these processes. GidA is a tRNA modifying enzyme potentially involved in translational regulation. In *E. coli,* GidA is required for addition of a carboxymethylaminomethyl group to uridine 34 which represents the first base of the tRNA anticodon. This modification is required for efficient anticodon-codon interaction among mRNA codons with an A or G at the wobble position. In order to address the effect of GidA on the proteome of *P. aeruginosa* we analyzed a PA14 *gidA* transposon mutant in comparison to the PA14 wildtype strain using the SILAC technique. Quantitative proteome data were analyzed by PseudoCAP enrichment test of regulated proteins and correlation analysis of protein changes in dependency of codon usage. Validation experiments were conducted to proof the hypothesis of GidA being a global regulator for translation in *P. aeruginosa*. The quantitative shotgun analysis resulted in 239 proteins with an increased abundance and 640 proteins with an reduced abundance in the *gidA* mutant. Mainly virulence-associated PseudoCAP groups like “Secreted factors” and “Two component regulatory system” were significantly enriched among proteins with lower abundance, whereas proteins belonging to “Translation, posttranslational modification, degradation” and “Transcription, RNA processing and degradation” were enriched among the proteins with higher abundance. Additionally, the higher frequency of codons for arginine and glycine with an adenine or guanine base in the wobble position in the group of lower abundant proteins indicates translational regulation due to GidA-mediated tRNA modification. Arginine and glycin codon-motif studies using GFP-reporterfusions in a +/-*gidA* background showed a *gidA*-dependent translational efficiency. Taken together, these data suggest the importance of GidA for the expression of genes containing a higher frequency of codons with adenine or guanine and propose a global translational regulation by selective codon usage in combination with tRNA modification in *P. aeruginosa*. The influence of GidA on the protein expression profile claims tRNA modification enzymes to be a promissing target for antibacterial treatment.

MPP39
Establishment of MEC1 cells as a new infection model for *Helicobacter pylori* pathogenesis in B cells

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Introduction: CagA is an important pathogenic factor of the human pathogen *Helicobacter pylori* (*Hp*), which induces severe gastric disorders such as gastritis, ulceration or gastric cancer. After translocation into gastric epithelial cells, CagA is phosphorylated by the host cell kinases Src and Abl in a hierarchical manner. Phosphorylated CagA (p-CagA) induces signal transduction pathways in the host cell and leads to drastic cell elongation combined with high epithelial motility. Compared to gastric epithelial cells, in which the pathogenic mechanism of *Hp* and the connected signaling of CagA are well investigated, less is known about the interaction of *Hp* with B cells.
Materials and Methods: We established MEC1 cells (B-cell line derived from B-CLL patients) as a new infection model to study the interaction between *Hp* and B cells. In kinetic experiments, MEC1 cells were infected for different time points with *Hp* wildtype and an isogenic mutant that lacks the *cagA*-gene. Injection of CagA and activated non-receptor tyrosine kinases were analyzed by Western blotting.
Results: We observed that CagA was injected and strongly phosphorylated after one, two and four hours of infection with *Hp*. To determine upstream signaling that led to the phosphorylation of CagA in MEC1 cells, specific inhibitors were used to target kinases of the Src and Abl family. We observed that p-CagA was strongly inhibited upon treatment with an inhibitor against Src (PP2) and slightly inhibited when an Abl inhibitor (STI571) was applied, whereas the addition of Dasatinib, which inhibited both kinases, led to a complete loss of p-CagA.
Discussion: In conclusion, the knowledge of the complex signaling network with CagA as a key molecule in B cells is crucially important to understand the *H. pylori* mediated gastric diseases such as gastric mucosa-associated lymphoid tissue (MALT) lymphoma. For further investigations, MEC1 cells is a suitable new infection model of *Hp*.

MPP40
Non-conventional secretion of membrane proteins through the type III secretion system of Salmonella

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Introduction: The type III secretion system (T3SS) of Gram-negative bacteria secretes effector proteins directly into the host altering host cell functions in order to promote bacterial survival. Usually, the T3SS effectors are soluble proteins acting from within the host cell cytoplasm, but a number of transmembrane domain (TMD) containing substrates were identified. Their existence raises the question of the discrimination of membrane protein targeting pathways inside the bacterial cell. Membrane proteins are typically secreted via the conserved Sec-pathway, in which the first TMD recruits the signal recognition particle (SRP). TMDs within T3SS substrates create a targeting conflict, as two sequential secretion signals for two different incompatible pathways are concatenated in the same protein: The SRP has the potential to target TMD-containing effector proteins to the inner membrane, from where they cannot be fed into the T3SS export apparatus, and hence are useless for the bacteria. From this, it becomes clear that membrane protein targeting for T3SS has to follow a novel mechanism that prevents SRP targeting and allows for the exclusive engagement of T3SS substrates with its cognate system.

Materials and Methods: We use three TMD-containing T3SS substrates: The SPI-1 translocon component SipB, which contains two TMDs, and the SPI-2 effector proteins SseF and SseG, predicted to contain four and three TMDs, respectively. We started out by analyzing the accumulation levels of SipB in the inner membrane of *Salmonella* by high resolution cell fractionation. To assess the SRP-targeting potential of these TMDs and the roles of their cognate chaperones in preventing erroneous targeting, we currently develop a test system that utilizes the well-studied SRP-target inverted leader peptidase (Lep-inv), which contains two TMDs. We have constructed Lep-inv chimeras where the SRP-binding first TMD has been replaced by the first TMDs of our T3SS substrates of interests.
Results and Discussion: Preliminary results show that SipB does not integrate into the bacterial inner membrane, neither in a T3SS proficient nor in a T3SS deficient environment. These results lead us to conclude that the avoidance of erroneous inner membrane targeting of SipB is very efficient. We also observe that SipB is rapidly degraded in the absence of its cognate chaperone SicA. If binding of the cognate chaperone plays a role in avoiding the binding of SRP to the TMDs of SipB, the rapid degradation of free SipB would close a potential loophole for mistargeting.

MPP41
Functional characterization of aggregative adherence fimbriae of *Escherichia coli*

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Introduction: Since the *Escherichia coli* O104:H4 outbreak in Germany in 2011 especially enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC) have come into focus of epidemic research. One of the most promising pathogenicity factors assumed to be involved in the pathogenesis of the O104:H4 outbreak strain compared, e.g. to the closely related EAEC O104:H4 strain 55989, are so called aggregative adherence fimbriae (AAF). Five different AAF types are known so far. The EHEC O104:H4 outbreak strain LB226692 possesses AAF type I, whereas 55989 expresses AAF/III fimbriae. All known AAF-encoding determinants are located on virulence plasmids (pAAs) and comprise four genes coding for the chaperone (AggD), usher (AggC), invasin (AggB) and fimbrial subunit protein (AggA). The *aggD*, *aggC* and *aggB* genes are highly conserved whereas the *aggA* genes differ notably between the different *aaf* types. The impact of the different AAF fimbrial variants on adhesive and aggregative phenotypes and thus on the pathogenicity of *E. coli* has not yet been comprehensively analyzed.
Materials and Methods: In order to analyze and functionally compare the five different AAF variants, the corresponding fimbrial gene clusters were cloned into an expression vector with inducible promoter and expressed in an identical *E. coli* K-12 strain background. The impact of the individual AAF variants on autoaggregation, adhesion to eukaryotic cells and biofilm formation was investigated.
Results: The five different *aaf* gene clusters could be successfully expressed in an identical *E. coli* K-12 strain background. Under the tested conditions, expression of AAF/I, AAF/III and AAF/V clearly contributed to the different phenotypes, whereas expression of AAF/IV and AAF/II fimbriae only had a weak impact on aggregative and adhesive phenotypes. Further studies will focus on AAF expression levels in the K-12 strain background as well as in the wild type strains.
Discussion: Interestingly, the recent O104:H4 EHEC outbreak strain and the closely related EAEC O104:H4 strain 55989 express different types of AAF fimbriae. It is currently unknown whether these individual AAF fimbrial variants confer different phenotypes or if they can be expressed interchangeably without affecting bacterial adherence or biofilm formation. Our results will allow for the first time to systematically compare the contribution of individual AAF variants and other pAA-encoded factors, e.g. Aap and AggR to bacterial adherence and biofilm formation.

MPP42
Delineating the interaction of pneumococcal surface proteins with the human adhesive glycoprotein fibronectin

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Introduction: *Streptococcus pneumoniae* (pneumococci) is a remarkable commensal and opportunistic bacterium employing various strategies to interact, adhere and invade into its human host. One of the striking strategies engaged by pneumococci to encounter the host is the diverse and marvelous repertoire of cell surface proteins. The adhesins are divided into three major categories: the LPxTG proteins, covalently anchored to the cell wall in sortase dependant manner; the choline-binding proteins (CBPs), non-covalently attached to the cell wall via interacting with phosphorylcholine (PCho) residues on wall teichoic acids (TAs) and lipoteichoic acids (LTAs); and the lipoproteins attached to the phospholipid bilayer of membrane. PavB is one of the LPxTG proteins, known to be a pneumococcal colonizing factor. The major part of the mature PavB consists of repetitive sequences termed as **S**treptococcal **S**urface **Re**peats (SSURE), whose number has been shown previously to be strain dependent. In this study the interaction of PavB with the host matrix glycoprotein fibronectin and its type III repeats thereof has been delineated in detail.
Materials and Methods: Recruitment of fibronectin by *S. pneumoniae* was assessed by immunoblots and flow cytometry. To study the interaction of PavB (SSURE fragments) with human fibronectin, far western blots were performed. To narrow done the specific binding domain of PavB in fibronectin, recombinant Fn type III domains were employed in binding assays. To identify critical amino acid sequences involved in the SSURE-Fn interaction, a synthetic peptide array consisting of immobilized 15mer Fn-peptides was screened with a PavB-SSURE domain. In addition, surface plasmon resonance (SPR) studies were used to show the differences in the interaction between PavB-SSURE and various Fn domains.
Results: Flow cytometric analysis shows that *S. pneumoniae* recruits fibronectin from human plasma in a dose-dependent manner. Far western blots further prove the interaction of SSURE domains and Fn and different FnIII repeats. Among the FnIII repeats, FnIII 7-10 (integrin binding domain) and FnIII12-14 (heparin binding domain) bound with highest affinity to the PavB-SSURE domain. This was further confirmed by SPR studies. A spot peptide array indicated finally the specific interaction of PavB-SSURE with various fibronectin type III repeats.
Conclusions: *S. pneumoniae* interacts specifically with human fibronectin via the SSURE domains of PavB. Protein-protein interactions reveal the high affinity of SSURE domain for different FnIII domains, most important being FnIII7-10 and 12-14. The interaction of pneumococcal PavB with the adhesive glycoprotein Fn has important implications in the pneumococcal-host interactions.

MPP43
S. aureus induced infective endocarditis investigated by MRI in a mouse model

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Introduction: Infective endocarditis (IE) is mainly caused by *S. aureus* and often leads to severe or life-threatening conditions. It is characterized by the presence of septic vegetations on the surface of the endocardium and the valves. So far the key players among the variety of different virulence factors and toxins to induce endocarditis are only partly understood. Here, we developed a mouse model of *S. aureus* induced IE. Together with state-of-the-art MRI (magnetic resonance imaging) which allows to characterize morphological and functional changes of infected cardiac valves we developed a tool kit to study the induction and progression of IE.
Materials and Methods: *Mouse model of endocarditis*: In the mouse model (CD1 mice), a 32-G polyurethane catheter was placed at the aortic root via the right carotid artery of the mouse, to induce a mechanical trauma of the endothelial layer of the aortic valves. Either 10^4,10^5 or 10^6 CFUs (colony forming units) of *S. aureus* bacteria (6850) were injected i.v. 24 h after catheter placement. A control group received sham-surgery only. *MRI protocol*: 2D self-gated cine UTE (ultra short echo time) images were acquired at 9.4 T on a Bruker BioSpec 94/20 followed by Reflection Point analysis.
Results: MR images of infected valves were scored according to (I) valve thickening, (II) functional defects leading to changes in the flow and (III) additional structures, such as vegetations and abscesses. IE revealed valve thickening in correspondence to the bacterial load. Compared to the control group which obtained the surgery only, substantially thicker valves were already observed for 10^4 bacteria (Fig.1). Blood agar cultures confirmed increased bacterial vegetations on the valves when higher bacterial concentrations were applied.
Discussion/conclusion: *S. aureus*-infected aortic valves could be visualized in the presented model using self-gated UTE MRI and Reflection Point

analysis and was confirmed by plating of infected heart tissue. This combination of methods allows for future assessment of disease course of endocarditis, bacterial patho-mechanisms and pharmacological studies of novel drugs.

Figure 1

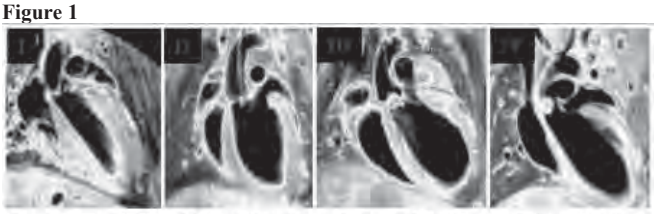

Fig.1: Sagittal UTE MRI of the mouse heart and Reflection Point analysis showing (I) non-infected and (II-IV) infected valves; infection with *S. aureus* using either (II) 10^4, (III) 10^5 or (IV) 10^6 CFUs. Substantial valve thickening was observed for infected valves.

MPP44

Role of PilY1 in adhesion to host cells and intracellular infection

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Legionella pneumophila is a Gram-negative bacterium which causes a severe form of pneumonia - Legionnaires' disease. A key feature of *L. pneumophila* pathogenicity is the ability to reprogram the intracellular trafficking and to evade the endocytic and lysosomal degradation. In previous work we have selected *L. pneumophila* transposon mutants that are attenuated in escaping the lysosomal degradation. One of the mutants that show significantly higher co-localization with lysosomal marker possesses several insertions in the *pilY1* gene. The *pilY1* gene is present predominantly in *L. pneumophila* strains and its knock out mutants show attenuated replication as well a reduced adhesion and uptake in THP-1 macrophage-like cells. Moreover these mutants have defects in twitching and sliding motility. The immunoblotting revealed that *pilY1* is exclusively expressed at the stationary phase, during which *L. pneumophila* expresses many other virulence factors. The protein represents a unique structure as it posseses a vWF domain at the N-terminus and a PilY1 domain at the C-terminus. The vWF domain is often involved in cell adhesion and is commonly found in extracellular eukaryotic proteins. In contrast the PilY1 domain shares homology with the virulence factor PilY of *P. aeruginosa* and PilC of *Neisseria* spp. and is proposed to influence type IV pilus biogenesis and stability. Genetic analysis revealed that PilY1 of *L. pneumophila* posseses a putative calcium-binding motif similar to *P. aeruginosa* and *Neisseria* spp. To prove this we expressed the recombinant C-terminal PilY domain and determined its calcium binding affinity. The function of calcium-binding as well as the influence of vWF domains during intracellular infection are discussed. Taken together, these results indicate a role of PilY1 as a new virulence factor of *L. pneumophila.*

MPP45

Genome sequence and phenotypic analysis of a new *Francisella species* (W12-1067) isolated in Germany

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Introduction: *Francisella* isolates from patients suffering from tularemia in Germany are generally strains of the species *F. tularensis* subsp. *holarctica*. To our knowledge, no other *Francisella* species are known for Germany. Very recently, a new *Francisella* species could be isolated from a water reservoir of a cooling tower in Germany.
Results: Strain W12-1067 was isolated from a water reservoir of a cooling tower of a hospital in Germany. The 16S rDNA of W12-1067 is 99% identical to the respective nucleotide sequence of the recently published new strain *F. guangzhouensis*. However, the overall sequence identity of the *fopA*, *gyrA*, *rpoA*, *groEL*, *sdhA* and *dnaK* genes is only 89%, indicating that strain W12-1067 is not identical to *F. guangzhouensis*. The whole genome of the strain was sequenced (~1.7 mbp, 32.2% G+C content) and the draft genome was annotated. Whereas various virulence genes common to the genus *Francisella* are present, the major virulence factor, the *Francisella* pathogenicity island (FPI), is missing. Instead, another putative type-VI secretion system is present within the genome of strain W12-1067 and the strain was able to replicate within a mouse-derived macrophage-like cell line. Furthermore, the growth optimum of the isolate is ~30 °C, is able to grow without additional cysteine within the medium and the strain is halotolerant.
Conclusions: Isolate W12-1067 is closely related to the recently described *F. guangzhouensis* species, exhibits a putative new type-VI secretion system and is able to replicate within eukaryotic host cells. Obviously, *F. tularensis* subsp. *holarctica* is not the sole species in Germany, therefore further research is needed to investigate the epidemiology, ecology and pathogenicity of *Francisella* species present in Germany.

MPP46

In vivo role of the mFPR2 receptor in *S. aureus* infection

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Virulence of the emerging Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) and other highly pathogenic *S. aureus* depends on Phenol-Soluble Modulin (PSM) peptide toxins, which combine the capacities to attract and lyse neutrophils and play a role in phagosomal escape of *S. aureus*. We have previously shown that PSM sensing by the human formyl-peptide receptor 2 (FPR2) leads to leukocyte activation and chemotaxis. However, mice have several potential FPR2 orthologs and it has remained unclear how FPR2 affects the course of *S. aureus* infections.
Here we demonstrate that the mouse mFpr-rs2 receptor (now referred to as mFPR2) is the functional FPR2 ortholog. It senses PSMs at nanomolar concentrations and initiates recruitment of leucocytes in response to infection with CA-MRSA *in vivo*. Use of mFPR2 knockout mice show that neutrophil activation and chemotaxis through PSM peptides and culture filtrates of highly pathogenic CA-MRSA occurred only in neutrophils of wild type mice and not in neutrophils of mFPR2 $^{-/-}$. Moreover in an *in vivo* peritonitis model we could show that FPR2 knock out leads to strongly reduced monocyte and neutrophil immigration after *S. aureus* infection. In contrast, no difference in leukocyte immigration between wild type and mFPR2$^{-/-}$ mice was observed after infection with an isogenic PSM deletion mutant. Thus, the innate immune system uses FPR2/mFPR2 to sense highly virulent bacterial pathogens. Targeting FPR2 may help to manage severe infections induced by *S. aureus*.

MPP47

Studies on the function of Autoinducer-2 of *Streptococcus pyogenes*

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The signal molecule Autoinducer-2 (AI-2) is a prevalent means for information exchange among multiple bacterial phyla. This information exchange, called quorum sensing (QS), plays an important role in the regulation of processes such as biofilm production or expression of virulence factors. AI-2 is produced by the enzyme LuxS which is also part of the activated methyl cycle (AMC). The AMC regenerates S-adenosylmethionine after acting as a donor for CH_3-groups. As part of the regeneration process LuxS catalyses the reaction from S-ribosylhomocysteine to homocysteine during which 4,5-dihydroxy-2,3-pentanedione (DPD) is released. DPD in turn is the common precursor molecule for the different types of AI-2. The aim of this study was to characterize the phenotype of a LuxS-deficient mutant of different strains of *Streptococcus pyogenes*, an exclusively human pathogen that can cause infections of the skin and the upper respiratory tract as well as streptococcal toxic shock syndrome and necrotizing fasciitis. Since QS via AI-2

contributes virulence factor production, gaining information about its function for *S. pyogenes* is of great medical importance. Thus, consequently, LuxS mutants were constructed for the serotypes *Streptococcus pyogenes* M1 Ferretti, M5 and M49. In addition to each deletion mutant, which caused an AI-2-deficiency and an interrupted AMC, two complementation mutants were constructed: A homologous complementation restoring the original phenotype after the deletion and a heterologous complementation with S-adenosyl-L-homocysteine hydrolase (SahH) from *Pseudomonas aeruginosa*. SahH bypasses the interrupted Pfs/LuxS-pathway within the AMC without restoring the AI-2 production. These complementation strategies allowed the discrimination between phenotypical changes caused by the AI-2-deficiency and changes caused by the interrupted AMC. With the wild type, the deletion mutant, and the two complementations of the strains M1 Ferretti, M5 and M49 growth experiments and assays to test survival of the cells in human blood were performed. Also, the effect of AI-2 on the human immune system was studied. Neutrophile granulocytes were isolated from human blood samples and incubated with fluorescent latex beads and 0 nM, 50 nM, 500 nM and 5000 nM AI-2. The observation was focused on changes in the granulocytes' phagocytosis of the latex beads. Since AI-2 is a common messenger of many bacteria and not produced by the host it is expected that the immune system shows adaptations to the molecule.
Our experiments showed that AI-2 production of *S. pyogenes* depends on growth phase, medium and serotype.

MPP48

Expression of IL-6 by monocytes activated by secreted proteins and surface peptides from *Streptococcus gallolyticus* subsp. *gallolyticus*

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Introduction: *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is an emerging pathogen in 20% of streptococcal-caused infective endocarditis (IE) cases. SGG-mediated IE can additionally be associated with colorectal cancer. Surface proteins are crucial for the accomplishment of SGG as a commensal and pathogen. These are involved in adhesion to and invasion of host cells and tissues, in biofilm formation and can cause or evade immune responses. Hence, in this study, proteins from SGG were analyzed on strain dependence and stimulation potential.
Materials and Methods: Four different isolates of SGG were digested with trypsin (20 µg/ml) or proteinase K (5 µg/ml) to generate "shaved" peptides or left untreated without proteases to get "shed" proteins for comparison. Present peptides in the supernatants were used for further analyses. These peptides were separated by SDS-PAGE and dyed with silver stain. Furthermore the peptides were used to stimulate the human monocyte cell line THP-1. Therefore 10^6 cells/ml were inoculated with 50 µg/ml peptide or PBS for 6 h. The IL-6 concentrations in the supernatants were measured afterwards.
Results: Silver stained SDS-PAGEs showed typical patterns of bands from shed proteins for each SGG isolate. The data of monocyte stimulation demonstrate differences in the potential of peptides from different SGG isolates to stimulate monocytes with regard to the secretion of IL-6 as inflammatory parameter. Peptides of the isolate 010672 did not stimulate monocytes, regardless of whether shed or shaved peptides were used. Marginal increases in IL-6 secretion were seen after stimulating monocytes with peptides from strain LMG17956 in comparison to controls. Trypsin-treated peptides led to the highest stimulation (1.6 times). Shed peptides and peptides after protease digestion from strain BAA-2069 stimulated THP-1 cells 2-2.5 times more than respective controls. Protease shaved peptides from strain UCN34 showed similar potential of stimulation to peptides from BAA-2069, but the highest stimulation was induced by shed proteins from this isolate. The IL-6 secretion was 4.5 times higher compared to controls.
Conclusion: Surface proteins and secreted proteins are distinct between different isolates of SGG as observed in SDS-PAGE and in their potential of stimulation. Apparently solely proteins of certain SGG strains are sufficient to cause an immune reaction. The native conformation seems to be crucial for recognition by monocytes because shed peptides of UCN34 stimulated more than shaved ones. Further investigations will focus on identifying the stimulating surface proteins of SGG.

MPP49

HtrA-mediated fragmentation of E-Cadherin

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Introduction: *Helicobacter pylori* (*Hp*) is a class-I-carcinogen that colonizes the human stomach. An important property of *Hp* is the disruption of the epithelial barrier function in the stomach. Epithelial cells show an apico-basolateral arrangement, which is maintained by tight junctions, adherence junctions and the actin-cytoskeleton. The transmembrane protein E-cadherin is an important component of adherence junctions, as it mediates properties counteracting the tumorigenic process. Bacterial high temperature requirement A (HtrA) has been identified as a serine protease contributing to the pathogenesis of *Hp* by cleaving the ectodomain of E-cadherin. Importantly, disruption of adherence junctions of epithelial cells by E-cadherin shedding might lead to the regulation of cancer- and migration-associated signal transduction pathways. Hence, aim of this study is a detailed analysis of HtrA-mediated E-cadherin fragmentation to identify possible cleavage sites in the E-cadherin molecule.
Materials and Methods: In infection experiments using human gastric carcinoma cells lines, E-cadherin fragment formation upon *Hp* infection was analyzed by Western blotting. Correlation of cleavage fragments to an E-cadherin domain was investigated using different antibodies recognizing different E-cadherin domains. The formation of cleavage fragments was also tested in in-vitro cleavage experiments by incubating recombinant E-cadherin with purified *Hp* HtrA. In-vitro cleavage experiments using other cadherin variants were performed to compare fragment formation and differences in fragment sizes. The results will be validated by deletion of defined E-cadherin domains in an expression plasmid.
Results: In infection experiments a stable ~90 kDa E-cadherin fragment was detected in the supernatant of infected cells. In contrast, in in-vitro cleavage assays using recombinant E-cadherin and HtrA three cleavage fragments as well as a coherent decrease in full-length protein were detected, which were partially different from cleavage fragments of other cadherins. According to the detected E-cadherin domains, modified E-cadherin deletion variants will be generated and overexpressed in gastric epithelial cells and tested for E-cadherin fragmentation.
Conclusion: A detailed characterization of HtrA-mediated E-cadherin cleavage is necessary for the successful generation of a protease-resistant E-cadherin protein, which allows analyzing the intracellular signal transduction pathways in response to E-cadherin cleavage during *Hp*-associated gastric cancer.

MPP50

Yersinia T3SS effectors as regulators for cellular integrity during T3SS transport? - A functional closeup with the *Yersinia* Toolbox approach -

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Introduction: A lot of enteropathogenic bacteria like *Shigella*, *Salmonella* and *Yersinia* are expressing a type 3 secretion system (T3SS). This needle complex enables - in an ATP dependent manner - translocation of several modulating bacterial effector proteins into the host cell in order to avoid bacterial clearance. For this, a translocator complex is inserted into the membrane of the targeted host cell, through which the effectors enter the cytoplasm. In *Yersinia enterocolitica* this translocator/pore-complex consists of YopB/D and can be detected by the inflammasome. In order to avoid bacterial recognition through this pathway, Yop-translocation must be a tightly regulated process. In this study, we want to analyze the effect on cytotoxicity of several Yop effector proteins (YopE/T/Q), interacting with the YopB/D-pore.
Materials and Methods: *Yersinia*-Toolbox (WA-314 pT3SS)-strains are used in all in-vitro infection experiments. Toolbox strains consist either of a modified virulence plasmid encoding YadA and the type 3 secretion system (pT3SS) alone or an additional plasmid (pACYC184) encoding one single Yop effector protein of interest (YopE/T/Q). To determine the dependency of Rho-GTPases (Mejía, Bliska et al. 2008) on cytotoxic effects, inactive and truncated Yop effectors were additionally generated. All developed strains were tested for Yop secretion and -translocation. HeLa cells as well as macrophages were infected and LDH-release was measured after 3h of incubation.
Results: HeLa cells, infected with strains translocating Yop-effectors (YopE/Q or T) of full length (e.g. WA-314 pT3SS, pYopE) showed all low levels of LDH-release (under 10%) compared to the control strain (WA-314 pT3SS without effectors: ~ 40%). In order to find the critical length of an

effector protein - necessary to inhibit cytotoxicity - strains with several truncated constructs (e.g. $YopE_{138}/E_{80}/E_{53}$; $YopQ_{138}$; $YopT_{138}/YopT_{106}$) were also analyzed. All truncated YopEs blocked LDH-release at comparable amounts as full length YopE. Infections with strains translocating $YopQ_{138}$ showed intermediate and truncated YopTs high levels of LDH-release. Quantification of released proteins via western blotting revealed that all truncated proteins are accepted substrates of the T3SS.
Conclusion: Our results indicate that membrane integrity at sites of the translocator complex is crucial for host cell survival. Yop translocation (YopE/Q/T) plays an important role to maintain this physiological membrane potential. The GAP domain of YopE is not necessary to avoid cytotoxic effects immediately after infection.

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MPP51
Disarming the host - *L. pneumophila* blocks retrograde trafficking to promote intracellular replication

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The Gram-negative bacterium *Legionella pneumophila* naturally replicates within environmental amoebae. By utilising a similar molecular mechanism, the pathogen also parasitises human alveolar macrophages, possibly leading to the severe pneumonia "Legionnaires' disease". Hereby, the formation of a replication-permissive compartment, the *Legionella*-containing vacuole (LCV), is a crucial process, which is dependent on the Icm/Dot type IV secretion system (T4SS). Approximately 300 different "effector" proteins are translocated by the T4SS into the host, where they subvert cell functions. Some effectors subvert the activity of small GTPases or the intracellular phosphoinositide (PI) pattern and thus affect host cell signaling and vesicle trafficking (*1*). We discovered that *L. pneumophila* interferes with the host retrograde vesicle trafficking pathway, wherein selected proteins, so-called cargo receptors, are recycled from the endosomes back to the Golgi. The key mediator of this process is the retromer complex, consisting of the heterotrimeric cargo-selective subcomplex (Vps26, Vps29, Vps35) and a heterodimeric membrane-deforming sorting nexin (SNX) subcomplex.
We showed that the T4SS effector RidL (Retromer interactor decorating LCVs) is needed for efficient intracellular replication and selectively binds both the Vps29 retromer subunit and the PI lipid PtdIns(3)*P*, which targets SNXs to the membranes (*2*). Although the effector did not affect the acquisition of the Vps cargo recognition sub-complex to the pathogen vacuole, the percentage of LCVs positive for retrograde cargo receptors and some SNXs was reduced in presence of RidL. Moreover, in macrophages *L. pneumophila* arrested retrograde trafficking of cholera toxin at the endosomal stage in a *ridL*-dependent manner, and in HeLa cells ectopically produced RidL blocked the retrograde transport of Shiga toxin. Finally, siRNA experiments revealed that a functional retrograde trafficking pathway restricts intracellular bacterial growth.
Taken together, these findings suggest that RidL inhibits retromer function to promote bacterial replication in host cells. We speculate that this might be due to a decreased lysosomal supply of acid hydrolases, bactericidal enzymes that are delivered by cargo receptors, which themselves depend on a functional retrograde pathway.

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MPP52
Metabolic marker genes of the microaerobic adaptation of *Pseudomonas aeruginosa* to the chronically infected cystic fibrosis lung

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Pseudomonas aeruginosa is the leading pathogen of chronic cystic fibrosis (CF) lung infection. Life-long persistence in the inflamed and ever fluctuating CF lungs results in the selection of a variety of changes in *P. aeruginosa* physiology. Accumulating evidence suggests that especially metabolic changes support the survival and growth of *P. aeruginosa* within the oxygen-depleted and nutritious CF mucus. To investigate if metabolic adaptations described for hypermutable *P. aeruginosa* from late CF lung disease (Hoboth et al., J. Infect. Dis. 2009:118-130) may represent changes specific to the microaerobic CF mucus, we determined the expression of selected genes during aerobic and anaerobic growth in LB and the artificial sputum medium ASM, with a focus on the differential regulation of the two isocitrate dehydrogenases Icd and Idh. Interestingly, both isoenzymes may replace each other under aerobic and anaerobic conditions. The NADPH- and RpoS-dependent Icd seems to be the leading isoenzyme under prolonged oxygen limitation and stationary growth phase. Knockout of the icd / idh genes lead to a defect in expression of different virulence associated genes. In summary, LacZ reporter analysis revealed that anaerobiosis increase the expression levels of *azu*, *cbb3-1*, *cbb3-2*, *ccpR*, *icd*, *idh* and *oprF* gene, whereas *himD* and *nuoA* are increasingly expressed only during anaerobic growth in ASM. Moreover, overexpression of the anaerobic regulator Anr improved the expression of *azu*, *ccpR*, *cbb*3-2 and *icd*. In summary, expression of *azu*, *cbb3-1*, *cbb3-2*, *ccpR*, *icd*, *idh*, *oprF*, *himD*, and *nuoA* appeared to be beneficial for the growth of *P. aeruginosa* under oxygen limitation so that these genes may represent marker genes for its microaerobic adaptation during chronic CF lung disease.

MPP53
The role of DNA methylation in *Pseudomonas aeruginosa* PAO1

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Pseudomonas aeruginosa is an opportunistic bacterial pathogen that causes severe acute and chronic infections. The ability of *P. aeruginosa* to form biofilms, to rapidly adapt to changing environments and to easily acquire antibiotic resistance are the main reasons for the increasing recovery rates of *P. aeruginosa* in the hospital setting. The role of restriction modification systems in bacteria is well known as a defense mechanism against foreign DNA. Furthermore, DNA methylation of adenine has been suggested to affect gene expression. In this study we analyzed DNA methylation and evaluated whether this affects transcription in *P. aeruginosa* as an option to respond to environmental changes. SMRT-sequencing technology was used to search for methylation motifs in *P. aeruginosa* PAO1. In addition, we established a mass spectrometry based approach (LC-MS) to analyze DNA methylation as an alternative strategy to validate sequencing results and to quantify methylation rate at different culture conditions. Additionally, a transcriptome analysis was performed to look for differential expressed genes.
Our results revealed that PAO1 has at least one active methylase related to a putative restriction modification system (hsdMSR). The corresponding motif was discovered with SMRT-sequencing and is located in the promotor region upstream of around 600 genes. The motif was confirmed using LC-MS. Therefore, genomic or plasmid derived DNA was isolated, digested and analyzed with LC-MS to quantify modified and unmodified adenines. We could show that the motif is extremely conserved because permutation of the motif abolished its methylation. Interestingly, methylation depended on temperature and growth phase: with increasing temperature the motif methylation rate decreased. At 42 °C and in stationary phase we detected a relative methylation of about 30 %. The finding, that the motif was not fully methylated implies a role for gene regulation. The transcriptome data

showed 11 differential expressed genes with the motif in the promotor region, among them were genes involved in iron metabolism and oxidative stress response.
In conclusion, our results demonstrate that DNA methylation affects gene expression in PAO1 and the putative hsd-system impacts on iron metabolism and oxidative stress response.

MPP54

Analysis of the assembly of bacterial type III secretion systems

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Introduction: Many pathogenic gram-negative bacteria use type III secretion systems (T3SS) to secrete effector proteins into target host cells. These proteins are able to modulate host immune responses or can lead to the uptake of bacteria into non phagocytic cells and by this lead to severe infections and cause enteric diseases. T3SS are highly conserved among all species expressing one of these systems. They are composed of over 20 different proteins and build a membrane spanning multi megadalton complex. Although progress concerning the structure and composition of T3SS is ongoing and gives deeper and deeper insights into the mechanism of action, the question how these systems assemble and thereby enable a functional secretion remains unclear.
Materials and Methods: A plasmid based in vivo photo-crosslinking system is used to find signature crosslinks for several protein-protein interactions between different T3SS components. In this method, the synthetic amino acid para-Benzoyl-phenylalanine (pBpa) is incorporated at specific positions of the target protein. After UV irradiation the benzophenon group of pBpa reacts to nearby C-H bonds and thereby links two interaction proteins covalently. Interaction partners are identified by western blot or mass spectrometry. The presence of signature crosslinks is tested in different genetic backgrounds, to get insights into the static assembly picture of T3SS. Furthermore, the goal is to analyze the dynamics of T3SS assembly. For this, the crosslinking approach is combined with a classical pulse chase labeling, which allows visualization of crosslinks over time.
Results: Preliminary results show different crosslinks for several proteins of the T3SS. The identified interactions were tested in the absence of different T3SS components.
Discussion: The Results show that the method of in vivo photo-crosslink can be an effective tool to study T3SS assembly and gives new insights into this complex machinery.

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MPP55

5' nucleotidases are involved in *Streptococcus pyogenes* virulence

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Introduction: *Streptococcus pyogenes* (Group A *Streptococcus*) is an exclusively human pathogen, which is responsible for diseases ranging from superficial infections of the skin and pharyngeal mucosal membranes up to severe systemic and invasive diseases and autoimmune sequelae [1]. As a human pathogen, *S. pyogenes* needs a variety of virulence factors to colonize the host tissue and to evade the immune system. Several studies reported that *S. pyogenes* possesses an immunogenic, surface associated, LPXTG-motif anchored protein with putative 5'-nucleotidase domains [2]. 5'-nucleotidases catalyze the hydrolysis of phosphate groups at the 5'-end of nucleotides and were shown to have important roles in virulence in several human pathogens [3, 4]. In our work, we investigated the involvement of a putative 5'-NT in the virulence of *S. pyogenes*.
Methods: We generated *S. pyogenes* 5'-NT gene deletions in M6, M18, and M49 serotype strains. Mutants and cognate wild type strains were analyzed for their ability to survive in human blood, for phagocytosis by neutrophiles and macrophages, for biofilm formation capability, and for their ability to escape *neutrophil extracellular traps* (NET). Furthermore, we heterologously expressed and purified the putative 5'-NT, applying StrepTag based affinity chromatography, for subsequent enzymatic characterization.
Results: The deletion of the 5'-NT in *S. pyogenes* M6, M18, and M49 serotype strains led to significantly decreased growth in human whole blood and increased killing by phagocytic cells. In *S. pyogenes* M6, 5´-NT deletion caused an increase in biofilm formation while in serotype M18 the 5'-NT deletion had the opposite effect. Furthermore, our data indicate that 5'-NT plays a role in NET-degradation.
Discussion: The differences in blood survival and phagocytosis of wild type and mutant strains by phagocytic cells indicate that 5´-NT is involved in virulence of GAS. It is likely that the 5´-NTs degrade ATP and thus prevent activation of purinergic receptors and consequently the production of pro-inflammatory substances.

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MPP56

Paracellular transmigration of *Campylobacter jejuni* across polarized epithelial cells and role of the secreted serine protease HtrA

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Introduction: Campylobacter jejuni is one of the most important bacterial pathogens of foodborne illness. Crossing the intestinal epithelial barrier and host cell invasion are primary reasons for tissue damage triggered by C. jejuni, but molecular mechanisms are widely unknown. We have recently shown that the serine protease HtrA (High temperature resistant protein A) of C. jejuni is secreted into the extracellular space, where it can cleave the ectodomain of the host cell adhesion protein and tumour suppressor E-cadherin. Aim of the present study was therefore to study in detail the transmigration process of C. jejuni by rigorous mutagenesis of HtrA and design of specific HtrA inhibitors.
Materials and Methods: Tight monolayers of polarized MKN-28 and MDCK epithelial cells in a transwell filter system were confirmed by fluorescence microscopy and measuring the transepithelial resistance (TER). We developed a genetic complementation system for studying htrA in C. jejuni. This allowed us to complement the wild-type htrA gene in trans, to exclude polar affects and to generate deletion and point mutants across the gene. Transmigration of wild-type C. jejuni and htrA mutants across MKN-28 and MDCK cells was studied by confocal laser scanning microscopy (CSLM), scanning electron microscopy (SEM), TER determination and computer-based inhibitor design.
Results: We confirmed that our DhtrA mutant is non-polar because complementation with the wild-type gene restored (i) expression of proteolytically active HtrA multimers, (ii) C. jejuni growth at high temperature (44°C) and (iii) growth under high oxygen stress conditions. Wild-type C. jejuni and complemented strains transmigrated efficiently in a time course up to 24 hours, while various htrA point and deletion mutants cannot. CSLM and SEM studies showed that the bacteria first adhere at the apical surface near the cell-to-cell junctions and then transmigrate by a paracellular pathway between neighboring cells, while leaving TER values unchanged. The extracellular domain of E-cadherin is cleaved-off by HtrA *in vitro* and during infection in vivo. In addition, we identified peptide inhibitors for E-cadherin processing by recombinant HtrA. One such peptide was also able to efficiently block E-cadherin ectodomain shedding and C. jejuni transmigration during infection.
Conclusions: In the present study we identified and characterized the serine protease HtrA of C. jejuni as a novel secreted virulence factor which opens the cell-to-cell junctions by cleaving the host cell adhesion protein and tumour suppressor E-cadherin. HtrA-mediated E-cadherin cleavage is

involved in rapid crossing of the epithelial barrier by C. jejuni via the paracellular route to reach basolateral surfaces. The results of this study advocate HtrA as a promising novel anti-infective drug target. The HtrA inhibitors identified serve as tool compounds for further studies on the HtrA-mediated pathogenicity of enterobacterial pathogens.

Infection Epidemiology and Population Genetics

MSP01

Comparison of methodological approaches for detection of major rifampin and isoniazid resistance conferring mutations in *Mycobacterium tuberculosis*

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Introduction: Emergence of multidrug-resistant *Mycobacterium tuberculosis* strains and their global dissemination necessitate development, evaluation and comparison of the rapid molecular tests that target genetic determinants of bacterial drug resistance. A wide range of such methods is available at present and the choice of those most appropriate is among the pertinent task of the National Tuberculosis Control Program.
Objectives: to compare different molecular methods for detection of the major rifampin (RIF) and isoniazid (INH) resistance conferring mutations in *M. tuberculosis* strains inBulgaria.
Materials and Methods: The study panel included 16 multi-drug resistant (MDR) and 20 susceptible *M. tuberculosis* clinical isolates. They were selected among 133 previously characterized strains (Valcheva et al., 2008) to represent the most prevalent mutations in Bulgaria i.e., *rpoB531* TCG>TTG *katG315* AGC>ACC, and *inhA* -15C>T. The drug susceptibility testing was performed by the proportion method.
Results: The results obtained by the previously validated reverse line blot hybridization (*rpoB351* and *inhA* -15) and PCR-RFLP (*katG315*) were considered as reference and were used for evaluation of the two real-time PCR (RT-PCR) methods: RT-PCR using FAM and JOE labeled TaqMan probes, and multiplex allele specific (MAS) RT-PCR kit. All methods correctly detected *rpoB531* mutations, clearly discriminating wild type and mutant alleles. Reverse hybridization method differentiated between *inhA* -15C/T alleles but not *katG315* alleles. Instead PCR-RFLP using *Msp*I digestion permitted to detect *katG315* AGC>ACC mutation. RT-PCR using classical labeled probes did not discriminate between wild type and mutant alleles of *katG315* AGC/ACC and *inhA* -15C/T alleles while use MAS RT-PCR successfully differentiated these alleles.
Conclusion: We conclude that MAS RT-PCR method best performed with all gene targets. Compared to reverse hybridization, this method, even if more expensive, provides faster and easier to use format for clear-cut detection of RIFand INH resistance-associated mutations in *M. tuberculosis* clinical isolates.

MSP02

Prevalence of PVL-positive *Staphylococcus aureus* in Bavaria - a retrospective survey

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Panton-Valentine Leukocidin (PVL) is a potent virulence factor associated with certain strains of *Staphylococcus* (*S.*) *aureus.* PVL can cause severe invasive infections like recurrent necrotic lesions or necrotic pneumonia, which can lead to death. Toxin production is known to occur in strains from clinical settings as well as in strains from the community. Pathogenicity and treatment can be complicated by distinct antibiotic resistance, however, outbreaks in both settings, with (MRSA) and without (MSSA) antibiotic resistance have been described. *S. aureus* is also known to persist in animals and has been isolated from food sources. However, a zoonotic potential or the frequency of PVL-positive strains among food isolates is unclear. We retrospectively screened 1306 *S. aureus* isolates collected and characterized during a Bavarian antibiotic resistance study in 2005/2006 for PVL potential. Screening was performed by targeting the PVL-marker genes *lukS* and *lukF* by an RT-PCR approach. PVL-positive isolates were characterized by PFGE-analysis and *spa*-typing and relatedness was calculated by UPGMA and BURP algorithms, respectively.
Prevalence rates for PVL-positive *S .aureus* were 3.9 % (26/661) for human isolates, 0.2 % (1/562) for veterinarian isolates and 0 % (0/83) for food isolates. The PVL prevalence rate of 3.9 % among human isolates did not differ for MSSA (21/535) or MRSA (5/126) strains. Genotyping by PFGE and *spa*-typing revealed two congruent clonal clusters of *spa*-CC159 (n = 10) and *spa*-CC310 (n = 5) which could be allocated to the clonal linages ST121 and ST2, respectively.
In summary, the results highlight the importance of the PVL virulence factor in human *S. aureus* strains. In contrast, PVL-positive strains can be neglected as a zoonotic reagent or in food sources. For both, human MRSA and MSSA, the determined prevalence rates for PVL were higher in Bavaria than rates estimated for Germany in the same timeframe. A lack of routine PVL-diagnostic will be discussed as a possible reason for underestimating true prevalence rates.

MSP03

Characterization of a novel thermostable nuclease (Nuc) homolog in a highly divergent *Staphylococcus aureus*-subclade recovered from non-human primates and bats in Africa

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Introduction: A highly divergent *Staphylococcus aureus* subclade based on concatenated sequences from multilocus sequence typing was recently detected in non-human primates and bats. Thermostable nucleases-encoding genes (*nuc*) have been used as targets for species identification of certain staphylococcal species, such as *S. aureus* and *S. intermedius*. Here, we aimed to characterize the sequence and structure of the thermostable nuclease (Nuc) of isolates of this divergent *S. aureus* subclade
Materials and Methods: Isolates belonging to the divergent *S. aureus* subclade were found in non-human primates and bats from Gabon (n=16), Nigeria (n=2), Côte d'Ivoire (n=11) and DR Congo (n=6). These isolates were compared with the *S. aureus* reference strains. Isolates were tested on DNAse agar to screen for the presence of nuclease activity. The *nuc* gene sequences were used to construct a neighbour-joining tree.
The deduced amino-acid sequences were aligned and the 3D structure was predicted using ESyPred3D. Nuc activities were spectrophotometrically measured by DNA digestion after heat treatment at 100°C for 20 min.
Results: The *nuc* gene was not detectable in the divergent subclade using published species-specific primers by Brakstad et al. (1992). However, nuclease activity due to a DNA digestion zone on DNAse agar was phenotypically shown. The suspected *nuc* gene region was sequenced by newly developed primers targeting conserved flanking regions. The *nuc* sequences of the divergent subclade and reference strains where separated in two groups in a neigbor-joining tree. Direct comparison of Nuc amino acid sequence between isolates of the divergent subclade and a *S. aureus* reference strain (N315) revealed a 78.1-80.4% Identity (92.4-94.1% similarity). The predicted 3D structures were also highly similar (Figure 1). Isolates of the divergent subclade and the reference strain (ATCC25923) showed similar thermostable nuclease activities mirrored by an increase of digested DNA (in absorbance units [AU]) after 10 min by 4.5±1.1 AU and 4.5±1.0 AU, respectively.
Conclusion: The divergent *S. aureus* subclade harbors a thermostable nuclease, which is highly divergent from Nuc of *S. aureus* reference strains on a genetic level. This explains a non-detection of the *nuc* gene using the standard *nuc* PCR by Brakstad et al. (1992). However, the deduced amino-acid sequence and tertiary structure are similar corresponding to a similar nuclease activity.
Since Nuc has been considered as an important virulence factor for infection (*i. e.* for invasion and immune evasion), our findings outline the pathogenic potential of this highly divergent *S. aureus*-subclade.

Figure 1

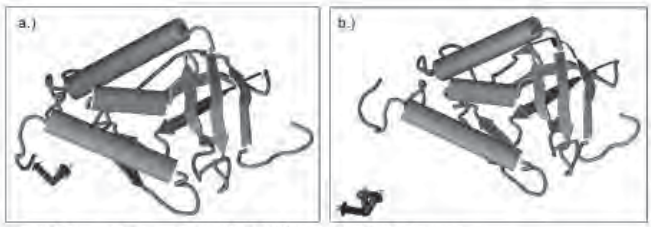

Figure: Predicted tertiary structure of the thermostable nuclease of monkey-associated *S. aureus* subclade (a.) and *S. aureus* ATCC49230 (b.). The 3D structure was predicted using ESyPred3D.

MSP04

Evidence of recombinational impact on evolutionary pattern of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* have a record of developing resistance rapidly to antibiotics, and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen. Since *Staphylococcus aureus* has showed great adaptability towards antibiotic selective pressure, by renovating its genome. Many of the basic biology questions about the population biology of *Staphylococcus aureus* and a better understanding of the origins and spread of MRSA clones could be answered if they could characterize unambiguously.
Materials and Methods: The issue was addressed in this study by differentiate 412 *Staphylococcus aureus* strains using multilocus sequence typing, recovered from tertiary care hospitals of the region.
Results: The isolates were uniformly disseminated between major and minor clonal complexes, signifying no noteworthy distinction in their tendency to cause illness. Examination of the sequence data suggests that most of the de novo nonsynonymous mutations are deleterious and hence eliminate earlier than permanent in the species.
Conclusion: In *S. aureus*, it might be one of the plausible mechanisms that most of the polymorphisms within clonal complexes have been eliminated in rare adaptive genotypes. Therefore, sporadically give rise to new clonal complexes as, among 21 new STs only two ST2556 and ST2402 were the resultant of new polymorphism.

MSP05

Rapid identification of enteroaggregative *E. coli* (EAEC)

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Objectives: Enteroaggregative *E. coli* (EAEC) are diarrheagenic pathogens responsible for both outbreaks and sporadic disease. The epidemiology of certain EAEC lineages in Europe remains unclear and their pathogenicity and distribution is possibly underestimated due to time consuming diagnostic methods unsuitable for routine testing. We established a database for future rapid characterization of EAEC clones by their *gnd* genotye. The *gnd* locus has been described to correspond to O serotype in other *E. coli* pathovars
Materials and Methods: For all *E. coli* strains included in this study, the presence of the *aggR* gene as a marker for EAEC was confirmed by PCR. In the first part of the study, 66 EAEC isolates of two strain collections from two different regions of Germany were typed using Multi locus sequence typing (MLST). A 643 bp region of the *gnd* locus was amplified for the establishment of the database. In a second part of the study, *gnd* sequences of 74 clinical EAEC strains isolated from patients with diarrhea were determined to be compared to sequences of the database.
Results: The 66 EAEC strains of the first part of the study were assigned to 19 different sequence types (ST) by MLST and 24 different *gnd* types (GTs), confirming the heterogeneity of EAEC in Germany. PCR for the *gnd* locus was negative in three out of 66 isolates, making them untypable by this method. Some MLST STs only included strains of the same GT, while other STs displayed a variety of GTs. Two potential local outbreaks were retrospectively identified, underlining the importance of a tool for rapid characterization of EAEC. The suspected outbreak strains were linked with regard to time and location of isolation and showed identical MLST STs and the same set of virulence factors by further PCR testing. These strains also displayed identical GTs. In contrast, EAEC of the same MLST ST that were otherwise unrelated fell into multiple GTs. In the second part of the study, 57/ 74 strains (77%) were successfully mapped to the database by GT. Of the remaining 17 strains, 3 were untypable, 3 showed an identical new GT and 11 had independent unknown GTs. *Gnd* typing was faster and more cost-effective compared with MLST as only a single gene locus had to be sequenced.
Conclusion *Gnd* typing is a rapid, reliable and robust method to identify EAEC clones. By the continuous addition of new sequences our database will greatly facilitate the detection of future EAEC outbreaks.
Supported by Deutsches Zentrum für Infektionsforschung (DZIF)

MSP06

Prevalence of intestinal parasite, Shigella and Salmonella species among diarrheal children in Jimma health center, Jimma southwest Ethiopia:a cross sectional study

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Introduction: Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries including Ethiopia. Globally, intestinal parasite, Shigella and Salmonella species remain major contributors to acute enteric infections. The study was aimed at determining the frequency of intestinal parasite, Shigella and Salmonella species identified from diarrheic children at Jimma Health Centre, Jimma south west Ethiopia.
Materials and Methods: A health institution based cross sectional study was conducted from March to November 2012. A structured questionnaire was used for collection of data on socio- demographic characteristics. Parasite and bacteria identification as well as susceptibility testing was done using standard parasitological and bacteriological procedures.
Results: A total of 260 diarrheal children were included in the study. A total of 129 (49.6%) samples were positive for intestinal parasite, Shigella and Salmonella species. Of these, 107 (41.1%), 6 (2.3%) and 16 (6.2%) samples were positive for intestinal parasite, Shigella and Salmonella species respectively. The dominant isolated parasite was G. lamblia with prevalence of 13.5% followed by A. lumbricoides (11.5%). The least identified parasites were Schistosoma mansoni and Taenia species accounting 0.4% each. Multiple parasitic infections were observed in 19 (7.3%) patients. Shigella species showed hundred percent resistances to ampicillin, amoxacillin, and cotrimoxazole.
All Salmonella isolates were resistant against amoxicillin. All Shigella and Salmonella species were susceptible to ceftriaxone, ciprofloxacin and gentamycin.
Conclusion: The presence of reasonably high amount of intestinal parasite and Salmonella and Shigella species that are drug resistance to the commonly prescribed drugs is a treat to the children and community at large. Therefore, measures including health education, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and antimicrobial surveillance is crucial.

MSP07

Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia

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Introduction: *S.* Concord in Ethiopia. The objective of this study was to determine the aetiology of febrile and diarrhoeic illness in Ethiopian children focussing on *Salmonella*.
Methodology: Paediatric patients (n = 1,225) presenting with diarrhoea or fever from the paediatric outpatient department of Tikur Anbessa University Hospital, Addis Ababa (n = 825), and Jimma University Hospital, South West Ethiopia (n = 400), were investigated for pathogens from January to August 2006.
Results: Parasites were detected in 337 cases, *Salmonella* in 65, and *Shigella* in 61. Serotyping of *Salmonella* (including 48 stored isolates) demonstrated the dominance of *S.* Concord: *S.* Concord (85), *S.* Typhimurium (7), *S.* Paratyphi B (2), *S.* Haifa (1), *S.* Typhi (2), *S.* Enteritidis (4), *S.* Butantan (2), *S.* Infantis (1), *S.* Pomona (1), *Salmonella* group M (28:y:-) (1), and *S.* Oskarshamn (1). Six isolates in serogroups B and D were untypeable. Of 81 *S.* Concord isolates, 30% were invasive, most (86.5%) were positive for ESBL production by E-test and 70% were multiply resistant to trimethoprim-sulphamethaxole, ceftriaxone, chloramphenicol and gentamicin, of which over one quarter (27%) also showed reduced susceptibility to ciprofloxacin.
Conclusion: Multi-drug resistant *S.* Concord was the major cause of salmonellosis in two regions of Ethiopia. The strain isolated was highly invasive, highly antibiotic-resistant, and represents a threat to heath care globally.

J Infect Dev Ctries 2011; 5(1):023-033.

MSP08

Methicillin-Resistant Staphylococcus Aureus in the Hospital Sector in Germany 2008-2011

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Introduction: Nosocomial infections are an increasing risk worldwide including in German hospitals. Methicillin-resistant Staphylococcus aureus (MRSA) infections are difficult to treat due to the resistance of the bacteria. As the S. aureus germ developed multi-resistance, the effectiveness of various classes of antibiotics is limited. Due to MRSA actions e.g. better infection control, improved hand washing of hospital staff or screening of patients a minimization could be reached (BMG, 2013). Currently, there are different sample-based MRSA-surveillance-systems providing an overview of the occurrence and distribution of MRSA (Chaberny et al., 2007).
Materials and Methods: Based on the German official hospital statistics 2008-2011 an analysis of secondary data was conducted. Encompassing about 17 million cases, the database includes all hospital patients in Germany i.e. also all MRSA cases. In addition, a dataset containing aggregate data at the regional-level and the state-level was compiled. The analysis was carried out using descriptive as well as inductive statistical methods including multilevel analysis and logistic regression.
Results: The prevalence of MRSA increased by 46% from 2008 (91,896 cases) to 2011 (134,129 cases). Men account for 56% and women for 44% of MRSA cases with an average age of 72 and 78 years, respectively. The median period of hospitalization decreased by three days from 2008 (15 days) to 2011 (12 days). The results of the multilevel analysis reveal that a higher number of beds per hospital, a higher share of elderly people, and a higher prevalence of antibiotics are associated with a higher share of MRSA in the region. The unemployment rate and the share of private hospitals are negatively related to the region's MRSA rate. Moreover, a rise in the prevalence of antibiotics is found to have a stronger positive effect on the share of MRSA in regions with a higher share of elderly people.
Discussion: Beside hospital hygiene and prevalence of antibiotics, there are other important factors influencing the occurrence of MRSA. Thus the regions' social, demographic, and health care structures are found to determine the distribution of MRSA. In addition there is evidence for a negative association between MRSA rates and the regions' share of private hospitals.

Chaberny I, Sohr D, Ruden H, Gastmeier P. 2007. Development of a Surveillance System for Methicillin-Resistant Staphylococcus aureus in German Hospitals. Infect Control Hosp Epidemiol. 28(4):446-52. BMG (Bundesministerium für Gesundheit). 2013. Bisherige Maßnahmen des Bundes zur Verbesserung der Infektionshygiene und zur Verhütung und Bekämpfung resistenter Krankheitserreger.URL:http://www.bmg.bund.de/praevention/krankenhaus nfektionen/aenderung-des-infektionsschutzgesetzes.html

MSP09

Detection of *Mycoplasma genitalium* in infertile and fertile women by PCR in Kurdistan, Sanandaj, Iran

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Object: The association between genital Mycoplasma infections and infertility has been reported in numerous studies. *Mycoplasma genitalium* the smallest known for a self-replicating microorganism-is associated with the genitourinary tract infections, tubal factor infertility, and neonatal morbidity and mortality. The aim of this study was to determine the prevalence of *M. genitaliumin* infertile and fertile women in in Kurdistan University of Medical Sciences Sanandaj, Iran.
Materials and Methods: In this study, 104 infertile women who attended for infertility Clinic-affiliated Besat hospital in Sanandaj (Kurdistan, Iran) from February to May 2013were selected as case group, and 104 fertile women with at least one child who referred to non-infertility clinics in the same time were included as control group. Dacron Swabs were used for sampling from endocervical canal of women. Cervical swabs were transported to laboratory in 5mL of Phosphate Buffered Saline (PBS) medium and were frozen at -20ºC until examination. DNA was extracted from samples using DNA extraction kit and subjected to Polymerase Chain Reaction (PCR) using *M. genitalium* specific primers. Statistical analysis was performed using the statistical package for the social sciences (SPSS) software.
Results: The age ranging for both groups was 14-40(average age 31.63 years old for fertile group and 29.16 years old for infertile patients).In fertile group 10 cases (9.6 %) were positive for *M. genitalium* while infertile group, *M. genitalium* was detected from 2 patients (1.98 %).
Conclusion: The prevalence of *M. genitalium* in the genital tract of infertile women in present study was fewer than other studies, while in the fertile group; the prevalence was higher than other reports. To better understand the association between *M. genitalium* and infertility in women, more epidemiological studies should be sought.

MSP10

The Saarland Nursing Home Multi-drug Resistant Organism (MDRO) Prevalence Study - First Data from the MRSAar Regional Network

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Introduction: Colonization and infection by multi-drug resistant organisms (MDRO) is a major, publicly recognized health concern, and unrecognized MDRO carrier status is thought to contribute to MDRO transmission between chronic and acute care institutions. Hence, the Saarland regional network for prevention and control of MDRO (MRSAarNet) has set out to determine the burden of MDRO throughout the Saarland health care institutions, and upon conclusion of the acute care hospital admission prevalence study (Herrmann et al., PLOS ONE, 2013), and of a dialysis center study, we here report the first results of the Saarland nursing home prevalence study.
Materials and Methods: Culture-based screening was performed with nasopharyngeal swabs for methicillin-resistant S. aureus (MRSA), and with rectal swabs for vancomycin-resistant enterococci and multidrug-resistant enterobacteriaceae (MRE), using selective media. This evaluation was accompanied by an ascertainment of risk factors using a standardized questionnaire.
Results: So far, of the 136 registered nursing homes in the State of Saarland, 64 (47%) participated in this ongoing study. Of 2705 residents screened, 134 residents (prevalence, 5.0/100 residents) were found to be colonized with

MRSA. Fecal swab specimen were found to be positive in 1.0/100 for vancomycin resistant enterococci (VRE), and 14,0/100 for enterobacteriaceae resistant to 3 antimicrobial compound classes (3MRGN) as defined by the Robert-Koch-Institute (RKI). Of these 3MRGN isolates, 81% were found to be *E. coli*, 16% were *Klebsiella* sp., and 2% belonged to the *E. cloacae* complex.
Discussion: The MRSA prevalence in nursing homes can be related to the findings of our acute care hospital admission prevalence study which indicated 2.2/100 MRSA admission patients positive for MRSA as well as an age pattern and risk factors thought to be frequent in the nursing home setting. These first data on the nursing home resident MDRO prevalence now strongly corroborate these findings. Further analysis upon study conclusion will allow to tailor strategies for prevention and control of MDRO, and particularly of MRSA, according to Saarland regional MDRO risk factor and distribution patterns.

MSP11

Prevalence of F5 fimbriae in *Escherichia coli* isolated from diarrheic and healthy calves since 1976- a meta-analytical approach.

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Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is one of the most common causes of diarrhea in calves. The adhesion of ETEC to intestinal epithelial cells is also mediated by F5 fimbriae. This fact was used for vaccine development and F5 fimbriae are one of the first antigens successfully applied in many studies to immunize dams, thereby providing a passive protection to neonatal calves. Here, we conducted a meta-analytical analysis on the occurrence of F5 fimbriae in *Escherichia coli* isolated from diseased and healthy calves during the last four decades to validate F5 as the appropriate vaccine antigen.
Materials and Methods: The PubMed database was searched for studies published before 31 of December 2013 with the following phrases: "*Escherichia coli* calves", "*Escherichia coli* calves virulence genes", "*Escherichia coli* calf" and "*Escherichia coli* calf virulence genes". A manual revision was conducted on all gathered publications and first selection based on title and/or abstract was done. From 2,552 citations identified via PubMed Search, 42 articles were identified as suitable for further analyses. Data extracted from these studies were stored in relational database created specifically for this research and analyzed with R software.
Results: In this study we found 42 publications suitable for further work, with 8,086 isolates from 21 countries investigated for presence of F5 fimbriae. 30 articles contained information about 4,983 isolates from diarrheic calves, 15 papers covered information about 2,424 isolates from healthy calves. The average of F5 fimbriae-positive strains isolated from diseased calves was 4.87 times higher than from healthy calves. Our study showed strong negative correlation (correlation coefficient: -0.479, p-value: 2.82×10^{-4}) between proportion of F5 fimbriae-positive isolates and the year in which research was performed for the whole population of examined animals. A more specific analysis showed that mentioned correlation is very significant in the group of diarrheic calves (correlation coefficient: -0.5432, p-value: 1.59×10^{-3}). No significant correlation was observed in the group of healthy calves (correlation coefficient: -0.4236, p-value: 0.116).
Conclusions: With the use of this meta-analysis we detected a changing prevalence of F5 fimbriae over time. A decreased prevalence of F5 fimbriae-positive strains isolated from diarrheic calves indicates a significant impact of vaccination against this antigen introduced in the past three decades. It also raises a need for search of new eligible antigens, which can improve protection of calves against pathogenic *Escherichia coli.* Systematic review of previously published data can accelerate and enhance this process.

MSP12

An automated analysis pipeline for whole genome MLST data using BioNumerics®

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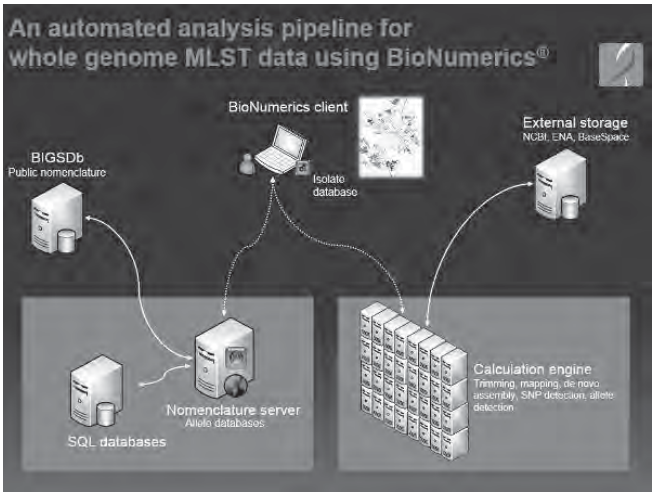
Objectives: For many years, Sanger sequencing based multilocus sequence typing (MLST) has been one of the standards in bacterial typing, characterizing isolates by looking at set of housekeeping genes (loci). As next-generation sequencing is increasingly replacing Sanger sequencing, conventional MLST can be extended to whole genome MLST (wgMLST), incorporating many more loci and thus providing higher resolution.
In this poster we present an easy to use high-throughput wgMLST data processing pipeline, built on the BioNumerics® software platform. A plethora of follow up tools is readily available to build gene-based evolutionary trees.
Materials and Methods: A wgMLST scheme is based on a set of reference sequences, from which all coding regions are extracted and used to create a set of discernible loci. This defines, for each locus, a set of variants and, where possible, *in silico* primers for validation purposes. The locus scheme, the alleles and their annotations are centralized in one nomenclature server. This server provides an automated curation system to manage and validate new alleles, ensures consistent internal naming, and remains synchronized with public nomenclature servers such as BIGsDB. Annotation of alleles, loci, sequence types and typing schemes are used in customized sample reporting.
Moving to sample processing, an assembly-free allele calling algorithm determines locus presence and detects allelic variants. This computationally inexpensive method is exhaustive for multi-copy loci, and provides an accurate idea of which loci are missing. The assembly-free called alleles are subsequently verified by *de novo*-based allele calling. To ensure ample calculation power, the assembly-free allele calling can be performed on the BioNumerics® calculation engine, which can be deployed locally or in the cloud. This calculation environment also offers trimming, mapping and *de novo* assembly algorithms. To enlarge the analysis space at hand, data from public or private repositories such as NCBI, ENA or BaseSpace can be used directly in the analysis.
Once allele assignments are complete, an extensive set of follow-up analysis tools becomes available within BioNumerics®. Starting from the wgMLST results, a plethora of (sub-)typing schemes can be defined, compared and analyzed easily (MLST, extended MLST, ribosomal MLST, core MLST, virulence genes, antibiotic resistance genes…)
The wgMLST analysis strategy will be illustrated by the analysis of surveillance data for *Listeria monocytogenes*.
Conclusion: Whole genome MLST provides a robust and high resolution picture of bacteria compared to traditional MLST, thereby spanning all the loci over the whole genome. The BioNumerics® 7.5 software (Applied Maths NV) offers a powerful platform where wgMLST schemes can be defined, automatically curated, analyzed and compared with historical data such as MLST or PFGE.

Figure 1



MSP13

PCR characterization of the *mec* element and identification of SCC*mec* structural types and variants in methicillin-resistant *Staphylococcus aureus* clinical strains isolated in Romania

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Introduction: The staphylococcal chromosomal cassette SCC*mec* element, which carries the determinant for "broad-spectrum" beta-lactam resistance in staphylococci, is a critical epidemiological marker for the characterization a discrimination of MRSA clones, through the identification of the structural types of this large and heterologous *genetic* element. The aim of the study was the genetic analysis of the *mec* element and the identification of the SCC*mec* types of MRSA strains among hospitalized patients from Romania.
Materials and Methods: The 39 MRSA analyzed strains were isolated during 2012 from different clinical samples (secretions, exudates, blood cultures) in patients hospitalized in the intensive care units of one hospital from Bucharest, the capital city of Romania. The suspected isolates were identified as *S. aureus* by using mannitol salt agar and soluble coagulase tests, as well as the automatik Vitek II system. Susceptibility to oxacillin and cefoxitin by disc diffusion were used for the detection of MRSA isolates. Multiplex PCR assay was performed for the detection and classification of currently described SCCmec types.
Results: The genetic characterization of the *mec* element using 13 pairs of primers targeting for the encoding regions, as well as for uncoding or open reading frames with unknown function of the SSCmec types I to VI allowed the typing of 50% from the analyzed strains. 17% of the typed strains harbored the SCC*mec* type III, as confirmed by the presence of *mecA* and *mecI* genes, as well as of some specific sequences located at the level of *J3* and *J1* regions. These strains also beared the *ccrC* gene (specific for *SCCmec* V) and the *dcs* region (characteristic to SCC*mec* types I, II, IV şi VI), demostrating the great mobility and instability of these genetic elements. One of the analyzed strains harbored concomitantly the *ccrB2* (specific for SCC*mec* II and IV) and *ccrC* genes, indicating the possible presence of SCC_{Hg} linked to SCC*mec* III. Four strains exhibited an uncomplete SCC*mec* III element, lacking the *mecI* gene and 11 strains exhibited SCC*mec* type IVa (harboring *mecA*, *ccrB2* and *dcs*). Nine isolates were *mecA*-negative, although bearing other elements specific for SCC*mec* (*dcs*, *mecI*, *ccrC, J*). In this case the MRSA phenotype could be assigned to many factors, such as: b- lactamase overproduction, methicillinase expression, aquisition of additional PBPs, different from PBP2a, the presence of SCV (***S**mall **C**olony **V**ariants*) with increased tolerance to beta-lactam antibiotics.
Conclusion. The analyzed de *S. aureus* strains harbor a heterogenous SCC*mec* structure, the majority being included in the SCC*mec* III and IV types, the last one characteristic to communitary aquired MRSA strains, supporting the current opinions highlighting the replacement of hospital associated-MRSA by CA-MRSA clones in clinical settings.
Keywords: CA-MRSA, structural SCC*mec* types, epidemiological marker SCC*mec*

MSP14

Genomic diversity of KPC-2/3 and OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in Germany

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Objective: Carbapenem-resistant *Klebsiella pneumoniae* are dreaded nosocomial pathogens. As a basis for future Next Generation Sequencing (NGS)-based outbreak analyses and the elucidation of dynamics of epidemic strain types, we analysed the genomic diversity of carbapenem-resistant *K. pneumoniae* circulating in German hospitals. Fifty *K. pneumoniae* isolates from both outbreaks and single cases which had been collected from hospital patients in different regions in Germany between 2008 and 2013, and which produced either KPC-2, KPC-3 or OXA-48 were included in this analysis.
Materials and Methods: All isolates were characterised previously by antimicrobial susceptibility testing as well as PCR and sequencing of different beta-lactamase genes. Bacterial DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN). The Nextera XT DNA Sample Preparation Kit was used to prepare the libraries. Whole genome sequencing was performed using the Illumina technology (MiSeq). Reads were mapped (BWA) onto a reference genome sequence (CP006923.1) and a maximum likelihood tree was constructed based on single nucleotide polymorphisms (SNPs).
Results: Whole genome sequence analysis revealed the presence of four major clusters, representing carbapenem-resistant *K. pneumoniae* multilocus sequence types (ST) known to be distributed internationally: KPC-3-producing ST 512, KPC-2-producing ST 258, OXA-48-producing ST 101 and OXA-48-producing ST 147. Genomic diversity within these four groups was extremely low, reflecting the clonal spread of few carbapenem-resistant *K. pneumoniae* strains in Germany.
Conclusion: Our baseline genomic sequence dataset will enable rapid identification and phylogenetic clustering of emerging strains, including those causing future outbreaks.

MSP15

Prevalence of Shiga toxin-producing, enterohemorrhagic and enteropathogenic *Escherichia coli*- a systematic review.

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Introduction: Calves are considered to be an important reservoir of Shiga toxin-producing, enterohemorrhagic and enteropathogenic *Escherichia coli* (STEC, EHEC, EPEC) and approximately 75% of EHEC outbreaks are linked to the consumption of contaminated bovine-derived products. The role of these pathotypes as calves pathogens is not elucidated. Fatal STEC infections in cattle were reported, despite cattle lack vascular endothelium with expression of globotriaosylceramide (Gb3), which is the receptor for Stx cellular internalization. A systematic review was performed to investigate the prevalence of aforementioned pathotypes in samples isolated from healthy and diarrheic calves.
Materials and Methods: The following phrases: "*Escherichia coli* calves", "*Escherichia coli* calves virulence genes", "*Escherichia coli* calf" and "*Escherichia coli* calf virulence genes" was searched in PubMed database. Obtained results were limited to studies published before 31 of December 2013 and revised manually basing on title and/or abstract. A total of 66 articles were identified as suitable for further analyses from 2552 citations selected by the Pubmed search. Data extracted from these studies were stored in a relational database created specifically for this research and analyzed with R software.
Results: A total number of 7066 STEC, 6151 EHEC and 5875 EPEC isolates was found in 59, 48 and 43 publications, respectively. The prevalence of EHEC and STEC significantly decreased over time, but prevalence of EPEC remained stable (STEC: correlation coefficient -0.45, $p<0.00005$; EHEC: correlation coefficient -0.38, $p<0.005$; EPEC: correlation coefficient -0.29, $p=0.019$). The chi-square test of independency showed that only EPEC appeared regardless of animals' health status (p-value: 0.31). EHEC and STEC were more prevalent in healthy compared to diarrheic calves ($p<10^{-10}$) and STEC and EHEC were isolated 1,5 times more frequently from healthy than from diarrheic calves.
Conclusions: Our research proves that three studied *E. coli* pathotypes play a small and uneven role in the etiology of the calf diarrhea. It also indicates a major role of calves as a reservoir of analyzed pathotypes. An unexpected observed decrease of EHEC and STEC pathotypes' frequency requires further investigations.

Antimicrobial Resistence and Drugs, Infection Prevention

PRP01
Bicarbonate Enhances the *In vitro* Antibiotic Activity of Kanamycin in Enteropathogenic *Escherichia coli*

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Abstract has been withdrawn.

PRP02
Mortality and length of hospital stay due to *Clostridium difficile* infections often overestimated? - A systematic review of attributable outcomes of CDI

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Introduction: Calculating the burden of healthcare-associated infections bears the risk of overestimation by not properly addressing the comorbidity of the affected population. We performed a systematic review to evaluate attributable mortality and attributable length of stay in hospital (LOS) as a basis for estimating the burden of *Clostridium difficile* infection (CDI).
Materials and Methods: Two authors independently searched for articles published since the year 2000 in Medline and EMBASE. Data regarding risk differences (RD) comparing patients with CDI and without CDI were extracted for the outcome mortality and for LOS. Inclusion criteria were among others an adjustment for comorbidities or severity of infection and consideration of the time-dependent bias.
Results: The search revealed 2227 articles, 136 of which were retrieved as full text. For the outcomes mortality and LOS we selected 4 and 6 studies, respectively. All of the 8 studies in total were cohort studies, 6 of 8 studies had a matched design. The attributable mortality was 0-11% whereas the attributable LOS in hospital ranged between 0-8 days. A statistically significant increase in mortality or LOS in CDI patients was reported only in 2 and 3 studies, respectively. One additional study that fulfilled the inclusion criteria showed a markedly higher attributable mortality and LOS (16.7% and 10.7 days). It was conducted in Canada during the epidemic caused by the hypervirulent *C. difficile* ribotpye 027 strain.
Discussion: Only half of the selected studies reported a significant attributable mortality and LOS of CDI. Therefore the attributable outcomes reported here differ from the common perception of CDI as a disease with substantial complications. Furthermore the distribution of ribotypes associated with an increased mortality and LOS may influence the burden of disease for CDI. Further research on risk differences is needed to estimate the real burden of CDI.

PRP03
Hydrolytic Detoxification of Albicidin.

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Introduction: *Xanthomonas albilineans* is a plant pathogen, which causes the leaf scald disease in sugar cane plant. It has been found that the major phytotoxin of X. albilineans is a small compound termed albicidin. Moreover, albicidin shows remarkable antibiotic activity at nanomolar range to various Gram-positive and Gram-negative bacteria.[1-3] Several bacterial resistance mechanisms against albicidin have already been described. One of the most potent is catalyzed by the *Pantoea dispersa* enzyme AlbD, which had been classified as an esterase and discussed as a possible approach to disease control.[4-6] AlbD hydrolytically detoxifies albicidin and attenuates thereby the pathogenicity of *X. albilineans*, yet the exact mechanism remained unknown due to the lack of the molecular structure of albicidin.
Materials and Methods: heterologous expression and purification of the resistance protein AlbD, site directed mutagenesis, *in vitro*-hydrolysis assay, HPLC-ESI-MS analytics
Results: Isolation of albicidin, gave us the opportunity to reinvestigate the molecular interaction of AlbD with albicidin. *In vitro*-assays showed that AlbD heterologously expressed in *E. coli* cleaves albicidin at an amide bond which leads to a complete loss of antimicrobial activity. Using truncated and modified substrates in hydrolysis assays with AlbD allowed for characterisation of the minimal binding motif around the cleavage site of AlbD. The core binding and recognition motif is assigned to a tripeptide moiety of albicidin. We could also show that different kinds of positively or negatively charged groups at different positions in derivatives can promote or abolish the cleavage reaction. By generation of gene inactivation mutants of AlbD, the functionality of the catalytic triad could be proven. Additionally, two conserved proline residues in the sequence of AlbD were investigated for their impact on albicidin hydrolysis, and indeed results showed their influence on the catalytic reaction.
Conclusion: We recommend the reclassification of AlbD as an endopeptidase, catalyzing the cleavage of albicidin and thereby diminishing the antimicrobial activity of albicidin. Furthermore, we obtained insights into the mechanistic principles of the hydrolysis. Future work will be

dedicated to solve the crystal structure of AlbD. Profound knowledge of the 3-dimensional protein character and further ligand-binding calculations, using the information we obtained earlier, will help to understand the exact mode of albicidin detoxification.

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PRP04

Non-thermal plasma as an effective method for the decontamination of surfaces *in vitro* and in vivo.

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Introduction: Non-thermal plasma (NTP) is an ionized gas with an ambient temperature. Its effects on biological objects are due to a combined action of charged particles, metastables, radicals and UV photons. The purpose of this study was to test the individual susceptibility of pathogenic bacteria to NTP, to measure the effectiveness of plasma treatment against bacteria in biofilms, on wound surfaces and in teeth root channels.
Materials and Methods: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterococcus faecium, Escherichia coli* were plated on agar and treated by argon plasma for 2 and 5 min, the amount of survived was counting in 18-24h. Bacterial biofilms were grown on glasses during 24-72h, then treated with plasma for 2 and 5 min and dyed with Life/Dead kit. A mouse model of infected *S. aureus* wounds was used to assess the efficiency of NTP treatment. For the investigation of effects onto bacteria in root channels the *Staphylococcus epidermidis* isolated from pulpitis was used. The biofilms were grown into root channels for 24h and treated with special device 'plasma jet' for 30, 60, 180, 240s.
Results: Gram-negative bacteria were more susceptible to NTP than Gram-positive ones: there were no survivors among the initial 10^5c.f.u. after 5 min treatment. The susceptibility of Gram-positive bacteria was species- and strain-specific.
5-min treatment of biofilms was bactericidal, the same time treatment of infected wounds reduced bacterial load to 10-100 fold and increased the wound healing in the first 3 days.
240 s treatment of bacteria in root channels by plasma jet caused 100% death of 10^9 bacteria.
Conclusion: NTP shows high bactericidal effect *in vitro* and *in vivo*.

PRP05

Comparison of EN standards used for evaluation of disinfectants and a fluorescence-based assay targeting tuberculocidal efficacy

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Introduction: Tuberculosis is one of the world's great public health threat, and in order to avoid device-related transmission of mycobacterial pathogens a reliable assessment of tuberculocidal or mycobactericidal efficacy of disinfectants is indispensable. Thus disinfectants are usually being tested in a suspension test (phase 2 step 1, EN 14348[1]) followed by a carrier test (phase 2 step 2, EN 14563[2]). However, these assays take at least 3 weeks because of the slow growth of the test organism *Mycobacterium terrae*. Recently, a rapid test system using fluorescent *M. terrae* for the evaluation of the tuberculocidal efficacy of disinfectants in the carrier test EN 14563 was published[3]. Results were obtained in a significantly shorter time than previously possible. Thus, the aim of this study was to compare the European standard system with the fluorescence assay and to validate the rapid test system including particularly the quantitative suspension test.
Materials and Methods: Quantitative suspension tests and quantitative carrier tests were carried out according to EN 14348 and EN 14563, respectively. Quantitative carrier tests and subsequent GFP-based determination of germicidal efficacy were carried out as described previously[3]. As a test germicide, a commercially available peracetic-acid formulation was used (gigasept® PAA concentrate; Schülke & Mayr GmbH, Germany).
Results: Testing of the germicide in the quantitative suspension test revealed tuberculocidal efficacy as required by EN 14348 (≥ 4 lg) at a concentration of 2% after 5 min. contact time under clean and dirty conditions. Accordingly, data from the quantitative carrier test EN 14563 under clean and dirty conditions demonstrated tuberculocidal efficacy (≥ 4 lg) at a concentration of 2% at 5 min. contact time. Data obtained from the fluorescence assay demonstrated a germicide concentration of 1% not to be effective within 5 min., which was indicated by an increasing fluorescence subsequent to the germicide treatment. However, at a concentration of 2% no increase in fluorescence was detectable after 5 min. contact time under clean and dirty conditions, indicating no live mycobacteria following this treatment.
Discussion: This data demonstrates, that identical in-use parameters for tuberculocidal efficacy were obtained by either applying the quantitative suspension and quantitative carrier tests according to EN 14348 and EN 14563 or by use of the GFP-based rapid test system. Thus, the rapid test system using fluorescent *M. terrae* compares well with the established European standard test procedure including both, phase 2 step 1 and phase 2 step 2 tests and provides a rapid and sensitive tool to test germicides for relevant in use-concentrations and contact times.

Literature:
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2. BS EN 14563: 2008-12
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PRP06

Two is better then one: The lantibiotic (auto) immunity system NisI and NisFEG

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Nisin, a 3.4 kDa antimicrobial peptide, is the most prominent member of the lantibiotic family, produced by some *Lactococcus lactis* strains. Nisin can inhibit cell growth and penetrates the target Gram-positive bacterial membrane by binding to Lipid II, an essential cell wall synthesis precursor. The assembled nisin-Lipid II complex forms pores in the target membrane. To gain immunity against its own-produced nisin, *Lactococcus lactis* is expressing two immunity protein systems, NisI and NisFEG. Here, we show that the NisI expressing strain displays an IC_{50} of 73 ± 10 nM, an 8-10-fold increase when compared to the non expressing sensitive strain. The cells expressing full-length NisI stop growing when the nisin concentration is raised above 70 nM rather than being killed. NisI is inhibiting nisin mediated pore formation, even at nisin concentrations up to 1 mM. This effect is induced by the C-terminus of NisI that protects Lipid II since its deletion showed pore formation again. The expression of NisI in combination with externally added nisin mediates an elongation of the chain length of the *Lactococcus lactis* cocci. While the sensitive strain cell-chains consist of mainly two cells, the NisI expressing cells display a length of up to 20 cells. The producing strain is also expressing an ABC transporter called NisFEG, which expels nisin from the membrane resulting in 6-8-fold more nisin that is needed to kill the cells. This immunity is mediated by the hydrolysis of ATP as shown by an ATP deficient mutant. The C-terminus of nisin is important for the interaction between nisin and NisFEG since deletion of the last six amino acids as well as of the last ring lowered the activity of NisFEG. Both results shed light on the mechanism of (auto)immunity of lantibiotic producer strains, and their surviving at high levels of their own lantibiotic in the habitat.

PRP07

Unusual development of penicillin-resistance in the *S. pneumoniae* clone Hungary^{19A}-6

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Introduction: The *Streptococcus pneumoniae* clone Hungary^{19A}-6 consists of high-level penicillin resistant strains of higher genetic diversity compared to other clones (1). Interestingly, one member, strain Hu15, is penicillin sensitive. The comparison and analysis of know penicillin resistance determinants documented that it differs from the resistant strain Hu17 only by genes encoding PBP2x, PBP2b and PBP1a. Moreover, *murM* and *ciaH*

were identical in both strains but differed from those of the sensitive laboratory strain *S. pneumoniae* R6.

Materials and Methods: We used this unique situation to study the contribution of resistance determinants in isogenic backgrounds, using either the laboratory strain R6, or penicillin sensitive strain Hu15 in transformation experiments as recipient and cefotaxime as the selective antibiotic. The obtained mutant strains were analysed and susceptibility to cefotaxime and oxacillin were tested. Furthermore, PBP profiles of the constructed strains were examined. The activity of CiaR-controlled promoters in *S. pneumoniae* R6 and Hu15 strains harbouring *ciaH232* allele were determined by measuring β-galactosidase activity (2).

Results and Conclusion: The $MurM_{Hu17}$ gene confers only very little increase in resistance of the R6 strain. As expected, successive transformation of $pbp2x_{Hu17}$ and $pbp1a_{Hu17}$ resulted in stepwise increasing cefotaxime resistance. Transformation of $ciaH_{Hu17}$ (*ciaH*232) resulted in further increase of cefotaxime resistance in the R6$pbp2x_{Hu17}pbp1a_{Hu17}$ background, suggesting that the CiaRH system is upregulated, and this could be verified using *lacZ*-reporter assays of genes controlled by CiaR.

There are three main results: (1) presence of the altered $murM_{Hu17}$ allele does not depend on resistance mediated via PBP genes; (2) Hu15-transformants expressed higher resistance level compared to R6-transformants, i.e. resistance mediated by the same resistance determinants depends on the genomic environment; (3) the presence of $ciaH_{Hu17}$ results in hyperactivation of the CiaR in response to $pbp1a_{Hu17}$ in R6$pbp2x_{Hu17}pbp1a_{Hu17}$.

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PRP08

Novel amino acid substitutions in the thymidine kinase and DNA polymerase of clinical HSV-1 strains isolated between 1973 and 2014

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Introduction: The standard treatment for herpes simplex viruses type 1 (HSV-1) infections has primarily been the administration of acyclovir (ACV). In case of clinical resistance, genotypic resistance testing by amplification and sequencing of the thymidine kinase (TK) and DNA polymerase (pol) genes has been recommended. However, the TK and DNA pol genes of HSV-1 show a significant variability. Despite numerous studies, the interpretation of genotypic results still has to be verified by phenotypic resistance testing.

Objective: The objective of this study was to examine the genotype of the TK and DNA pol in 297 clinical HSV-1 strains, which were isolated between 1973 and 2013. When novel or unclear mutations were found, the resistance phenotype to ACV or foscarnet (FOS) was analyzed.

Materials and Methods: 297 routinely collected HSV-1 strains from 280 patients were included to examine the TK and DNA pol genes for amino acid (aa) changes or frameshifts. After amplification and sequencing of DNA fragments, the sequence data were compared to that of the HSV-1 reference strain 17 of GeneBank (NC_001806). Ninty-four strains which contained unknown or novel non-synonymous mutations were subsequently tested for their sensitivity to ACV and FOS applying tetrazolium reduction assay.

Results: In the TK, 31 novel natural polymorphisms and three unknown ACV resistance- associated mutations could be detected. For the DNA pol gene, 73 novel polymorphisms, two deletions without premature stop codons and two aa changes related to ACV resistance were analyzed. Four aa changes in both the TK and DNA pol, which have not been described so far, could not be characterized as natural polymorphism or related to resistance.

Conclusion: In this study, a high number of aa changes associated with the natural polymorphism of TK and DNA pol genes could be detected in ACV-sensitive HSV-1 isolates. The data will significantly improve the interpretation of genotypic findings in the context of ACV resistance testing of HSV-1.

PRP09

Assembly of the nisin maturation complex *in vitro*

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Nisin is an antimicrobial peptide secreted by *Lactococcus Lactis*. It has been used in food industry for the past five decades [1] and contains unique post-translational modifications [2]. The dehydratase NisB, catalyses the dehydration of serines and threonines in the core peptide to di-dehydroalanine and di-dehydrobutyrine, respectively [3,4]. The typical (methyl)lanthionine rings are installed in a regio- and stereospecific manner by the cyclase, NisC [3]. The fully modified prenisin is then exported by the ABC-Transporter NisT [5], and the protease NisP cleaves off the leader; thereby producing biologically active nisin [6]. It is proposed that the dehydration and the cyclation are catalysed by a complex of the modification enzymes, NisB and NisC, in an alternating fashion [7]. It was shown that these enzymes were also able to process therapeutic peptides *in vivo* [8]. For this reason a better understanding of these modification complexes would enable *in vitro* modifications of peptides containing serine and threonine residues. Upon combining multi-angle-light-scattering with size exclusion chromatography (MALS-SEC), we are able to demonstrate the first *in vitro* assembly of the nisin modification complex. This modification complex is only formed in the presence of a prenisin. However, complex formation is dependent on the modification state of the core peptide and on the bound leader peptide. Additionally, the unmodified and the dehydrated prenisin form the complex in the same manner. Interestingly, the modified prenisin, having all (methyl)-lanthionine rings installed, does not form any complex. The highly conserved -FNLD- box in the leader peptide [9] was mutated to AAAA. No complex formation was seen indicating that, this box is essential for the complex formation. Finally, MALS-SEC provided the first quantitative data about the stoichiometry of the nisin modification complex comprising a dimer of NisB, a monomer of NisC and a single molecule of prenisin.

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PRP10

Evaluation of the Anti-*Listeria* potentials of some plant-derived triterpenes.

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Introduction: *Listeria monocytogenes* is the causative agent of the fatal disease listeriosis. The pathogenicity of some *Listeria* species such as *Listeria grayi* and *Listeria ivanovii* especially to immune-compromised individuals has been reported. The increase in antibiotic resistance and the intracellular life cycle of the pathogenic *Listeria* limits the treatment options and this calls for research into alternative treatments. In this study 2 triterpenes (3β-hydroxylanosta-9,24-dien-24-oic acid and methyl-3β-hydroxylanosta-9,24-dien-21-oate) isolated from the plant *Protorhus longifolia* and an acetyl derivative of ursolic acid (3β-acetylursolic acid) isolated from the plant *Mimusops caffra* were assessed for their anti-*Listerial* activities *in-vitro*.

Materials and Methods: The broth microdilution assay was used to determine the MIC's of the triterpenes against *Listeria monocytogenes* ATCC- 19115 and environmental isolates; *Listeria grayi* (LAL 3) and *Listeria ivanovii* (LDB 11).The chequer board method was used to determine the interactions between the triterpenes and conventional antibiotics. The lactate dehydrogenase assay was used to determine membrane damaging potentials of the triterpenes.

Results: The MIC values were found to range from 0.185 to 3.33 mg/ml. Interactions involving 3β-hydroxylanosta-9,24-dien-24-oic acid were mainly additive with ampicillin and synergistic with neomycin, gentamicin and penicillin G. Interactions involving methyl-3β-hydroxylanosta-9,24-dien-21-oate were mainly antagonistic with ampicillin, indifferent with neomycin, ranging from synergistic to indifference with gentamicin and synergistic with penicillin G. Interactions involving 3β-acetylursolic acid were mainly indifferent with ampicillin, synergistic with neomycin and gentamicin while ranging between synergistic and additive with penicillin G. Low quantities of cytosolic lactate dehydrogenase were released from the cells treated with 4× MIC concentration of the triterpenes in comparison to the cells treated with 3%Triton X-100 as a positive control.
Conclusions: This study shows the potential that these plant triterpenes have in listeriosis chemotherapy especially as shown by the favourable interactions they had with penicillin G, the antibiotic of choice in listeriosis treatment.

PRP11
Characterization of a putative cryptic RND efflux pump in *Acinetobacter baumannii*

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Objectives: The multidrug resistance phenotype of *Acinetobacter baumannii* is often associated with overexpression of resistance-nodulation-division (RND) efflux pumps that have broad substrate specificity. Three RND pumps have been described in this species; AdeABC, AdeIJK, and AdeFGH. Through data mining the published *A. baumannii* genomes we have identified an RND pump (*A. baumannii* ATCC 17978 locus_tag A1S_2660) showing 49% identity to the RND pump AcrB of *E. coli*. The objective of this study was to identify expression and substrates of this pump.
Materials and Methods: To determine the natural expression of A1S_2660, we created a reporter system using the predicted promoter region and its first 21 nucleotides, and cloned this in frame to a promoterless β-galactosidase gene residing on shuttle plasmid pJN17/04. Our reporter assay was performed on solid media supplemented with X-gal; gene expression was detected by blue colonies. *A. baumannii* transformants were tested with the following compounds incorporated in gradient agar plates or by disc diffusion; β-lactams, chloramphenicol, fluoroquinolones, gentamicin, sulbactam, benzalkonium chloride, sodium chloride, sodium deoxycholate, sodium salicyclate, SDS, ethanol and organic dyes. For overexpression of the pump, the complete A1S_2660 gene was cloned in frame to an IPTG-inducible lac-promoter encoded on shuttle plasmid pBA03/05. To identify substrates of the pump, antibiotic susceptibility to β-lactams, chloramphenicol, erythromycin, fluoroquinolones, gentamicin and was performed by disc diffusion supplemented with 0.005, 0.01, 0.05 and 1 mM IPTG. Expression of the pump was determined by qRT-PCR.
Results: The pump was not constitutively expressed or induced when grown in the presence of the majority of tested compounds as visualised by white colonies on agar. However, chloramphenicol, ertapenem, imipenem, meropenem, sulbactam, ethanol and sodium chloride induced expression of the pump which was considered low as the colour change was visible after ≥ 24 hours incubation. Construction of the expression plasmid was confirmed by sequencing. The presence of IPTG in solid media had no impact on bacterial growth with the parent strain or out-of-frame construct. However, IPTG concentrations of ≥0.01 mM led to markedly reduced bacterial growth suggesting overexpression of the pump was toxic. With the addition of 0.005 mM IPTG no impact on the growth was observed. qRT-PCR showed expression of the pump, however no change in antimicrobial susceptibility to any of the tested antibiotics was seen using 0.005 mM IPTG.
Conclusion: While expression of the cryptic A1S_2660 RND efflux pump was weakly induced in the presence of various unrelated compounds, including carbapenems, its overexpression did not affect the antimicrobial susceptibility of *A. baumannii* ATCC 17978. Compounds other than the tested antibiotics may be substrates of this pump.

PRP12
A *Staphylococcus aureus* mutant highly tolerant to daptomycin

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Bacterial persister cells represent a subpopulation of phenotypic variants within an isogenic bacterial culture. They can be distinguished by a reversible drug-tolerant phenotype commonly attributed to decreased physiologic activity and growth (1). Challenge of planktonic stationary *S. aureus* HG003 cultures by the lipopeptide antibiotic daptomycin usually leads to the isolation of less than 0,1% persister cells tolerant to the drug (2). Knowledge on the phenotypes and genetic factors governing high-persister (*hip*) mutants producing elevated levels of drug-tolerant cells is to date mostly confined to Gram negative bacteria, and especially to *Escherichia coli*. We hence aimed to select an *S. aureus hip* strain to investigate associated genetic mechanisms and population dynamics. To this end, *S. aureus* HG003 cultures grown in rich medium *in vitro* were treated with 100-fold MIC of daptomycin for up to ten consecutive cycles, in which 7.5 h of drug exposure each was followed by 16 h cultivation in non-selective medium. Persister levels were quantified in retrospect by calculating colony-forming units of samples spotted on non-selective agar. After the sixth cycle a mutant strain (designated HG003D6) was obtained producing more than three orders of magnitude more cells, tolerant to daptomycin among the population in stationary growth phase. Notably, MIC and MBC values of HG003D6 were unaffected, ruling out daptomycin-resistance. Time-dependent monitoring of persister production of HG003D6 revealed a rapid boost in persister levels at the onset of stationary growth phase. Illumina HiSeq2500 sequencing of HG003D6 unraveled that a single nucleotide polymorphism within a putative membrane associated transporter, resulting in an amino acid exchange is responsible for the observed phenotype. The mechanism leading to the observed phenotype needs to be clarified.

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PRP13
Adaptation of translation upon peptide deformylase inhibition by actinonin in *Bacillus subtilis*

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In bacteria, translation is initiated with N-terminally formylated methionine (fMet) as starting amino acid. From newly synthesized proteins the formyl residue is removed by peptide deformylase (PDF) followed by removal of Met from some proteins by Met amino peptidase (MAP). Both, removal of the formyl and Met can be crucial for correct protein folding and function. PDF has been identified as promising antibiotic target, complementing a whole suite of targets in bacterial translation. Though actinonin is a potent inhibitor of PDF, it affects growth of the gram-positive model organism *B. subtilis* only moderately. To analyze the adaptation mechanisms mediating growth in presence of actinonin, a global quantitative LC-MS-based approach was used, which allowed monitoring of the protein's N-termini as well as protein regulation. As expected, we found that PDF inhibition leads to accumulation of proteins retaining fMet at the N-terminus.
Actinonin-resistant *B. subtilis* mutants have previously been shown to be impaired in a pathway supplying the formyl donor for $tRNA^{fMet}$ synthesis [1]. These mutants are thought to bypass fMet-dependent translation initiation by initiating with Met. We found enzymes of the formyl donor supply pathway to be down-regulated in response to actinonin suggesting that this bypass is part of the metabolic adaptation. Inhibition of the tetrahydrofolate (THF) biosynthesis pathway by trimethoprim also limits the supply of the formyl donor and was previously shown to lead to fMet-independent translation initiation in *B. subtilis* [2]. In agreement with the results of Arnold [2], the growth defect caused by trimethoprim can be annulled by supplying thiamine, methionine, glycine, adenosine and guanosine, which are products of THF-dependent biosynthesis pathways. In contrast we show that the moderate growth defect of actinonin cannot be cured by addition of these supplements. This underlines that for *B. subtilis*, in contrast to other bacteria, adaptation by fMet-independent translation initiation is not associated with a considerable fitness cost. Interestingly, we observed that proteins normally processed by MAP accumulate as proteoforms with non-formylated Met upon actinonin treatment. MAP being unable to remove the N-terminal Met from its regular substrates provides evidence that PDF inhibition by actinonin leads to secondary effects hindering further N-terminal protein processing.

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PRP14

In search for novel antimicrobial substances for *Chlamydia trachomatis*

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The gram-negative obligate intracellular human pathogen *Chlamydia trachomatis* is one of the main causative agents of sexually transmitted disease and infection of the upper inner eyelid (trachoma). *Chlamydia* feature a biphasic developmental cycle, in which the elementary bodies (EBs) initiate the infection, develop within an inclusion into the replicative form called reticulate bodies (RBs) that differentiate back to EBs at the end of the developmental cycle. RBs can also convert to a persistent non-replicative state. Re-infection and persistence are the reason for prolonged therapy of chlamydial infections and make *C. trachomatis* an interesting model organism in search for novel classes of antimicrobial agents. We have tested different types of compounds, which included sterol- and quinone-based substances and the derivatives of pipecolic acid. Several of these compounds showed strong inhibitory effect on the growth of chlamydial inclusions. Where tested, the inhibitory effect of the substances on the ability of *C. trachomatis* to create viable progeny was even more pronounced, whereas general cytotoxicity for the host cell was not observed. The future perspectives include identifying the targets of these substances and increasing their efficiency through chemical modifications.

PRP15

tRNA-dependent aminoacylation of phosphatidylglycerol: Three-dimensional structure of two bacterial resistance proteins

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Bacteria adapt to changing environmental conditions by controlling the properties of their membranes. The aminoacylation of the polar head group of the phospholipid phosphatidylglycerol (PG) is catalyzed by Lys-tRNA^{Lys}-dependent lysyl-phosphatidylglycerol synthase (L-PGS) or by Ala-tRNA^{Ala}-dependent alanyl-phosphatidylglycerol synthase (A-PGS) and enables microorganisms to cope with substances that are harmful for the integrity of the cell membrane. Therefore, aminoacyl-phosphatidylglycerol synthases (aa-PGS) from varying microorganisms function as virulence factors and thus have been also termed as ´multiple peptide resistance factors´ (MprFs). In the Gram-positive pathogen *Staphylococcus aureus* MprF/L-PGS mediates nonsusceptibility to human antimicrobial peptides [1]. For the opportunistic pathogen *Pseudomonas aeruginosa*, aminoacylation of PG was also found *e.g.* in response to acidic environmental conditions which also points towards a more general function in *P. aeruginosa* lipid homeostasis [2,3].
Aa-PGS proteins consist of a two-domain architecture: the C-terminal catalytic domain shows full enzymatic activity and the N-terminal transmembrane domain harbors a flippase activity which translocates the aminoacylated PG from the inner to the outer leaflet of the cytoplasmic membrane [4,5].
This work reveals the X-ray crystallographic structures of the catalytic domains of the tRNA-dependent A-PGS from *P. aeruginosa* and the L-PGS from *Bacillus licheniformis* in complex with the substrate analogue lysine amide at a resolution of 2.3 Å and 2.1 Å, respectively. Both proteins feature a central tunnel, which facilitates the binding of the polar aminoacyl-tRNA molecule and of the hydrophobic lipid substrate PG at opposite sites of the enzyme. The substrate analogue lysine amide is surrounded by a network of polar interactions formed by highly conserved amino acid residues. The catalytic relevance of these residues was confirmed in an extended mutagenesis study [5]. Furthermore, mutational studies of amino acid residues located at the bottleneck of the central tunnel reveal the potential binding of the PG substrate.
The three-dimensional structures of A-PGS and L-PGS in combination with biochemical experiments using a series of artificial aminoacyl-tRNA or lipid substrates allows for the molecular understanding of aa-PGS substrate recognition which is a basis for the future development of new antimicrobial compounds.

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PRP16

Aganocides-A New hope to Antibiotic Resistance and beyond

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The development of antibiotic has created a new world for antimicrobial therapy, but the current rise of antibiotic resistant ‘super bugs’ has put a big question mark on anti microbial research ?And compelled to re-look ,re-examine the longstanding approaches not only for prevention but also strategies for treatments. There is an urgent need to develop non antibiotic antimicrobial agents which must have potential to reduce dependence on antibiotics and provide a new front line of defense as well as effective treatment to a wide spectrum of infectious diseases. To hold such views together a new class of compounds have been identified as “Aganocides” which are non antibiotic in nature ,these novel pharmaceuticals has been develop based on molecules involvement in host defense and present with in white blood cells and to kill captured microorganisms. N-Halo moiety is the pharmacophore of Aganocides group. Currently perhaps there are no literature records of resistance to this class of molecules .N -halo moiety comprises of chloro, bromo and iodo constituents.
Aganocides have potential to replace antibiotics and antiseptics as first order therapy against bacteria, viruses and other pathogens in topical application. In general, antibiotics besides killing bacteria and viruses also damage or destroy human cells .The other line of antiseptic like iodine and alcohol containing solutions has only preventive role and are restricted in their use for infections on tissue surfaces and cavities. However Aganocides are much more effective than these agents and 400-500 times less toxic to animal tissues thus providing higher therapeutic index (the ratio of effective dose to toxic dose) translates directly to patient benefit subsequently higher or similar microbial kill rates. In general Aganocides kill on contact all bacteria, viruses, fungi and protozoa more rapidly in minutes taking very less time as antibiotics takes. On these lines N-Chloro taurine found in white blood cells has been recognized for treating infectious conjunctivitis, as a natural antiseptic as well as a novel topical antimicrobial agent. The late studies further introduce many others like N-bromo taurine, N, N dichloro taurine and many more. The conventional therapies for treatment of intra and extra cellular diseases has in existence for many years but several noticeable complication and complexity compelled to update the current strategies and to cope the new emerging challenges of this new millennium the answer lies in new era of Nanotechnology which is perhaps one of the most effective gift of science to mankind. The unique physical and chemical properties of nano particles, particularly their small size and high surface to volume ratio allow this technology to surpass barriers and to gain assess easily to bio-molecules and also to biological systems .In principle manipulation of nano size particles is possible and its size, shape and chemical parameters can be altered in order to facilitate molecular interaction more effectively such development may lead to design engineered vehicles to carry various therapeutic or diagnostic agents which may be potentially useful for medical application including targeted drug delivery, gene therapy ,cell labeling as well as development of new drug/pharmaceutical/medicine ,and such agents development through this new technology are designated with new term nano drug/Nano pharmaceuticals/Nano medicine..There are number of taurine containing nano drugs/medicine for immune-modulator to energy provider as well for human skin care. While Aganocides may provide an ideal therapy for surfaces and cavities throughout the body and could also participate in reducing the contact time, common pathogens have with antibiotics ,thus they provide less chance for bacteria to develop resistance ,can reverse antibiotics for effectively treating systemic infections their by potentiating their ability to save life with more efficiently. If these Aganocides can join “Umbrella of Nano Technology” perhaps it may constitute much desired and need of hour “our dream antimicrobial agents”.

PRP17

Heavy metal tolerance of *Staphylococcus aureus* isolates from patients treated with silver coated wound dressings

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Introduction: Silver coated wound dressings are a new useful tool to fight bacterial infections. The use of these is already established in a few ambulatories in Austria. *In vitro* studies with heavy metal surfaces showed good results but only by direct contact with the patch and with a dry metal surface. But the efficacy of the coatings seems to be less effective over a longer application time. One reason for the decrease of the antimicrobial effect could be an adaptation of the involved strains.
The aim of the study was to test heavy metal tolerance of *S.aureus* wound isolates that had contact to silver coated wound dressing in comparison to isolates that had not.
Materials and Methods: 40 *S.aureus* isolates collected from 2012 and 2013 were tested for their copper tolerance. Strains were analysed in a Bioscreen devise over a period of 72 hours. Optical density was determined every 5 minutes. Strains were incubated with copper concentrations up to 20 mM copper chloride.
Results: Some isolates of both groups showed high tolerance levels for copper chloride. But both groups had the same distribution in the low, medium and high tolerance group. Interestingly some of the high tolerance strains show a massive very early lag-phase over days.
Discussion: The investigation of 40 preliminary isolates showed interesting results in terms of long term survivors. But the comparison of the isolates has to be extended to allow a more valid data interpretation.

PRP18

Biosynthesis of silver nanoparticles by *Crocus sativa* bulbs and Antimicrobial effects on four common pathogens

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Introduction: In this study, silver nanoparticles were biosynthesized from saffron bulb extract and their antibacterial effects were examined on four common pathogens.
Materials and Methods: The production of silver nanoparticles via the UV-vis spectrum, TEM and XRD was confirmed once the color of solution in question changed to brown. Subsequently, the antibacterial effects of the aforementioned nanoparticles were tested on the pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* using the agar well diffusion method.
Results: The results showed nanoparticles that varied in size. Furthermore, their antimicrobial effects proved to be strong.
Conclusion/Discussion: It is hypothesized that the antimicrobial properties offered by the nanoparticles are due to their ability to change bacterial membrane permeability and cellular respiration.

PRP19

Investigation of the mechanism involved in tigecycline resistance in *Enterococcus* spp.

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Introduction: In November 2007 tigecycline (TGC), a tetracycline derivative, was approved in Germany. Tigecycline represents one of the last-line therapeutics to combat even multi-drug resistant bacterial pathogens. In January 2007, the first *Enterococcus faecium* TGC-resistant isolate was identified. Since then, the National Reference Centre for *Staphylococci* and *Enterococci* received 27 TGC-resistant *E. faecium* and *E. faecalis* isolates. Although the precise mechanism of how Enterococci become resistant to TGC remains to be determined, it has been demonstrated for *Staphylococcus aureus* that decreased TGC susceptibility is associated with up-regulation of certain efflux pumps. Thus, the aim of this study is to investigate the contribution of efflux pumps to the development of a TGC resistance phenotype in clinical isolates of *Enterococcus* spp.
Materials and Methods: The minimum inhibitory concentration (MIC) of TGC was determined by microbroth dilution (MD) and Etest®. Further, we compared the effect of six different efflux-pump inhibitors (EPI) on TGC resistance of selected *E. faecium* isolates. Moreover, high and low level TGC-resistant strains were analyzed regarding putative genome and transcriptome differences by means of whole genome sequencing and qRT-PCR.
Results: As analyzed by MD experiments, varying levels of resistance to TGC exist for the 27 strains investigated. From the selected EPIs, which inhibit different classes of efflux pumps, paroxetin decreased the MIC in 8 of 27 isolates. This suggests the involvement of an efflux system of the MATE or MFS type. Comparative genome analyses of three clonally identical strains, but showing different levels of TGC resistance, further support our hypothesis by revealing an MFS efflux pump as possible drug resistance protein. Subsequent qRT-PCR analyses showed a 6-fold up-regulation of the respective ORF in the "native" isolate compared to the isogenic TGC-sensitive strain. The effect was even more pronounced by comparing the TGC-sensitive to the isolate with a very high TGC MIC, hence demonstrating a 43-fold increase in transcription of the putative MFS pump.
Discussion: Our preliminary results indicate the involvement of both an MFS-and/or a MATE-pump in efflux of TGC from the enterococci cell cytoplasm. This most likely depends on the genetic prerequisite of the strain, as not all isolates were tested positive for this MFS exporter. Functional analyses of the respective MFS pump are part of current investigations, in order to elucidate its impact on mediating TGC resistance.

PRP20

Validation of the new real-time PCR kit Check-Direct CPE for the detection of KPC, NDM-1/VIM and OXA-48 in Enterobacteriaceae.

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Introduction: The emergence and dissemination of Carbapenemase-producing Gram-negative bacteria poses a serious impact on the health care system. Specific and reliable detection is crucial to prevent the spread of these organisms. According to this background we validated the new real-time PCR kit Check-Direct CPE (Check-Points, Netherlands) for the detection of KPC, NDM-1/VIM and OXA-48 in Enterobacteriaceae. The test can be applied on rectal and perianal specimens and on overnight grown bacterial colonies.
Materials and Methods: The Check-Direct CPE assay was validated for 28 reference strains of Enterobacteriacae. These strains were characterized by PCR, MALDI-TOF, phenotypic methods (VITEK2, E-test, Hodge-test) and sequencing. The panel included isolates with known carbapanemases (KPC,OXA-48, VIM and NDM-1), carbapenem-resistant isolates where resistance is mediated by combinations of ESBL/AmpC enzymes plus porin-loss and carbapenem-susceptible isolates. DNA was extracted from overnight colonies using crude DNA extraction method with a heating step at 98°C. Subsequently, the assay was validated with swabs spiked with reference strains (500-50000 CFU/ml) to assess the capability of the test for use with direct swab specimens (e.g. rectal swabs). DNA extraction was done with the Easy MAG system (bioMérieux). The Real-Time PCR was performed on the LightCycler 480 (Roche).
Results: 25 Enterobacteriaceae reference strains with characterized carbapenem-resistance mechanism (KPC n=6, NDM-1 n=5, OXA-48 n=11, VIM n=3) were tested with the Check Direct CPE-assay. All (100%) of the carbapenemase-positive strains were correctly detected with the assay. The characterized carbapenemase-negative strains also showed a correct-negative result. The LoD of the Check Direct assay was determined by using spiked swab samples and ranged from 19 to 37 CFU/ PCR for10 reference strains tested.
Conclusions: The Check-Direct CPE (Check-Points) for the detection of KPC, NDM/VIM and OXA 48 showed fast and reliable results when tested with characterized reference strains. Spiking experiment proved the capability of the test for the use on direct swabs specimens.

PRP21

ESBLs producing shiga toxigenic *Escherichia coli* (STEC) from diarrhoeic patients in India

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Introduction: Shiga toxin producing *Escherichia coli* (STEC) causes a spectrum of human sufferings like bloody diarrhea and even life threatening conditions such as hemolytic uremic syndrome (HUS). Although STEC are not considered as reservoir of ESBLs, recently a few reports have been published on the association of STEC isolates with ESBL genes.
Materials and Methods: *E. coli* were isolated from 180 non-replicating fecal samples from individual human patients with the history of diarrhea from different hospitals in Mizoram, India. Antimicrobial susceptibility test and confirmation for ESBLs production was done by DDST method. All the ESBLs producing isolates were serotyped for their O- serogroups. $bla_{CTX\text{-}M\text{-}1}$ and/or bla_{SHV} genes were detected by PCR assay. The transmission of antibiotic resistance genes was done by *in vitro* conjugation study. Multiplex PCR was done for detection of stx_1, stx_2, *eae* and *ehxA* genes. Isolates positive for ESBLs and STEC marker genes were characterized by the ERIC-PCR, REP-PCR and RAPD-PCR.
Results: A total of 333 *E. coli* isolates were recovered. Altogether, 41 isolates were confirmed as ESBLs producer, of which 29 belonged to 3 serogroups (O64, O89 and O91) and the remaining 12 were untypable. O64 (16/29) was the predominant serogroup. Altogether 36 (10.81%) and 5 (1.5%) isolates were found to be positive for $bla_{CTX\text{-}M\text{-}1}$ and bla_{SHV} ($bla_{SHV\text{-}12}$) gene, respectively. The resistance trait from any of the isolates could not be transferred to the recipient host by conjugation method. A total of 8 (2.40%) and 3 (0.90%) isolates were recorded as STEC and EPEC, respectively. Two (0.60%) isolates belonging to serogroup O64 and positive for $bla_{CTX\text{-}M\text{-}1}$ gene were also positive for stx_1 gene. Both the isolates revealed identical banding patterns by RAPD-PCR, REP-PCR and ERIC-PCR.
Conclusion and Discussion: The antimicrobial resistance among the enteric bacteria, especially the production of ESBL is rising worldwide, albeit to a lesser extent in STEC. This is probably the first information on association of serogroup O64 *E. coli* carrying both ESBLs and STEC genes. Serogroup O64 is not considered as potential human pathogen, but detection of such organisms with a combination of ESBLs and STEC marker genes is suggestive of higher propensity of this O serogroup in human and/or animal population. The future studies should be directed to find out the selective pressure, which may promote such combinations of virulent and resistant pathogens.

PRP22

Identification of new lantibiotic gene clusters in *Bacillus thuringiensis* DSM 2046 and the haloarchaeon *Haloferax mediterranei* DSM1411

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The rise of antimicrobial drug resistance has recently led the WHO to declare a postantibiotic era, in which even minor infections can become life threatening again, "a very real possibility" [1]. Our project focuses on the identification and characterization of novel antibiotic post-translationally modified peptides (**RiPPs**) by genomic data mining and heterologous expression. Up until now the biosynthesis of lanthionine containing antimicrobial peptides (lantibiotics) has only been described for bacteria. Using BLAST searches employing the characteristic lantibiotic modifying enzyme LanM we have found a putative lantibiotic biosynthesis gene cluster in *Haloferax mediterranei* DSM 1411, a member of the halophilic archaea. This organism produces an antimicrobial activity against another halophilic archaeon, *Halobacterium salinarum* DSM3754, when growing in proximity on an agar plate. Several Gram-positive halophilic bacteria species were not affected by this substance. The predicted gene cluster encodes for two class II lantibiotic modifying enzymes as well as proteins involved in producer self-protection and two possible prepeptides. The focus of our study lies on the correlation between the antimicrobial activity and the predicted gene cluster. Additionally we found a putative lantibiotic biosynthesis gene cluster in *Bacillus thuringiensis* DSM 2046. The predicted prepeptide contains a lipid II binding motif which is characteristic for class II lantibiotics. These lantibiotics inhibit cell wall biosynthesis by binding to the cell wall precursor lipid II. In order to determine whether this lipid II binding motif is essential for the antimicrobial activity of the novel lanthipeptide we have constructed two mutant peptides with a predicted higher and lower affinity for lipid II. We want to assess the antimicrobial activity of these altered peptides using heterologous expression of the lantibiotic modifying enzyme and the lantibiotic peptide in *E. coli.*

[1] WHO, 30.04.2014: Antimicrobial resistance: global report on surveillance 2014. ISBN 978 92 4 156474.

PRP23

Characterization of thymidine kinase and DNA polymerase genes in clinical herpes simplex virus type 2 isolates

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Introduction: The thymidine kinase (TK) of herpes simplex virus (HSV) is the main target for resistance to acyclovir (ACV). Furthermore, mutations in the DNA polymerase (DNA pol) can confer resistance against ACV and foscarnet (FOS). The impact of amino acid (aa) substitutions on enzyme activity and potential drug resistance is important for a successful antiviral therapy. However, only limited data are available especially for HSV type 2 (HSV-2).
Objective: In this study, the TK and DNA pol gene of 83 clinical HSV-2 isolates, which were collected routinely between 1973 and 2013, were examined.
Materials and Methods: Fragments of TK and DNA pol genes were amplified and sequenced. Sequence data were compared with that of the reference strain HG52 (GenBank Acc. No. X14112). Resistance phenotype against ACV and FOS was analyzed if novel, so far unclear or resistance-related mutations were found. In addition, phylogenetic analysis of the TK and DNA pol genes was carried out including sequences of the study and GenBank.
Results: Four novel TK aa changes and the substitution T131M, which was unclear to date, were characterized as natural polymorphisms. Furthermore, 17 unknown and the so far unclear substitution R628C within the DNA pol gene were analyzed as natural polymorphisms, too. Three isolates could be tested as resistant, whereby the origin of ACV resistance was unclear for one isolate. Additionally, one isolate showed a weak susceptibility to ACV and there was no FOS resistance. Phylogenetic analysis revealed a hierarchy of the TK mutations G39E, N78D and L140F. A mutation cluster could not be analyzed for the DNA pol of HSV-2.
Conclusion: The study supports once more the importance of the phenotypic adjustment of genotypic findings to enrich the knowledge about natural gene polymorphisms and resistance-associated mutations in HSV-2 strains to improve the interpretation of genotypic findings.

PRP24

Molecular Characterization of Antibiotic resistance of Helicobacter pylori clinical strains isolated from Jordanian patients with Gastrodeudenal diseases

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Introduction: Gastritis and peptic ulcer are considered major health problems worldwide. It has been reported that more than 80% of chronic active gastritis are due to the pathogenic bacterium *Helicobacter pylori* where persistent infection remains for decades. Successful treatment of *H. pylori* routinely requires the use of multiple agents with different mechanisms including compounds inhibiting acid secretion in conjunction with Antibiotics. However, recent data showed the emergence of resistant clinical strains particularly against metronidazole and clarithromycin. The aim of this study is to determine the prevalence of and the susceptibility of *H. pylori* isolates recovered from patients with chronic gastritis or peptic ulcer to several antimicrobial agents *in vitro*.
Materials and Methods: A prospective study has been conducting on Jordanian patients attended the gastrointestinal unit of the Jordan university hospital starting from May 2012 with gastroduodenal problems. Both Antral and corpus mucosal biopsies from the stomach of each patient were obtained, processed and tested by rapid urease test and cultured on selective media (Columbia blood Agar containing 7% laked horse blood and dent selective supplement). Presumptive *H. pylori* colonies were subsequently

confirmed by biochemical tests including catalase, oxidase and rapid urease tests in addition to the standard 16S rDNA PCR assay. The antimicrobial susceptibility testing was performed by standard agar diffusion methods according to CLSI. Subsequently, MICs were determined by E test and microbroth dilution methods.
Results: Among 72 symptomatic patients, 13 (23%) patients showed positive *H. pylori* infection by both rapid urease test and culture. The antibiotic susceptibility profile of 12 clinical strains were tested and showed that all of the isolates were sensitive to amoxicillin. Resistance to ciprofloxacin (MIC ≥ 4.0 ug/ml) and clarithromycin (MIC ≥ 2.0 ug/ml) were observed in 23% and 8% of the isolates respectively while 85% of the strains were resistant to metronidazole (MIC ≥ 8 ug/ml).
Conclusion: The present study showed that the prevalence of metronidazole resistance among clinical isolates of *H. pylori* is very high. Lower resistance to other antibiotics are reported. Concern should be taken into consideration when metronidazole is used for the treatment of *H. pylori* in our region.

PRP25
Susceptibility of NanA towards neuraminidase inhibitors is associated with its primary sequence and the binding mode

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Streptococcus pneumoniae is responsible for the majority of pneumonia cases. Spread of colonized pneumococci leads to diseases that range from a mild upper respiratory tract infection to severe and potentially life-threatening conditions. As one of the most conserved surface-associated proteins of *S. pneumoniae*, neuraminidase (NanA) catalyzes the removal of terminal sialic acid residues from various glycoconjugates of the eukaryotic cell surface to reveal receptors for adherence. It therefore promotes the bacterial colonization and biofilm formation. The predominance of NanA, corresponding to its essential roles in pathogenesis of pneumococcal strains, renders it an attractive target for therapeutic intervention of neuraminidase inhibitors (NAI). 15 *nanA* genes from Jena hospital and DSMZ pneumococcal isolates were amplified and sequenced. Based on the alignment of the deduced protein sequences, NanAs of various origins were divided into several clades. The major residue substitutions are located in three domains, namely, the carbohydrate binding module, the catalytic domain and the C-terminal anchor region. To associate these differences of NanAs with the property of the enzyme and their susceptibility towards NAI, domain(s) of three representative NanAs were expressed and purified as recombinant proteins from *E. coli*. Using a chemiluminescent-based assay, the susceptibility of these recombinant enzymes towards NAI was evaluated. The inhibition values obtained from the highly specific and competitive NAI oseltamivir clearly demonstrated that only the variations in the catalytic domain of NanA contributed to the significant difference in IC_{50}. Further, the inhibitory effects of two plant isolates (the diarylheptanoid katsumadain A and the isoprenylated flavone artocarpin, respectively) were compared towards differentially constructed recombinant NanAs. The IC_{50} values of these natural compounds presented another pattern than that of oseltamivir, which implies an alternative binding mode or an additional target for interaction apart from the catalytic center, *e.g.* the carbohydrate binding module of NanA.
Despite the highly conserved enzymatic activity of NanA, our phylogenetic analysis of the primary sequence of NanA revealed the evolutionary diversity of this enzyme. Moreover, this diverseness leads to the distinct susceptibility of the NanA towards different NAIs. Our findings therefore warrant further investigations to shed light on ligand-target interactions and putative correlations to their anti-pneumococcal profile.

PRP26
Investigation of antibiotic prescription in a veterinary teaching hospital: Results from a three-month retrospective survey

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The number of disease-causing bacteria resistant to one or more antimicrobial agents in human and veterinary medicine is rising constantly, especially with regard to potentially zoonotic pathogens. Whereas the inappropriate use of antimicrobials in veterinary medicine is assumed to substantially contribute to this alarming expansion, data concerning the common antibiotic prescription pattern in small animal medicine are so far missing. Here we present antibiotic prescription data obtained for all dogs and cats treated at the Small Animal Clinic of the FU Berlin within a three-month period in 2013. Individual patient parameters, medical history and initial diagnoses were recorded for a total number of 2,923 initial treatments. In case of antibiotic prescription, detailed information on the therapy (i.e. agent, route of administration, duration, change of agent) was registered. Based on the body site initially associated with the reason for visiting the clinic, all recorded treatments were categorized into 12 groups.
In total, 2,134 treatment cases of dogs [73%] and 789 of cats [27%] were analyzed. Regarding treatments of dogs, the three most frequently recorded presentation reasons were categorized as follows: diseases of the gastrointestinal tract (20%) skin disorders (18%) and diseases of the musculoskeletal system (16%). For treatments of cats, the diseases of the gastrointestinal tract (19%) were followed by systemic disease (14%) and skin disorders (12%). In 1,079 [37%] of all recorded treatments, the clinical conditions of the patient led to the prescription of at least one antimicrobial agent, including 817 antibiotic treatments of dogs [28%] and 262 cats [9%]. With regard to the 12 treatment groups, wounds were among the three most common reasons for antibiotic prescription (41 of 67 presentation; 61%), followed by diseases of the genitourinary system (124 of 233; 53%) and diseases of the respiratory system (77 of 162; 48%).

PRP27
Virtual screening and *in vitro* evaluation of inhibitors for different metallo-β-lactamases

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Introduction: Antibiotic resistance in bacterial pathogens is one of the major threats regarding human health. An alarming trend is the spread of metallo-β-lactamases (MBLs) among Gram-negative pathogens that transfer resistance against almost all β-lactams including carbapenems. The development of new anti-infective agents remains one of the most significant demands in modern medicine. To overcome MBL-mediated resistance, a combination of β-lactam and β-lactamase inhibitor, which protects the β-lactam antibiotic against the activity of the β-lactamase, is urgently needed. The identification of such MBL inhibitors is the focus of our recent work.
Materials and Methods: Virtual screening for inhibitors of different class B1 MBLs, i.e. NDM-1 (New Delhi metallo-β-lactamase 1), VIM-1 (Verona integron-encoded metallo-β-lactamase 1) and IMP-1 (Imipenemase 1), were performed by filtering different vendor libraries according to the Astex 'rule of 3', followed by consensus docking into the crystal structures of the targeted proteins using Molecular Operating Environment (MOE), Genetic Optimization for Ligand Docking (GOLD) and (Protein Ligand ANT System) PLANTS software suites. The top-scored fragments were manually selected and evaluated in a fluorescent assay system with purified recombinant protein. Possible binding was confirmed by STD-NMR.
Results: We present a computer-aided approach to screen for inhibitors of three different class B1 MBLs: NDM-1, VIM-1, and IMP-1. We were able to identify primary inhibitors of NDM-1, VIM-1, and IMP-1 by means of virtual screening of a fragment library with more than 200.000 compounds and molecular docking. We established a fluorescence based assay system with the purified proteins and proved the inhibitory activity of identified fragments.
Discussion: The growing prevalence of metallo-β-lactamase producing clinical isolates calls for the urgent development of new anti-infective agents. Pomising fragments can now serve as starting point for further development of potent MBL inhibitors by chemical modification of the evaluated fragments.

PRP28

Mode of action of carolacton against *Streptococcus pneumoniae*

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Streptococcus pneumoniae commonly inhabits the human naso-oropharynx as an asymptomatic commensal. Nevertheless, the bacterium can cause severe non-invasive (e.g. otitis media, sinusitis, bronchitis) and invasive (e.g. pneumonia, bacteremia, meningitis, pericarditis) diseases and is responsible for approx. 5 million deaths worldwide annually. An increased number of clinical isolates of *S. pneumoniae* present a wide range of antibiotic resistances. - Carolacton, a macrolidic ketocarbonic acid synthesized by the myxobacterium *Sorangium cellulosum*, disturbs cell division, resulting in formation of chains and elongated or bulging cells. This effect could be observed for all bacterial species tested so far. - The mode of action of carolacton has been studied in the oral pathogen *Streptococcus mutans*. Treatment with carolacton killed cells under conditions of cell envelope stress, such as in biofilms which develop localized very low pH. The eukaryotic-like serine/threonine kinase (eSTK) PknB and the cysteine metabolism regulator CysR were discovered to mediate the lethal effect of carolacton in *S. mutans*[1,2], but the relationship between those two and the molecular target of carolacton are unknown.
In this study, the mode of action of carolacton in *S. pneumoniae* and the unknown components involved in the cellular response to carolacton are investigated. The response to carolacton was studied in the highly virulent *S. pneumoniae* strain TIGR4 and the less virulent strain D39 in different media and under different growth conditions. A Δ*stkP* deletion mutant in the TIGR4 background showed severe deficiencies in growth, but remained slightly sensitive to carolacton.- In order to further investigate the mechanism of carolacton in *S. pneumoniae*, RNA sequencing will be used to analyse changes in the transcriptome of the wild-type, the Δ*stkP* mutant and the Δ*cysR* mutant at defined time points after carolacton treatment. A comparative analysis of the transcriptome changes in *S. mutans* and *S. pneumoniae* will provide new information on conserved cellular processes influenced by carolacton and may reveal novel putative cellular targets. - Identification of differentially expressed transcripts will then be used for the construction of fluorescence-based transcriptional reporter strains for single cell analysis. Moreover, a possible interaction of StkP and carolacton will be examined *in vitro* using Surface Plasmon Resonance (SPR) spectroscopy.

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PRP29

Frequency of detection of multi-resistant Pseudomonas aeruginosa in hospital plumbing units

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Introduction: Both water supply and waste water installations can be persistently contaminated with multi-resistant Pseudomonas aeruginosa (MR P.a.). This contamination might be the source of nosocomial spread of the pathogens. In this study, we screened individual rooms at a University Hospital for presence of MR P.a. after hosting a respective patient between 2007 and 2013.
Materials and Methods: Hand washing sink traps, shower traps and toilets were swabbed in 2013 according to a standard operating procedure. 21 wards from 8 departments were included. P.a. resistant towards ureidopenicillins, cephalosporins, carbapenems and gyrase inhibitors were isolated using selective agar plates. Presence of vim metallo-β-lactamases was tested by PCR.
Results: 47 rooms, which had hosted an MR P.a. positive patient between 2007 and 2013, were analyzed. As controls, 94 rooms were selected from corresponding wards without history of hosting of such patients (2 control room per test room). 9 of 47 test rooms were positive for MR P.a. and 9 of 94 control rooms. The difference in proportion was not significant. The time span between accommodation of a positive patient and sampling period was 5 to 37 months (median 21 months). Duration of patient was 5 to 68 days (median 14). 9 of 18 strains were vim-2 metallo-β-lactamase positive.
Conclusions: The results do not imply that recent or previous history of accommodation of an MR P.a. positive patient is correlated with persistent contamination in the plumbing unit. However, the analysis might have been hampered by a lack of data in the control group, since there was no screening policy. It is interesting that appr. 12% of rooms harbored MR P.a. in their waste water systems despite the fact that there was no ongoing outbreak and that water supply was free of P. aeruginosa. Further studies are needed to support the direct contribution of waste water systems to the endemic persistence of MR P.a. in a hospital. Nevertheless, the data suggest that policies are needed to prevent contamination of patients using plumbing units. Molecular typing is on the way to unravel the maximum duration of persistence. Comparative analysis in other hospitals should be conducted.

PRP30

Remarkable antimycobacterial activity of new diastereoisomeric ethambutol-like squaramides against $H_{37}Rv$ and MDR strains

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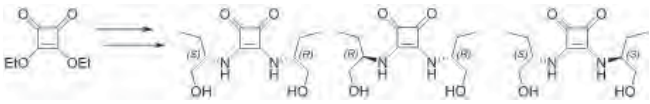
Introduction: The long current drug regiments, the emergency of drug resistant strains and HIV co-infection have resulted in resurgence in research efforts to address the urgent need of new anti-tuberculosis drugs. Ethambutol (EMB) is one of the frontline agents recommended by the WHO for the treatment of tuberculosis.
Objectives: To evaluate the influence of configuration of the molecules for *in vitro* antimycobacterial activity of a 3 novel 2-aminobutanol derived diastereoisomeric squaramides as near structural EMB analogues.
Methods: Susceptibility testing for new compounds was carried out by the recommended by WHO proportional method of Canetti on Lowenstein-Jensen medium. The critical concentrations for the compounds were 5, 2, 1, 0.2, 0.1 and 0.05 mg/ml.
Results and Conclusions: Using commercially available chemicals, 3 new 2-aminobutanol derivatives bearing squaric acid moiety have been easily synthesized in high yields and diastereoisomerically pure form. We focused on all possible isomers, inspired by early studies concerning antitubercular activity of EMB-diastereoisomers (Shepherd C *et. al.* 1966), as well as by our recent work in this topic (Dobrikov G. *et al.* 2012). The *in vitro* activity of the compounds against *Mycobacterium tuberculosis* ($H_{37}Rv$ and MDR strains) was evaluated. All compounds showed remarkable activity - up to 40 fold higher than EMB. It was found clear dependence between configurations of the molecules ant their activity against MDR-strain. These results can be considered an important starting point for further investigation of the newly synthesized compounds.

Figure 1



PRP31

ESBL- and AmpC-beta-lactamase-producing Enterobacteriaceae in imported reptiles

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Introduction: The global emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) and AmpC-β-lactamases is a major concern in human and veterinary medicine. To which extent reptiles imported from foreign countries maybe involved in the spread of these bacteria, i.e. due to asymptomatic colonization and transient shedding, is currently unknown.
Materials and Methods: Between July and December 2013 85 fecal samples as well as shedding remains from different reptile species imported from 16 non-European countries were taken during routine border controls at Frankfurt Airport. Samples were cultivated on 5%-blood agar, Gassner agar, McConkey agar with 1mg/L Cefotaxim and in liquid nutrient broth with 10µg Meropenem. Species differentiation was performed by MALDI-

TOF analysis. The combination disc method was used for detection of ESBL/AmpC phenotypes according to CLSI protocols. MIC-testing was performed by broth dilution using the Micronaut test kit. The determination of beta-lactamase-genes was done by PCR and sequence analyses, MLST analysis was performed according to the PubMLST protocol.

Results: Out of 85 samples, >470 Enterobacteriaceae isolates could be cultivated (thereof 85 *E. coli*, 56 *K. pneumoniae*, 59 *C. freundii* and 57 *Enterobacter* spp.). In 17 (20%) samples we identified 21 ESBL- and 11 AmpC-producing isolates originating from different reptile species and from various countries, such as Vietnam, Colombia, Uzbekistan, Guatemala, and the USA. A number of β-lactamase-genes, such as $bla_{\text{CTX-M-3,-14,-15,-27,-55,-65}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{CMY-2}}$, less often genes for bla_{DHA}, und bla_{EB}, were detected. All strains were susceptible to carbapenems. MLST revealed a high diversity among *E. coli* isolates and showed STs that frequently appear in ESBL-producing *E. coli* worldwide, such as ST10, ST38, ST131 and ST167, but also STs which have been rarely encountered in Europe.

Discussion: This study shows that reptiles frequently carry multidrug-resistant bacteria and that their transport may contribute to bacterial dissemination across borders, such as described for humans travelling over long distances. Beside ESBL-types pandemicly occuring in Central Europe, such as CTX-M-15, types rather uncommon for this geographic region were also imported, probably giving rise to their spread in a new geographic niche. Moreover, reptiles turned out to be a vector not only for the dissemination of ESBL-associated STs, such as the human pandemic clone ST131, but also for *E. coli* genotypes that so far haven´t been recognized in Europe. A better understanding of the epidemiological impact of „travelling" reptiles on the dissemination of multidrug-resistant bacteria may be desirable.

PRP32

Electrochemical biosensors of susceptibility of E.coli JM109 cells to antibiotics.

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Introduction: Infectious diseases are a serious problem of public health services. With broad usage in medical practice of antibiotics and the subsequent formation of resistance to them of microorganisms there was a necessity of an estimation of sensitivity of activators to the given preparations as reference point for a choice of antibiotic chemotherapy. Existing modern methods of antibiotic susceptibility, despite the doubtless advantages, don't allow quickly and to conduct qualitatively research on antibiotic resistance of microorganisms. To accomplish a sensitive monitoring, there still is a high need for sensitive, simple, rapid, cost-effective, label-free, field-ready and portable detection methods for the identification of microbial pathogens and their viability. All it testifies to necessity of creation of the new methodical approaches, allowing to judge degree of sensitivity of microbial cells to antibiotics in a shorter term after the research beginning. The aim of this work was the development of electrochemical biosensors for determining the sensitivity microorganism antimicrobial direct method using nanostructured electrodes.

Materials and Methods: Electrochemical measurements were carried out using a PGSTAT12 µAutolab. Electrochemical studies of E. coli JM 109 strain were done in 0.1 M potassium-phosphate buffer containing 0.05 M NaCl, pH 7.4 in volume=1 ml. A typical screen-printed electrode involved the working carbon/graphite (d=2 mm), the auxiliary graphite and the Ag/AgCl reference electrodes. Electrochemical activity of bacterial cells detected by methods of cyclic voltammetry(CV) and square-wave voltammetry(SWV). The following reagents were used: didodecyldimethylammonium bromide (DDAB) to modify the surface of working electrode, Cefepime , Amikacin- Vial, Ampicillin, Erythromycin.

Results: Electrochemical activity was registered of the microorganism. The reductive peak current of SWV found to be increased with the increasing cell number (Fig. 1). Electrochemical properties of E.coli JM109 strain were used for investigation of susceptibility of bacterial cells to antibiotics. DDAB/E.coli JM109 electrodes were incubated with cefepime (1.04 mM), ampicillin (1.43 mM), amikacin (0.852 mM), or with erythromicine (13.6 µM). Maximum cathodic peak current reduction for cefepime and amikacin was 92% and 81%, respectively for 5 h period of exposition to drugs. Ampicillin, a bactericide which inhibits cell growth by inhibiting the production of cell wall, give 95% of inhibition for 4 h of incubation. In the presence of erythromicine, cathodic peak current grows up to 225% during 5 h (Fig. 2). Erythromicine (13.6 µM) did not inhibits cell growth on electrode. The relative standard deviation (R.S.D.) of 7% was calculated for n=3.

Conclusion: The present study shows that the biosensor based on modified electrodes printed DDAB immobilized on the working electrode surface with biological material - E.coli JM109 can be used to determine the sensitivity of the microorganism to a number of antibiotics.

Figure 1

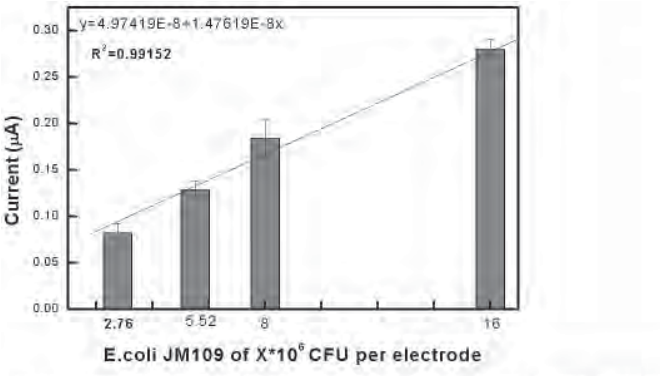

Fig.1 Plots of mean peak currents obtained from SPE of E.coli JM109 at different cell number of 2.76×106 to 16×106 CFU electrode. Calibration curve of E.coli JM109 ranging from 2.76×106 until 16×106 CFU per electrode. Error bars represent ±standard deviation of the mean(n=3)

Figure 2

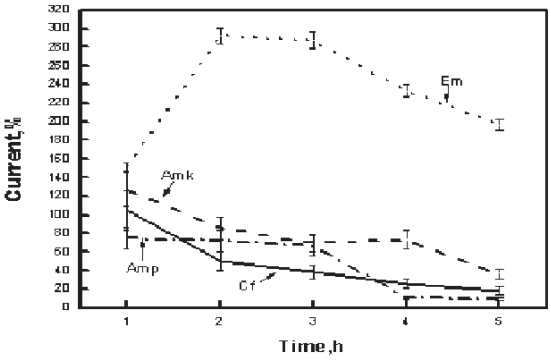

Fig.2 Electrochemical response of SPE/DDAB/E.coli JM109 to different antibiotics: Amp(1.432mM), Cf(1.04mM), Amk(0.852mM), Em(13.6µM). Data are the mean ±standard deviation of three different experiments.

PRP33

Bacterial response to small molecule inhibitors acting on trans-translation

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Antibiotic research becomes of increasing urgency in the light of development and spread of resistant pathogens especially in hospitals and resulting ineffectiveness of antibiotics currently in clinical use. Unfortunately, approval of structurally new antibiotics is decreasing and most substances in clinical trials are derivates of marketed antibiotics. As a result, many antibiotics have the same bacterial target, which in turn promotes the development of cross-resistances. Therefore, the exploitation of underexploited bacterial targets is highly desirable. Proteomic approaches are useful in elucidating antibacterial mechanisms [1], because bacteria react to environmental stimuli by adapting their proteome to meet the physiological challenges. In case of antibiotic stress, the induced proteins are often indicative of the damage caused by the antibiotic and reflect bacterial attempts of compensation of target inhibition. We have established a proteome profile library of *Bacillus subtilis* treated with different antibiotics [2]. Proteins significantly induced serve as marker proteins and represent a specific proteome profile for each antibiotic. This library allows a comparative analysis of the mode of action of novel antibiotics. Recently, a study investigated the process of *trans*-translation as a potential antibacterial target and found small molecule inhibitors of this pathway [3]. *trans*-translation is a ribosome rescue mechanism releasing ribosomes stalled at the 3' end of incomplete or damaged mRNAs. SmpB and tmRNA are the key elements in *trans*-translation. During *trans*-translation, the nascent polypeptides are tagged for proteolysis and the corrupt mRNA is degraded

so that the stalled ribosomes can be released [4]. The absence of *trans*-translation in humans and the reduced viability and virulence of *trans*-translation mutants make this pathway a promising antibacterial target in many pathogens. We used the small molecule inhibitors KKL-35 and KKL-40 to expand our proteomic profile library by addition of a proteome signature for *trans*-translation inhibition and to analyze the bacterial response to these inhibitors. Based on the proteomic signature for *trans*-translation inhibitors structurally new inhibitors of *trans*-translation can be rapidly identified. Furthermore, the library allows us to compare the marker proteins for *trans*-translation with already existing proteome profiles of various antibiotics inhibiting different steps of protein biosynthesis to elucidate the bacterial response to *trans*-translation inhibition. In addition, based on specific marker proteins the exact inhibited step, at which *trans*-translation is inhibited, can be determined.

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PRP34

In vitro testing for synergism of antibiotic triple combination against multi-resistant Gram-negative pathogens

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Objectives: The worldwide increase of multi-resistant Gram-negative bacteria (MRGN) represents a major challenge for clinicians. A recent major retrospective study observed a significantly decreased 30-day mortality in patients with KPC-producing *K. pneumoniae* blood stream infections who were treated with meropenem/tigecycline/colistin triple combination compared to monotherapy [1]. However, the mechanism of the clinically observed synergism is still elusive. Therefore, we aimed to investigate the *in vitro* synergistic activity of this triple combination against clinical isolates of MRGN with different, molecularly defined mechanisms of beta-lactam resistance.
Materials and Methods: Twenty-four carbapenems non-susceptible clinical isolates (*K. pneumoniae*, n=12; *E. coli*, n=1; *A. baumannii*, n=2; *E. cloacae*, n=1) were studied. All strains harbored different types of beta-lactamases. In addition, some strains also exhibited loss of porins (OmpK). Measuring of MIC was performed according to EUCAST guidelines by broth microdilution. The assay was modified for triple checkerboard testing. The fractional inhibitory concentration (FIC) for a single antibiotic was calculated as follows: FIC of drug A = MIC of drug A in combination/MIC of drug A alone. FIC index was calculated as sum of each single FIC value and interpreted as follows: FIC index ≤ 0.5 was defined as synergy, FIC index > 4 was defined as antagonism and indefinite interaction was defined as an FIC index > 0.5 - 4.
Results: Synergism (FIC value ≤ 0.5) of combinations was detected only against *K. pneumoniae* isolates, particularly against isolates with high MICs (interpreted as resistant, according to EUCAST clinical breakpoints) for individual antibiotics of the combination. Highest synergism (i.e. lowest FIC values) was found for the triple combination and for the double combination of colistin/tigecycline. Synergism was not associated to expression of a specific beta-lactamase.
Conclusion: Our *in vitro* results are in line with the clinical observations that describe decreased mortality in patients treated with tigecycline/colistin/meropenem compared to monotherapy. The triple combination seems to be particularly useful in *K. pneumoniae* isolates with high MICs against individual antibiotics and seems not to depend on the mechanism of resistance.

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PRP35

Age-related resistance rates of Staphylococcus aureus and Escherichia coli: data from the German Antibiotic Resistance Surveillance System (ARS) from 2009-2012

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Objectives: Resistance rates may differ according to different age groups.Data from the German Antibiotic Resistance Surveillance system (ARS) are presented.
Materials and Methods: ARS is a voluntary laboratory based surveillance system collecting resistance data of all clinical pathogens and sample types. Data are transmitted electronically to the central data-base of the national public health institute. The development of resistance-rates of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) against oxacillin (OXA) and cefotaxim (CTX), respectively, are presented for the time period from 2009 to 2012. Resistance-rates have been calculated separately for the following age groups: <1 year, 1 to 5 years, 6 to 15 years, 16 to 59 years, ≥60 years.Copy strains and screening samples have been excluded. Data are presented separately for hospital and ambulatory care.
Results: From 2009 to 2012, in hospital and ambulatory care, the overall-resistance of *S. aureus* against OXA decreased significantly from 23.3% to 18.2% and from 12.8% to 10.3%, respectively (Table 1). In children (age groups: <0/1-5/6-15 years) we saw the lowest percentages of OXA-resistance, the level of which did not differ between hospital and ambulatory care. In contrast, in adults (age groups: 16-59/≥60 years) resistance-rates differed significantly between both health care types. In hospital care as well as in ambulatory care, resistance rates showed a continuous and significant decrease from 2009 to 2012 in the age group ≥60 years. The development of the resistance-rates of the other age groups only showed slight changes comprising a decrease in hospital care and an increase or stable course in ambulatory care without achieving the significance level. In *E. coli* the overall resistance against CTX rose significantly from 7.8% in 2009 to 10.2% in 2012 and from 3.2% to 6.1%, respectively, in hospital and ambulatory care. With the exception of the age group <1 year, the resistance-rates of the different age groups are throughout around twice as high in hospital care as compared to ambulatory care. In hospital care, the age group <1 year did not show a change of resistance-rates, whereas all other age groups indicate an increase that was most prominent in children of the age groups 1-5 and 6-15. In ambulatory care, the resistance-rates nearly doubled from 2009 to 2012 throughout all age groups.
Conclusion: The drop of the overall OXA-resistance in *S. aureus* was ascertained in patients ≥60 years but could not be proved in other age groups. An increase of CTX-resistance of *E. coli* was observed throughout all age groups except of the age group <1 year (hospital care) showing stable values over time. Age-related resistance-rates from different health care sectors may help to inform decisions about empirical antibiotic therapy and contribute to a better understanding of the epidemiology of resistant pathogens.

Figure 1

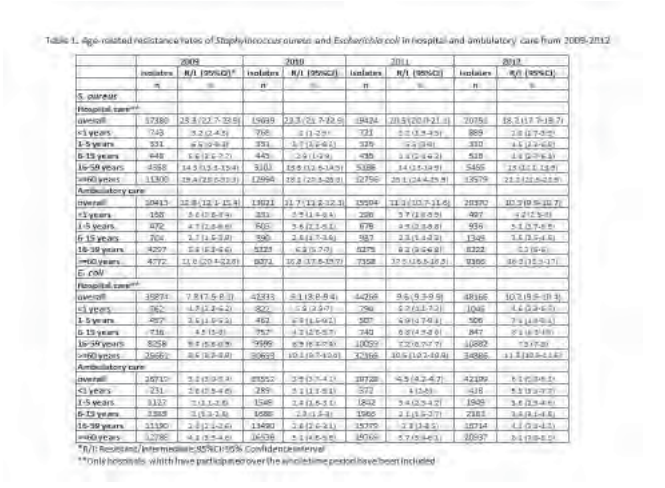
Table 1. Age-related resistance rates of *Staphylococcus aureus* and *Escherichia coli* in hospital and ambulatory care from 2009-2012

	2009		2010		2011		2012	
	isolates	R/I (95%CI)*	isolates	R/I (95%CI)	isolates	R/I (95%CI)	isolates	R/I (95%CI)
	n	%	n	%	n	%	n	%
S. aureus								
Hospital care**								
overall	17180	23.3 (22.7-23.9)	19819	23.3 (22.7-23.9)	18424	20.5 (20.0-21.1)	20756	18.2 (17.7-18.7)
<1 years	743	5.2 (3.4-7.5)	768	3 (1.7-4.9)	721	3.5 (2.3-5.1)	889	2.8 (1.7-4.4)
1-5 years	931	4.5 (3.2-6.2)	931	3.7 (2.6-5.1)	929	3.3 (2.3-4.5)	930	4.1 (2.9-5.6)
6-15 years	648	5.6 (3.9-7.7)	645	2.9 (1.7-4.5)	635	3.3 (2.1-4.7)	518	3.3 (2.1-5.1)
16-59 years	4988	14.5 (13.5-15.4)	5101	15.6 (14.6-16.5)	5188	14 (13.1-14.9)	5466	13 (12.1-13.9)
≥60 years	11300	28.4 (27.5-29.3)	12966	28.1 (27.3-28.9)	12756	25.1 (24.4-25.9)	13579	21.3 (20.6-22.0)
Ambulatory care								
overall	10413	12.8 (12.1-13.4)	13021	11.7 (11.2-12.3)	15504	11.2 (10.7-11.6)	21370	10.3 (9.9-10.7)
<1 years	158	3.4 (0.8-8.4)	231	3.9 (1.4-8.4)	298	3.7 (1.6-7.1)	497	4 (2.1-6.9)
1-5 years	872	4.1 (2.8-5.8)	965	3.6 (2.5-5.1)	878	4.3 (3.1-5.8)	936	5.1 (3.7-6.9)
6-15 years	704	3.7 (2.1-5.9)	990	2.6 (1.7-3.6)	987	2.3 (1.4-3.5)	1349	3.6 (2.5-4.8)
16-59 years	4297	5.6 (4.9-6.4)	5223	6.2 (5.7-7)	8275	6.2 (5.6-6.8)	8202	6.2 (5.6-6.8)
≥60 years	4772	21.9 (20.9-23.0)	6071	19.2 (18.4-19.9)	7358	17.5 (16.8-18.2)	9386	16.2 (15.5-17)
E. coli								
Hospital care**								
overall	15874	7.8 (7.5-8.1)	17233	8.1 (7.8-8.4)	16259	9.6 (9.3-9.9)	18166	10.2 (9.9-10.5)
<1 years	662	4.8 (3.3-6.7)	822	5.5 (3.9-7)	798	6.9 (5.1-9.1)	1046	4.6 (3.4-6.1)
1-5 years	857	3.6 (2.6-5.1)	962	4.9 (3.6-6.5)	887	4.9 (3.7-6.5)	926	7.5 (5.8-9.5)
6-15 years	736	4.5 (3-6.5)	757	4.2 (2.8-5.9)	740	6.8 (4.9-9.1)	847	8.5 (6.4-10.9)
16-59 years	8258	9.4 (8.6-10.2)	9586	8.9 (8.3-9.6)	10059	9.2 (8.6-9.7)	10882	9.3 (8.8-9.9)
≥60 years	5366	8.6 (7.8-9.6)	6063	10.1 (9.3-10.9)	5716	10.5 (9.7-11.4)	5465	11.3 (10.5-12.1)
Ambulatory care								
overall	26710	3.2 (3.0-3.4)	29552	3.9 (3.7-4.1)	28728	4.5 (4.2-4.7)	42189	6.1 (5.8-6.4)
<1 years	231	2.6 (0.8-6.1)	289	3.1 (1.3-6.1)	372	4 (1.9-7.3)	428	5.3 (3.3-7.9)
1-5 years	1137	2.1 (1.3-3.3)	1346	3.4 (2.5-4.5)	1362	3.4 (2.5-4.5)	1949	3.4 (2.8-4.2)
6-15 years	1363	1.5 (0.9-2.3)	1688	2.3 (1.7-3.1)	1966	2.1 (1.5-2.7)	2383	3.6 (3.1-4.3)
16-59 years	11190	2.3 (2.1-2.6)	13490	3.4 (3.2-3.7)	15370	3.7 (3.4-3.9)	18714	4.7 (4.5-4.9)
≥60 years	12789	4.3 (3.9-4.6)	16539	5.1 (4.8-5.5)	15019	5.7 (5.3-6.1)	20937	9.1 (8.8-9.5)

*R/I: Resistant/ intermediate; 95%CI: 95% Confidenceinterval
**Only hospitals which have participated over the whole time period have been included

PRP36

Silver tolerant *E. coli* display active efflux of Ag^+ and low-level cross-resistance to certain antibiotics

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Antibiotic resistance is a major public health threat and a continuing challenge in order to assure antimicrobial chemotherapy of infectious diseases. Heavy metals and natural antibiotics are substances that microorganisms face in many environmental niches. Therefore, multidrug efflux mechanisms are broadly conserved in various bacteria.
In a previous study we observed that stepwise exposure to increasing concentrations of heavy metals, such as silver, resulted in bacterial adaptation and tolerance that enabled growth in high concentrations of the respective biocide. This adaptation was characterized by an increase in the expression of efflux pump genes.
The aim of the study was to induce silver-tolerance in *E. coli* in order to investigate the impact of the resulting adaptation mechanisms on the susceptibility to certain antibiotics. For this purpose a silver-susceptible strain of *E. coli* was adapted to growth in the presence of silver by stepwise increasing the silver concentration in the culture medium. As in gram-negative bacteria, at least two general mechanisms for effectively blocking drug access exist, i.e., the outer membrane (OM) permeability barrier and active efflux systems, expression of presumable silver-tolerance related genes *ompA* and *cusC* was quantified for both strains using Real-Time qRT-PCR analysis. In addition, the susceptibility to certain antibiotics was determined for the original, silver-susceptible strain as well as for the silver-tolerant strain.
Quantification of the expression of genes *cusC* and *ompA* revealed a strong up-regulation in the adapted *E. coli* strain, indicating to an overproduction of the respective proteins. Furthermore, low-level cross-resistance was observed as indicated by a decrease in the susceptibility to ampicillin and ciprofloxacin for the adapted strain. In conclusion, the results from this study suggest that an increased expression of efflux pumps due to the presence of silver ions may exert influence on the antibiotic susceptibility of silver-tolerant bacteria. However, the contribution of biocides to the development of bacterial antibiotic resistance has to be further investigated and additional research is required to better characterize potential cross-resistance with antibiotics.

PRP37

gamma-Glytamyl-Transpeptidase of *Francisella tularensis*: enzymatic characterization and drug target for a new class of anti-infectives

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Introduction: *Francisella tularensis* is a facultative intracellular, anaerobic, Gram negative coccobacillus and the causative agent of tularemia. Infections caused by this bacterium lead to several clinical symptoms ranging from localized cutaneous ulcerations at the site of infection and swelling of local lymph nodes to fatal pneumonia or septicemia with a lethality >30% in humans. Due to its particularly high virulence (10-50 bacteria are sufficient for infection) *F. tularensis* is classified as category A select agent. Treatment options, however, are more and more limited by increasing antibiotic resistance. Hence, new anti-infective drugs are required to meet this medical need.
The g-Glutamyl-Transpeptidase of *F. tularensis* (FtgGT) is the key enzyme for generating the essential amino acid cysteine from glutathione in *F. tularensis*. DgGT deletion mutants of *F. tularensis* were unable to grow or infect macrophage cell lines *in vitro* (Alkhuder et al., 2009), and were strongly attenuated in mouse infection models (Kadzahev et al., 2009). Therefore, FtgGT is a promising drug target for a pathogen specific anti-infective therapy with reduced risk of (cross-)resistance development.
Materials and Methods: We have recombinantly produced and purified the gGTs of different *Francisella* subspecies using the SUMO-tag /SUMO protease system. The correct folding and stability of the enzymes were monitored using Sypro-orange unfolding assays. Moreover, enzymatic activity was confirmed by the gGpNA based colorimetric enzyme activity assay in which the g-glutamyl moiety of the substrate is translocated onto Glygly as acceptor and the liberation of the p-Nitroanilid is monitored at 405 nm.
Results: On the basis of the colorimetric activity assay the FtgGT was characterized in terms of pH optimum and acceptor substance. Successful inhibition of enzyme activity by the reference inhibitor Acivicin (Stole et al., 1994) was shown, confirming the usefulness of this biochemical assay for compound screening.
Discussion: By characterization of the gGT of *F. tularensis ssp. holarctica* in terms of pH optimum and acceptor substance a solid basis for the development of species specific inhibitors was laid. Due to their specificity these inhibitors are expected to exert reduced side effects and are less prone to induce resistance in antiinfective therapy.

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PRP38

Inhibition of streptococcal invasion by bacterial natural compounds - a possible way to encounter persistence and dissemination

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Millions of people are affected by streptococcal diseases every year. The spectrum can range from common throat infections to life-threatening diseases like sepsis or necrotizing fasciitis. Furthermore, streptococcal post-infection sequelae represent a serious health hazard in certain geographic areas.[1] As antibiotic resistance is emerging, it is necessary to find new antibiotics which do not pose this problem. A key pathogenic mechanism is invasion of eukaryotic cells: after invasion dissemination is possible as well as persistence which can cause recurrent infections.[2]Therefore, several extracts and purified compounds derived from myxobacteria were tested with the aim to find a molecule inhibiting invasion. *S.pyogenes* strains A40 and A8 were used for cell infection assays. In each assay compounds were added to analyze possible inhibitory effects. To estimate the range of inhibition of invasion double-immunofluorescence staining and examination via electron microscopy after an infection experiment were used.[3] By using a bacterial invasion assay intracellular surviving bacteria were determined.[4] Moreover, cytotoxicity was assessed by using an MTT assay under infection conditions. Two compounds show inhibition of invasion of Group A streptococci into different types of epithelial cells. As the streptococcal strains used in the assays exhibit representatives for two important invasion mechanisms of streptococci[5], it can be shown that these different pathways can be inhibited by the natural compounds quite efficiently. Additionally, the compounds do not show cytotoxic effects under infection conditions. If invasion can be inhibited, it will be a first step to encounter persistence and dissemination: if streptococci are disabled to invade cells, the reservoir required to cause recurrent infections is reduced. Moreover, dissemination will be hampered as they will only be able to attach to cells. Additionally, killing of streptococci using antibiotics such as penicillin will be facilitated as the bacteria cannot hide in an intracellular compartment anymore.

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PRP39

Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and the CIT-type AmpCs

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In Gram-negative bacteria, the production of beta-lactamases represents the most important contributing factor to resistance against beta-lactam antibiotics. During the last couple of years increasing numbers of antibiotic-resistant bacteria have become a challenge to infection control. Particularly in Enterobacteriaceae, extended-spectrum (ESBL)- and AmpC-type ß-lactamases play an important role. Most of their genes are plasmid-encoded and the expressed enzymes lead to reduced susceptibility to broad spectrum beta-lactams. For this reason a major interest in efficient and reliable methods for rapid screening of high sample numbers is recognizable. Therefore, a multiplex real-time PCR was developed to detect the predominant class A beta-lactamase genes $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM} and CIT-type AmpCs in a one-step reaction. A set of 114 Enterobacteriaceae containing previously identified resistance gene subtypes and in addition 20 undefined animal and environmental isolates were used for the validation of this assay. To confirm the accessibility in variable settings, the real-time runs were performed analogous in two different laboratories using either the Roche Lightcycler 480II or the BioRad CFX96. The obtained results showed complete accordance between the real-time data and the predetermined genotypes. Even if sequence analyses are further necessary for a comprehensive characterization, this method was proofed to be reliable for rapid screening of high sample numbers and therefore could be an important tool for e. g. epidemiological purposes or support infection control measures.

PRP40

Occurrence of enterobacteria producing broad-spectrum beta-lactamases in hospital wastewater

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Introduction: Bacterial resistance to antibiotics has emerged as a global concern. Healthcare facility wastewater may act as a source of resistance genes with potential spread to environment. For this reason, additional research is needed in order to determine the prevalence of multiresistant bacteria in hospital wastewater.
Materials and Methods: Three wastewater samples were collected in 2013. Cellulose swabs were immersed into wastewater in a hospital sewage treatment plant with subsequent inoculation on three types of cultivation media - blood agar, MacConkey agar and desoxycholate citrate agar. In the same period, enterobacteria producing broad-spectrum beta-lactamases were collected from clinical samples of hospitalized patients. After identification using MALDI-TOF MS, beta-lactamase production was determined by relevant phenotyping tests. Genes responsible for production of TEM, SHV, CTX-M beta-lactamases, AmpC enzymes and Qnr proteins were established using PCR. The pulsed-field gel electrophoresis (PFGE) was applied for comparison of genetic relatedness of selected isolates from wastewater and patients. Another material collection is planned for July 2014.
Results: Over the study period, enterobacteria with production of extended spectrum beta-lactamases or AmpC enzymes were detected in hospital wastewater as well as in clinical specimens from patients. Using PCR bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$ and genes encoding AmpC enzymes (CIT and DHA types) were identified. Resistance to fluoroquinolones was found in 31 (75.6%) isolates from hospital wastewater, of which 20 carried the *qnrB* gene. Two identical strains of *Klebsiella pneumoniae* were determined using PFGE method, one from permanent urine catheter of a hospitalized patient and another isolated from wastewater.
Conclusion: Hospital wastewater represents a potential reservoir for multiresistant bacteria.

Supported by the grant IGA MZCR NT/14398 and LF_2014_021.

PRP41

Identification of key factors of the pIP501 encoded type IV secretion system

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Spread of antibiotic resistances among microorganisms through conjugative type IV secretion systems (T4SSs) is an increasing issue for human health. Our working model for research of T4S in Gram-positive bacteria is the plasmid pIP501. Nosocomial *Enterococcus faecalis* and *Enterococcus faecium* strains often contain this broad-host range plasmid encoding 15 putative transfer genes in a single operon for a T4SS multiprotein complex (TraA-TraO). TraA has been characterized as a relaxase which negatively autoregulates the pIP501 *tra* operon. The Tra proteins TraE (ATPase), TraJ (coupling protein) and TraG (muramidase) show significant sequence similarities to the well understood and characterized Gram-negative T4SS from *Agrobacterium tumefaciens*. Other putative key members of the conjugative transfer complex are TraL, TraM (putative channel factors) and TraO (surface adhesin) [1]. The function of most of the transfer proteins is not fully understood and it is not known in detail how the conjugative T4SS works. Our goal is to knockout *tra* genes to get more information about T4S in Gram-positive bacteria. To generate knockout mutants in *E. faecalis* pIP501 we use a method based upon homologous recombination described by Kristich et al. (2007) with minor modifications [2]. For complementation of the knockout mutants we clone the affected wild type gene in the *E. coli*/*E. faecalis* shuttle vector pEU327. Conjugation assays will show if the individual deleted genes are essential for T4S. The first knockout mutant that we generated is the deletion mutant *E. faecalis* pIP501Δ*traG*. Biparental matings with the *traG*-knockout strain showed that this peptidoglycan-degrading enzyme is essential for pIP501 conjugative transfer [3]. Further studies of *tra* gene knockout mutants will enable to complete the model of the pIP501 conjugative transfer machinery which would represent the first T4SS model of a broad-host-range plasmid originating from Gram-positive bacteria.

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PRP42

Description of KHM-2, a novel metallo-beta-lactamase found in a multidrug-resistant *Pseudomonas aeruginosa* isolate from Germany

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Objectives: The worldwide increase of multidrug-resistance in gramnegative bacteria has become an important clinical challenge. Carbapenem resistance can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. A worrying trend is the dissemination of Ambler class B metallo-β-lactamases (MBL). Here we describe a novel KHM-type MBL, KHM-2, that was found in a multidrug resistant *P. aeruginosa* isolate in Germany.
Materials and Methods: Multilocus sequence typing (MLST) was performed by amplification and sequencing of seven *P. aeruginosa* housekeeping genes. Susceptibility to antibiotics was determined by disk

diffusion, Etest and microdilution. The presence of a MBL was determined by EDTA combined-disc-tests, MBL Etest and by a bioassay based on cell-free extracts. A modified Hodge-Test was performed. PCRs and subsequent sequencing were performed for VIM, IMP, NDM, GIM, SIM, SPM, AIM, DIM and KHM. Total DNA of *P.aeruginosa* NRZ-03096 was digested with *Mbo*I and cloned into the pBK-CMV vector. Recombinant clones were sequenced. The KHM-2 encoding sequence was cloned into the pBK-CMV vector and transformed into *E. coli* TOP10 for activity analysis. Localisation of the gene was determined by PFGE and Southern blotting. The bla_{KHM-1} gene was also cloned into the pBK-CMV vector and expressed in *E. coli* TOP10 to serve as a reference for MIC studies.

Results: A multidrug-resistant *P. aeruginosa* isolate from northern Germany was sent to the National Reference Laboratory for Multidrug-resistant Bacteria for further characterisation. The isolate was resistant to piperacillin, piperacillin/tazobactam, amoxicillin, amoxicillin/clavulanic acid, ceftazidime, cefepime, aztreonam, imipenem, meropenem and doripenem as well as to gentamicin, tobramycin, amikacin, ciprofloxacin and levofloxacin, according to EUCAST criteria. The modified Hodge-test was positive for imipenem, meropenem and ertapenem. The isolate showed synergy with EDTA in the combined disk test and the MBL Etest. An unspecific result due to toxicity of EDTA was excluded by a positive bioassay based on cell-free extracts. Sequencing of recombinant plasmids revealed the expression of a novel metallo-β-lactamase, KHM-2, with 74% similarity to KHM-1. *E. coli* TOP10 producing KHM-2 showed increased MICs for beta-lactams, except aztreonam.

Conclusion: *P. aeruginosa* NRZ-03096 harboured a novel metallo-β-lactamase, KHM-2. As this is the only known member of the KHM group except from KHM-1, it is likely that the bla_{KHM-2} gene has been recently mobilized from a still unknown source. With only 74 % similarity to KHM-1, KHM-2 showed distinct differences regarding the amino acid sequence. This further underlines the growing diversity of carbapenemases and the continuing spread of Ambler class B metallo-β-lactamases in clinical *P. aeruginosa* strains.

PRP43

Acquisition of ESBLs in *Salmonella* spp. and *Shigella* spp. under systemic cephalosporin therapy

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Introduction: *Salmonella* and *Shigella* are a widespread cause of foodborne gastrointestinal infections. Here we report on emergence of extended-spectrum beta-lactamases (ESBLs) in these species isolated from three patients under cephalosporin therapy.

Materials and Methods: Patient 1 was a 15 year old boy who with a presumed exacerbation of ulcerative colitis (UC). Patient 2 was a 21 months old infant with diarrhoea due to a foodborne disease, and patient 3 was a 4 year old neuroblastoma patient with gastroenteritis. In all patients several stool samples were screened for enteric pathogens. Species identification, antimicrobial susceptibility testing and *Salmonella* serotyping were performed. Beta-lactamase genes were identified by PCR and sequencing. ESBL gene transfer was tested by broth mate conjugation experiments, and the plasmids were characterized (S1-nuclease PFGE).

Results: In patient 1 we found 5 isolates: *Salmonella* serovar Shubra (n=3), *E. coli* (n=1) and *Aeromonas hydrophila* (n=1). Culture of samples from patient 2 resulted in *Shigella boydii* (n=3) and *E. coli* (n=1). In patient 3 we found *Salmonella* serovar Manhattan (n=2), *E. coli* (n=2), *Citrobacter freundii* (n=2) and *Klebsiella pneumoniae* (n=1). The initial *Salmonella* and *Shigella* isolates of all patients were susceptible to 3rd generation cephalosporins. Thus, the patients received systemic cephalosporin treatment. However, all isolates from samples collected 6 days (patient 1), 9 days (patient 2) and 6 weeks (patient 3) later showed the ESBL-phenotype. We identified CTX-M-14 in *S.* Shubra, *E. coli* and *A. hydrophila* from patient 1. For patient 2 we found CTX-M-15-producing *S. boydii* and *E. coli*. Patient 3 was positive for SHV-12-producing *S.* Manhattan, *C. freundii*, *E. coli* and *K. pneumoniae*. ESBL genes $bla_{CTX-M-14}$ and bla_{SHV-12} were located on conjugative plasmids of 65 kb and 50 kb size, respectively. Isolates of patient 2 harboured a 100 kb plasmid carrying $bla_{CTX-M-15}$.

Conclusion: In all three cases the ESBL gene carrying plasmids in *Salmonella* and *Shigella* isolates were identical to plasmids in other ESBL-producing enterobacterial species that were present in the gut. Antibiotic treatment may enhance the selection of pathogens harbouring the resistance gene carrying plasmid. These cases demonstrate exemplarily the importance of *in vivo* plasmid transfer for the spread of antibiotic resistance genes among gram-negative bacteria of the human microbiome.

PRP44

Nitroxoline inhibits *in vitro* adherence to and invasion of human urinary bladder epithelial cells by uropathogenic bacteria.

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Urinary tract infections are the most frequent bacterial infections in industrialized countries. Nevertheless, there is no suitable vaccine available for prophylaxis and antibiotic therapy frequently fails due to resistant strains. In this study the ability of the antibiotic Nitroxoline was tested for its ability not only to inhibit bacterial adherence to human bladder epithelial cell line T24 of Nitroxoline-sensitive but also Nitroxoline-resistant uropathogens. In addition a potential effect of Nitroxoline on bacterial invasion of T24 cells was investigated. Adherence was quantified in the absence and presence of Nitroxoline after infection of T24 wells with bacteria for 2h, removal of non-adherent bacteria, methanol fixation, Giemsa staining and microscopic examination of the slides. Invasion efficiency was quantified by the Gentamicin protection assay, i.e. infection of T24 cells for 2h in the presence or absence of Nitroxoline (25 µg/ml), withdrawal of the medium was followed by 1 h incubation in the presence of 100µg/ml Gentamicin followed by removal of the gentamicin containing medium, lysis of the epithelial cells, plating of suitable dilutions and enumeration of CFU by plate count after incubation overnight. Intracellular bacterial survival was determined by infection of T24 cells for 2 h, subsequent incubation in the presence of 100 µg/ml Gentamicin and 25 µg/ml Nitroxolin for 1 h. Numbers of intracellular bacteria was enumerated as for invasion efficiency. Adherence to T24 cells of uropathogenic *E. coli* strains CFT073, 536 and NU14 and of uropathogenic *Klebsiella pneumonia* strains 3091 and of uropathogenic *Citrobacter freundii* strain 3009 was more than 90 % reduced in the presence of 25 µg/ml Nitroxoline. In addition, adherence of the Nitroxoline-resistant mutants was also inhibited by 25 µg/ml Nitroxoline, although to a lesser extent.

Invasion of T24 cells was determined for two Nitroxoline-resistant uropathogenic *E. coli* strains (536r, NU14r). Nitroxoline inhibited invasion of both strains by more than 90 %.

Finally, the effect of Nitroxoline on intracellular survival of Nitroxoline-sensitive bacteria was determined. Numbers of bacteria inside T24 cells differed not significantly between Nitroxolin- treated and untreated cells for the uropathogenic *E. coli* strains 536 and NU14 as well as for *C. freundii* strain 3009. We conclude that Nitroxoline inhibits *in vitro* adherence of Nitroxoline-sensitive as well as Nitrxoline-resistant uropathogenic bacteria. Invasion of host cells by Nitroxoline-sensitive uropathogenic *E. coli* is also strongly inhibited by Nitroxoline. However, the number of already internalized Nitroxoline-sensitive uropathogenic *E. coli* is not affected by Nitroxoline.

PRP45

Murgocil is a Highly Bioactive Staphylococcal-specific Inhibitor of the Peptidoglycan Glycosyltransferase Enzyme MurG

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Staphylococcus aureus is the leading cause of serious gram-positive infections in developed countries and the emergence of antibiotic resistance seriously challenges treatment options. This alarming development makes the search for new antibacterial agents with unprecedented mechanisms of

action essential. Peptidoglycan (PG) biosynthesis represents a most attractive target pathway for antibiotic intervention, although no compound targeting an intracellular step of PG synthesis, except for fosfomycin, has been developed so far. Here we describe the identification of a compound that augments the activity of imipenem against methicillin-resistant Staphylococcus aureus in a β-lactam potentiation screen and showed peptidoglycan biosynthesis inhibitory effects in whole cell assays. Genetic, biochemical and structural modeling data revealed that the small molecule inhibitor specifically targets the glycosyltransferase MurG, involved in the synthesis of the central cell wall building block Lipid II [1].

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PRP46

Identification of nasal colonization with atypically resistant Enterobacteriaceae in inpatients of an Infectious Disease Department in Madagascar

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Introduction: The increasing resistance of bacterial pathogens to antimicrobial drugs does not spare tropical countries. Atypically resistant strains are prevalent in various parts of Subsaharan Africa. Here we assessed the nasal colonization with atypically resistant Enterobacteriaceae in patients of an Infectious Disease Department in a Madagascan hospital.
Materials and Methods: Nasal swabs were taken from 169 inpatients of the Infectious Disease Department of the University Hospital Joseph Raseta de Befelatanana, Antananarivo, Madagascar directly at admission over a period of 6 months. All included patients answered a questionnaire to provide information on gender, residence, professional contact with animals, chronic diseases, past hospitalizations and antibiotic treatment. Enterobacteriaceae were isolated from chromogenic Brilliance ESBL selective agar. Subsequently, assessment of piperacillin, ceftazidim, meropenem and ciprofloxacin resistance by E-testing, of ESBL- and AmpC-expression by ABCD-testing, and of CTX-M ESBL genotype by commercial hyplex ESBL PCR were added.
Results: A total of 13 out of 169 patients (7.1%) showed nasal colonization with ESBL-positive strains or strains with resistance against at least three out of the four tested bactericidal antibiotic drugs. All 13 respective Enterobacteriaceae isolated from the chromogenic Brilliance ESBL selective agar were resistant against ceftazidim. A proportion of 11 out of these 13 were resistant against at least three out of the four tested bactericidal antibiotic drugs. No specific risk factors were determined, although inhabitants of the capital Antananarivo showed even a lower relative risk of being colonized compared with patients from rural Madagascar.
Conclusion: A relevant proportion of nasal colonization with cephalosporin-resistant Enterobacteriaceae was demonstrated in Madagascan hospital patients, which might lead to endogenous infections resulting from this colonization. The results suggest that a continuous surveillance of atypically resistant Gram-negative pathogens could be beneficial in Madagascan hospitals.

PRP47

New Delhi Metallo-beta-Lactamase 1 substrate binding site mutant K211E: substrate and Zn^{2+} dependent activity

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Introduction: Being discovered in 2009, the New Delhi Metallo-beta-Lactamase 1 (NDM-1) has reached immediate attention due to its exceptionally broad substrate promiscuity conferring resistance to all beta-lactam antibiotics and its rapid dissemination both across the globe as well as between bacterial species. Moreover, being frequently plasmid encoded in combination with various resistances against most other classes of antibiotics, NDM-1 positive bacterial pathogens are extremely difficult to treat and pose an enormous threat to human health.
Inhibition of the NDM-1 activity is expected to broaden the treatment options against NDM-1 positive pathogens and thereby meet this growing medical need. However, this approach is impeded as no Metallo-beta-Lactamase inhibitors are in clinical use so far and as the development of NDM-1 specific inhibitors is hampered by the exceptionally large active site. For obtaining structural information about the required active site geometry we recombinantly produced both wild type and K211E substrate binding site mutant and compared their enzymatic activities using different beta lactam substrates at various Zn^{2+} concentrations.
Materials and Methods: NDM-1 WT and K211E mutant were recombinantly expressed and purified using a SUMO tag / SUMO protease system. Appropriate protein folding was confirmed via CD analysis. The activity of both NDM-1 forms was monitored at various Zn^{2+} concentrations in a colorimetric assay in which the hydrolysis of the structurally related substrates CENTA and Nitrocefin (Cephems) as well as Imipenem (Carbapenem) was monitored. Moreover, the inhibitory effect of Captopril, a published NDM-1 inhibitor, was determined with different substrates and Zn^{2+} concentration.
Results: The absolute and relative activity of WT versus K211E mutant NDM-1 differ substantially depending on the type of substrate and Zn^{2+} concentration used in the biochemical assay. Even within the Cephem class of beta-Lactam substrates the WT enzyme shows clear differences in the specific activity, with CENTA being a more efficient substrate than Nitrocefin. In contrast, the K211E mutant, which is completely inactive with Imipenem as substrate, shows limited activity with CENTA, but exceeds WT activity with Nitrocefin at increasing Zn^{2+} concentrations. Moreover, the inhibitory effect of Captopril, a published inhibitor of NDM-1, clearly depends both on the substrate and Zn2+ concentration used.
Conclusions: Enzymatic activity of both NDM-1 forms and the inhibitory effect of Captopril markedly depend on the type of substrate and the Zn^{2+} concentrations used. This has strong implications on screening procedures for NMD-1 inhibitors for clinical use. Besides using clinically relevant beta-Lactams as substrates, physiological Zn^{2+} concentrations have to be considered when searching for NDM-1 inhibitors using biochemical beta-Lactam hydrolysation assays.

PRP48

Prevalence of MRSA and gram-negative bacteria with extended-spectrum beta-lactamases(ESBLs) and carbapenemases in Libyan patients from a German hospital

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative bacteria producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases are an emerging threat in German hospitals. Patients from abroad, especially from countries with high prevalence of colonization with MRSA, ESBL- and carbapenemase-producers, are suspected to be asymptomatic carriers of multidrug-resistant pathogens. Here we show the results of a bacterial screening (January 2012 - December 2013) of Libyan patients admitted to the Charité university hospital, a tertiary care center with over 3.200 beds.
Materials and Methods: 278 patients were admitted due to secondary treatment of war injuries suffered during the Libyan civil war which ended in October 23rd, 2011. Rectal and nasal swabs from the patients were

screened for multidrug-resistant bacteria. Species identification and antimicrobial susceptibility testing were performed. Beta-lactamase genes were identified by PCR and sequencing. MRSA were characterized by spa-typing and a multiplex PCR for detection of markers for common CA-MRSA lineages.
Results: The patients were predominantly male (79.5%) with a median age of 36 years (IQR 27-45). 50 (17.9%) patients we found colonized with multidrug-resistant isolates: MRSA (n=23), carbapenem-resistant *Pseudomonas aeruginosa* (n=4) *and Acinetobacter baumannii* (n=4), ESBL-producing *Escherichia coli* (n=29), *Klebsiella pneumoniae* (n=11) and *Enterobacter spp.* (n=3). 14 (5%) patients were colonised with multidrug-resistant bacteria of two or more different species. The dominant ESBL-type was CTX-M-15 (63%). ESBL-producing *E. coli/K. pneumoniae* from 5 patients additionally harboured carbapenemase OXA-48. Overall, prevalence of MRSA, ESBL-E and carbapenem-resistant bacteria were 8.3%, 12.9% and 2.2%, respectively. Molecular typing revealed MRSA, which were different from MRSA clones circulating in German hospitals. Most of them were grouped to clonal complex 5 and exhibited a narrow resistance phenotype (n=11). But among these strains were also multidrug-resistant MRSA ST239 (n=4) and CA-MRSA of clonal lineages ST1, ST80 and ST97 (n=4).
Conclusion: This study showed that 8-13% of the patients from Libya were colonised with MRSA and ESBL-producing *Enterobacteriaceae*, but only a minority was positive for carbapenem-resistant bacteria. The prevalence rates were significantly higher than in German patients. Our work demonstrated the importance of microbiological screening to detect colonisation with multidrug-resistant organisms at an early stage. As a result hygiene measurements could be introduced to prevent further spread of these bacteria.

PRP49
Characterization of OXA-321, a novel carbapenem hydrolyzing variant of OXA-10 in *Klebsiella pneumoniae*

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Introduction: Resistance against betalactam antibiotics, particularly carbapenems, caused by betalactamase-production in Enterobacteriaceae contributes to the growing challenge nosocomial infections pose to general healthcare. Here we report the detection of a novel carbapenem hydrolyzing variant of the OXA-10 betalactamase, from an isolate of *Klebsiella pneumoniae*, belonging to Sequence Type ST-11. The isolate yielded resistance against penicillins and carbapenems, but not cephalosporins. Betalactamase activity could be inhibited by clavulanic acid but not by EDTA. Shotguncloning experiments revealed that the distinctive resistance phenotype was caused by a $bla_{\text{OXA-10}}$-like Gene that differed from $bla_{\text{OXA-10}}$ only by a single mutation resulting in a single amino acid substitution in the Protein. The novel betalactamase was named OXA-321.
Materials and Methods: Subsequently, OXA-321 and OXA-10 were heterologously expressed in *Escherichia coli* Top 10, followed by comparative MIC studies by microdilution, Etest and agardiffusion in isogenic strains expressing OXA-321, OXA-10 and no betalactamase at all. The OXA-321 Protein was afterwards overexpressed, purified by FPLC and subjected to biochemical characterization.
Results: The comparative MIC studies showed carbapenem resistance of strains expressing OXA-321. The biochemical characterization demonstrated hydrolysis of Ertapenem identifying OXA-321 as a carbapenemase. Exploration of the genetic context of $bla_{\text{OXA-321}}$ showed that the $bla_{\text{OXA-321}}$ gene was located in a class I Integron that was associated with a transposable element of the IS91-Family. Furthermore localization of $bla_{\text{OXA-321}}$ on a plasmid and horizontal genetic mobility could be demonstrated.
Discussion: This study shows once more that betalactamases that do not confer carbapenem resistance to Enterobacteriaceae, such as OXA-10, can provide a foundation from which by as few as one point mutation, novel enterobacterial carbapenem resistance can arise. Mobile genetic elements associated with such genes might care for their spread resulting in quick emergence of carbapenem resistance in clinically relevant bacteria, from unsuspected betalactamases not usually screened for in diagnostic PCR.

PRP50
Molecular Identification and Characterization of Drug-Resistant *Mycobacterium tuberculosis* using Hydrogel DNA Microarray

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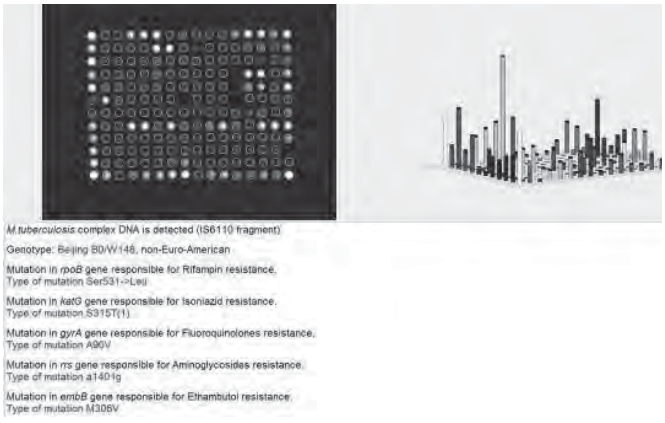
Question: The prevalence of drug-resistant strains of *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), requires development of new measures to control this threat. Rapid and accurate diagnosis of the pathogen and its drug susceptibility pattern are both essential for timely initiation of treatment, and for limiting the spread of resistant strains among the population.
Materials and Methods: A molecular assay based on original technology of hydrogel microarrays has been developed for fast identification of multidrug (MDR) and extensively drug resistance (XDR) TB. The microarray comprised of immobilized oligonucleotides based on the corresponding sequences of the IS6110 region, the sequences of *rpoB, katG, inhA, ahpC, gyrA, gyrB, rrs, eis, embB* genes with the mutations that act as markers for identification of MDR and XDR TB, and the sequences of SNPs that identify different TB genotypes. The procedure included multiplex amplification of analyzed fragments of mycobacterial genome with simultaneous fluorescent labeling of PCR-products followed by hybridization on the microarray. Hybridization images were processed with fluorescence reader equipped with proprietary software (Fig.1).
DNA samples from collection of respiratory specimens and clinical isolates were used for evaluation of the assay. Strains resistance to rifampicin, isoniazid, fluoroquinolones, aminoglycosides, ethambutol was determined using a Bactec MGIT 960 system.
Results: A developed method provides the detection of *M. tuberculosis* complex DNA with the simultaneous identification of 114 genetic markers of the resistance to rifampin, isoniazid, fluoroquinolones, aminoglycosides, ethambutol and determination of clinically relevant TB genotypes, such as Beijing, Beijing B0/W148, Haarlem, LAM and Ural. Compared to conventional drug susceptibility testing, the sensitivity and specificity of the assay were 97.4% and 96.2% for rifampin; 98.0 and 91.5% for isoniazid, 94.1% and 85.2% for fluoroquinolones; 95.1% and 86.4% for aminogycosides; and 67.4% and 90.5% for ethambutol, correspondingly.
Conclusions. The assay allows one to perform analysis of the *M. tuberculosis* genome with simultaneous drug resistance profiling and determination of endemic and clinically relevant genotypes of TB causative agent. The format of the test as 'one sample - one PCR tube - one microarray' enables parallel analysis of dozens of specimens within a single day. The test has been implemented into several institutions of the TB control system of Russia, and it has shown itself to be a reliable and inexpensive method for fast identification of drug-resistant TB strains.
Figure legend. Analysis of DNA from XDR TB strain using the developed assay. A bar graph of normalized signals of microarray elements is shown on the right.

Figure 1



PRP51

Effect antibiotics consumption for E.coli resistance on medicine wards of Dietrich Boenhoffen Klinikum

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Escherischia coli is not only common commensal of digestive tract but also often facultative pathogen responsible for various spectrum communicable diseases: sepsis, wound and urinary tracts infections. Since 1995 till 2013 it was observed E.coli resistance upward trend, which reverse at the end of year. Hospitals antibiotics consumption is nowadays not the most important factor of antibiotics resistance, but we wish to know how strong effect has it in our hospital. We have analyzed E.coli resistance on 3 general medicines wards of our hospital between 2011 and 2013, and antibiotics consumption in 2012 -2013. There was not observed any statistic significance between departments towards antibiotic resistance against groups with seldom use: ureidopenicillins, cephalosporins, aminoglicosides, carbapenems and co-trimoxasole. Only aminopenicillines and fluochorochinolones resistances were presented significance differences between wards. Both groups are the most popular in our hospital. Relationships between resistance and DDD100 are not linear. FL consumption seems to have stronger and rapid effect. Although AP wards resistance trend mostly duplicated general it can find also periods with a difference. That suggests weak but present long term effect. That has proved that antibiotic consumption remain still important risk factor. Consumption changings seems to be an independent factor

PRP52

Inducible high-level beta-lactam resistance in *mecC*-carrying *Staphylococcus aureus*

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Introduction: Recently, a novel low-affinity penicillin-binding protein (PBP) encoded by *mecC* (PBP2c) mediating methicillin-resistance in *Staphylococcus aureus* was discovered. The *mecC* gene shares only 70% DNA homology to *mecA* and is embedded within the chromosomal cassette SCC*mec* type XI (along with the regulatory elements *mecRI/mecI*). We proofed that *mecC* is the genetic determinant providing beta-lactam resistance in SCC*mec* type XI-harboring *S. aureus* strains. However, *mecC*-MRSA isolates were shown to exhibit comparatively low MICs for beta-lactam antibiotics compared to *mecA*-MRSA carrying other SCC*mec* elements.
Materials and Methods. A *mecC*-harboring *S. aureus* strain (w44646, *spa* type 843 and MLST type ST130) was grown in the presence of incrementally increased oxacillin concentrations starting at sub-inhibitory concentration (approx. 0.5 MIC of the clinical isolate). The oxacillin concentration was increased up to 100 x MIC of the parent strain. The resulting stable high-level resistant strains were analyzed by whole genome sequencing and transcriptional analyses of *mecC* and its regulators *mecRI* and *mecI* were performed.
Results: The stepwise increase of the oxacillin concentration led to a highly resistant strain with a MIC of >256 µg/ml for oxacillin and cefoxitin, respectively. Interestingly, whole genome sequencing revealed neither a mutation within *mecC* nor a mutation within its regulators or elsewhere within the SCC*mec* XI element. Nevertheless, in the induced strain, the expression of *mecC* was strongly up-regulated by oxacillin induction compared to its parent strain (443-fold compared to 92-fold). Also, the expression of both *mecI* and *mecR* was up-regulated by oxacillin induction (15-fold and 14-fold, respectively).
Conclusion. We showed that a *mecC*-mediated MRSA is capable to develop and exhibit *in vitro* a highly resistant phenotype comparable to *mecA*-mediated MRSA. Besides altered β-lactam affinity properties of PBP2c, our results indicate that the regulation of the protein expression may play an essential role in the development of this resistant phenotype. Although *mecC*-harboring *S. aureus* strains exhibit frequently MIC values below or just above the breakpoint for oxacillin and/or cefoxitin, consequently, a beta-lactam antimicrobial chemotherapy should not be taken in consideration. Further studies are warranted to identify the mechanisms involved in *mecC* regulation and its role in the MRSA resistance phenotype.

PRP53

Susceptibility of non E. coli uropathogens to nitroxoline and the *in vitro* pH-activity relationship

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Introduction: Urinary tract infections (UTI) are one of the most common infectious diseases in humans, with approximately 150 million cases yearly worldwide (1). The treatment of UTI in outpatients has become more complicated because of rising antimicrobial resistance (R) in E. coli (ECO) and other uropathogens (2, 3, 4). Nitroxoline (5-nitro-8-hydoxyquinoline, NTX) has become an attractive drug for the treatment of acute and recurrent UTI (5). In a previous study, NTX showed uniform *in vitro* activity against a German collection of ECO urine isolates, irrespective of the R profile (6). The objectives of the present study were i) to evaluate the *in vitro* activity of NTX in comparison to nitrofurantoin (NIT) against various uropathogens other than ECO and ii) to define the relationship between pH and the *in vitro* activity of NTX.
Materials and Methods: MICs of NTX and NIT were determined by the microdilution method according to the standard ISO 20776-1 (7) for a panel of 211 organisms including 101 Proteus mirabilis (PMI), 30 Klebsiella pneumoniae (KPN), 30 Morganella morganii (MMO), 20 P. vulgaris (PVU), and 30 Staphylococcus saprophyticus (SSA). In addition, MICs of NTX and NIT were determined against 27 selected strains (including ECO) at pH values of 5.5, 7.4 and 8.0.
Results: MIC-50/90 values (mg/L) of NTX for PMI, KPN, MMO, PVU and SSA were 8/8, 4/8, 4/8, 4/4 and 8/8, while those of NIT were 128/128, 64/128, 32/32, 64/64 and ≤16/≤16, respectively. The highest observed MICs were 8 mg/L for NTX and ≥512 mg/L for NIT. MICs of both drugs determined at the three pH values are given in the Table. NTX activity was increased at the acidic pH and slightly decreased at the alkaline pH, compared to activity at pH 7.4. However, the magnitude of difference in activity varied between species, with the greatest shift seen for PMI and staphylococci. NIT also tended to be most active at the acidic pH.
Conclusion: NTX showed almost identical *in vitro* activity against the five uropathogenic bacterial species tested and was up to 16-fold more active than NIT. NTX exhibited optimal antibacterial activity at acidic pH.

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Figure 1

Table: MICs of NTX and NIT at pH 5.5, 7.4 and 8.0 (mg/L)

Species (n)	pH 5.5		pH 7.4		pH 8.0	
	NTX	NIT	NTX	NIT	NTX	NIT
E. coli (9)	0.5 – 4 (2)*	≤16 – 128 (≤16)	2 – 8 (2)	≤16 – 256 (≤16)	2 – 8 (4)	≤16 – 256 (32)
K. pneumoniae (9)	2 – 32 (4)	≤16 – ≥512 (64)	2 – 32 (8)	≤16 – ≥512 (64)	2 – 32 (8)	32 – ≥512 (64)
K. oxytoca (1)	4	32	8	32	16	64
P. mirabilis (3)	0.25 – 0.5	32 – 64	8	128	8 – 16	256
S. aureus (2)	0.5 – 2	≤16	2 – 8	≤16	8	32
S. saprophyticus (3)	1 – 4	≤16	8 – 16	≤16	16 – 32	≤16

*median MIC in parentheses

PRP54

Occurrence of CTX-M-15 producing Escherichia coli belonging to clonal group O25B:H4-ST131 in Germany, 2010

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Introduction: CTX-M-15 extended-spectrum-beta-lactamase (ESBL) producing Escherichia coli (ECO) strains belonging to clonal group O25b:H4-ST131, have emerged worldwide among the last decade (1). However, data on the occurrence of these isolates in Germany are scarce. The objective of this study was to evaluate the prevalence of CTX-M-15 producing strains belonging to the ECO clonal group O25b:H4-ST131 in Germany.
Materials and Methods: 505 ECO (diverse specimens) from hospitalized patients and 499 ECO (urine isolates only) from outpatients collected in 43 medical laboratories during a German resistance surveillance study in 2010 were analysed. Susceptibility of strains was tested for a panel of antibiotics according to the standard ISO 20776-1 and interpreted by EUCAST criteria (v4.0) (2, 3). ESBL-producing ECO were confirmed according to the procedure described by the CLSI (4). Isolates with an ESBL phenotype were further characterized by molecular biological technics. CTX-M-15 producing ECO were screened for the presence of the *rfbO25b* gene that is associated with the clonal group O25b:H4-ST131.
Results: 91 (18%) and 40 (8%) ECO from in- and outpatients were ESBL producers, respectively. CTX-M ESBLs were present in 84/91 (92.3%) ESBL-producing isolates from hospitalized patients and in 39/40 (97.5%) ESBL-producing isolates from outpatients. One clinical isolate harboured a new variant, CTX-M-143, that differed from CTX-M-15 in only one amino acid substitution (Asn173Ser). A CTX-M-15 ESBL was found in 44/91 (48.4%) hospital isolates and in 27/40 (67.5%) community isolates. Screening for the *rfbO25b* gene was positive for 47.7% (21/44) and 70.4% (19/27) of $bla_{\text{CTX-M-15}}$ strains isolated from in- and outpatients, respectively. All 21 hospital isolates of clonal group O25b:H4-ST131-CTX-M-15 were non-susceptible to fluoroquinolones (FQ) and 3rd generation cephalosporins (3GC), while 38.1%, 52.4%, 57.1%, 95.2%, 100% and 100% were susceptible to aminoglycosides, piperacillin-tazobactam, co-trimoxazole (SXT), fosfomycin (FOS), colistin, and carbapenems, respectively. Of the 19 outpatient isolates belonging to O25b:H4-ST131-CTX-M-15, all were non-susceptible to 3GC, while susceptibility rates were 5.3%, 15.8%, 94.7%, and 100% for FQ, SXT, FOS and nitrofurantoin, respectively.
Conclusion: ECO isolates belonging to clonal group O25b:H4-ST131 seem to be widely disseminated among CTX-M-15-producing strains recovered from patients in Germany.

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PRP55

Cumulative Antimicrobial Susceptibility Reports - Analyses of the Influence of Different Parameters

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Introduction: Many clinical microbiology laboratories generate cumulative antimicrobial susceptibility reports on a regular basis. Criteria for selection of tests aggregated by these reports, however, are often obscure. The CLSI has published a guideline for presentation of these data. We sought to describe the influence of the different parameters suggested by this document.
Materials and Methods: Susceptibility data of microorganisms were stored in a database and program scripts were developed to assess the consequences ensuing from the various algorithms suggested for selection of isolates.
Results: The most pronounced effect was caused by exclusion of screening cultures for MRSA which decreased the MRSA rate in some cases to one fourth. Whereas inclusion of the first isolate only of a species during the time period analyzed stresses the susceptibility results for bacteria that are present at patient admission, this strategy underestimates the resistance burden added by nosocomially acquired organisms. This difference was most often below 5% but could be as high as 10%. Not unexpectedly, this effect was absent for organisms that are usually acquired pre-clinically (e.g. *S. pneumoniae*).
Conclusion: We recommend exclusion of screening cultures and suggest the laboratories communicate the strategy used for strain selection.

PRP56

How dead is dead in biofilm-associated infections?: Investigation by fluorescence in situ hybridization (FISH)

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Introduction: Biofilm-associated infections pose a significant risk for patients, since they tolerate higher concentrations of antibiotics than measured as minimal inhibitory concentrations (MIC). Standard clinical diagnostics comprise cultivation and testing for antibiotic susceptibility. However, these tests imply disintegration of the biofilm and growth of the bacteria *in vitro*. Therefore the routine procedures might miss bacteria in stationary phase, presumable persister cells and give no information about spatial distribution of the viable cells or active biofilm layers.
Materials and Methods: 16S rRNA directed fluorescence in situ hybridization (FISH) allows visualization and quantification of bacterial ribosome-containing, metabolically active cells in situ within biofilms. We used FISH to visualize the effect of antibiotics and antiseptics and measured the amount and distribution of viable and active bacterial cells in the biofilms. *In vitro* results were compared to in vivo grown staphylococcal biofilms (animal model), and also from clinical samples, to translate laboratory research results to the clinical situation.
Results: FISH allowed to quantify and localize the effect of antibiotic and antiseptic treatment on *in vitro* and in vivo grown staphylococcal biofilms using FISH. FISH showed ribosome-containing, metabolically active bacterial cells in biofilms, which were treated according to MIC-measurements and which remained culture-negative. We found a statistically significant correlation between antibiotic and antiseptic treatment duration/type of antibiotic and amount of FISH-positive cells in the biofilms.
Discussion: Biofilm characteristics are important features in antibiotic susceptibility of infections in the patients. Our findings confirm the higher tolerance of bacteria toward antibiotic treatment in biofilms both *in vitro* and in the clinical setting. They stress the point that our current diagnostic techniques regarding cultivation and antibiotic resistance testing *in vitro* are not satisfactory. In situ techniques might further our knowledge about the efficiency of antibiotic treatment on biofilms in vivo.

PRP57

Report of the National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria on Carbapenemases in Germany in 2013

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Objectives: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drugs against gramnegative bacteria will be introduced into clinical practice within the next years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is challenging for the microbiological laboratory.
Materials and Methods: The National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts allows the detection of still unknown β-lactamases.
Results: A total of 3430 isolates were investigated for carbapenemases in the National Reference Laboratory in 2013. Carbapenemases were found in 699 Enterobacteriaceae strains (38.2%), 215 *P. aeruginosa* (21.4%) and 420 *A. baumannii* (94.6%). The most frequent carbapenemases in Enterobacteriaceae were OXA-48 (41.3%), VIM-1 (16.3%), KPC-2 (15.5%), NDM-1 (12.7%) and KPC-3 (7.3%). In *P. aeruginosa* VIM-2 was the most frequent carbapenemase (83.3%).

OXA-23 was the most frequent carbapenemase in *A. baumannii* (71.4%) followed by OXA-72 (12.6%) and OXA-58 (11.2%).
Conclusions: Almost all carbapenemases found worldwide also arrived in Germany. However, the molecular epidemiology in Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Israel or the USA. Compared to data from the previous years the frequency of NDM-1 producing *Enterobacteriaceae* increased considerably. An ongoing surveillance of resistance determinants is necessary, especially for infection control and diagnostics.

PRP58

Resistant bacteria in water environments - Quantification, identification and susceptibility testing of bacteria in municipal and hospital effluents

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Resistant bacteria in water environments are an increasing concern. The question has been raised about the clinical impact of environmental resistance and currently little is known about the dynamics of resistant bacteria and genes encoding resistance in sewage treatment plants.
We compared different effluents by selective, non-selective, and enrichment culture (Table1). The number of at all cultivable bacteria, enterobacteria, extended spectrum ß-lactamase (ESBL) and carbapenemase producing bacteria (CRB), MRSA and vancomycin resistant enterococci (VRE) were determined. The water samples were taken from a (i) central municipal sewage treatment plant with incoming water of about 24.6 Mio m3/yr (CME_IN)and respective outflow (CME_OUT), (ii) a defined urban effluent (DUE) of solely human origin, (iii) and an effluent (420.000 m3/yr) of a 1,200-bed university hospital (HOE). Samples were taken over a period of two years and more than 3000 cultured bacteria were identified by MALDI-TOF MS fingerprinting with Maldi Biotyper (Bruker). Respective resistances were confirmed by Etest® (Oxoid), ESBL detection disks (MAST) or carbapenemase identification disks (Rosco) and Check-MDR®-Array (Check-Points).
According to Table1 (**see appendix1**) counts of at all cultivable bacteria and enterobacteria were nearly stable in all locations tested except for CME_IN with at least 10-fold higher concentrations in spring and autumn compared to samples taken in summer. Highest concentrations were found in CME_IN, lowest in CME_OUT. Thereby Enterobacteria emerged in a 10-fold lower concentration than at all cultivable microorganisms. ESBL were detected in all samples (except CME_OUT). Compared to the counts of overall enterobacteria, ESBL occurred in a 100- to 1000-fold lower concentration. All isolates tested by Check-MDR-Array were detected as CTX-M1. CRB were rarely isolated, those found were only isolated after enrichment culture; none were found in all samples CME_OUT even after enrichment. After sewage treatment process no resistances were found, indicating that no or few resistant bacteria are discharged into receiving water environment. Of note is that VRE are less present in DUE of solely human origin than in HOE and CME_IN consisting also of other environmental sewage and agricultural waste water also of animal origin. Therefore, VRE seem to be less prevalent in wastewater of defined urban origin. Direct plated samples and enrichment culture of CME_OUT revealed several enterobacteria isolates but none of them could be determined as ESBL. Most probably, this is caused by non-selective sewage treatment conditions and a resulting loss of plasmids. Furthermore no cultivable MRSA were found in any sample analyzed, although mecA-PCR showed positive results indicating that MRSA might be in a non-cultivable state.

Figure 1

Table 1 Bacterial counts revealed by direct plating on different agar media per millilitre

	CME_IN	DUE	HOE	CME_OUT
Summer 2012				
CASO 37°C	8.73E+05	Not done	8.59E+05	1.02E+03
Lactose-TTC	Not done	Not done	Not done	Not done
chrom ESBL	2.65E+03	Not done	1.90E+03	n.a.e
MacConkey+Ertapenem	n.a.e	Not done	n.a.e	n.a.e
chrom MRSA	n.a.e	Not done	n.a.e	n.a.e
chrom VRE	1.60E+03	Not done	3.60E+03	n.a.e
Autumn 2012				
CASO 37°C	3.15E+07	6.40E+05	1.33E+06	6.50E+02
Lactose-TTC	1.71E+06	2.74E+04	6.66E+05	5.83E+02
chrom ESBL	2.53E+03	2.23E+03	2.66E+04	n.a.e
MacConkey+Ertapenem	o.a.e.	o.a.e.	o.a.e.	n.a.e
chrom MRSA	n.a.e	n.a.e	n.a.e	n.a.e
chrom VRE	1.45E+03	o.a.e.	5.80E+03	n.a.e
Spring 2013				
CASO 37°C	4.10E+07	3.20E+05	1.93E+06	1.20E+02
Lactose-TTC	9.50E+05	1.33E+05	2.42E+05	1.03E+02
chrom ESBL	5.41E+03	1.70E+02	9.25E+03	n.a.e
MacConkey+Ertapenem	o.a.e.	o.a.e.	o.a.e.	n.a.e
chrom MRSA	n.a.e	n.a.e	n.a.e	n.a.e
chrom VRE	2.80E+03	n.a.e	4.90E+03	n.a.e
Summer 2013				
CASO 37°C	7.38E+06	1.18E+06	7.40E+06	8.40E+02
Lactose-TTC	2.44E+06	4.98E+05	2.54E+05	6.48E+02
chrom ESBL	7.21E+04	1.15E+02	1.22E+04	n.a.e
MacConkey+Ertapenem	n.a.e	n.a.e	o.a.e.	n.a.e
chrom MRSA	n.a.e	n.a.e	n.a.e	n.a.e
chrom VRE	1.06E+04	2.45E+02	2.20E+03	n.a.e

o.a.e. only after enrichment *n.a.e. none after enrichment*

PRP59

Survival of *Escherichia coli* on copper surfaces is enhanced by glutathione

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Copper surfaces in contrast to stainless steel or plastics surfaces for instance leads to rapid killing of microorganisms. This effect has been known for long times and could nowadays be important for public health especially in hospitals, preventing distribution of multiple-drug resistant bacteria. The killing process has been shown for Gram-negative bacteria such as pathogenic *Escherichia coli* (1), *Pseudomonas aeruginosa* (2), *Burkholderia cepacia* (3), *Salmonella enterica* (4) as well as for the Gram-positive *Staphylococcus aureus* (5), for viruses (6) and for yeasts (7). Direct contact of the living cells to the copper surface, followed by copper ions released from this surface are the assumption for the antimicrobial properties. The inactivation process: contact to copper, release of copper ions and killing of bacteria by these ions depends on different parameters such as temperature, humidity, ionization/ corrosion, dry or wet copper surface and organic material. If using copper surfaces as antimicrobial agent in hospital settings, the question arises which molecular mechanisms are responsible for killing microorganisms. This knowledge is essential to optimize handling and cleaning of copper surfaces and to avoid formation of copper-surface-resistant pathogenic bacteria. The feature of Dps (DNA binding protein of starved cells) to help microorganisms to tolerate copper ions and metallic copper surfaces was determined. This cytoplasmic protein which is abundant in stationary phase cells in *E. coli* protects the DNA by binding non-specifically and sequesters iron in the interior of the dodecameric protein (8). If DNA is the target of killing cells at copper surfaces in proteobacteria, Δ*dps*-mutants should therefore show enhanced killing on copper surfaces, especially in cells without copper efflux systems such as the coppertransporting P-type ATPase CopA. While Dps did not contribute to copper tolerance, we could demonstrate that glutathione, which is also involved in copper ion resistance (9), increased survival of *E. coli* on solid copper surfaces. Since proteobacteria contain glutathione but most Gram-positive bacteria not (10), presence or absence of this important cellular thiol compound explains the fundamental difference in the way how Gram-negative and -positive bacteria were killed on solid copper surfaces.

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PRP60

A new antimicrobial coating killing antibiotic resistant pathogens

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Microbial biofilms seriously affect human health and cause bio-corrosion and biodegradation of almost all kinds of materials. The International Space Station (ISS) is a confined habitat which provides a special environmental niche for microorganisms with direct or indirect impact on health, safety and performance of the crew as well as on the stability of the structural material. Biodegradation of critical materials may result in system failure and this may jeopardize the crew. Studies performed in Mir indicated that microbial damage to polymers and metals resulted in malfunctioning and even breakage of air conditioners and water recycling systems (1,2). In search of an antimicrobial material which can be used on the ISS, we studied the antimicrobial activity of a novel antimicrobial surface coating based on micro galvanic elements formed by silver and other precious metals with surface catalytic properties.
The antimicrobial coating efficiently inhibited growth of multiple antibiotic resistant strains of the opportunistic pathogens, *Staphylococcus aureus*, *Staphylococcus epidermidis, Enterococcus faecalis* and *Enterococcus faecium* as shown by growth inhibition tests on agar surfaces and in batch cultures. It also strongly reduced biofilm formation of diverse clinical *Enterococcus* and *Staphylococcus* isolates as monitored by Scanning Electron Microscopy (SEM). Growth and biofilm formation of *Legionella* in a long-term experiment in a simulated drinking water pipeline inoculated with *Legionella* was also strongly reduced by the novel antimicrobial coating. The antimicrobial material was implemented in the BIORISK experiment on the ISS and showed significantly higher antimicrobial activity after one year exposition on the ISS than an electroplated silver coating applied onto a stainless steel surface. In contrast to the silver coating, from the novel antimicrobial material no bacteria could be cultured. Only after activation of the microorganisms detached from the antimicrobial coating by the addition of medium, some bacteria became culturable. 16S rRNA gene sequencing affiliated the majority of them to the genus *Staphylococcus* (~ 60%), followed by bacilli (~ 18%) and enterococci (~12%).
In summary, the antimicrobial coating kills pathogenic bacteria irrespective of their Gram behavior. Long-term maintenance-free activity of the antimicrobial coating on the ISS proved its successful application in spacecrafts. A putative mechanism of action of the antimicrobial coating is presented.

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PRP61

Antibiotic Resistance Patterns of Methicillin- resistant *Staphylococcus aureus* (MRSA) from blood cultures from hospitals in North Rhine-Westphalia, 2011-2013

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Introduction: Bloodstream infections with MRSA are potentially life-threatening and associated with higher mortality when compared to methicillin-susceptible isolates. MRSA are usually resistant to different antibiotic classes, which reduces treatment options. Therefore monitoring of resistance patterns is important for the design of treatment regimens. Here we report data on antibiotic resistance in a large representative set of MRSA bacteraemia isolates from North Rhine-Westphalia (NRW).
Materials and Methods: Microbiological laboratories in NRW were asked to send all MRSA bacteraemia isolates obtained from patients in NRW between 12/2011 and 10/2013 to a central typing facility together with information on the patient's age, sex and geographical information. Isolates (n=1906) were subjected to molecular typing, to susceptibility testing by means of microbroth MIC (panel with test substances for 14 antibiotic substance classes) and to PCR for *mec* genes. Results were interpreted according to the EUCAST standard, for mupirocin isolates exhibiting MIC`s > 32 mg/ were recorded. For selected isolates PCR for resistance genes was performed.
Results: Frequencies of resistance in addition to oxacillin were: ciprofloxacin (CIP) 96%, moxifloxacin (MFL) 96%, erythromycin (ERY) 77,7%, clindamycin (CLI) 69,7%, mupirocin (MUP) 4,4%, tetracycline (TET) 3,6%, gentamicin (GEN) 3,5%, rifampicin (RAM) 2,0%, fusidic acid (FUS) 1,5%, fosfomycin (PHO) 1,0%, daptomycin (DAP) 0,4%, tigecyclin (TGC) 0,2%, linezolid (LNZ) 0,05%, cotrimoxazole (SXT) 0,26%, and teicoplanin (TPL) 0,05%. There was no resistance to vancomycin (VAN) and MIC of 2 mg/l was found in only 5 isolates (0,26%). The most frequent multiresistance phenotype was PEN, OXA, ERY, CLI, CIP, MFL (60% of all isolates); isolates mostly belonged to the predominant HA-MRSA clonal complexes CC22 (40%) and ST225 (48%). Remarkably, 18% of all isolates were only resistant to PEN, OXA, CIP, MFL; the majority of these were attributed to CC22 (82,7%), CC45 (8,3%) and ST225 (5,2%). Resistance patterns exceeding 5 antibiotic substance classes were rare (0.2%) such as PEN, OXA, GEN, ERY, CLI, TET, CIP, MFL, RAM, PHO, TGC, TPL and attributed to ST239. The only linezolid resistant isolate did not contain the transferable *cfr*-gene.
Conclusions: Resistance to antibiotics which are relevant for treatment of invasive infections such as septicemia and pneumonia and recommended by expert panels of ESCMID und ISC[1], is still rare in MRSA isolates from blood cultures. The same applies to isolates with vancomycin MIC`s above 2 mg/l. Isolates attributed to MRSA ST239 which exhibit a broad pattern of multiresistance are rare in Central Europe so far. Import from countries where they are prevalent (Turkey, East Asia) requires special attention.

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PRP62

Biosensoric detection of antibiotic traces by a GFP-based reporter system

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Objectives: The increasing prevalence of antibiotic resistant bacteria poses a serious health threat. This is predominantly ascribed to an increased clinical and non-clinical use of antibiotics.
This study aimed at developing a biosensoric system to detect traces of antibiotics in the clinics and the environment by GFP-based reporter systems. Thus, assuming a postulated common bactericidal mechanism for different antibiotic classes, an antibiotic hypersusceptible *tolC* mutant of *E.coli* K-12 C600 was generated and transformed with reporter plasmids carrying antibiotic-inducible promoters coupled with *gfpmut2* to determine fluorescence of recombinant cells grown in the absence and presence of different antibiotics at sub-MICs.
Materials and Methods: The *tolC* deletion was introduced by a "gene doctoring" protocol. The identification of antibiotic-responsive promoters (e.g. precA for quinolone activity) was based upon qRT-PCR data for selected indicator genes. Fluorescence was measured from recombinant cells grown to early log-phase after addition of subMICs of antibiotics representing different classes.
Results: Since the initial approach to identify a universal antibiotic-inducible promoter by qRT-PCR failed, a set of reporter plasmids was developed using promoters of genes encoding proteins involved in essential metabolism, DNA damage repair, and oxidative stress response, respectively.
Representative data obtained with a precA-GFP reporter plasmid in C600 *tolC* show a detection limit for norfloxacin at 0,0064 µg/ml corresponding to 0.2 x MIC.
Discussion: GFP-based reporter systems are useful tools for rapid and sensitive detection of traces of antibiotics in order to screen environmental samples for the presence of novel antibiotic activities, in clinical (urine, blood) and in non-clinical (e.g. food) samples. It permits the classification of new potential antibacterials with respect to a proposed mode of action by

their drug specific expression pattern. Another advantage is the easy performance with potential for automatic detection and high through-put screening.

PRP63

Whole-transcriptome sequencing of *Klebsiella pneumoniae* clinical isolates

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Introduction: Resistance to available antimicrobial therapies continues to rise at an alarming rate and poses a serious threat to human health. The clinical management of *Klebsiella pneumoniae* infections is hindered due to the high prevalence of multi drug resistant strains. *K. pneumonia* is an important human pathogen causing nosocomial infections with high morbidity and mortality.
We used whole-transcriptome sequencing (RNA-Seq) to combine quantitative data on gene expression profiles with qualitative information about sequence variations to study global genotype-phenotype correlations in clinical *K. pneumoniae* isolates. Based on these findings we establisheda mass spectrometry (Sequenom MASSarray®) assay to rapidly identify genetic markers that determine pathogenicity and antimicrobial resistance.
Materials and Methods: The transcriptomes of 35 clinical *K. pneumoniae* isolates and three reference strains were sequenced on an Illumina HiSeq 2500. To be independent from strain specific genome content, a pan-genome was created by using bidirectional best BLATS hits and sequence reads were mapped against this reference with STAMPY. Consensus sequences were extracted to identify single nucleotide polymorphisms (SNPs) and for *in-silico* MLST typing. Reads not mapping to the reference were extracted and the accessory transcriptomes were assembled using Oases. Acquired resistance determinants were identified via BLAST search and antibiotic susceptibility was determined using the Vitek2 system (bioMérieux).
Results: We identified several chromosomal mutations leading to antibiotic resistance (e.g. gyrase and topoisomerase mutations) as well as several transferable resistance markers, like β-lactamases of the types KPC, IMP, CTX-M, OXA, SHV, TEM and VIM or aminoglycoside modifying enzymes (AAC, APH, ANT). An *in-silico* MLST typing revealed that the large majority of these isolates belong to the high risk and wide spread sequence types ST101 (n=13), ST258 (n=10) and ST512 (n=3). Our Sequenom MASSarray® assay detected SNPs with very high sensitivity and specificity and furthermore, the experimental work-flow has been improved to ensure that 10 hours are sufficient to obtain reliable results for approximately 50 isolates.
Discussion: The use of RNA-Seq enables studying mutations, differentially expressed genes and the occurrence of transferable resistance determinants in a single approach. The in-depth analysis revealed the presence of several resistance markers which could be linked to the antibiotic susceptibility profile of the clinical isolates. We used this information to establish a fast and reliable re-sequencing approach to rapidly identify the presence of SNPs and extra chromosomally encoded resistance determinants like β-lactamases and aminoglycoside modifying enzymes.

PRP64

Innovative whole cell-based screening technologies - "SmartScreens"

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Multidrug-resistant pathogens have emerged rapidly in the clinic and cases of community-acquired infections increased worldwide, necessitating new antimicrobial drugs. Unfortunately, new antibiotics with unprecedented mechanisms and targets have been proven difficult to find with current methods, so innovation in screening technology and prediction of suitable targets is needed.
Aim of this project is to develop whole cell-based screenings with increased sensitivity and intrinsic information on the target and MoA of compounds.
Selected promoter-reporter fusions, specific for major biosynthesis pathways, i.e. DNA, RNA, protein and cell wall biosynthesis, were constructed in different gram positive species using different reporters.
Antisense (AS) RNA strains were utilized to achieve specific knockdowns combining the power of whole cell assays (bioactivity) and sensitive target identification. Specific AS-RNA mediated gene depletion confers selective antibiotic hypersensitivity, since it results in a lower cellular pool of the cognate protein and thus sensitizes cells to inhibitors of this specific target
Furthermore, phenotypic potentiation screenings were established to identify synergistic activities of compounds with β-lactams to re-sensitize methicillin resistance in *S. aureus* (MRSA).
Moreover, we use the `essential gene paradox` of wall teichoic acid (WTA) biosynthesis to screen for late stage WTA inhibitors. Late stage WTA biosynthesis genes are essential for cell viability, whereas early steps (*tagO* and *tagA*) are not. Further, late stage genes are conditionally essential since they are dispensable in a *tagO* or *tagA* deletion background, referred to as "essential gene paradox".

PRP65

Complete sequence of a TnGBS-like multi resistance integrative conjugative element from *Streptococcus pyogenes*

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Introduction: Integrative and conjugative elements (ICEs) play an important role in the evolution of bacteria. They contribute substantially to intra-species as well as inter-species lateral gene transfer. ICEs may carry genes that provide a selection advantage to the bacteria, e. g. useful metabolic functions, resistance to physical or chemical stress, antibiotics or the immune system of an infected host. Most ICEs encode phage-like integrases for excision and integration of the element from and into the genome, respectively. In a minority of ICEs the integrase gene is replaced by a DDE transposase gene, for instance, in the TnGBS family of ICEs. Here we describe a novel TnGBS-like DDE transposase carrying ICE in the human pathogen *Streptococcus pyogenes* that carries multiple antibiotic resistance genes.
Results: Genome sequencing of the *S. pyogenes* isolate A1148, originating from an invasive infection, identified a TnGBS-like multi resistance ICE. This ICE integrated into the same chromosomal site as TnGBS2.5 in *Streptococcus agalactiae* and ICE*Sp1116* in *S. pyogenes*. The identified DDE transposase is almost identical to the DDE transposases of TnGBS2.5 and ICE*Sp1116*. In contrast to the *S. agalactiae* TnGBS family of ICEs, which lack typical cargo genes, TnGBS-like ICE in *S. pyogenes* carry antibiotic resistance genes. In addition to *erm*B and a truncated inactive *tet*M*, which were also reported for ICE*Sp1116*, we identified the resistance genes *aph*(3')-IIIa, *aad*E, *cat*(pC194), *dfr*F and a truncated inactive *sat*4* in the ICE of *S. pyogenes* isolate A1148. The isolate is resistant to the following antibiotics (minimum inhibitory concentrations are given in parentheses): streptomycin (>2048 mg/l), kanamycin (>2048 mg/l), erythromycin (>32 mg/l), trimethoprim (>512 mg/l), chloramphenicol (32 mg/l) and tetracycline (16 mg/l). Transfer of the ICE was shown by intra-species mating experiments with an average transfer frequency per donor of $3.8*10^{-4}$.
Discussion: Several TnGBS-like ICEs encoding a DDE transposase have been identified by systematic genome analysis in many streptococcal genomes, however, much more often in commensal than human pathogenic streptococci. These ICEs lack typical cargo genes. Our findings and the report of ICE*Sp1116* indicate the presence of TnGBS-like ICEs in the human pathogen *S. pyogenes* that carry antibiotic resistance genes. We observed an accumulation of multiple resistance genes in a TnGBS-like ICE, which can be readily transferred between different *S. pyogenes* strains. Although *S. pyogenes* has remained widely susceptible to beta-lactam antibiotics, the potential spread of multiple antibiotic resistances is a serious matter of concern, as it may drastically reduce therapeutic alternatives in cases of treatment failure and intolerance to beta-lactams.

PRP66

Development of resistance management strategies using evolutionary principles

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The alarming spread of resistance is threatening to render even the most routinely applied antibiotic therapies ineffective. Nosocomial strains such as MRSA or Pseudomonas aeruginosa may be well on their way to becoming the major cause of morbidity and mortality, even in the developed world. The gravity of the situation is emphasized by the 2014 WHO report on antibiotic resistance, which explicitly forwards antibiotic resistance as a major threat to public health. The traditional reply to rising resistance is the development of novel compounds, e.g. through more efficient screening methods, or the identication of new scaolds or targets. However, these approaches do not touch on the heart of the matter: Resistance is an evolutionary response to the use of antibiotics. Therefore, the introduction of novel therapeutics will inevitably be met with new resistances. In the long term, this approach is bound to fail. I will present our research group's program aimed at exploiting evolutionary insights to develop sustainable resistance management strategies. Using high-thoughput *in vitro* experiments with E. *coli* and *Pseudomonas aeruginosa*, we investigate the longer-term effects of existing therapies such as synergistic combination therapy or antibiotic rotations. Understanding the molecular mechanisms and evolutionary trajectories underlying fast resistance evolution will helpnding intelligent ways of applying existing antibiotics such that the evolution of resistance is minimized. For example, we have shown that synergistic combination therapy maximizes selection for resistance and may lead to relapse of a fully resistant bacterial population within 36 hours. The resistance was realised in E. coli through a duplication of a large genomic region containing the e ux pump acrAB. Intriguingly, the copy number of this genomic amplication increased with antibiotic concentration. These results illustrate that during combination therapy bacteria evolve resistance mechanisms that are functionally different from those they evolve during the corresponding monotherapies. Therefore, the assumption that combination therapies are preferred over monotherapies because resistance mutations are less likely, does not hold. We feel the importance of evolution in the spread of antibiotic resistance is still widely underestimated or misunderstood. Our ideas and results on antibiotic resistance evolution are therefore extremely relevant for specialists in healthcare, the pharmaceutical industry and research. The DGHM - VAAM conference would be an ideal platform to exchange ideas across disciplines, and would help spreading this important message.e pharmaceutical industry and research. The DGHM - VAAM conference would be an ideal platform to exchange ideas across disciplines, and would help spreading this important message.

MICROBIOTA, PROBIOTICS AND HOST

PWP01

The microbiome of urine and vaginal fluid in bacterial vaginosis

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Bacterial vaginosis (BV) is a disease of the female genital tract which is characterised by a shift from commensal Lactobacilli to biofilm forming anaerobic, Gram negative bacteria such as *Gardnerella vaginalis* and *Atopobium vaginae*. The symptoms are odor, discharge and itchiness, but BV is also associated with an increased risk of preterm birth and acquisition of HIV. The common treatment for BV is Metronidazole. However, due to the bacterial biofilm mode of growth the disease has a high rate of recurrence, so there is a need for an approach targeting biofilm formation of bacteria. As part of a pilot study for a subsequent clinical study that aims at finding a medical product against recurrent BV, patient samples of urine and vaginal fluid, collected from healthy and diseased women, were analysed with Illumina high throughput sequencing.
Lactobacillus was identified as the most abundant genus in urine and vaginal fluid, both in health and disease. However, the microbial communities differed in urine and vaginal fluid with respect to the next most abundant genera in health and disease. Generally, the BV communities in disease showed a significantly larger diversity of genera compared to healthy conditions. A principle coordinate analysis separated samples from health and disease, but not from urine and vaginal fluid. Most relevant for the clustering of samples in BV were the genera *Atopobium*, *Gardnerella*, *Prevotella* and *Peptoniphilus*. On the species level, main players in BV due to their significant increase in abundance were *A. vaginae* in urine and *A. vaginae*, *G. vaginalis* and *Prevotella timonensis* in vaginal fluid. By contrast, *L. crispatus* was identified as species with a protective effect since its number was decreased significantly in both, urine and vaginal fluid, in BV. Simultaneously, significant decreases were observed for *Streptococcus agalactiae*, *Escherichia coli* and *Enterococcus faecalis* in urine and *S, mutans* and *Escherichia* sp. in vaginal fluid.
Taken together, in BV the bacterial load of *A. vaginae* was significantly increased and the number of *L. crispatus* was significantly decreased in both, urine and vaginal fluid samples. Therefore, these two species can be used as biological markers for BV. Furthermore, these findings will be implicated in an *in vitro* multispecies biofilm model in order to test substances that can influence the integrity of a biofilm to find possible future drugs against recurrent BV.

PWP02

***Galleria mellonella* as alternative model to study influence of bacterial factors on innate immunity**

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Several factors are thought to be involved in the development of IBD, genetic susceptibility of the host and environmental factors as well as intestinal microbiota and the host immune system. Focus on bacteria-host as well as bacteria-bacteria interaction during colonization will help to better understand composition of intestinal microbiota and their role in induction or prevention of IBD and other autoimmune diseases.
In previous work our group assessed the influence of different lipopolysaccharide (LPS) endotoxicity on maintenance of gut homeostasis and inflammation in different mouse models.
We want to generate a high throughput model apart from the common mouse models to assess the impact of those bacterial strains and intend to use the lepidopteran insect model *Galleria mellonella*, which is widely used to study host-microbe interactions. It is especially interesting as its innate immune system shows high similarity with the mammalian system and if it produces comparable Results:

PWP03

Bacterial influence on gut homeostasis and inflammation

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The interaction and competition among commensal bacteria plays an important role during development of IBD. In healthy hosts, the well-balanced interplay between non-pathogenic symbionts supports maintenance of gut homeostasis and contributes to intestinal immunity. In genetically predisposed hosts, pathobionts - a certain group of commensals - can accumulate and trigger inflammation. However which factors cause and favour such accumulations is unknown.
In our model *E. coli* mpk can cause colitis in germ-free *IL-2*$^{-/-}$ mice due to a yet unknown mechanism while *B. vulgatus* mpk - another commensal - can in turn prevent colitis during *E. coli* mpk and *B. vulgatus* mpk co-colonization. After whole genome sequencing we identified different genes that might play a role in competition among those bacteria or become important during inflammation. To study those candidate gene sets *in vivo* we will use an invertebrate animal model, the larvae of the greater wax moth *Galleria mellonella* as it shares homology with the mammalian innate immune system.

PWP04
Fluorescent bifidobacteria for analysis of host-microbe interaction

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Bifidobacteria are an important component of the human gastrointestinal microbiota and are frequently used as probiotics. The genetic inaccessibility and lack of molecular tools has hampered a detailed analysis of the factors and mechanisms of bifidobacteria involved in adaptation to, colonization of, and interaction with the host. In the present study, a range of molecular tools were developed that will allow to close some of the gaps in functional analysis of bifidobacteria. A number of promoters were tested for transcriptional activity in *B. bifidum* S17 using pMDY23, a previously published promoter probe vector. The promoter of the *gap* gene (P_{gap}) of *B. bifidum* S17 yielded the highest reporter gene activities among the tested promoters.
Thus, P_{gap} and the pMDY23 backbone were used to construct a range of vectors for expression of different fluorescent proteins (FPs). Successful expression of green, cyan, yellow and red FPs was successfully shown for three strains representing three different *Bifidobacterium sp*. The red fluorescent *B. bifidum* S17 pVG-mCherry was further used to demonstrate application of fluorescent bifidobacteria for adhesion assays. Furthermore, this strain was successfully detected in human primary macrophages generated by *ex vivo* differentiation of monocytes. This demonstrates that detection of bifidobacteria inside relevant host cell populations is possible.
Plasmid pMGC-mCherry was cloned combining a chloramphenicol resistance marker and expression of the mCherry FP. This plasmid was successfully used to study host colonization and gastrointestinal transit time of *B. bifidum* S17 in C57BL/6J mice under specific pathogen free (SPF) as well as under germ free (GF) conditions. This revealed that *B. bifidum* S17 pMGC-mCherry is not able to stably colonize the gastrointestinal tract of SPF mice. By contrast stable colonization could be demonstrated in GF animals mice with up to 10^9 colony forming units per g feces for more than 30 days. Competition experiments revealed that *B. bifidum* S17 pMGC-mCherry is outcompeted by a normal bacterial microbiota within 3-4 days.

PWP05
The microbiome of early childhood caries

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Dental caries, also known as “tooth decay” or “cavities” is one of the most abundant chronic infectious diseases among children. Early childhood caries (ECC) can be caused by several reasons (for instance baby-bottle syndrome), but in every case it is influenced by the dental plaque on the tooth surface composed of bacteria and fungi. Since it is known, that there are more than 700 different microorganisms living in the human oral cavity, the etiology of caries is still not understood. In ECC, several conditions are especially extreme and it is known that ECC-associated bacteria are acidogenic (Palmer *et al.* 2010). As it is known that the fungus *Candida albicans* is involved in ECC-formation (Raja *et al.* 2010) and interacts with the caries-associated bacterium *Streptococcus mutans* (Sztajer *et al.* 2014), this eukaryote will also be included in the metatranscriptome analysis. Therefore we are planning to set up a cohort-study and to recruit at least 400 children from the Kindergartens (aged between three years and six years) in Braunschweig/Germany. These children will be sent to the dentists, who will collect samples from the dental plaque and saliva of healthy children and ECC-patients. The parents will get a questionnaire where we will find out the dietary habits, teeth-brushing habits and migration background. Using Illumina deep sequencing, we will analyze the taxonomic composition and the metatranscriptomes of dental plaque of children carrying dental caries compared to healthy children.
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PWP06
The probiotic *E. coli* Nissle 1917 does not engage in conjugation with EHEC?

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The probiotic *Escherichia coli* strain Nissle 1917 (EcN) has been successfully used for almost a century in the treatment of several intestinal diseases, e.g. diarrhea, ulcerative colitis and constipation. An important feature for its application as a drug is its biological safety. During co-cultivation experiments we observed that EcN does not act as recipient for a conjugative, virulence-associated plasmid of EHEC O104:H4 strains in contrast to other *E. coli* strains. To prove EcN’s genetic stability liquid mating assays were performed. Spontaneous rifampin-resistant mutants of EcN and *E. coli* K-12 strains (MG 1655, DH5α and HB101) were selected for differentiation. The EHEC O104:H4 strain TY3730 which harbors a conjugative plasmid conferring resistance against extended-spectrum beta-lactamases (pESBL) was used as donor. Co-cultivation experiments were performed at a ratio of 10:1 (recipient:donor) for 24 hours and transconjugants were selected on LB-agar plates containing rifampin and ampicillin. Preliminary results showed no transconjugants for EcN. In contrast, the conjugation rate of *E. coli* MG 1655, DH5α and HB101 was 1.4 %, 2.3 % and 0.01 %, respectively. Furthermore, an involvement of EcN’s cryptic plasmids and capsule could be ruled out by testing corresponding isogenic mutants of EcN for their recipient ability. Other factors that might be involved in this effect are currently under investigation. Additionally, the observed conjugation resistance of EcN will be reconfirmed by using other conjugative plasmids as well as by performing surface mating experiments.

PWP07
Evidence that the gastrointestinal mucus layer interferes with colibactin activity of *Escherichia coli* Nissle 1917

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Recent publications reported the probiotic *E. coli* strain Nissle 1917 (EcN) to harbor the *pks*-island which encodes the genotoxin colibactin and induces fatal DNA double strand breaks (DSB) in eukaryotic cells. In order to investigate the effect of mucus non-mucus producing HeLa, moderate amounts of mucus producing LS174T and the heavy mucus-producing HT29 MTX-E12 cell line was employed.
In a first approach LS174T cells were cultivated in DMEM with 10% FCS or with 50% FCS or with 10% FCS plus 1 mM propionate and infected with EcN. The latter two culture conditions induced enhanced mucus production and subsequently a smaller number of cells which suffered from DSB as demonstrated by CometAssay®. In a second approach cell viability of EcN infected HeLa and HT29 MTX-E12 cells as the consequence of DSB was analysed by flow cytometry after propidium iodide and Hoechst 33342 staining. After 24, 48 and 72 hours post EcN infection cell viability was highest for HT29 MTX-E12 cells which had produced a thicker and denser mucus layer than LS174T cells. In contrast, the number of dead cells was highest for the non-mucus producing HeLa cells.
These results reveal a protective effect of the mucus (layer) most likely due to the inability of *pks*-positive *E. coli* to get into direct contact with the epithelial cells because of the mucus barrier. This is in line with earlier studies in which a direct contact between the colibactin producer and the epithelial cells was proven to be essential for the induction of DSB. Future plans are to enhance mucus production of HT29 MTX-E12 cells by culturing in semi-wet interface with mechanical stimulation and subsequent analysis of DSB via a γH2AX phosphorylation assay.

PWP08
Effects of bacterial isolates from murine feces in activation and maturation of BMDC

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The gut microbiota plays an important role in the development of inflammatory bowel disease (IBD). The intestinal immune system is permanently exposed to a high variety of commensal bacteria. It could be shown that bone marrow derived dendritic cells (BMDC) will maturate completely when stimulated with *Escherichia coli* mpk. In contrast, BMDC will arrest in a semi-mature state after stimulation with commensal *B.*

vulgatus. No further maturation was detected after restimulation with *E. coli* mpk. To analyze whether this effect is specific for *B. vulgatus* we isolated three bacterial isolates (presumable *Bacteroides vulgatus, Bacteroides sartorii, unknown gram-negative bacteria*) from murine feces and differentiated them according to activation and maturation of BMDC. The idenfitication of the unknown species via biochemical and MALDI-TOF analysis failed. It could also not be identified by sequencing 16s rDNA.
The unknown bacteria revealed proinflammatory effects compared to *B. vulgatus* or *B. sartorii*. As shown by further experiments we will characterize the unknown bacteria via T-cell proliferation assays, analysis of proinflammatory effects using a T-cell transfer model of chronic colitis and whole genome.

PWP09
Role of the ethanolamine utilization pathway in bacterial competition

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The microbiota plays a central role in many physiological reactions and disruptions can lead to diseases such as Inflammatory Bowel Disease (IBD). The underlying molecular mechanisms however are still not understood. The main challenge is often the vast number of microorganisms underlying interbacterial interactions as well as their effects on host immunity.
Caenorhabditis elegans is potentially useful to study the role of certain microbes in IBD given the combined power of bacterial and nematode genetics. However, knowledge on the effects of bacteria on its immune system is still rudimentary e.g. it is debated whether pathogenic bacteria have a metabolic advantage when colonizing the intestine thereby contributing to perturbation.
We therefore want to investigate the immune response of *C. elegans* after exposure to different bacteria strains. In further experiments we want to use this system to examine various metabolic pathways specific to pathogenic bacteria or pathobionts (commensals with a pathogenic tendency) to evaluate their role in the colonization process. In previous studies our lab identified the ethanolamine utilization cluster as promising candidate pathway that could enable bacteria to use ethanolamine as a non-fermentable carbon source accessible during inflammation.

PWP10
Exploring the role of the urinary tract microflora on the outcome of urinary tract infections

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Acute cystitis is one of the leading bacterial infections in otherwise healthy women. It is primarily caused by uropathogenic *E. coli* (UPEC). Treatment of urinary tract infections (UTIs) normally necessitates antibiotic therapy. However, this becomes increasingly more difficult due to the emergence of antibiotic resistant bacteria and the ability of UPECs to hide intracellularly. Of note, recent studies disproved the commonly accepted theory that urine is sterile in the UT. Instead, various uncultivable bacterial species in addition of bacteria of the vaginal flora, e.g. lactobacilli, were commonly found to colonize the UT. The role of the bacterial community in the establishment of UTI however is yet unknown. In addition, there is increasing interest in how the gastrointestinal microbiota impact on the emergence of diverse bacterial infections and diseases of the UT. In the present study, we sought to investigate a potential beneficial contribution of probiotic lactobacilli on the prevention of UTI. For this purpose, we established a mouse model to characterize the murine microbiota in healthy individuals and during acute UTI by means of 16S rDNA PCR. Furthermore, we analyzed the role of lactobacilli on the severity of UTI. Unraveling the impact of the probiotic microflora on UPEC during the course of UTI will be crucial for the development of effective alternative antibiotics-free prevention strategies to combat recurrent UTI.

PWP11
Optimization of RNA-based stable isotope probing to link structure and function of microbial communities in the intestinal tract of mice

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RNA-based stable isotope probing (SIP) is a promising technique to identify microorganisms with particular physiological traits within a complex microbial community. Tracking an isotopic label down into the ribosomal RNA provides phylogenetic insight into active microbial populations which have assimilated the labeled tracer substrate into their nucleic acids under *in situ* conditions [1, 2]. The technique has been successfully applied to intestinal ecosystems before [3]; we use it to identify carbohydrate-degrading bacteria in the intestinal tract of mice. Clearly, successful detection and identification of active community members necessitates a clear separation of isotopically labeled RNA molecules by isopycnic density gradient ultracentrifugation from the abundant, unlabeled background RNA [4]. However, previous studies also reported that contamination of labeled RNA with unlabeled RNA cannot be completely avoided [5, 6], probably due to RNA secondary structures impeding the centrifugation-based separation.
To implement the SIP-technology in the lab, experiments were performed with ^{13}C-labeled *E. coli* RNA, extracted from *E. coli* K12 cells grown in a minimal medium with ^{13}C-glucose as sole carbon source. When centrifuged separately, labeled and unlabeled RNA nicely showed peak concentrations at densities of ~ 1.82 g ml^{-1} and ~ 1.79 g ml^{-1}, respectively. However, when mixed in equal ratios prior to centrifugation, no clear separation was observed and the RNA concentration peaked at medium densities. Based on the literature [5, 6], addition of formamide and a reduction of the total RNA load in the centrifugation step might alleviate this artifact. However, using less RNA means a loss of sensitivity, and formamide is a toxic compound. Preliminary experiments suggest that addition of 1M urea, a compound well known to denature RNA secondary structures, improves the separation of isotopically labeled and unlabeled RNA during simultaneous centrifugation in CsTFA. Ongoing research aims to substantiate this finding, using not only *E. coli* RNA but also RNA extracted from mouse fecal samples, and to evaluate potential effects of urea on the subsequent amplification of precipitated RNA from the centrifugation gradients by RT-PCR.

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PWP12
Identifying single bacterial species which can interfere with *Salmonella* Typhimurium induced colitis

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The mammalian gut harbors a highly diverse microbiota with more than 500 different species. They contribute to nutrient supply, immune modulation and interfere with enteric infections (colonization resistance). The interactions between the host and its microbiota leading to protection against infections are highly complex and therefore not fully understood. To investigate those interactions we have made use of a reductionist gnotobiotic mouse model system based on a defined and low complex microbiota. These mice are highly susceptible to enteric infection with the human pathogen *Salmonella enterica* serovar Typhimurium and develop colitis within 12 hours after oral infection. In contrast, mice with a complex microbiota show no or delayed colitis. *Salmonella* Typhimurium is evading the host defense mechanisms by exploiting the inflammatory response of the host for its own growth by using mucus derived carbohydrates as energy source and a variety of anaerobic electron acceptors. Our aim is now to investigate if single bacterial species from the microbiota are sufficient to prevent colitis or if a consortium of several species is required for protection.

PWP13
Revealing the response of the active mucosal microbiota from the rat colon to a change in diet

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The intestinal microbiota is a densely inhabited microbial community that provides many functions for the host including the degrading of non-digestible nutrients into useful metabolites, the synthesis of vitamins and the regulation of the immune system. The microbiota is known to evolve over the lifetime of the host and to respond to different environmental influences. The focus of the study was to observe the response of the mucosal microbiota from a high-fat diet rat model as well as from a chow fed diet rat model to a change in diet. To analyse the response of the gut microbiota to a switch in diet 16S rRNA gene pyrosequencing and LC-MS/MS metaproteomic analysis hyphenated with protein-based stable isotope probing (protein-SIP, ^{15}N-fully labelled diet) was performed. As a result, we were able to decipher the mucosal colon microbiota community structure in regard to taxonomy, enzymatic functionalities and active taxa related to nitrogen utilisation from the feed over a three day period. Microbial active taxa in regard to nitrogen utilisation belonged to the abundant phyla like *Firmicutes*, *Proteobacteria* and *Bacteroidetes* as well as those from low abundant phyla like *Spirochaetes*, *Deinococcus-Thermi* and *Planctomycetes*. In addition, we observed rapid changes in the community composition including a decline of *Enterobacteriaceae* and *Streptococcaceae*. Identified proteins were assigned to functional categories of which replication, transcription, signal transduction as well as carbohydrate and amino acid metabolism were overrepresented.
The integrated data analysis opens the path to understand the complex gut microbiota in more detail using protein-SIP to identify the active taxa for specific substrate utilisation.

PWP14
Study of Mare's Milk Enzyme Profiles along Three Months of Lactation

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Introduction and Objective. In the present study ten raw milk samples from two different mares were analyzed from an enzymatic point of view. The aim of this work was the determination of mare's milk enzyme profiles, as well as observation of the changes in the profile along the mare lactation
Materials and Methods: The samples were drawn by hand in five shots over three months of lactation. The aim of this work was the determination of mare's milk enzyme profiles, as well as observation of the changes in the profile along the mare lactation. The profiles were characterized by the API ZYM ® (bioMérieux, Marcy l' Etoile, France) semiquantitative method. It is a quick, simple, specific and sensitive system used for the identification of microorganisms which provides consistent and reproducible Results: Its application to the study of enzyme profiles of raw milks opens a new possibility of identification other than the classical use for bacteria, such as the monitoring of enzyme activities in a certain food matrices over time, allowing its stability evaluation.
Results and Discussion: The first mare, the enzymes leucine arylamidase, esterase lipase esterase, naphthol-AS-bi-hydrolase, valine arylamidase, alkaline phosphatase, α-galactosidase, acid phosphatase, N-acetyl-β-glucosaminidase and α-fucosidase showed a persistent activity over time the time, in decreasing order of intensity. For the second mare enzymes showed higher activity were, in decreasing order: leucine arylamidase, esterase, lipase esterase, naphthol-AS-bi-hydrolase, valine arylamidase, alkaline phosphatase, α-galactosidase, acid phosphatase, N-acetyl-β-glucosaminidase and α-fucosidase. These activities have been described previously in milk, mainly in livestock species such as cattle, sheep and goats Conclusion: The most prominent activity was leucine arylamidase, also known as leucine aminopeptidase (LAP), an enzyme present in the Gram-positive cocci, especially in *Streptococcus* spp. The milk sampled had levels of microorganisms of the genus in microbiological counts parallel to this test. Streptococci were detected by inoculating Shape Man Rogosa agar (MRS). Characterization of enzyme profiles using API ZYM ® is presented as a rapid, simple, specific and sensitive system. Its application to the study of enzyme profiles of raw milks opens new possibilities of use, such as the monitoring of enzyme activities in a certain food matrices over time, allowing the knowledge of its stability.

PWP15
Addressing the influence of *S.* Typhimurium induced gut inflammation on the Oligo-Mouse-Microbiota

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Salmonella Typhimurium (*S.* Tm) is a food-borne pathogen and is among the most clinically important serotypes that cause salmonellosis in humans with tens of millions of infections every year worldwide. The infection is usually self-limiting, however in very young, old or immunocompromised patients the *S.* Tm infection can become life-threatening (more than hundred thousand deaths per year, WHO 2013).
S. Tm induced acute gut inflammation is followed by changes in composition and relative abundance of the bacteria that constitute the resident gut microbiota (commonly detected on phylum level) as well as *Salmonella* overgrowth (Stecher *et al*., Plos Biology 2007). *S.* Tm benefits from inflammatory host responses which decrease colonization resistance mediated by the beneficial anaerobic microbiota. The mechanisms how *S.* Tm takes advantage of inflammation and thereby outcompetes the microbiota during gastroenteritis are just beginning to be explored. So far, it could be shown that anaerobic electron acceptors (e.g. nitrate and tetrathionate), iron availability and neutrophils play a role in *S.* Tm dominance over the resident microbiota (Winter *et al*., Embo Rep. 2013).
In order to further investigate and prioritize those mechanisms as well as to characterize the shifts in microbiota composition during *S.* Tm induced gut inflammation at a single species level we use a gnotobiotic mouse model based on a novel consortium of mouse-adapted strains, the Oligo-Mouse-Microbiota (Oligo-MM). The Oligo-MM comprises 12 isolates from 5 eubacterial phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*) which stably colonize the murine gut. In addition, we developed highly strain specific molecular tools (qPCR and FISH) to monitor bacterial composition over time. This makes the Oligo-MM a valuable tool for studying the influence of the mucosal immune system and other environmental factors on microbial ecology in the gut.

PWP16
Resuscitation of VBNC lyophilized probiotic bacteria with eukaryotic cell culture liquid.

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Introduction: Bovine fetal serum (BFS) and passage through animal organism are considered as the most effective factors, promoting resuscitation of VBNC (Viable But Non Culturable) bacteria. The purpose of research was to check capability of CHO eukaryotic cells (EC) and mouse myeloma (MM) culture liquids (CL) to resuscitate VBNC cells of commercial of lyophilized probiotics.
Materials and Methods. In our resuscitation experiments on VBNC cells of lyophilized *Bifidobacterium bifidum* (BB) and *Lactobacillus acidophilus* (LA) semi-solid Elliker medium (EL) was supplemented with DMEM

medium-based CL (1, 5, 10%) after 2 days of MM cells cultivation (controls - DMEM, DMEM with 10% BFS) or RPMI 1640 medium-based CL with 5% BFS and gentamicin (10 µg/ml) after 7 days of CHO cells cultivation (controls - RPMI 1640 with the same additives). Tests of probiotic bacteria viability in probiotics were conducted in EL without supplements. All bacterial dilutions were incubated within 7days at 30°C. CFU/ml values were taken periodically, starting from first 24hrs of incubation. Total number of cells was counted in Thoma counting chamber by means of Zeiss microscope (Germany). Total % of viable cells in population was defined with Live/Dead® dye kit (Baclight™) in luminescent microscope (OPTON, 8×40 times magnification). Number of VBNC cells was calculated as difference between amounts of viable cells and CFU/ml.

Results: In BB population we revealed 96.23% VBNC cells, 82.82% viable and 17.18% dead cells. DMEM with 10% BFS and 2 days EC cultivation (CL) were confirmed as promoting factors of VBNC cell resuscitation (compared to DMEM without supplements). So, typical colony growth was registered in BB dilutions (from 10^{-1} to10^{-6}) of 1 treatment dose; there were no active growth in 10^{-7}. In dilutions starting from 10^{-8} we observed emergence of considerably smaller colonies (0,8-1,0 mm in diameter, compared to typical size of 3mm). Their numbers decreased with further dilution of the probiotic. Similar effect was detected in experiments with RPMI 1640 (with 5% BFS and CL of CHO cells). Mentioned smaller size colonies are of great interest, apparently representing delayed growth of resuscitated VBNC cells. Each supplement and its concentration had own special effect on colony formations. Same effect was registered during experiments with LA VBNC cells of lyophilized probiotics.

Conclusion. In our opinion, revealed effect of resuscitation on VBNC cells of commercial lyophilized probiotics by CL depended, besides BFS, on eukaryotic cells CL type. We expect comparison of derived results with experimental data on resuscitation VBNC cells of other probiotic bacteria.

PWP17

Quantitative *in vitro* synthesis and purification of antimicrobially active microcin S

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Introduction: Probiotic bacteria should possess specific mechanisms to protect themselves against pathogens and to mediate a health benefit to their host. In the gastrointestinal tract such a beneficial mechanism may be the secretion of substances with an antimicrobial effect. Some of those bacteriocins, which are synthesized ribosomally by enterobacteria, are called microcins due to their small size. Such a microcin producing strain is *Escherichia coli* G3/10, a component of the probiotic drug Symbioflor2. Among others, this microcin S (MccS) suppresses the adherence of enteropathogenic *E. coli in vitro* [1].

Since the antimicrobial effect of microcins is directed against closely related species they represent promising alternatives to classical antibiotics. Considering the current spread of antibiotic resistances, especially in Gram-negative pathogens, the search for new antimicrobial substances is of high priority to ensure containment of this development. Against this background we have started a detailed investigation of structural and functional aspects of microcin S.

Therefore, MccS with a C-terminal His-tag was synthesized in high quality and quantity in a cell-free *in vitro* system to further investigate its antimicrobial activity.

Materials and Methods: The *mcsS* gene of *E. coli* G3/10, coded on the megaplasmid pSYM1, was fused with a C-terminal 6 x His-tag and cloned into different expression vectors with a T5 or T7 promoter. To induce synthesis of *mcsS* in small scale, the resulting plasmids were applied in a cell-free *in vitro* system using the EasyXpress Protein Synthesis Kit (RiNA GmbH, Berlin). Expression was controlled via dot blot followed by immune detection using a monoclonal antibody against the 6 x His-tag. Upscaling of *in vitro* expression was performed gradually using the vector-promoter system with the highest quantity of expressed MccS. Chromatographic methods were implemented for separation of pure MccS. Antimicrobial activity against sensitive strains was determined via agar diffusion tests.

Results: High quantities of His-tagged MccS, synthesized with different expression vectors, could be shown by dot blot. Only minor differences between T5 and T7 promoter systems were noticeable. Immuno blotting of the protein mixture revealed a 6 x His-tagged MccS with the expected molecular weight of 13.3 kDa. Subsequently, upscaling and purification procedures yielded milligram quantities of protein.

Discussion: We could show that a cell-free expression system is better suited for recombinant synthesis of novel microcin S in high quantities, than inducible expression in bacteria. The concentrations of MccS reached with this system are necessary for further investigations to get a better insight into structure and the antimicrobial mode of action of this potentially new antibiotic agent.

Literature

[1] Zschüttig, A. *et al.* (2012), PLoS One 7(3):e33351.

QUALITY MANAGEMENT IN DIAGNOSTIC MICROBIOLOGY

QSP01

Stabilization of bacterial titers for head-to-head comparison of methods for the detection of bacterial contamination in platelet concentrates

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Introduction: Bacterial contamination of platelet concentrates (PCs) is the most frequent infectious complication in transfusion therapy in developed countries. Bacterial detection methods (rapid detection methods (e.g. flow cytometry, NAT), culture methods) are available to increase blood safety, but the evaluation of these methods is a complicated process. Since some detection principles required bacterial vitality and bacteria are capable to grow in PCs, variable titers prevent an objective comparison. In this context, a proficiency panel (RfB) with stabilized sample material was established. Currently three independent collaborative trials were performed to evaluate the diagnostic sensitivity of different bacterial detection methods.

Materials and Methods: Three different modules were available in the collaborative trial: 1) application of rapid methods, 2) application of culture methods, 3) bacterial identification. The stability of bacterial titres between sample set-up and execution of analysis was assured by the addition of antibiotics, temperature control, and a maximum transportation period of 20 h to obtain the highest reproducibility of Results: Each of the 3 trials included six to eight samples which were analyzed with three different rapid methods (BactiFlow flow cytometry, 16S rDNA NAT, Q-MAP-Bakt (Attolab)) and culture methods. The sample set-up included six different bacterial strains, 2 negative samples and 4-6 positive samples with bacterial titers in three different concentrations (app. 1,000 CFU/ml, 10,000 CFU/ml, 100,000 CFU/ml).

Results: The setting of the collaborative trial proved sufficient prevention of bacterial growth, the supplementation of antibiotics did not influence bacterial detection using the different methods. The results of the three collaborative trials for rapid screening methods showed that samples spiked with bacteria in the range of 100,000 CFU/ml obtained positive results with all rapid screening methods. Samples spiked with 1,000 CFU/ml showed a lower number of correctly identified positive results, the diagnostic sensitivity ranged from 100% (n = 3) for BactiFlow, followed by NAT (89-100 %, n = 3) and Q-MAP-Bakt (33.4 %, n = 1). The application of culture methods for detection and identification of bacteria showed a diagnostic sensitivity of 100%, all participants detected and identified the samples inoculated with transfusion-relevant bacteria correctly.

Conclusion: The collaborative testing proved successful for the three offered modules. The head-to-head comparison demonstrated a good performance of NAT and BactiFlow, showing a slightly advantage of the BactiFlow assay. This proficiency panel enables the verification of the analytical sensitivity of rapid bacterial detection systems under controlled routine conditions and represents an important contribution for blood safety.

QSP02

Platelet transfusion transmitted relevant bacteria strains - Growth ability testing in Platelet Concentrates

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Introduction: Bacterial contamination of platelet concentrates (PCs) still remains a persistent problem in transfusion medicine. In order to validate and to compare methods of bacteria detection in PCs, it is crucial to use bacterial strains which are likely to contaminate and able to proliferate PCs during storage. In 2010 the first WHO Repository for Platelet Transfusion-

Relevant Bacteria Reference Strains were established. The repository consists of four bacteria strains (*Staphylococcus epidermidis* (PEI-B-P-06), *Klebsiella pneumoniae* (PEI-B-P-08), *Streptococcus pyogenes* (PEI-B-P-20) *and Escherichia coli* (PEI-B-P-19) which were included in an international collaborative study in order to evaluate their potential in setting up Blood Bacteria Standards (BBS) as a tool for objective validation and assessment of microbiological methods for blood safety. The next step will be to enlarge this bacteria panel and to approve more strains as BBS. Different isolates of the same bacteria species may vary in their behaviour in PCs. They can be inactivated by self-sterilisation effects of blood, may persist or grow in PCs during storage. The growth potential of the bacteria is supposed to be on strain level not on species level.
Materials and Methods: Dedicated bacterial strains (i.e. Morganella morganii) were evaluated under "real life" and routine conditions (inoculated in PC-bags) regarding their ability to proliferate in platelet concentrates after low spiking (< 1 Colony Forming Unit (CFU)/ml)). Microbial count was performed at day 1, 2, 4 and 7.
Results: Whereas one isolate showed a logarithmic growth in PCs, two other isolates remained on a low microbial count / were not detected at all during 7 days. The data (Morganella morganii) provide indication that the potential of growth in PCs is on strain level.
Conclusion: Blood Bacteria Standards (BBS) are a feasible tool for objective validation and assessment of detection methods for contamination in blood components. There are indications that the growth ability is related to the strain level.

QSP03
Non Invasive pH- Measurement - A Promising Approach for Monitoring Bacterial Growth in Platelet Concentrates.

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Introduction: Determination of pH in Platelet Concentrates (PCs) is an established parameter for quality control in blood transfusion services. It is well known that the drop in pH value may be triggered by the increase of CO_2 due to microbial growth. In previous studies (Montag et al: Time Course of pH in Platelet Concentrates after Bacterial Contamination, ISBT 2008) the proof of this principle was already shown for PCs: in all so far tested bacterial strains an obvious drop in pH value occurred after a sufficient bacterial count was reached in the preparation.
Materials and Methods: Pooled PCs (PPC) were spiked with low levels of four different platelet transfusion relevant bacteria species (*Bacillus thuringiensis, Morganella morganii, Serratia marcescens, Enterobacter cloacae*) in order to confirm the conclusion of previous pH studies (s. poster ISBT 2008). In defined intervals the bacterial count was determined by streaking samples on agar plates in order to picture the strain specific growth curve in PCs. Additionally, pH was measured at the same time points with the BCSI pH1000 (Blood Cell Storage Inc. (BCSI), Seattle, USA), and negative controls were processed in parallel.
Results: In the former study the suitability of the BCSI 1000 method could be shown with six bacteria strains and one yeast strain. The extension with four further strains (as mentioned above) showed similar Results: All species showed a characteristic growth curve with subsequent drop, followed by a re-increase in pH. It could be verified that microbial growth can be monitored by non-invasive continuous measuring of pH value in PCs.
Conclusion: Non invasive pH-measurement is a suitable method for microbial screening of PCs and could be a useful tool for blood transfusion services to prevent the transfusion of contaminated PCs, if the pH measurement is conducted continuously.

QSP04
Establishment of Production and Determination of *Bacillus subtilis* Endospores for the use as Bioindicator with comparable resistance properties

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Bacterial endospores are preferred for the validation of disinfection and sterilisation procedures because of their high resistance. Typically spore suspensions with defined resistance properties are commercially available; however they are often too expensive for the utilisation in routine applications. Thus, the production process of *Bacillus subtilis* spores was optimised for the utilisation of spores as bioindicators.

First, the influence of the pH-value on the spore production was studied. Therefore a constant pH of 5.6 during the fermentation was investigated and showed a maximum spore concentration of $9.5*10^8$ Spores/ml, which was similar to the spore concentration obtained in the unregulated control. With this strategy the maximum concentration in the regulated fermentation was achieved 25 hours earlier.
Secondly, investigations to improve the cultivation temperature led to a significantly increased cell proliferation using 38 °C and 40 °C. Moreover, the sporulation started 8 hours earlier at 38 °C compared to the conventional temperature of 30 °C.
Third, the addition of glucose at the start of sporulation led to a doubling of the cell number but delayed the sporulation process by 20 hours. However, glucose increased the spore yield at the end of the fermentation process to $1.35*10^9$ spores/ml, which was 3.5-fold higher compared to the spore yield in the control fermentation.
The optimisation of the medium showed a spore fraction of 100 % in chemically defined fed-batch medium after 30 hours bioreaction. In contrast a 100 % spore fraction was obtained after 55 hours bioreaction using conventional 1xCDS-Medium, although both media led to a similar spore yield of $1*10^9$ spores/ml. Using peptone as an additive to fed-batch medium, the final spore concentration permitted $2.3*10^9$ spores/ml which represents a 50-fold increase compared to the complex Standard-1 medium.
Moreover, a method to quantify spores via the dipicolinic acid content was established and revealed for the first time that the cultivation medium influences the amount of dipicolinic acid inside the spore. This exhibits a big disadvantage in the comparison of resistant properties of spores if they were produced in different media and shows the necessity to use the same chemically defined medium for the spore production if they should be used in comparative studies.
Overall, the obtained results showed that regulation of the pH-value and the temperature as well as modifications in the medium composition during fermentation accelerates the sporulation process and increases the spore yield of *B. subtilis*.

National Reference Laboratories and Consiliary Laboratories

RKP01
Effects of 5 years of immunization with higher valent pneumococcal conjugate vaccines in German children

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Introduction and aims: A general recommendation for vaccination with pneumococcal conjugate vaccine (PCV) was issued for German children ≤2 years in 2006. In 2009, two higher-valent PCVs (PCV10, PCV13) were licenced in Germany. Here, we present data on invasive pneumococcal disease (IPD) -cases sent in for serotyping in the eight years following the start of PCV-vaccination.
Materials and Methods: Pneumococcal isolates recovered from children with IPD were sent to the GNRCS. Serotyping was performed using the Neufeld-Quellung-reaction.
Results: From July 2012 to June 2013, an increase in IPD cases among children <2 years was observed for the first time since the introduction of childhood vaccination (98 vs. 75 cases in 2011-2012). This change in trend persisted in July 2013 - April 2014, and is caused by an increase in non-PCV13 serotypes.
Cases with PCV7 serotypes decreased by over 90%, while cases with non-PCV7 serotypes more than doubled. The six new serotypes increased after PCV7 introduction but decreased after higher-valent vaccine Introduction: Less cases caused by serotypes 1(-89%), 3 (-57%), 7F (-94%) and 19A (-81%) were reported from July 2013-April 2014 as compared to the same time periods in 2009-2010 and 2010-2011 for children <2 years of age. Non-PCV13 serotypes significantly increasing among children are: 6C, 10A, 11A, 12F, 15A/B/C, 22F, 23B, 24F, 33F, 35B, 35F and 38.
Conclusions: Eight years after the general vaccination recommendation reported IPD-cases in children <2 years of age caused by all serotypes have decreased by almost 50%. An increase in non-vaccine serotypes has become apparent in 2012-2013, however, a net reduction of cases was still observed.

RKP02

A New Consultant Laboratory on Anthrax in Germany

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Anthrax is a worldwide occurring but, at least in Germany, rare zoonotic disease. However, an outbreak of anthrax in drug users has been emerging in Germany and other European countries in the years 2009-2012. Livestock also became affected during the past years. The causative bacterium *Bacillus anthracis* is considered as a biological agent which could potentially be misused on purpose. The spores of the agent are extremely resistant to environmental conditions and chemical disinfectants.
Single cases and outbreaks of anthrax as well as samples of suspected intentional release are raising questions on diagnostics as well as on laboratory and clinical management. Consequently, the first consultant laboratory for anthrax in support of the Public Health sector in Germany has been appointed in May 2014. It is located at the Division of Highly Pathogenic Microorganisms (ZBS 2) at the Centre for Biological Threats and Special Pathogens of the Robert Koch Institute. The consultant laboratory offers diagnostic and scientific support by applying accredited microbiological, molecular and serological methods, typing of strains, and providing recommendations and advice, e.g. on disinfection and decontamination. In addition, the laboratory is active in research projects on anthrax, including the investigation of *B. anthracis* strains having caused the outbreaks among heroin users, as well as the ones of *Bacillus cereus* biovar *anthracis* representing a new variation in the *Bacillus cereus* group found so far in different regions of Africa. Furthermore, the laboratory supports the establishing of quality assurance schemes, including anthrax diagnostics, at national and international level, fulfilling the role of main coordinator of the European Joint Action "Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens" (QUANDHIP), which brings together 29 laboratories from the European Network on Highly Pathogenic Bacteria (ENHPB) and 8 laboratories from the "European Network of P4 Laboratories" (ENP4 Lab) from altogether 22 European countries. Beyond External Quality Assurance Exercises for highly pathogenic bacteria and viruses, the bacterial part of the project has additionally focussed the following special topics: Antimicrobial susceptibility testing, MALDI-TOF analysis, evaluation of hand-held test kits, and the generation of qualitative and quantitative reference samples. http://www.rki.de/

RKP03

Prospective multicentre study on antimicrobial resistance of *Helicobacter pylori* in Germany

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Objectives: Antimicrobial resistance of *Helicobacter pylori* endangers the successful eradication of the bacteria. The aim of this prospective surveillance study (*ResiNet*) is to continuously keep antimicrobial resistance of *H. pylori* in Germany under surveillance and to identify risk factors for its development.
Materials and Methods: From July 2001 until December 2012, a total of 2762 patients were prospectively enrolled. We gathered standardized clinical and epidemiological data, carried out antimicrobial susceptibility testing, identified risk factors for the development of resistance and analysed the clinical outcome of the study patients in a follow-up study.
Results: 1651 *H. pylori* positive patients with complete data sets were worked up. Primary resistance rates were 29.4% for metronidazole (MTZ), 6.7% for clarithromycin (CLR) and 3.1% for both antimicrobials (MTZ/CLR). Prior unsuccessful eradication therapies, female sex and country or continent of origin were identified as independent significant risk factors for developing resistance. 45.7% (n=755) of patients were followed up. The majority of patients benefitted a therapy as measured by a subjective reduction or disappearance of their symptoms.
Conclusions: Patients without any previous eradication therapy can be treated empirically; in patients with prior therapy failures and in patients who frequently suffer from unrelated bacterial infections antimicrobial susceptibility testing is recommended.

RKP04

A novel duplex PCR for simultaneous detection of *Borrelia burgdorferi* s.l. and *Borrelia miyamotoi*

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Introduction: *Borrelia miyamotoi*, first described in 1995 in Japan, is the first relapsing-fever spirochete that occurs sympatrically with Lyme borreliosis spirochetes in *Ixodes* ticks in Asia, Europe and USA. First human cases were described in Russia in 2011. Subsequently, human cases were detected in Europe and the USA suggesting that *B. miyamotoi* is an emerging human pathogen. Because of the sympatric occurrence of *B. burgdorferi* and *B. miyamotoi* in ixodid ticks, diagnostic tools that detect and distinguish the two pathogens are desirable.
Materials and Methods: We have developed a real-time duplex PCR based on the flagellin genes that enables us to detect and differentiate at the same time *B. burgdorferi* s.l. and *B. miyamotoi*. The analytical specificity and sensitivity was tested with dilution series of various genospecies of *B. burgdorferi* and *B. miyamotoi*, *Leptospira* spp. and *Treponema phagedenis*. Specificity was confirmed by multilocus sequence typing (MLST) using eight housekeeping genes. We tested tick DNA samples from the Siebengebirge near Bonn (n=840) and from Southern Germany (n=700).
Results: In total, 12 *B. miyamotoi* and 69 *B. burgdorferi* s.l. positive ticks were found in the Siebengebirge. CT values for *B. miyamotoi* and *B. burgdorferi* s.l. ranged between 22 and 37 and between 29 and 40, respectively. Two of the *B. miyamotoi* positive samples were confirmed by *glpQ* PCR and sequencing. Samples from Southern Germany are currently under investigation.
Conclusion: We have shown that the real-time duplex PCR allows simultaneous and sensitive detection of *B. burgdorferi* s.l. and *B. miyamotoi* in environmental and potentially clinical samples. Prevalence of *B. miyamotoi* in the Siebengebirge near Bonn and in Southern Germany was found to be approximately 1 %. Duplex PCR did not show any loss in sensitivity compared to single PCR. Therefore, this method appears to be a promising approach for detecting *Borrelia* species in environmental and clinical samples.

RKP05

Is there a correlation between sequence type and disease manifestation in Lyme borreliosis?

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Introduction: Spirochetal bacteria of the *Borrelia burgdorferi* sensu lato species complex are causative agents of Lyme Borreliosis (LB), a tick-borne zoonotic disease. In Europe, five species, including *B. garinii* and *B. bavariensis,* are known to be human pathogenic. Symptoms associated with LB induced by *B. garinii* and *B. bavariensis* can vary in different patients, for example some patients may develop erythema migrans (EM) while in others it may cause symptoms known as neuroborreliosis (NB). Studies in the United States suggested that within *B. burgdorferi* sensu stricto lineages may exist that exhibit different invasive properties in humans (1).
Materials and Methods: Using multilocus sequence analysis (MLSA) on eight chromosomally located housekeeping genes (*clpA, clpX, nifS, rplB, recG, uvrA, pyrG, pepX*), we investigated whether a similar phenomenon can be observed in the European *Borrelia* species *B. garinii* and *B. bavariensis*. The genes were PCR amplified in isolates from human patients with different symptoms (EM, NB) as well as from isolates of questing ticks from Germany.
Results and Discussion: Our data show that there was no apparent correlation between sequence type and geographic origin of the sample. Furthermore, we did not find evidence for an association of sequence type and clinical symptoms. Further investigations like whole genome sequencing or on less conserved genes on plasmids are required to reveal whether a correlation exists between distinct genotypes of *B. burgdorferi* and symptom manifestation in LB.

[1] Hanincova et al. (2013) Multilocus Sequence Typing of *Borrelia burgdorferi* Suggests Existence of Lineages with Differential Pathogenic Properties in Humans. PLoS ONE 8(9): e73066. doi:10.1371/journal.pone.0073066

RKP06

Detection of Sabin like polioviruses among asymptomatic Syrian refugees and asylum seekers, Germany, 2013/2014

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In Germany, assurance of polio free status in the context of Global Polioeradication is based on enterovirus (EV) surveillance, which focuses on patients with signs of aseptic meningitis/encephalitis or acute flaccid paralysis, representing the key symptoms of poliovirus (PV) infection. In response to the wild poliovirus (WPV) outbreak in Syria 2013 and high number of refugees coming from Syria to Germany, RKI additionally advised stool screening for EV of asymptomatic Syrian refugees/asylum seekers aged <3 years, possibly lacking polio immunization due to stop of routine vaccination program during civil war.
Enterovirus (including PV) diagnostic of stool samples was performed at the National and WHO Reference Laboratory for Poliomyelitis and Enteroviruses at RKI (NL/RRL) and three labs of the established German EV-laboratory network using molecular and virological methods. Characterization of EV-positive samples as well as differentiation between vaccine and wild PV was performed at the NL/RRL.
From Nov 2013 to Apr 2014, stool samples from 629 Syrians were tested. Overall, 93 (14.8%) were positive in an EV specific PCR. Of these, 12 could be identified as PV. Intratypic differentiation revealed Sabin-like PV in all cases indicating a recent oral polio vaccination (OPV). Isolates were retrieved from nine samples; failure of isolation from the other three is most likely explainable by longer time distance to OPV vaccination resulting in non-infectious particles. All PV isolates were confirmed by serotyping using monospecific antibodies. The remaining 81 samples were characterized as non-polio enteroviruses (NPEV) representing several members of group A, B and C.
WPV was not detected via stool screening over a period of six months indicating a very low risk for importation by Syrian refugees and asylum seekers at that time. Due to OPV vaccination campaigns implemented in Syria and neighboring countries, presence of Sabin-like polioviruses in arriving refugees/asylum seekers was expected. Possibly underlying WPV infection in recently OPV immunized persons could be excluded by cell-culture based methods. Results further showed that highly sensitive PCR systems might be useful tools to supplement WHO-recommended cell-culture techniques to identify poliovirus.

RKP07

Colonization of inhabitants in nursing homes by *Clostridium difficile*

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Introduction: *Clostridium difficile* is the most prevalent pathogen causing diarrhoea in hospitals. However, there is also emerging relevance of *C. difficile* infections (CDI) in nursing homes. Although the exact root of *C. difficile* infection remains elusive in the majority of cases inhabitants of nursing homes are assumed to be potential sources for transmissions.
Materials and Methods: In the present point prevalence study anal swabs from people in nursing homes (State of Saarland) were routinely screened for *C. difficile* by anaerobic culture using selective media (Chrom-Agar, MAST DIAGNOSTICA). Samples were incubated in anaerobic atmosphere for 3 to 5 days (GENbox anaer; Biomerieux) and isolates were identified by MALDI-TOF (Bruker Daltronics). Then the isolates were characterized by PCR-ribotyping using capillary gel electrophoresis and automated pattern recognition analysis (Bionumerics), by the detection of toxin genes (tcd A, tcd B, cdt A and B) using multiplex-PCR and by anaerobic resistance testing using E-test and agar diffusion assay (Liofilchem).
Results: A total number of 707 anal swabs from 22 different nursing homes were currently included. Thereof 36 (5,1%) were tested positive on *C. difficile*; however, the *C. difficile* rate was different between the various nursing homes. Toxigenic ribotypes 001 (n=4, 15%), 014 (n=5, 19%) and 027 (n=2; 8%) were predominantly found while other toxigenic ribytypes as 002, 012, 017, 126 and 328 sporadically occurred (n=1, 4%). The most abundant non-toxigenic ribotypes 010 and 140 were absent in the present cohort, however, six non-toxigenic isolates were found with still unclassified ribotypes (23%). Interestingly, also unclassified ribotypes with positive binary toxin were identified which was similar to toxin profiles of 027 strains. All isolates were sensitive to rifampicin, vancomycin (MHK median: 0,38 mg/l, range: 0,25-2 mg/l) and metronidazol (MHK median: 0,50 mg/l, range: 0,25-8 mg/l); antibiotic resistance to moxifloxacin and clarithromycin could be assigned to specific ribotypes.
Conclusion: Overall, the prevalence of *C. difficile* colonized inhabitants of nursing homes was 5,1 % which is similar to the general population. The predominant ribotypes included also hypervirulent 027 strains and corresponded to the ribotypes in hospitals. Therefore asymptomatic *C. difficile* carriers may contribute to transmission of nosocomial ribotypes in their local environment.

Figure 1

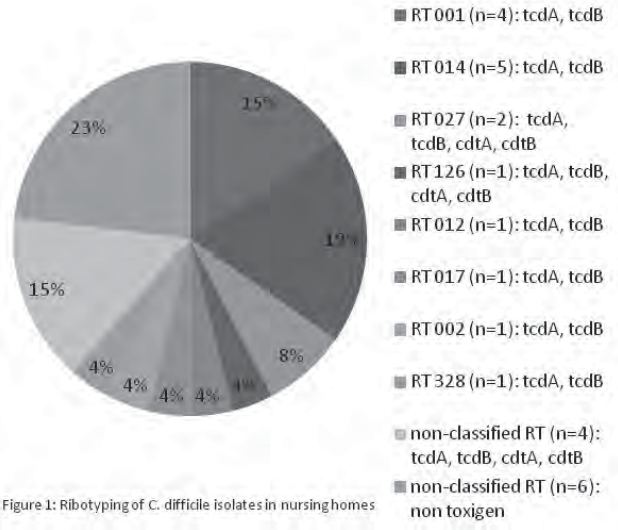

Figure 1: Ribotyping of C. difficile isolates in nursing homes

RKP08

Five years of *H. influenzae* laboratory surveillance: invasive isolates in Germany 2009 to 2013

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Introduction: The German Reference Laboratory for Meningococci and *Haemophilus influenzae* (NRZMHi) at the Institute for Hygiene and Microbiology in Würzburg has started laboratory surveillance in 2008. Here, we present epidemiologic surveillance data from 2009 to 2013.
Materials and Methods: Data were collected from survstat@rki and the database of the reference laboratory, which receives blood culture- or CSF-isolates from laboratories on a voluntary basis. The NRZMHi reports the data to the laboratories and since 2011 to the public health service. Capsular serotyping was performed by slide agglutination and PCR. Enhanced surveillance included a telephone contact of public health offices asking laboratories to send strains to the NRL
Results: Incidence rates for invasive infections reported to the RKI have increased from 0.23/100,000 to 0.51/100,000 during the observation period. Rough comparison of the number of submitted isolates to the NRZMHi and the notification data using survstat@rki suggest a coverage of initially 60% in 2009 up to 73% in 2013. The majority of invasive *H. influenzae* infections typed at the NRL (n=1001) was caused by unencapsulated isolates (nontypeable *H. influenzae*, NTHi) in adult patients of age. The serotype distribution in Germany for the five years of observation was as follows: NTHi 80.2% (n=803); Hib 4.0% (n=40); Hif 12.8% (n=128); others 3.0% (n=30). Patients aged ≥ 65 years from whom isolates were submitted to the NRZMHi represented 60.6% (n=608), whereas young children and toddlers (aged ≤ 4 years) represented only 10.6% (n=106). The proportion of ampicillin resistant strains was 13.2%, beta-lactamase was found in 9.7% of all tested isolates (n=999).

Enhanced surveillance in collaboration with the health authorities of the Federal State of Baden Württemberg was initiated in 2009 to study whether submission rates could be increased by implementing active feedback to the laboratories (manuscript in preparation). Enhanced surveillance resulted in considerably higher coverage compared to other Federal States.
Discussion: NTHi disease in the elderly is emerging in Germany. The laboratory coverage can be increased by enhanced surveillance. Matching of the German statutory notification dataset with the laboratory dataset has been established for meningococci and will now be implemented for *H. influenzae*. This procedure will allow the determination of precise coverage rates as well as the improvement of the Federal dataset.

RKP09

Frequency of Species, Serogroups, Monoclonal Subgroups and Sequence Types among Legionella Strains Isolated From Patients in Germany

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Introduction: According to CAPNETZ 10000 to 20000 pneumonias in Germany are caused by *Legionella spp*. per year. The aim of this study was to analyze the occurrence of species, serogroups, mAb-subgroups and sequence types among clinical isolates of *Legionella pneumophila* in Germany. Furthermore, characteristics in the regional distribution and the genetic diversity among the population of *Legionella pneumophila* in Germany were investigated. To assess these findings in relation to the worldwide population of *Legionella pneumophila*, strains from the database of the European Working Group for Legionella Infection (EWGLI) were analyzed as well.
Materials and Methods: Clinical isolates of *Legionella pneumophila* were collected until 7th August, 2013 by the Institute of Medical Microbiology Dresden (n_G=408) and the EWGLI (n_I=4374).
Genotyping was performed with the established 7-gene-SBT-scheme. Phenotypic characterizations were conducted with monoclonal antibodies of the Dresden Panel. Phylogenetic analyses were implemented with Bionumerics Version 7. Statistical analyses for differences in distribution were operated with χ^2 and Fisher's exact test.
Results: Serogroup 1 isolates were most frequent in Germany (n_G=319; 78,2 %) and worldwide(n_I=3888; 88,9 %), followed by serogroup 3 (n_G=18; 4,4 % or n_I=77; 1,8 %) and serogroup 6 (n_G=15; 3,6 % or n_I=59; 1,3 %). MAb 3/1 positive strains were the most common cause for community acquired and travel associated pneumonias in Germany and worldwide ($p<0,001$). Both in Germany and abroad, mAb 3/1 negative isolates and isolates of serogroups 2 to 15 were significantly more frequent among nosocomial infections ($p<0,001$). The most common sequence type in Germany was ST 1 (n_G=69; 16,9 %), whereas the international ST 47 (n_I= 463; 10,8 %) was isolated only thrice. The second most common sequence type in Germany was ST 182 (n_G=47; 11,5%) and it was almost exclusively restricted to the area of Berlin-Brandenburg (n_G=40; 85,1 %). Phylogenetic analyses of German isolates revealed 18 clonal groups. Most frequent STs (e.g. ST1, ST 182, ST 62, ST 23) did not cluster in a common group.
Conclusion: Differences and similarities in the population of clinical strains in Germany and worldwide were illustrated. As frequent STs did not cluster together, no shared virulence-associated gene was detected by the 7-gene-SBT-scheme. To conclude, attention should be paid to infections caused by strains with common detected sequence types as they seem to have a more pathogenic potential.

RKP10

Advancement of a German Molecular Surveillance Network for EHEC infections

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The Molecular Surveillance of infection pathogens completes the surveillance of infectious diseases and contains a wide spectrum of methods. Scientific progress has led to the development of new methods of the further typing including the comprehensive sequencing of infection pathogens whose use makes an improvement in protection and prevention of infectious diseases possible. The fast outbreak detection in cooperation with different facilities of the Public Health Services is on the aim of these methods for the description of important determinants of the medically relevant and epidemiologically meaningful pathogens at a national level.
Basis for a systematic Molecular Surveillance is the isolation of bacterial pathogens in every case which is seldom realized by the medical labs in the course of modern diagnostics methods. A concept for the Molecular Surveillance was already worked out at the NRC for *Salmonella* and other Enteric Bacterial Pathogens at RKI in cooperation with 6 different labs of the German Public Health Offices to clear this condition for EHEC infections certainly.This provides that labs in connection with the obligation to registration pass certain isolates on to defined laboratories for systematic analyses.

RKP11

Investigation of human pathogenic sequence types of the species *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* using multilocus sequence analysis (MLSA)

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Introduction: Lyme borreliosis (LB) is a tick-borne zoonitic disease that occurs in the temperate zones of the Northern Hemisphere. The causative agents of LB are members of the *Borrelia burgdorferi* sensu lato species complex which currently consists of 20 species. In Europe, five species are known to be human pathogenic including *B. burgdorferi sensu stricto* (s.s.) , *B. afzelii, B. garinii, B. bavariensis* and *B. spielmanii*. Previous work suggested a correlation between *Borrelia* species and disease manifestation in humans. To investigate in more detail (i) whether there is a correlation between *Borrelia* species and disease manifestation and (ii) whether intraspecific differences exist, we employed multilocus sequence analysis (MLSA), a sensitive method for bacterial strain typing.
Materials and Methods: Eight genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB, uvrA*) located on the main linear chromosome of *Borrelia* were amplified using conventional PCR from patient isolates of *B. burgdorferi* s.s. and *B. afzelii* (n=110) from Europe. For comparative purposes we used MLSA data from ticks from Europe which are available via the MLST database (www.borrelia.mlst.net).
Results: Our results show that sequence types from patients with neuroborreliose cluster closely together with sequence types from patients with erythema migrans. It was particularly interesting to find *B. burgdorferi* s.s. sequence types that have not been described in European ticks or patients but in ticks and patients in the USA.
Conclusion: The results obtained in our study allowed development of new hypotheses with respect to pathogen acquisition and the population structure of *B. burgdorferi* s.s..

RKP12

In vitro cultivation and diagnostic tools for the emerging pathogen *Borrelia miyamotoi*

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Introduction: The genus *Borrelia* encompasses the group of relapsing-fever spirochetes and the *Borrelia burgdorferi* sensu lato (s.l.) species complex that harbors the causative agents of Lyme borreliosis. *Borrelia miyamotoi,* first described in 1995 (1), represents a relapsing-fever spirochete which is transmitted by ixodid ticks and occurs sympatrically with *B. burgdorferi* s.l. in Asia, Europe and USA. In 2011, the first human cases of *B. miyamotoi* infection and associated disease were reported (2) suggesting that this species represents an emerging human pathogen. Although *Borrelia* are known to be fastidious bacteria, strains of this species have proven particularly difficult to adapt to culture conditions. Here, we report a method for successful long-term *in vitro* cultivation of the emerging pathogen *B. miyamotoi*.
Materials and Methods: Based on the MKP-medium different modifications were tested like aerobe, microaerophilic or anaerobe

conditions, different temperatures and different relative amounts of human-, mouse-, or bovine serum. Furthermore, a real time duplex PCR targeting the p41 was developed for detection and differentiation of *B. miyamotoi* and *B. burgdorferi* s.l. in parallel.
Results and Discussion: The type and quantity of serum as well as the atmosphere were critical for successful *in vitro* cultivation. The generation time ranged from 7 to 24 h. Maximum density of bacteria reached $2.5x10^7$/ml. Analytical sensitivity is between 1-100 genome copies per PCR-reaction for the different *B. burgdorferi* s.l. species and 10 for B. miyamotoi. These methods enable future studies on the completely unclear incidence of this emerging disease.

(1) Fukunaga et al. 1995 Genetic and Phenotypic Analysis of *Borrelia miyamotoi* sp. nov., Isolated from the Ixodid Tick Ixodes persulcatus, the Vector for Lyme Disease in Japan, Int J System Bacteriol 45(4), 804-810
(2) Platonov et al. 2011 Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg Infect Dis. 17(10), 1816-23.

RKP13
Isolation and Functional Characterization of a Novel *Clostridium botulinum* Neurotoxin A Subtype

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Introduction: Botulinum neurotoxins (BoNTs) are secreted by different Clostridium species in association with non-toxic complex proteins and are the causative agents of botulism, a severe paralytic illness in men and animals. In recent years it has become increasingly clear that the BoNT molecules are a complex family of neurotoxins comprising seven distinct serotypes of BoNT (named BoNT/A-G) and more than 30 subtypes which can be distinguished based on their amino acid sequence. While it is well acknowledged that sequence differences of BoNT subtypes can affect detection or therapy, their relevance in terms of function and epidemiology is basically not understood. Here, we present the identification and characterization of a novel BoNT/A subtype designated BoNT/A8.
Materials and Methods: The novel strain was isolated from a case of food-borne botulism in Chemnitz, Germany (strain Chemnitz). The strain was subjected to whole genome sequencing and conventional Sanger sequencing. Functional characterization included analysis of binding to mammalian surface receptors (gangliosides, protein receptors), detection of endopeptidase activity by mass spectrometry and testing of biological activity in the mouse phrenic nerve hemidiaphragm assay.
Results: Comparison of the BoNT/A8 gene sequence with published sequences identified it as a novel subtype within the BoNT/A serotype. The toxin is located within a ha-/orfX+ cluster and is on the protein level most similar to BoNT/A2 and BoNT/A5. Unexpectedly, we found an arginine insertion located in the HC domain of the heavy chain which is unique compared to all other BoNT/A subtypes known so far.
Functional characterization revealed that its binding characteristics to mammalian surface receptors are slightly affected. When comparing the enzymatic activity of either the recombinant light chain or the natural, full-length neurotoxin of BoNT/A8 to BoNT/A1 in different endopeptidase assays we found BoNT/A8 to be significantly less active than BoNT/A1. Finally, using a classical mouse phrenic nerve hemidiaphragm assay it turned out that the overall toxicity of BoNT/A8 is reduced compared to BoNT/A1. Still, despite its reduced activity the novel BoNT/A8 subtype is of physiological relevance, since it caused severe botulism in a 63 year old male.
Conclusion: This is the first description and a comprehensive characterization of a novel BoNT/A subtype which combines genetic information with functional analysis using different technical approaches. Our results show that subtyping of BoNT is highly relevant and necessary to effectively fight the sophisticated BoNT molecule. Novel BoNT/A subtypes might pave the way to new therapeutics for different indications and medical applications.

RKP14
Identification and characterization of vaccine-derived Polioviruses in foreign children with severe immune defect

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An essential requirement for eradication of poliomyelitis is the elimination of circulating vaccine derived polioviruses (cVDPV) and polioviruses excreted by chronically infected individuals with immunodeficiencies (iVDPV). Since attenuated oral poliovirus vaccine (OPV) is still used in some regions of the world, risk for rapid reversion to neurovirulence can occur. Long term shedding raises risk of transmission to unprotected contacts. In Germany, surveillance of circulating enteroviruses mainly targets patients with signs of aseptic meningitis/encephalitis and acute flaccid paralysis (AFP), the key clinical symptoms of poliovirus infection. We here report incidental finding of VDPV type 2 in asymptomatic children from Eastern Mediterranean Region after polio vaccination with severe combined immunodeficiency (SCID) in Germany.
Two patients being under medical treatment for bone marrow transplantation (BMT) in German University hospitals (Ulm, Bonn) were routinely tested for the presence of gastrointestinal viruses. PCR and virus isolation subsequently resulted in further typing identifying poliovirus type 2. Samples were sent to the National Reference Centre for Poliomyelitis and Enteroviruses at the Robert Koch-Institute for differentiation between polio wild-/vaccine viruses. Samples from contact persons and follow up samples from patients were analyzed.
Sequencing of complete VP1 region showed a highly evolved VDPV type 2 with 5% divergence to parental vaccine strain in patient 1 and conversion from Sabin 2 to VDPV type 2 in patient 2 during time of shedding. Shedding occurred in both cases for at least three month. After BMT poliovirus clearance could be documented. All contact persons were tested poliovirus negative and interventional vaccination with inactivated polio vaccine (IPV) was performed in medical and laboratory personnel as well as in other persons at risk of infection. Additionally, norovirus and rhinovirus were detected by PCR in patient 1 and patient 2, respectively.
Routine screening of SCID patients represents a powerful tool to identify chronic poliovirus carrier. Those patients can pose a risk for contact persons with insufficient poliovirus immunization. Detection of VDPV represents a public health alert at the final stage of polioeradication since VDPVs have been shown to cause outbreaks in underimmunized groups. Therefore complete polio vaccination of hospital staff is required. BMT has been shown to stop virus shedding.

Regulation and Signaling (incl. Stress Responses)

RSP01
Transcriptomic Monitoring of *Escherichia coli* Growth.

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Introduction: Analysis of the transcriptome is a powerful tool for understanding genetic regulatory processes. Prerequisite to any transcriptomic analysis is biological sampling and statistical data analysis. Exemplified by transcriptomic monitoring of bacterial growth we established a data processing pipeline for the analysis of RNASeq data. The goal was to find strongly regulated genes during exponential growth of *Escherichia coli*.
Materials & Methods: *Escherichia coli* strain K12 (DSM-No 498) was grown as batch culture with DSM medium 1 at 37°C in Erlenmeyer flasks. Cultures were shaken at 150 rpm. Three biologically replicates were incubated from one inoculum (OD_{600} of 1.4; 1:10) and sampled at four different time-points (45 min, 3 h, 5 h, 7 h) covering major growth states at average OD_{600} of 0.14, 0.80, 1.69, and 1.78, respectively. mRNA extraction, sequencing and annotation was performed following standard protocols and software (as outlined in the presentation).
Results & Discussion: The OD_{600} of all three biological replicates ranged between 1.76 and 1.81 after 7 h of growth. For a first insight into gene regulatory processes with focussed our analysis on expressed enzymes, i.e. proteins with allocated EC numbers. 204/257 individual enzymes were

found to be significantly up/down-regulated between time-point 45 min and 5 h (see Fig. 1, more details will be presented). These could be allocated to 97 pathway categories.
Fig. 1: Excerpt from the Boehringer biochemical pathway map with highlighted up (red) and down (green) regulated enzymes.
The clustered expression pattern of time-course changes are shown in Fig. 2. Interpretation of the results will be presented.
Fig. 2: Clusters of the most strongly regulated genes. To be plotted here, the log2 fold change has to be > 7 or < -7 at any time-point compared to 45 min.

Figure 1

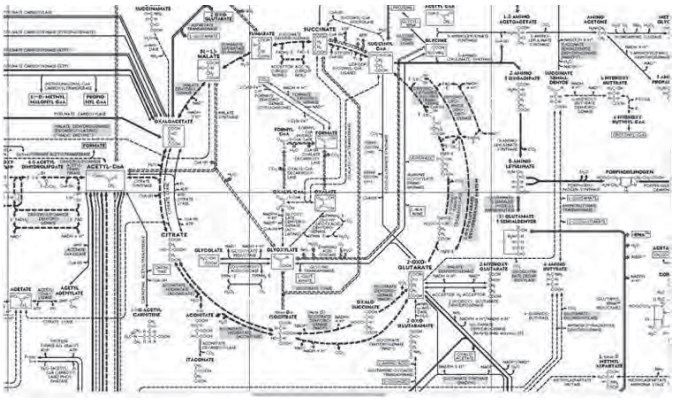
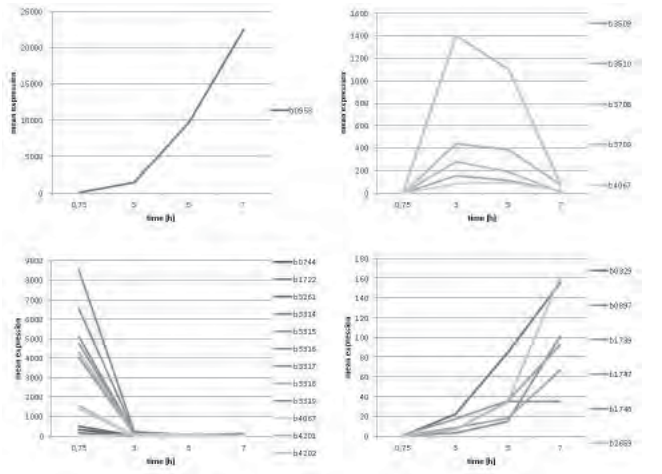


Figure 2



RSP02

Factors facilitating genome evolution in *Bacillus subtilis*

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The soil bacterium *Bacillus subtilis* lives in a constantly changing environment. Maladapted bacteria can adapt to a given ecological niche by (i) adjusting gene expression, (ii) by posttranslational control of protein activities, and (iii) by the accumulation of beneficial mutations that provide the bacteria with a selective growth advantage (1). A genomic alteration that falls into the latter category and restores fitness can be observed in a *B. subtilis* mutant lacking a functional glutamate dehydrogenase (GDH). This mutant has a severe growth defect on complex medium. However, the growth defect can be suppressed by the rapid excision of one part of a direct repeat (DR) present in a second, cryptic GDH gene (2,3). Since repetitive DNA sequences such as DRs are mutational hotspots they play an important role in bacterial adaptation. We are interested in factors facilitating evolution of the *B. subtilis* genome. We provide evidence that the transcription-repair coupling factor Mfd is needed for the high mutation rate of the GDH gene. Moreover, we found that the three RNase H enzymes of *B. subtilis*, which remove the RNA part of RNA:DNA hybrids are involved in the mutagenesis event. The lack of RNase H activity drastically increases the stability of the DR in the cryptic GDH gene. Finally, we observed that transcription strongly affects the mutation rate. We are currently working on the identification of additional factors that are involved in DR modification in *B. subtilis*. The understanding of the underlying molecular mechanism of DR instability in bacteria as well as in eukaryotes is very important because the instability of consecutive DNA repeats accounts for some severe human diseases (4). Moreover, the inactivation of factors causing DNA instability may help to engineer stable production strains for industrial applications.

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RSP03

Effect of cadmium on physicochemical surface properties and antioxidative enzymes system of *Cupriavidus metallidurans* CH34 and *Pseudomonas putida* mt2

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Introduction: *Cupriavidus metallidurans* CH34 and *Pseudomonas putida* mt2 were used as cadmium (Cd) resistant and sensitive bacteria respectively to study the effect of Cd on physicochemical surface properties and antioxidative enzymes. Physicochemical surface properties include the study of surface charge and hydrophobicity.
Materials and Methods: MOPS milieu medium (pH 7.0) was used as growth medium to conduct all the experiments. The method of Shamim and Rehman (2013a) was followed for analysis of physicochemical surface properties. The method of Shamim and Rehman (2013b) was followed for antioxidative enzymes profiling.
Results: In this research work, effective concentration 50 (EC_{50}) was calculated for both isolates in presence of Cd (*C. metallidurans* CH34 = 2.5mM; *P. putida* mt2 = 0.25mM). The zeta analysis did not show any significant change in surface properties of both isolates under Cd stress. Cd made *P. putida* mt2 surface to behave as intermediate hydrophilic (θ_w=25.32°) while *C. metallidurans* CH34 as hydrophobic (θ_w=57.26°) at their respective EC_{50}. Phospholipids fatty acids profiling showed that change from cis to *trans* fatty acids was observed in mt2 strain (0.45%) but not in CH34 strain (0%). Cyclopropane fatty acids expression was observed more in mt2 strain (0.06 to 0.14%) but less in CH34 strain (0.01 to 0.02%). Degree of saturation of fatty acids decreased in *P. putida* mt2 (36.8% to 33.75%) while increased in *C. metallidurans* CH34 (35.6% to 39.3%). *C. metallidurans* CH34 used only catalase (CAT) whereas *P. putida* mt2 used superoxide dismutase (SOD) and ascorbate peroxidase (APOX) to combat Cd stress.
Conclusion: In order to cope up with Cd stress, the cell surfaces of both isolates behaved in different ways. As CH34 is a known metal resistant isolate, so it only expressed CAT under Cd stress where mt2 used SOD and APOX to survive in Cd contaminated environment.
Keywords: Cadmium; physicochemical surface properties; antioxidative enzymes.

RSP04

Post-transcriptional regulation of surface pathogenicity factors in the enteropathogen *Yersinia enterocolitica*

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Introduction: The Gram-negative bacterium *Yersinia enterocolitica* is a gastrointestinal pathogen that drastically remodels its envelope in response to temperature and pH, including the deployment of several adhesins, type III secretion and lipopolysaccharide (LPS) modifications. To quickly adapt to changes in environmental conditions many bacteria produce small RNAs and the RNA chaperone Hfq that modulate the stability and translation of mRNAs. In this study, we investigated whether post-transcriptional regulation mediated by the RNA chaperone Hfq has an impact on the expression of pathogenicity factors in *Y. enterocolitica*.
Materials and Methods: Following gene inactivation in two strains of *Y. enterocolitica* serotype O:8, we compared the *hfq* mutants with their isogenic parents using immunoblotting and LPS staining. Gene regulation was assessed by RT-qPCR as well as by translational fusions with green fluorescent protein. Bacterial fluorescence was assessed by flow cytometry.
Results: Strains mutated in *hfq* exhibited increased production of the adhesin Ail, Ail-like OmpX, the Myf fimbriae, the siderophore receptor FyuA and LPS with long O-antigen chains. Using RT-qPCR and reporter fusions, we could show that expression of *ail* and *ompX* is indeed increased

in *hfq*-negative strains. Moreover, reporter fusions carrying a heterologous promoter for *ompX* still exhibited Hfq-mediated regulation, indicating that Hfq inhibits expression of *ompX* at the post-transcriptional level.
In contrast, production of the non-fimbrial adhesins InvA and YadA was decreased in the *hfq*-negative strains, suggesting that Hfq promotes the production of two major virulence factors in *Y. enterocolitica*. Using reporter fusions, we could show that Hfq promotes the expression *invA* most likely at the transcriptional level. Consistent with this, we observed changes in expression of the *invA* transcriptional regulators *rovA*, *ompR* and *phoP* in *hfq*-negative strains.
Conclusions: Our results show that the RNA chaperone Hfq has a profound impact on the composition of the bacterial envelope, in particular on the production of surface pathogenicity factors. Hfq inhibited expression of Ail-like OmpX post-transcriptionally, and modulated the expression of several transcriptional regulators controlling the composition of the bacterial envelope. Further work will aim to identify and characterize Hfq-dependent small RNAs that participate in remodeling the surface of *Y. enterocolitica*.

RSP05
Role of oxygen and the OxyR protein in the response to iron-limitation in *Rhodobacter sphaeroides*

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High intracellular levels of unbound iron can contribute to the production of reactive oxygen species (ROS) in the Fenton reaction, while depletion of iron limits the availability of iron containing proteins, some of which have important functions in the oxidative stress defence. Vice versa increased ROS levels lead to damage of proteins with iron sulfur centres[1]. Thus organisms have to coordinate and balance their responses to oxidative stress and iron availability. Our knowledge on the molecular mechanisms underlying the co-regulation of these responses is still limited. To discriminate between a direct cellular response to iron-limitation and indirect responses, which are the consequence of increased levels of ROS, we compared the response of the α-proteobacterium *Rhodobacter sphaeroides* to iron-limitation in presence or absence of oxygen.
One third of all genes with altered expression under iron-limitation showed a response, which was independent of oxygen availability. The other iron-regulated genes showed different responses in oxic or anoxic conditions and were grouped into six clusters based on the different expression profiles. For two of these clusters induction in response to iron-limitation under oxic conditions was dependent on the OxyR regulatory protein. An OxyR mutant showed increased ROS production and impaired growth under iron limitation. In addition to protein coding genes three sRNAs showed iron-dependent expression.
Some *R. sphaeroides* genes respond to iron-limitation irrespective of oxygen availability. These genes therefore reflect a "core iron response" that is independent of potential ROS production under oxic iron-limiting conditions. However, regulation of most genes is biased by oxygen availability. Most strikingly, the OxyR-dependent activation of a subset of genes upon iron limitation under oxic conditions, including many genes with a role in iron metabolism, reveals that elevated ROS levels are an important trigger for this response. OxyR thus provides a regulatory link between the responses to oxidative stress and to iron-limitation in *R. sphaeroides*. The highlighted sRNAs provide regulatory links between iron metabolism and photosynthesis and between iron metabolism and sulfur metabolism.

[1] Touati, D. (2000). Iron and oxidative stress in bacteria. *Arch Biochem Biophys* 373, 1-6.

RSP06
Identification of sRNA-binding proteins in *Streptococcus pyogenes* M49

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Introduction: Small non-coding RNAs (sRNAs) are a novel type of regulators that were found in several bacterial species. For the Gram-positive human pathogen *Streptococcus pyogenes* a few sRNAs like FasX are already identified and several putative candidates exist. In order to bind their target mRNA some sRNAs need a chaperone. In enteric bacteria the chaperone Hfq has been described and is required to support the base pairing between trans-encoded sRNAs and their target mRNA. No *hfq* homologue could be detected in *Streptococcus pyogenes*. Therefore, the aim of this work was to identify an Hfq-anologue in *S. pyogenes* M49 using the sRNA FasX as bait.
Materials and Methods: *E.coli* BL21 was transformed with a plasmid containing the gene for a MS2-MBP fusion protein. Induction of gene expression was followed by protein purification with an amylose resin column. An *in vitro* synthesis of the T7-MS2-aptamer-tagged FasX sRNA with T7 RNA polymerase was performed. To detect potential RNA-protein interactions the MS2-aptamer tagged FasX was incubated with *S. pyogenes* M49 cell lysate followed by an affinity chromatography.
Results: A pull down assay was conducted to identify sRNA-binding proteins from *S. pyogenes*. Two FasX bait constructs were designed to determine on which end of the sRNA the potential proteins would bind. Through fusion PCR FasX sRNA was MS2-aptamer tagged at the 3' and 5' end respectively. For affinity purification of MS2-aptamer tagged FasX-protein complexes, MS2-MBP fusion protein was produced in *E.coli*. The yield was 0.5 mg recombinant protein/l culture at a purity of approximately 90 %. The bait was incubated with *S. pyogenes* M49 crude extract. Using an MS2-MBP amylose column, MS2-tagged RNA-protein complexes were isolated. Elution fractions were analyzed by SDS-PAGE and compared to a mock purification lacking the tagged RNA.
Conclusion: A pull down assay was employed to enrich sRNA-binding proteins from *S. pyogenes* M49. Proteins that bound specifically will be identified by mass spectrometry. The detection of RNA protein interactions will help to identify potential sRNA chaperone molecules or other RNA-protein-complexes involved in sRNA-mediated regulation.

RSP07
Muropeptides (oligosaccharide-oligopeptides) are the factors of the dormant mycobacteria resuscitation

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Since the R. Koch's discovery of the causative of tuberculosis - *Mycobacterium tuberculosis* in 1882, the world tuberculosis pandemic is still a crucial problem of the society. In spite of the fact, that chemotherapeutic approaches have already been known for 50 years, one third of population carries tuberculosis infection in a latent state, being in a constant risk of transformation of the latent disease into the active form. Few years ago in our lab a protein Rpf (Resuscitation promoting factor), that stimulates reactivation of the "non-culturable" mycobacteria (incl. *M. tuberculosis*) into the active state was discovered. It had been proven, that Rpf possess enzymatic activity, namely - peptidoglycanhydrolase. But then the question arises - how both activities - resuscitation and enzymatic are bound together. We supposed first, that low-molecular weight metabolites oligosaccharide-oligopeptides might be released in milieu as a result of peptidoglycan Rpf hydrolysis. These products presumably could serve as a signal directly toward the cell and the other surrounding cells. Though, it was unclear, which compounds are releasing during peptidoglycan Rpf treatment and which of them directly induce reactivation. In the current study we demonstrated the ability of Rpf to hydrolyze mycobacterial cell wall, and it was shown that, as low molecular weight, as well as high molecular weight products were able to stimulate resuscitation of the dormant mycobacteria (in-lab model of mycobacterial dormancy). High molecular weight products of PG hydrolysis served as a substrate for Rpf, with subsequent release of the products with the lower molecular mass. We identified that the final product of mycobacterial PG joint degradation by Rpf with the other PG endopeptidase - RipA (resuscitation promoting factor interacting protein) is N-acetylglucosaminyl-(ß1→4)-N-glycolyl-1,6-anhydromuramyl-L-alanyl-D-glutamin (1,6-anhydro-GMDP). We synthesized this product and tested its resuscitation activity toward the dormant cells of *Mycobacterium smegmatis*. Comparison of the resuscitation activity of the 1,6-anhydro-GMDP with some other synthetic derivatives of peptidoglycan remodeling (incl. GMDP: N-acetylglucosaminyl-(ß1→4)-N-glycolylmuramyl-L-alanyl-D-glutamin, disaccharide: N-acetylglucosaminyl-(ß1→4)-N-glycolylmuramic acid, Dipeptide: L-alanyl-D-glutamin, MUDP: N-acetylmuramyl-L-alanyl-D-glutamin) allowed us to suggest, that it is 1,6-anhydro-disaccharide-dipeptide is a key molecule triggering resuscitation process in the dormant forms of mycobacteria, consequently interacting with mycobacterial intracellular protein-kinase PknB. Knowledge of the

resuscitation pathway among *Mycobacteria* is a key for synthesis and development of new anti-resuscitation drugs of pathogenic species of mycobacteria.

RSP08

The *Corynebacterium glutamicum* vanillate utilization system: Repression of the *vanABK* operon by VanR, a PadR-like regulator

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Corynebacterium glutamicum utilizes a variety of aromatic compounds in soil, *e.g.* the products of lignin degradation, such as ferulate, vanillate, and protocatechuate [1]. Genome sequencing and functional analysis of *C. glutamicum* revealed an operon consisting of three genes, *vanABK*, encoding the vanillate utilization system. Accordingly, vanillate is taken up via VanK, the specific transporter, and converted to protocatechuate by vanillate *O*-demethylase, whose subunits are encoded by *vanA* and *vanB* [2]. The *vanABK* operon is regulated by a specific repressor encoded by *vanR*, which is located upstream of *vanABK* in a reverse orientation. This was shown by fusion of the promoter of *vanABK* (P_{vanA}) to *eGFP* and measurement of the fluorescence intensity in the presence and absence of VanR. Heterologous expression of the possible *vanR* gene transcripts in *E. coli* indicated that a VanR variant consisting of 192 amino acids was the functional form of protein. Besides, size-exclusion chromatography showed that VanR molecules formed dimers. *In vivo*, vanillate induced the P_{vanA} activity similar to ferulate and vanillin. *In vitro* analysis by thermal shift assay confirmed vanillate as the main effector of VanR. To identify the VanR binding site, DNA footprinting was performed and the region between +9 and +45 with respect to transcription start site of *vanA* was detected as the VanR binding site. Deletion of nucleotide blocks inside the VanR binding site almost rendered P_{vanA} constitutive. Finally, by fusion of the VanR binding site and T7 promoter, the exact inverted repeat forming the binding site of VanR was detected. Interestingly, two inverted repeats (similar to the AACTAACTAA sequence) overlapping each other were found. Mutation of these nucleotides and electrophoretic mobility shift assay suggested that two VanR dimers are bound to the DNA for repression of the P_{vanA} transcription.

[1] Shen *et al.* (2012). **Appl Microbiol Biotechnol.** 95:77-89.
[2] Merkens *et al.* (2005) **Curr Microbiol.** 51:59-65.

RSP09

Temperature reduction stimulates proteomic changes enhancing biofilm formation of *Neisseria meningitidis*

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Introduction: The human pathogen *Neisseria meningitidis* (N.m., meningococci) frequently colonizes the human nasopharynx. Biofilm formation is thought to support colonization. Within the nasopharynx, meningococci encounter temperatures below 37°C. The adaptation of Nm to reduced temperatures is unexplored.
Materials and Methods: Using stable isotope labeling of Nm with ^{15}N and highly sensitive and highly accurate mass spectrometry, we compared the proteomes of Nm grown at 37°C and 32°C.
Results: We found the highest rate of deregulated proteins between 37°C and 32°C among outer membrane (OM) and periplasmic proteins, whereas inner membrane and cytosolic proteins were rarely affected. Nm grown at 32°C showed markedly elevated levels of biofilm formation and autoaggregation. By testing knockout mutants we found that the three highly upregulated OM proteins all contributed to biofilm formation and autoaggregation. Furthermore, an autotransporter protease, which suppresses Nm biofilm formation by the cleavage of adhesins, was less expressed at 32°C. Finally, we showed that increase of biofilm formation at slightly decreased temperatures was a general phenomenon of Nm and the related species Neisseria gonorrhoeae and Neisseria lactamica.
Conclusion: Temperature reduction from 37°C to 32°C influences the OM proteome, e.g. the vaccine antigen NHBA. The changes consecutively elevate biofilm formation and autoaggregation in Nm. Temperatures in the human nasopharynx might therefore serve Nm as a signal for the recognition and colonization of its desired niche.

RSP10

Identification of genes of *Pseudomonas aeruginosa* influencing pyocyanin production in a synthetic bacterial community

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Polymer-degrading bacteria face exploitation by opportunistic bacteria that grow with the degradation products without investing energy into production of extracellular hydrolytic enzymes. This scenario was investigated with a co-culture of the chitinolytic bacterium *Aeromonas hydrophila* strain AH-1N and *Pseudomonas aeruginosa* strain PAO1 as opportunist with chitin as sole source of carbon, nitrogen, and energy. During the co-culture *P. aeruginosa* progressed from a commensal to a parasitic growth strategy by producing quorum sensing-controlled secondary metabolites, among them the redox active pigment pyocyanin. Pyocyanin inhibited the enzyme aconitase of *A. hydrophila* through the production of reactive oxygen species causing a block of the citric acid cycle. This led to a massive acetate release by *A. hydrophila*, which supported substantial growth of *P. aeruginosa* [1].
To investigate the molecular mechanisms of this growth strategy transposon mutagenesis of *P. aeruginosa* was carried out to identify mutants unable to produce pyocyanin in co-culture. Several mutants with a transposon insertion in the gene cluster PA1421-1415 were isolated. A defined deletion of PA1421, encoding a guanidinobutyrase, completely abolished pyocyanin production in co-culture and led to reduced pyocyanin production in single culture. This was linked to a reduced transcription of both the *pqsABCDE* and the *phzA1-G1* operons in the mutant as investigated with transcriptional *lacZ* fusions.
Furthermore, several auxotrophic mutants were isolated that were unable to produce pyocyanin in co-culture even if tryptone was added as source of amino acids. Pyocyanin production could not be restored by overexpression of PqsE. In single culture, however, these mutants produced pyocyanin when grown in the presence of tryptone, and the production could be enhanced by PqsE overexpression.
Screening in co-culture led to the identification of *P. aeruginosa* mutants exhibiting a phenotype specifically under these conditions. Thus, this system is appropriate for identifying regulation levels of virulence factor production that are influenced by interactions with other microbes.

[1] Jagmann et al. (2010), Environ Microbiol 12(6): 1787-802

RSP11

Revisiting the Expression of the *atp* Operon in *Escherichia coli*

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The *atp* operon in *E. coli* consists of nine genes (*atpIBEFHAGDC*), encoding the structural subunits of the F_1F_o-ATPase and the dispensable protein AtpI. The F_1F_o-ATPase is one of the central parts of oxidative phosphorylation by using the proton motif force generated by the respiratory chain to synthesize ATP (Senior, 1988).
Four promoters have been described or predicted for the *atp* operon: a dominant promoter preceding the operon, two weak promoters inside the coding sequence of the first gene, *atpI* (Nielsen *et al.,* 1984), and a promoter inside the coding sequence of *atpD* (Huerta & Collado-Vides, 2003). According to the first gene on the corresponding transcript these promoters will be labeled *atpIp*, *atpBp1, atpBp2* and *atpCp* in this work.
Reporter plasmids were generated for all four putative promoters and for different combinations of them. QuickChangemutagenesis was used as described in Zheng *et al*. 2004. to alter regulatory elements. Construction of *phoPQ* and *rne131* deletion strains was performed according to Datsenko and Wanner 2000. β-Galactosidase activity was determined as described in Wiesemann *et al*. 2013.
We present data that demonstrates the activity of all four putative promoters under different physiological conditions including different carbon sources and pH values as well as in regard of the influence of *phoPQ* and *rne131* deletions. Additionally, we examined the influence of internal promoters, stem loops and restriction sites on the expression of *atpI* and *atpB*.
In this study, we demonstrate the activity of all four putative promoters including the two *atpB* promoter whose existence and contribution has been disputed (Kasimoglu *et al.*, 1996; von Meyenburg *et al*., 1982) and for the first time for the fourth promoter, *atpCp*. We further show that the stemloop structures inside of *atpI* affect the expression from promoters located upstream of them.

RSP12

Comparative proteomic profiling of *Leishmania tropica*: Investigation of a case infected with cutaneous and viscerotropic leishmaniasis by 2-dimentional electrophoresis (2-DE) and Liquid Chromatography (LC)-Mass Spectrometry

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Viscerotropic leishmaniasis caused by *Leishmania tropica* is a significant problem in the diagnosis and treatment management. This infection differs from classical visceral leishmaniasis caused by *L. infantum* in terms of clinical pathology, lacking hepato-splenomegaly and lower titer of anti-leishmania antibody. Since differential gene expression is more important in outcome of the infection, we employed proteomic approach in order to identify potential proteins implicated in disseminating of *L. tropica* from cutaneous to the viscera. Two-dimensional electrophoresis and mass spectrometry were used for comparing proteome profiling of cutaneous and visceral forms of *L. tropica* isolates. Out of about 700 protein spots revealed by 2DE map, 56 proteins were confidently identified and classified into 12 categories according to the biological process. The largest groups consist of proteins involved in carbohydrate metabolism (18%) and protein synthesis (16%). Most of the identified proteins which implicated in energy metabolism, cell signaling and virulence such as triosephosphate isomerase, calmodulin-like preotein and elongation factor (elf1-alpha) were down-regulated in visceral form. Whereas expression of some proteins that have a role in protein folding, antioxidant defense and proteolysis such as co-chaperon, tryparedoxin and ubiquitin were enhanced in visceral form. Our results suggest that *L. tropica* probably uses different mechanisms for surviving in viscera to establish viscerotropic leishmaniasis. The current study, for the first time, has gained a new insight into the mechanisms underlying the dissemination of *L. tropica.*

RSP13

Genome analyses of nine Janthinobacteria underline the importance of the ketone-based quorum sensing systems in nature.

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We have isolated, sequenced and characterized nine purple-pigmented bacterial strains from rainwater cistern samples, belonging to the genus Janthinobacteria. The draft genomes of HH100 - HH107 and the complete genome of HH01 average 6.7 Mbp, with 63.4 % GC content and each genome encode for about 6,000 ORFs. Most remarkably, all isolates code for a significant number of NRPS gene clusters and all isolates synthesize violacein. We previously have shown that the violacein biosynthesis in HH01 is in part controlled by the primary autoinducer (JAI-1) present in HH01 [1]. Interestingly, none of the sequenced isolates encode for autoinducer I- and II synthase genes (i.e. *luxI*- and *luxS* homologs), instead they carry a single copy of the *Vibrio cholerae* and *Legionella pneumophila* CQS/LQS quorum sensing systems [1,2]. The janthinobacterial quorum sensing system (JQS) consists of an autoinducer synthase (JqsA), a sensor kinase/phosphatase (JqsS) and a response regulator (JqsR), like the CQS system of *V. cholerae* [1,2]. *In silico* analysis indicate the corresponding JAI-1 autoinducer to be 2-aminopentadec-2-en-4-one, deviating in the head group and chain length to the autoinducers CAI-1 and LAI-1 synthesized by *V. cholerae* and *L. pneumophila*. Since the JQS system is present in all analyzed genomes, we conclude ketone based quorum sensing is characteristic for the genus *Janthinobacterium* and does play an important role in these microorganisms. Besides an effect of JAI-1 on the violacein synthesis in HH01, RNA-seq technology showed 34 genes were significantly up-regulated and 57 down-regulated in the background of the mutant strain vs. the control strain. Among the most strongly regulated genes were those linked to the autoinducer synthase JqsA, the nitrite reductases AniA and NirBDBC, the polyketide synthase Jab_2c35360, as well as the non-ribosomal peptide synthase Jab_2c35400. Ongoing work in the laboratory focuses on detailed analysis of the JAI-1 regulatory circuit in HH100-107 and its general importance for the interaction of this microbe with its environment.

[1] Hornung C, Poehlein A, Haack FS, Schmidt M, Dierking K, et al. (2013) The *Janthinobacterium* sp. HH01 Genome Encodes a Homologue of the *V. cholera* CqsA and *L. pneumophila* LqsA Autoinducer Synthases. PLoS ONE 8(2): e55045. doi:10.1371/journal.pone.0055045
[2] Tiaden A, Spirig T, Hilbi H (2010) Bacterial gene regulation by alphahydroxyketone signaling. Trends Microbiol 18: 288-297.

RSP14

Self-Regulation of Exopolysaccharide Production in *Bacillus subtilis* by a Tyrosine Kinase

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We report that the *Bacillus subtilis* exopolysaccharide EPS is a signaling molecule that controls its own production. EPS synthesis depends on a tyrosine kinase that consists of a membrane component, EpsA, and a kinase component, EpsB. EPS interacts with the extracellular domain of EpsA, which is a receptor, to control kinase activity. In the absence of EPS, the kinase is inactivated by autophosphorylation. The presence of EPS inhibits autophosphorylation and instead promotes the phosphorylation of a glycosyltransferase in the biosynthetic pathway, thereby stimulating the production of EPS. Thus, EPS production is subject to a positive feedback loop that ties its synthesis to its own concentration. Tyrosine kinase-mediated self-regulation could be a widespread feature of the control of exopolysaccharide production in bacteria.

RSP15

Regulation of oxidative stress response in *M. avium* ssp. *paratuberculosis* (MAP) by the ferric uptake regulator FurA

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Mycobacterium avium ssp. *paratuberculosis* (MAP), the causative agent of Johne's disease (JD) in ruminants, persists and replicates in subepithelial macrophages of the intestine, accompanied by metabolic adaptation to host cell defense mechanisms, such as oxidative stress. A persistent exposure of MAP to oxidative stress during infection was concluded from the enhanced expression of SodA and KatG, enzymes responsible for the neutralization of reactive oxygen and nitrogen species (Weigoldt et al. 2011, 2013).
The ferric uptake regulator FurA is known to be involved in iron homeostasis in many bacteria. The role of FurA in mycobacteria is still unclear; however, it is proposed to contribute to stress response regulation. A direct proof for this hypothesis was missing so far.
To gain insight in the functional role and regulation of FurA in MAP, we constructed a *fur*A deletion strain (MAPΔ*fur*A) by specialized transduction. RNA deep sequencing analyses revealed the FurA regulon, comprised of 13 higher and 35 lower expressed genes. Most of them are predicted to be involved in general stress response or virulence. No genes related to metal homeostasis were found to be affected by *fur*A deletion. Exposure of MAP to iron starvation or oxygen stress and qRT-PCR analyses revealed an iron-dependent activity of FurA. FurA seems to act as repressor in a Fe^{2+} loaded form, whereas gene activation occurs in the apoform. Furthermore, regulation of *fur*A was not iron dependent but affected by peroxide stress.
The role of FurA in intracellular survival was monitored in J774 macrophages. MAPΔ*fur*A showed significantly enhanced survival rates compared to the wildtype and the complemented strain.
Overall, these analyses indicated a considerable involvement of MAP FurA in the adaptation of MAP to the host cell environment and intracellular survival.

Weigoldt,M., Meens,J., Bange,F.C., Pich,A., Gerlach,G.F., and Goethe,R. (2013) Metabolic adaptation of *Mycobacterium avium* subsp. *paratuberculosis* to the gut environment *Microbiology* **159**: 380-391.
Weigoldt,M., Meens,J., Doll,K., Fritsch,I., Mobius,P., Goethe,R., and Gerlach,G.F. (2011) Differential proteome analysis of *Mycobacterium avium* subsp. *paratuberculosis* grown *in vitro* and isolated from cases of clinical Johne's disease *Microbiology* **157**: 557-565.

RSP16
Global analysis of *Clostridium difficile* stress response to environmental conditions and infection

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The bacterium *Clostridium difficile* is currently a major cause of high morbidity and mortality in Germany with several hundreds if not thousand patients dying per year. *C. difficile* associated diarrhea (CDAD) has caught increasing attention as one of the most deadly, hospital-acquired diseases. Almost nothing is known about the gene regulatory, protein and metabolic networks involved in the host associated life cycle of *C. difficile*. Our work aims to determine the gene regulatory network in response to general and specific stress factors as displayed during the infection cycle. In *Bacillus subtilis* the general stress response is mediated by the alternative sigma factor B (σ^B). However, the role of σ^B in *C. difficile* remains still unclear. To get a deeper insight into the stress response of *C. difficile* we analyzed σ^B, perR and fur mutants. We therefore applied a systems biology approach using different omics technologies and infection studies. The changes of interconnected networks in response to different environmental conditions (i.e. heat shock, oxygen exposure and iron limitation) will provide a detailed molecular insight into the *C. difficile* infection process. This constitutes a solid basis for the development of novel prevention, diagnosis and therapeutic strategies.

RSP17
Impact of the envelope stress response on production of pathogenicity factors in *Yersinia enterocolitica*

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Introduction: Upon ingestion of contaminated food or water, enteropathogenic *yersiniae* can colonize the intestinal tract, invade the host epithelium and proliferate in lymphoid tissue. To mount a successful infection the bacteria need to adapt quickly to changing environmental conditions. A key post-transcriptional regulator of adaptation is the RNA chaperone Hfq which is required for virulence and modulates the expression of many pathogenicity factors associated with the surface of *Y.enterocolitica*. In some Gram-negative bacteria the inactivation of *hfq* leads to an activation of envelope stress response (ESR) pathways, which are known to be important for bacterial envelope homeostasis. In Gram-negative bacteria the extracytoplasmic stress regulator RpoE and the two component system CpxRA govern two important ESR pathways. In this study we assessed whether a *Y. enterocolitica* strain mutated in *hfq* exhibits elevated ESR gene expression and the consequence of transient overexpression of RpoE and CpxR on production of surface pathogenicity factors.
Materials and Methods: Differential gene expression between wildtype and *hfq*-mutant was assessed by RT-qPCR. To achieve transient overexpression, ESR regulators were cloned under the control of an inducible promotor. Immunoblotting and translational fusions with GFP were used to assess expression of pathogenicity factors.
Results: Using RT-qPCR we could show increased abundance in RpoE-dependent genes (e.g. *degP, rpoE, fkpA*) in *hfq*-mutants grown at 27°C and 37°C but only a modest increase in CpxR-dependent transcripts (e.g. *cpxR, cpxA, ppiA*). Upon induction of the ESR regulator of CpxR, we observed a decrease in production of the major adhesion YadA. Overexpression of RpoE was accompanied by a decrease in YadA and OmpX. In contrast RpoE promoted a production of the adhesin Invasin and the chaperone DegP.
Discussion: Our results provide evidence that post-transcriptional regulation by the RNA chaperone Hfq affects the ESR in *Y.enterocolitica.* Moreover we observed that the ESR pathways modulate the production of pathogenicity factors. However the effects by ESR only partly overlap with the effects seen in *hfq*-negative strains.

RSP18
Identification of the *accessory gene regulator* (*agr*) signal peptide of *Listeria monocytogenes*

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Listeria monocytogenes is a ubiquitous Gram-positive food-borne pathogen, causing listeriosis in risk patients. Since *L. monocytogenes* is able to grow both as a saprophytic organism organized in biofilms and as an intracellular pathogen, the different factors needed for survival in these totally different habitats have to be regulated. One regulatory mechanism of *L. monocytogenes* involved in adaptation to both habitats is the *accessory gene regulator* (*agr*) system (Riedel *et al.*, 2009). This autoregulatory pathway consists of a pre-peptide (AgrD), which is processed by the peptidase AgrB into the autoinducing peptide (AIP). The peptide then activates the two-component system AgrCA. An *agrD* deletion mutant showed attenuated virulence *in vitro* and *in vivo* as well as reduced biofilm formation. Additionally, during *in vitro* growth on complex medium, > 600 genes were differentially expressed in the Δ*agrD* mutant compared to the wildtype. With this study we aim to identify the structure of the native listerial AIP. Luciferase reporter strains were created by cloning the promoter upstream of the *agr* operon (P_{II}) in front of the genes for the luciferase reporter in the promoter probe vector pPL2*lux* (Bron *et al.*, 2006). Following electroporation and chromosomal integration of the resulting vector in *L. monocytogenes* EGDe wildtype and Δ*agrD* both strains were assayed for P_{II} activity. As expected P_{II}-driven luciferase activity could be measured in the wildtype but not the Δ*agrD* mutant suggesting positive auto-regulation. Surprisingly, no AIP could be detected in culture supernatants of the wildtype strain using this reporter system. In an attempt to demonstrate AIP-driven activation of P_{II}, *agrBD* was overexpressed in *E. coli*. Supernatants of the recombinant *E. coli* host contained considerable *lux* reporter activity and the listerial AIP could be purified from these supernatants by HPLC. In a complementary approach, potential AIPs of *L. monocytogenes* were designed based on *in silico* analysis of the deduced amino acid sequence of the *agrD*-encoded AIP and compared to known AIPs of other Gram-positive organisms. These potential listerial AIPs were synthesized and assayed for P_{II} luciferase reporter activity. This led to the identification of both P_{II} activating and inhibitory peptides.

RSP19
Sensing of C4-Dicarboxylates in the DcuB/DcuS and the DctA/DcuS Sensor complexes.

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In absence of the aerobic transporter DctA and the anaerobic transporter DcuB, the C4-dicarboxylate sensor DcuS is constitutively active in an effector independent manner [1,2]. A role for both transporters as cosensors under aerobic and anaerobic conditions, respectively, was therefore assumed and it was hypothesized that transport activity of DcuB and DctA is necessary for signal perception [3].
To test this hypothesis the role of the transporters and of DcuS in the DctA/DcuS and the DcuB/DcuS sensor complexes were analyzed: (i)Transport substrates of both transporters were determined by a competitive uptake assay. The substrate spectra were compared with the substrates inducing a DcuB/DcuS or DctA/DcuS dependent reporter gene (*dcuB-lacZ*). Effectors like L-malate and fumarate were substrates of both sensor complexes as well as of the transporters. In contrast, citrate and maleate induced the expression of *dcuB-lacZ* but were neither recognized by DcuB nor by DctA. (ii) The variant DctA(S380) was able to restore C4-Dicarboxylate responsiveness to DcuS but was deficient in growth on fumarate and transport. Therefore transport and regulatory function of DctA could be uncoupled, which was previously demonstrated for DcuB [2]. (iii) Since induction of *dcuB-lacZ* requires approx. 10-30 fold higher effector concentrations than transport by DcuB (K_m= 100 μM) or DctA (K_m = 30 μM) different binding sites for transport and signal perception can be assumed. (iv) DcuS was titrated with DctA and with increasing expression levels of *dctA* C4-dicarboxylate responsiveness of DcuS was restored.
The results demonstrate that the transporters DcuB and DctA transfer the sensor DcuS to a C4-dicarboxylate responsive state, but do not directly contribute to signal perception. This constitutes a mechanism by which cellular levels of DctA and DcuB are kept constant in dividing cells, ensuring basal expression levels in absence of C4-dicarboxylates.

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RSP20
The FNR-like Transcription Factor FLP Regulates Cytochrome *d* Oxidase Synthesis in *Xanthomonas campestris* pv. *vesicatoria*

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The plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is an obligately aerobic, oxidase-negative γ-proteobacterium that causes bacterial spot disease on pepper and tomato plants. It grows in the intercellular space between plant cells in a biofilm where it must deal with variable oxygen concentrations that depend, for example, on whether the plant is exposed to light or not. The bacterium also has to cope with reactive oxygen species (ROS), which potentially form part of the host defense response. The genome of *Xcv* encodes a protein we term FLP, which is a FNR-like protein. Members of the FNR superfamily are dimeric, redox-sensitive iron-sulfur cluster (FeS)-containing transcription factors that sense oxygen and ROS directly. Normally, FNR-like proteins are activated under anaerobic or oxygen-limiting conditions where they regulate gene expression either positively or negatively. Oxygen destroys the integrity of the FeS cluster causing conversion of the dimeric transcription factor to a monomeric species, which is incapable of binding DNA or regulating gene expression.
It was initially surprising to find a predicted FNR-like protein in an obligate aerobic bacterium like *Xcv*. Bioinformatic and transcriptomic studies identified a minimal FLP-regulon in *Xcv*, whereby principally the *cydABX* operon, which encodes a high affinity cytochrome *d* oxidase, is positively regulated by the transcription factor. The DNA-binding domain of FLP is essentially identical to that of the *E. coli* FNR protein and this is consistent with a consensus FNR-recognition motif (TTGAT-N_4-ATCAA) located at postion -41.4 bp upstream of the transcription start site in the regulatory region of the *cydABX* operon. Quantitative RT-PCR experiments confirmed that in a *flp* mutant expression of the *cydABX* operon was reduced, but not abolished, indicating another as yet unidentified regulator is involved in controlling expression of the operon. Physiological analyses indicate that rather than having an overt phenotype, a *flp* mutant grows slightly better in axenic cultures compared with the wild type *Xcv* strain and overproduction of FLP results in poorer growth than the wild type in minimal medium. A similar growth phenotype was also observed for the mutant during growth on tomato plants suggesting that the cytochrome *d* oxidase has an important role in controlling the rate of oxygen consumption by the bacterium and it does this by governing oxidase preference. The importance of cytochrome *d* oxidase is underscored by the fact that the *cydABX* operon appears to be essential in this important phytopathogen.

RSP21
A sRNA in *Rhodobacter sphaeroides* which is processed upon $^1O_2$ exposure

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Exposure to oxygen and light generates photooxidative stress by the bacteriochlorophyll *a* mediated formation of singlet oxygen ($^1O_2$) in the facultative photosynthetic bacterium *Rhodobacter sphaeroides* (1). A differential RNA-sequencing identified RSs0682 as most abundant regulatory small RNA (sRNA). This sRNA derives from a terminator structure in the 5'UTR of *mraZ*, the first gene of the *dcw* (division cell wall) gene cluster, and features an altered processing in response to $^1O_2$ and in stationary phase. Most likely this sRNA influences gene expression at the post-transcriptional level for the benefit of the stress response under these particular growth conditions. The RNA processing site has been determined by 5'RACE. The 3'-segment of the full-length sRNA displays the processed sRNA and is highly conserved between different *Rhodobacterales* species (2). Moreover processing was not observed in an *hfq* deletion strain of *R. sphaeroides* and the interaction of the full-length sRNA as well as the 3'-segment with Hfq was proven by co-IP experiments. This indicates an important role of the RNA chaperone (3). To investigate the impact of the sRNA a RSs0682 overexpression strain was used for Microarray analysis. In addition, the search for putative targets was completed by a screening for potential interactions of RSs0682 with mRNAs by the use of IntaRNA. Finally we plan to identify the RNase that catalyses the RSs0682 cleavage. Using knock-out strains it was already shown in vivo that the processing is RNase III- and RNase J-independent.

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RSP22
Characterization of the photolyase/cryptochrome family protein CryB in the purple bacterium *Rhodobacter sphaeroides*

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The cryptochrome- and photolyase related protein CryB of *R.sphaeroides* was identified as a blue light receptor with signaling and regulating functions, but also shows a contribution to light-dependent photoreactivation after damage by UV-light *in vivo*, attributed to photolyases. These enzymes specifically recognize and repair defined types of DNA damages, usually two different versions of dimerized neighboring thymine bases. CryB differs from other members of its family in several aspects, most strikingly concerning the composition of the cofactors. Cryptochromes and photolyases bind two light absorbing chromophores. The photo-redox responsive FAD (flavine adenine dinucleotide) is a conserved cofactor and found in all members of this family, and a second variable cofactor serves as an antenna to absorb additional light energy. The antenna in *R.sphaeroides* was identified as 6,7-dimethyl-8-ribityl-lumazine, DLZ, and represents a new cofactor. Additionally, an iron-sulfur cluster was identified as a third cofactor besides FAD and lumazine, which is unique for this group of proteins, termed CryPro family [1].
We have constructed amino acid substitution variants of CryB by site-directed mutagenesis at relevant cofactor binding residues. These versions have been inserted into a *cryB* knockout strain of *R.sphaeroides* on a plasmid. The strains were analysed for their ability to survive exposure to UV light and subsequent white light illumination to investigate the light-dependent activity of photolyases. A version which is locked in the oxidized state of FAD is still capable of restoring photoreactivation in ΔcryB (survival rate of 70-80% in wild type and complementation strains compared to non-stressed cells). Lack of the antenna cofactor DLZ has the same effect, while a double mutant shows an impaired photoreactivation comparable to the *cryB* knockout strain (20% survival).
To investigate the mechanism of photorepair, further amino acid substitutions will be tested, as well as complementations with characterized photolyases of other organisms. This might reveal details about a possible additional photolyase activity besides the regulatory functions as a blue light receptor.

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RSP23
Dynamic interactions within a bacterial two-component system

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Two-component systems (TCSs) are the main mechanisms by which bacteria sense and respond to environmental stimuli [1] and consist of a sensor kinase (SK) and a response regulator (RR). Some TCSs are additionally modulated by an accessory protein [2]. However, how these accessory proteins modulate the response is for most TCSs not known.
We use the Cpx-TCS as a model to investigate signal recognition and transduction in TCS signaling [3]. It consists of the SK CpxA, the RR CpxR and the periplasmic accessory protein CpxP. CpxP is a Cpx-TCS dependent factor that counteracts extracytoplasmic protein-mediated toxicities [4, 5]. Moreover, for misfolded proteins derived from the P pilus of uropathogenic *E. coli* CpxP appears to act as an adaptor protein for the periplasmic protease DegP [4]. On the other hand, *cpxP* overexpression results in reduced Cpx-response [6]. Thereby, CpxP inhibits autophosphorylation of reconstituted CpxA [7]. Physical interaction between CpxA and CpxP was predicted as a requirement for this regulatory interaction, but never shown. Now, we demonstrated physical interaction between CpxP and CpxA in unstressed cells using bacterial two hybrid assay (BACTH) and membrane-Strep-

tagged protein interaction experiments (mSPINE) [8]. Moreover, mSPINE displayed that this interaction is detached by high NaCl concentration and misfolded pilus subunit PapE. Overall, our study emphasizes a model in which the inhibitory and supporting functions of CpxP for envelope stress response are linked: In unstressed cells, CpxP associates with CpxA to shut off the Cpx-TCS. Envelope-stress conditions induce the displacement of CpxP from CpxA resulting in Cpx-TCS activation. Together, our results suggest that CpxP modulates the activity of the Cpx-TCS by dynamic interaction with CpxA in response to specific stresses.

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RSP24

Physiological stratification and differential spatial distribution of amyloid curli fibres, cellulose and flagella in *Escherichia coli* macrocolony biofilms

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Introduction: Bacteria preferently live in structured communities known as biofilms. Although it is widely recognized that biofilm formation depends on bacterial self-produced matrix components such as adhesins, amyloid fibres and exopolysaccharides, the precise location, organization and structural function of these components inside biofilms and the link to bacterial physiology are poorly known. *Escherichia coli* produces flagella during the post-exponential phase, when nutrients become limiting, but the rod-shaped cells still grow. Upon entry into stationary phase, cells stop producing flagella, become small, ovoid and multiple stress resistant and generate amyloid curli fibres and the exopolysaccharide cellulose. At the regulatory level, the synthesis of curli and cellulose depends on the biofilm regulator CsgD.
Materials and Methods. Using flagella, curli fibres, cellulose, cell morphology and visualized expression of *csgD* as 'anatomical' hallmarks in fluorescence and scanning electron microscopy, we seek to elucidate the spatial order of physiological stratification and matrix distribution in *E. coli* biofilms at high resolution.
Results: Two physiologically distinct cell layers and three differential patterns of matrix distribution were distinguished at high resolution in macrocolonies of AR3110, a K-12 strain with restored capacity to produce cellulose. Macromorphologically, AR3110 macrocolonies are only about 60 micrometer thin, large and exhibit radial ridge-like structures formed by vertical buckling of the biofilm. The lower layer (about 20 micrometer thick) features bacteria that resemble those in post-exponential phase with entangled flagella as a single and characteristic matrix component. In sharp contrast, the upper layer of flat sectors and ridges (about 40 micrometer thick) contains starving stationary phase-like bacteria surrounded by (i) cellulose sheets/filaments in the inner zone, at the boundary with the lower layer, and (ii) a dense nanocomposite of curli and cellulose in the outermost zone. This differential pattern of matrix composition in the upper layer (cellulose only vs cellulose/curli composite) precisely correlates in space with bimodal expression of *csgD*.
Conclusion/Discussion: These results contribute to our understanding to how physiologically distinct subpopulations of bacteria arise within a community and how these subpopulation dictate a precise spatial order of matrix synthesis that ultimately defines the biofilm architecture.

RSP25

Transcriptional Regulation of Reductive Dehalogenase-Encoding Genes in *Dehalococcoides mccartyi* by a MarR-Type Regulator

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Reductive dehalogenases catalyze the reduction of halogenated terminal electron acceptors during organohalide respiration. Only a subset of the 32 reductive dehalogenase-encoding genes (*rdhAB*) in the genome of *Dehalococcoides mccartyi* strain CBDB1 is expressed on the protein level suggesting a specific regulation in response to halogenated electron acceptors. The *rdhAB* genes *cbdbA1453/52* and *cbdbA1455/54* are located directly upstream of a divergently oriented gene *cbdbA1456*, which encodes a MarR-type regulator. Its role in the transcriptional regulation of both *rdhA* genes was investigated by quantitative reverse-transcription PCR in strain CBDB1 and by *in vivo* and *in vitro* interaction studies of the heterologously produced MarR regulator.
Both *rdh* genes and the *marR* gene were transcribed during growth on 1,2,3-trichlorobenzene. The promoters of the *rdhA* genes and the *marR* gene were introduced as singly copy promoter-*lacZ* fusions onto the chromsome of *E. coli*. All three promoters were recognized by the host RNA polymerase as revealed by β-galactosidase activity measurements. When transformed with a MarR-encoding plasmid the β-galactosidase activity of both *rdhA*-promoter *lacZ* fusions was strongly repressed, whereas that of the *marR* promoter was not. The transcriptional start sites of *cbdbA1453* and *cbdbA1455* were mapped to to the vicinity of a 13 bp direct repeat, which was conserved in both predicted promoters. A specific interaction of MarR with both *rdhA* promoters (but not with its own promoter) was confirmed using electrophoretic mobility shift assays and was narrowed down to the 13 bp direct repeat by DNase I footprinting.
The results demonstrate that MarR acts as a transcriptional repressor of *rdh* operon expression and probably plays a role in the halogenated compound-specific expression of selected *rdh* genes during organohalide respiration.

RSP26

Osmotic stress tolerance of *Acinetobacter baylyi*: Identification of a choline - glycine betaine pathway gene cluster and its transcriptional regulation

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Soil dwelling bacteria such as *Acinetobacter baylyi* are faced with perpetual changes in the environment. Changes in the osmotic pressure of the biotope pose a particular challenge to the cells. In previous work we showed that *A. baylyi* accumulates the compatible solute glycine betaine to cope with hypersaline environments [1, 2]. We now elucidated a two-step pathway whereby *A. baylyi* is able to synthesize glycine betaine from its precursor choline. The genes encoding a potential choline oxidation pathway (*betA*, *betB*) are closely associated with two choline transporters (*betT1*, *betT2*) and a potential transcriptional regulator (*betI*). To elucidate the function of the distinct genes, we generated mutants and analyzed their potential to oxidize choline by thin layer chromatography and choline-dependent oxygen reduction. The studies provided clear evidence that *betA* encodes a choline-dehydrogenase and that *betB* encodes a betaine-aldehyde-dehydrogenase. In order to gain insight into the regulation of the genes encoded in the *bet*-cluster, we investigated their transcriptional organization. We found that *betA*, *betB* and *betI* form an operon whereas the two transporter genes *betT1* and *betT2* are independently transcribed. Quantitative Real-Time PCR analyses revealed that the transcript levels of the *bet* genes are dependent on salinity and on choline content of the growth medium. Electrophoretic mobility shift assays led to the identification of two BetI binding sites within the *bet* gene-cluster and the affinity of BetI to the binding sites was modulated by choline. Taken together these data give first insights into the role of BetI in the transcriptional regulation of the choline and osmo-regulated glycine betaine pathway in *A. baylyi*.
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RSP27

FurC regulates expression of *zupT* encoding the central zinc importer ZupT of *Cupriavidus metallidurans.*

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The β-proteobacterium *Cupriavidus metallidurans* is a model organism for bacterial resistance to transition metals. Survival of this bacterium in environments with high concentrations of metal ions such as Zn^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+} is made possible by an arsenal of metal efflux systems like the chromosomally encoded P-type ATPases ZntA and CadA or those encoded by resistance determinants on the megaplasmids pMOL28 and pMOL30. Zn^{2+} acts as cofactor for several essential enzymes like alcohol dehydrogenase, carbonic anhydrases or carboxypeptidases. Therefore, constant zinc uptake is necessary, and in *C. metallidurans* this function is performed by a battery of redundant and rather unspecific secondary import systems, namely ZupT, CorA1-3, and PitA for metal-phosphate complexes.
Zinc-dependent regulation is required to maintain zinc homeostasis by orchestrating uptake and efflux reactions. Regulators of the Fur family act as transcriptional repressors that bind metal ions. Most prominent is the Fur repressor of iron uptake, but other proteins might interact with zinc (Zur), manganese (Mur) or nickel ions (Nur). *C. metallidurans* contains three members of the Fur protein family, designated FurA, FurB and FurC, all encoded on the two chromosomes of the bacterium.
The function of the three *fur*- orthologs were investigated with single Δ*fur* mutants by growth experiments, observation of the expression level of zinc dependent import- and efflux systems, and by *in vitro* studies with heterologously expressed and purified *strep*-tag-Fur proteins.
This study determines the role of FurC as zinc uptake regulator that controls the expression of one of the central players of the zinc homeostasis, *zupT*. In addition, FurA is shown to be the central iron-uptake regulator of *C. metallidurans*. FurB may be a second classical Fur specifically required under iron-starvation conditions, or may provide a link between iron and zinc homeostasis.

RSP28

Phosphorylation signaling through the Legionella quorum sensing histidine kinases LqsS and LqsT converges on the response regulator LqsR

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The environmental bacterium *Legionella pneumophila* causes a life-threatening pneumonia termed Legionnaires´ disease. *L. pneumophila* uses for cell-cell communication the autoinducer LAI-1 (3-hydroxypentadecane-4-one), which is produced and detected by the Lqs (*Legionella* quorum sensing) system. The system comprises the putative sensor kinases LqsS and LqsT, the prototypic response regulator LqsR and the autoinducer synthase LqsA. Lqs-regulated processes include pathogen-phagocyte interactions, production of extracellular filaments and natural competence for DNA uptake [(1)]. Using biochemical approaches, we found that LqsS and LqsT are autophosphorylated by [γ-^{32}P]-ATP at a conserved histidine residue (H_{200} or H_{204}) located in their cytoplasmic histidine kinase domain. Immuno-precipitation revealed that LqsS and LqsT are bound by the response regulator LqsR as well as by phospho-LqsR. LqsR prevented autophosphorylation of either sensor kinase in a manner dependent on the conserved receiver domain aspartate (D_{108}). Moreover, the response regulator catalyzed the dephosphorylation of phospho-LqsS or phospho-LqsT, and efficient dephosphorylation also required D_{108}. LqsR was phosphorylated at D_{108} *in vitro* with acetyl-phosphate, and the phosphorylation by either acetyl-phosphate or phospho-LqsT caused dimerization of the response regulator. Finally, LqsT (but not LqsS) was autophosphorylated by ATP upon heterologous production in *E. coli*. Taken together, these results indicate that phosphorylation signaling through the *Legionella* quorum sensing histidine kinases LqsS and LqsT converges on the response regulator LqsR [(2)].

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RSP29

Small RNAs expressed in *Burkholderia cenocepacia* biofilms

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Burkholderia cenocepacia is a member of the *Burkholderia cepacia* complex (Bcc), a group of closely related opportunistic pathogens able to infect cystic fibrosis patients and immunocompromised individuals. Bcc infections are very difficult to treat due to the high innate antimicrobial resistance of this group of bacteria. Biofilm formation has been reported for many Bcc strains, further contributing to their recalcitrance.
In recent years, an increasing number of small non-coding regulatory RNAs has been discovered in prokaryotes. Among the regulatory cascades influenced by small RNAs are global developmental processes such as biofilm formation.
Small RNAs of *Burkholderia* spp. are to date largely unidentified. We aim to identify small RNAs involved in *B. cenocepacia* biofilm formation. For this purpose, RNA from biofilm grown cells was sequenced using differential RNA sequencing. This method combines 5'-end sequencing of total RNA with terminator exonuclease treatment of a portion of the RNA sample and allows precise mapping of transcription start sites.
Transcription start sites were then screened for putative small RNAs, based on their position relative to annotated genes, secondary structure formation, presence of a terminator and conservation within genomes of sequenced *Burkholderia* sp. More than 60 putative new small RNAs were identified using this approach.
Most small RNAs are expressed only under specific growth conditions. Therefore, a subset of putative small RNAs with relatively strong expression in biofilms compared to planktonically grown cultures was selected for further characterisation. The relevance of selected putative small RNAs for growth, biofilm formation and stress resistance was investigated by expressing antisense RNA on a rhamnose-inducible vector. Preliminary results indicate that these small RNAs play a role in stress resistance.

RSP30

Improvement of competence in *Bacillus subtilis*

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Bacillus subtilis takes up extracellular DNA in a physiological state, called competence. However, only one fifth of the cell population of a wild type strain becomes competent. To facilitate the genome manipulation and industrial application, it is important to alter the genome of *B. subtilis* in order to obtain a higher level of transformable cells in the bacterial culture. Development of competence depends on ComK, the major transcriptional regulator of DNA-binding, -uptake and -recombination genes (*comG* operon).[1] ComK is protected by ComS from degradation by inhibiting the formation of a stable ternary ComK/MecA/ClpC complex.[2] Besides expression of *comK* is regulated by additional factors, such as sporulation regulator (Spo0A), nutrition limitation regulator (CodY), transition state regulator AbrB, degradation regulator DegU, and Rok, the ComK repressor. The *comG* operon encodes a pilin-like structure for binding and uptake of extracellular DNA.[3] After transformation, the assimilated single strand DNA is protected by various single strand binding proteins as DprA.[4] To improve competence, *comK*, *comS*, *dprA* and combinations of them were integrated into the *B. subtilis* 168 genome under control of P_{mtlA} and strains were transformated using a newly designed method with LB medium. Transformation efficiency was compared with *B. subtilis* 168 using the established technique in Spizizen's minimal medium. Expression of *comK* together with *comS* resulted in the highest level of competence, which was increased by 64-fold. Since high level expression of *comK* arrested the growth after dilution into fresh LB medium, cells were constructed with a deletion of original *comK* and *comS*. After *comS* deletion, competence was improved by 71-fold but still with cell arrest, whereas *comK* deletion compensated cell arrest, but competence was just improved by 10-fold.

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RSP31

Actinobacterial Intelligence: a comprehensive survey of signal transduction in this phylum

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Signal transduction is an essential process in bacterial homeostasis that allows bacteria to sense their complex and ever changing environment and adapt accordingly. Four distinct types of Signal Transducing Proteins (STPs) can be distinguished: one-component systems (1CSs), two-component systems (2CSs), chemotaxis-related proteins and ECF sigma factors (*1*). *Actinobacteria* are particularly rich in STPs and the genomes of many members of this phylum are particularly rich in ECF sigma factors (*2*). For this reason, we have comprehensively investigated the abundance and diversity of STPs encoded in 119 actinobacterial genomes, based on the data stored in the Microbial Signal Transduction (MiST) database.
Overall, we have observed an approximately linear correlation between genome size and the total number of encoded STPs. While such a correlation is also maintained for 1CSs, it does not hold true for 2CSs or ECF sigma factors. In case of 2CSs, this deviation is mostly due to the fact that larger genomes encode an excess of histidine kinases (HKs) over response regulators (RRs), which suggests an increased ability to integrate distinct environmental signals onto single outputs. A detailed analysis of the domain architectures of HKs and RRs has allowed us to identify novel proteins that are only found in actinobacterial genomes. Additionally, almost 500 previously unclassified ECF sigma factors were classified into 21 new groups. This classification reflects not only the sequence diversity of their conserved regions but also their genomic context conservation, putative target promoter motifs as well as the presence of group-specific anti-sigma factors. Three of these novel groups contain ECF sigma factors with C-terminal extensions, indicative of a fusion between ECF sigma factors and the corresponding anti-sigma function.
This comprehensive survey demonstrates that actinobacterial genomes encode previously unknown STPs, which may represent new mechanisms of signal transduction and regulation. This information not only expands our knowledge on the diversity of bacterial signal transduction but also provides clear and testable hypotheses on their mechanisms, which can serve as starting points for experimental studies.

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RSP32

Phosphosugar stress causes EII^{Glc}-dependent inhibition of the PTS phosphorylation cascade in *Corynebacterium glutamicum*

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The Gram-positive bacterium *Corynebacterium glutamicum* co-metabolizes most carbon sources such as glucose, fructose and sucrose. The phosphotransferase system (PTS) of this organism catalyzes the uptake and concomitant phosphorylation of those three sugars and possesses specific permeases for each of them - EII^{Glc}, EII^{Fru} and EII^{Suc}, respectively. The required phospho-groups are delivered by a phospho-relay between phosphoenolpyruvate and the common PTS components EI and HPr. Deletion of *pgi*, encoding the enzyme phosphoglucoisomerase, blocks the first step of glycolysis and directs the glucose-driven carbon flux towards the pentose phosphate pathway. *C. glutamicum* Δ*pgi* grows poorly with glucose as a sole substrate as the PTS-mediated glucose uptake is drastically reduced to avoid accumulation of glucose-6-phosphate (1). Growth of the mutant with sucrose as a sole carbon source is not affected. However, glucose addition to sucrose-cultivated *C. glutamicum* Δ*pgi* cells immediately arrested their growth. In detail, addition of glucose caused within 15 sec inhibition of the EII^{Suc}-mediated sucrose uptake which could not be prevented by transcriptional or translational inhibitors. Similar growth and rapid sucrose uptake inhibition could be observed after addition of maltose, which is not transported by the PTS but is also metabolized to glucose-6-phosphate. EII^{Glc} is required for the initiation of the inhibitory mechanism as no negative effects by the addition of glucose or maltose were observed for the EII^{Glc}-deficient strain *C. glutamicum* Δ*pgi* Δ*ptsG*.
Here, we investigated the target of the mechanism causing rapid uptake inhibition. Glucose is not *per se* toxic for *C. glutamicum* Δ*pgi* as the mutant grew well with glucose plus ribose, which is a non-PTS substrate entering the central metabolism at the end of the oxidative part of the pentose phosphate pathway. Furthermore, we showed that the addition of glucose or maltose rapidly inhibits the uptake mediated by the third, fructose-specific PTS permease EII^{Fru}, as well. This indicated that the general PTS activity of the cell might be object of the regulatory mechanism. Indeed, analysis of the phosphorylation state of HPr - the last common component of the PTS phosphorylation cascade - indicated that the reason for the uptake inhibition is a rapid depletion of phosphorylated HPr. The addition of maltose or glucose to sucrose-cultivated *C. glutamicum* Δ*pgi* caused decrease of the HPr-P / HPr ratio in the cells. These results show that the *ptsG*-encoded EII^{Glc} is part of a novel mechanism for perception of phosphosugar stress which leads to instantaneous inhibition of the PTS phosphorylation cascade in *C. glutamicum*.

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RSP33

Cross-talk between the envelope stress Cpx-TCS and the redox responsive Arc-TCS

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The two-component signal transduction systems (TCSs) permit bacteria to sense and response to a large variety of environmental stimuli. They consist of a membrane bound sensor kinase (SK) and a cytosolic response regulator (RR). Inducing stimuli lead to SK autophosphorylation which further transfers a phosphoryl group onto its cognate RR. The activated RR then causes the appropriate cellular response [1]. However, there is no detailed knowledge about the mechanisms how bacteria coordinate simultaneously the specific activities of the many different but highly related TCSs in one cell [2].
A physiological relevant cross-talk was suggested between the CpxAR-envelope stress TCS and the ArcBA-redox stress TCS in *E. coli*. The current model suggests a cross-phosphorylation between the SK CpxA and the non-cognate RR ArcA during antibiotic treatment resulting in the generation of oxygen radicals (ROS) that finally causes bacterial cell death [3].
We now show physical interaction using the bacterial two-hybrid system (BACTH). In addition, we demonstrate functional interaction between in nano-disc reconstituted full length CpxA and ArcA by phosphotransfer assays *in vitro*. These studies are currently accomplished by the determination of the affinities between CpxA and ArcA as well as between ArcA and its respective sensor kinase ArcB. Furthermore co-localization studies using super resolution microscopy will be presented for CpxA and ArcA under various conditions to confirm the assumed physiological relevance of this proposed TCS cross-talk *in vivo*.

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RSP34

Fine-tuning of sulfur metabolism and glutathione levels by a small RNA upon singlet oxygen stress in *Rhodobacter sphaeroides*

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The photosynthetic model bacterium *Rhodobacter sphaeroides* faces photooxidative stress due to the bacteriochlorophyll-mediated generation of singlet oxygen ($^1O_2$) in the light. Our aim is to investigate the underlying response mechanisms, focusing in particular on the involvement of small RNAs.
The alternative sigma factor RpoE, which is on top of $^1O_2$-dependent regulation, induces the 219 nt long sRNA RSs0019 (1). RSs0019 contains a small ORF (150 nt), which is translated under $^1O_2$ stress. Over-expression of RSs0019 negatively affected mRNA levels for several genes involved in sulfur metabolism as well as for the hypothetical protein RSP_0557, as shown by microarray analysis. To distinguish between peptide- versus sRNA-driven effects, several RSs0019 mutant variants were over-expressed and compared to the genuine sRNA by real time RT-PCR. These experiments suggested RSs0019 to be a potential bifunctional RNA, as loss of peptide expression did not affect gene regulation by RSs0019. We used a

lacZ-based *in vivo* reporter system to further uncover the effect of RSs0019 ORF expression on the potential target RSP_0557. These studies also suggested RSs0019 to be Hfq-dependent and binding of RSs0019 to Hfq was shown by co-immunoprecipitation.
Our data provide evidence that both RSs0019 and RSP_0557 act as negative regulators of glutathione levels and together create an incoherent feed-forward type of regulation. This interplay might fine-tune glutathione levels under conditions of $^1O_2$ stress.

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RSP35
Regulation of anaerobic hydrocarbon-degradation in *Aromatoleum aromaticum* EbN1

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Aromatic hydrocarbons like benzene, toluene, ethylbenzene and xylenes (BTEX) along with phenolic compounds including phenol and ethylphenol are used in large amounts in industrial processes and therefore lead to widespread environmental pollution. The genome-sequenced β-proteobacterium *Aromatoleum aromaticum* strain EbN1 is capable to degrade the hydrocarbons toluene and ethylbenzene as well as different phenolic compounds anaerobically [1, 2]. Our main aim is to clarify the mechanisms involved in discrimination of these very similar aromatic substrates. In EbN1 two separate gene clusters can be identified for the degradation of phenol and ethylphenol, which indicate a σ^{54}-dependent regulation due to conserved -24/-12 promoter sequences and characteristic XylR-like regulators adjacent to the clusters. The genes coding for the enzymes of anaerobic toluene metabolism are induced co-ordinately in the presence of toluene, whereas those coding for the enzymes of anaerobic ethylbenzene metabolism are induced sequentially in the presence of ethylbenzene and the intermediate acetophenone, respectively. Primer extension analysis was used to determine the start sites of RNA transcription. Three operons coding for two-component regulatory systems were identified in the genome sequence of EbN1 as possible candidates for affecting the induction of all toluene-catabolic genes (*tdiSR*), ethylbenzene-catabolic genes (*ediSR*) and acetophenone-catabolic genes (*adiRS*). Each two-component regulatory system is composed of one sensory histidine kinase and a response regulator with considerable similarity to each other. Here we show a way to produce and purify the regulatory protein components of EbN1 as soluble proteins in *E. coli*. The functions of these gene products are investigated by genetic and biochemical studies. Deletion mutagenesis by homologous recombination showed that the *adiRS* operon is indeed involved in the acetophenone-dependent induction of gene expression. Moreover, the predicted acetophenone-sensing histidine kinase (AdiS) and the corresponding response regulator (AdiR) were purified and results on their biochemical properties will be shown.

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RSP36
Local c-di-GMP signalling by an EAL domain trigger enzyme in *E. coli* biofilm control

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Introduction: C-di-GMP—which is produced by diguanylate cyclases (DGC) and degraded by specific phosphodiesterases (PDEs)—is a ubiquitous second messenger in bacterial biofilm formation. In *Escherichia coli*, several DGCs (YegE, YdaM) and PDEs (YhjH, YciR) and the MerR-like transcription factor MlrA regulate the transcription of *csgD*, which encodes a biofilm regulator essential for producing amyloid curli fibres of the biofilm matrix. Since the c-di-GMP controlling module of YdaM/YciR is highly specific for regulation of *csgD* transcription, we investigated a potential local signalling by the global second messenger.
Materials and Methods: For determination of *csgD* transcription regulation, we performed genetic epistasis experiments by measuring gene expression using *lacZ* reporter gene fusions in various mutation backgrounds. Protein interaction studies *in vitro* and *in vivo* show detailed protein domain contacts. Furthermore, we confirmed altered protein activity by complex formation using enzymatic and DNA binding assays.
Results: Here, we demonstrate that this system operates as a signalling cascade, in which c-di-GMP controlled by the DGC/PDE pair YegE/YhjH (module I) regulates the activity of the YdaM/YciR pair (module II). Via multiple direct interactions, the two module II proteins form a signalling complex with MlrA. YciR acts as a connector between modules I and II and functions as a trigger enzyme: its direct inhibition of the DGC YdaM is relieved when it binds and degrades c-di-GMP generated by module I. As a consequence, YdaM then generates c-di-GMP and—by direct and specific interaction—activates MlrA by altering its DNA-binding which leads to stimulation of *csgD* transcription.
Conclusion: Altogether, we present a novel concept of local c-di-GMP signalling where not only a GGDEF domain protein acts as a co-activator for transcription and as a DGC, but where also an EAL domain protein acts as a trigger enzyme. Bifunctionality, i.e. the combination of DGC/PDE activity with highly specific protein-protein interactions, may represent a general principle in local c-di-GMP signalling.

RSP37
A physiological two-layer architecture is essential for the structural integrity of bacterial macrocolony biofilms

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Introduction: Bacterial macrocolonies represent biofilms that can produce intricate three-dimensional structures. Macrocolony microarchitecture and morphology depends on an extracellular matrix of adhesins, amyloid fibres (e.g. curli) and exopolysaccharides (e.g. cellulose). Within *E. coli* macrocolony biofilms, vegetatively growing cells are located in the bottom layer and in the outer growth zones, whereas stationary phase cells completely surrounded by amyloid curli fibers and cellulose are observed in the top layer. Since synthesis of curli fibres and cellulose is under the control of a transcriptional cascade involving the stationary phase sigma factor RpoS and the biofilm regulator CsgD, this two-layer structure indicates physiological zones with RpoD and RpoS being the dominant sigma subunits of RNA polymerase in the bottom and top layers, respectively (Serra & Hengge. 2014. Env. Microbiol). This raised the question whether the physiological two-layer structure with matrix production only in the top zone is just a side-product of nutrient gradients that necessarily built up in macrocolonies and affect global gene expression or whether this stratification is essential for biofilm architecture and integrity.
Materials and Methods: In order to verify that the two-layer architecture of macrocolony biofilms reflects distinct sigma factor dominance, we designed artificial vegetative and RpoS-dependent promoters not affected by any transcription factors and fused these to Gfp as a visual marker in cryosections through macrocolonies. Moreover, we constructed a strain in which CsgD is under vegetative control which should lead to curli fibre production (detectable by the fluorescent dye thioflavin S) in the vegetative bottom layer and studied the consequences for macrocolony architecture and integrity by fluorescent microscopy of cryosections and scanning electron microscopy.
Results: Using the synthetic promoter-Gfp fusions as a tool we can assign RpoD- and RpoS-dependent gene expression to distinct layers within macrocolony biofilms. Reprogramming the synthesis of the biofilm regulator CsgD under the control of RpoD resulted in vegetative expression of amyloid curli fibers also in the bottom layer. This resulted in altered morphology of the macrocolonies with the two now very rigid curliated layers breaking apart.
Conclusion and Discussion: These data demonstrate a clear physiological stratification of macrocolony biofilms that is based on nutrient gradients leading to a differential use of vegetative and stationary phase sigma subunits of RNA polymerase. Moreover, RpoS-driven massive matrix production only in the top layer of the two strata is essential for macrocolony integrity and morphogenesis.

RSP38

Mutations in the small RNA GlmY/GlmZ cascade drastically increase susceptibility of *Escherichia coli* to antibiotics targeting glucosamine-6-phosphate synthase

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Glucosamine-6-phosphate (GlcN6P) synthase (GlmS) is an essential enzyme that catalyzes a key reaction in the pathway for cell wall biosynthesis: synthesis of GlcN6P. In *Escherichia coli*, synthesis of GlmS is activated upon GlcN6P shortage by base-pairing of the cognate transcript with small RNA GlmZ [1]. When GlcN6P levels are sufficient, GlmZ is recruited to RNase E by adapter protein RapZ for processing and decay. This process is counteracted by the homologous sRNA GlmY which accumulates upon GlcN6P depletion. GlmY acts as decoy and sequesters RapZ by an RNA titration mechanism [1,2]. In turn, GlmZ is stabilized and activates GlmS synthesis adjusting its levels to the concentration of its enzymatic product, procuring GlcN6P homeostasis. Due to its pivotal role in cellular physiology, GlmS is a potent target for microbial warfare as well as antimicrobial chemotherapy. Many Gram-positive bacteria excrete tri-peptides such as Bacilysin, which inhibit GlmS and thereby growth of competing microbes. Nva-FMDP is a synthetic Bacilysin derivative with improved growth inhibitory properties [3]. Nonetheless, Gram-negative bacteria such as *E. coli* and *Salmonella* show a high intrinsic resistance against this antibiotic for so far unknown reasons [3]. Here we show that Bacilysin and Nva-FMDP cause strong overexpression of the GlmS enzyme in *E. coli*, which overcomes inhibition of its enzymatic function. This is the consequence of GlcN6P depletion, which up-regulates *glmS* expression through activation of the GlmY/GlmZ pathway. In agreement, mutation of GlmY or GlmZ renders *E. coli* highly susceptible to the antibiotics. Thus, the GlmY/GlmZ system represents an attractive target for future antimicrobial chemotherapy as its inhibition strongly increases the growth-inhibitory potential of Bacilysin and related antibiotics.

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RSP39

A *Legionella pneumophila* Lqs-regulated SinR-like transcription factor controls pathogen-host interactions, biofilm formation and competence

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Legionella pneumophila is an amoeba-resistant opportunistic pathogen that promotes cell-cell communication through the signalling molecule 3-hydroxypentadecan-4-one (LAI-1, *Legionella* autoinducer-1). The Lqs (*Legionella* quorum sensing) system comprises a gene cluster encoding the LAI-1 autoinducer synthase LqsA, the cognate sensor kinase LqsS as well as the response regulator LqsR, and additionally, it includes the 'orphan' sensor kinase LqsT [1, 2]. The system controls *L. pneumophila*-phagocyte interactions, production of extracellular filaments and natural competence for DNA uptake. In line with the different phenotypes of the *ΔlqsT* and *ΔlqsS* strains, transcriptome studies revealed that 90% of the genes down-regulated in absence of *lqsT* are up-regulated in absence of *lqsS,* suggesting a partially antagonistic function of LqsT and LqsS. Reciprocally regulated genes encode translocated effector proteins implicated in virulence as well as components of a 133 kb genomic 'fitness island'.
As a part of the 133 kb, putatively mobile genomic element, we recently identified a SinR-type transcription factor orthologous to the master regulator of biofilm formation in *Bacillus subtilis*. *L. pneumophila sinR* was up-regulated in a strain lacking *lqsS*, and in turn, regulated the expression of *lqsA* and *lqsR*. Compared with wild-type *L. pneumophila*, a *sinR*-deficient strain showed greatly enhanced natural competence for DNA acquisition, impaired host cell uptake and intracellular replication, as well as reduced competitiveness against wild-type bacteria upon co-infection of amoebae. Moreover, in contrast to strains lacking *lqsS* or *lqsR*, *ΔsinR* was impaired for biofilm formation but did not produce extracellular filaments. Together, these results indicate that SinR is linked to the complex regulatory network encompassing the Lqs system and coordinates distinct processes implicated in pathogen-host cell interactions, biofilm formation and competence of *L. pneumophila*.

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RSP40

Functional and structural analyses of the *Vibrio vulnificus* stressosome

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The stressosome, a 1.8 MDa protein complex, controls the activity of the general stress sigma factor, SigB, in the Gram-positive soli bacterium *Bacillus subtilis*. Comparative genomics has revealed that the gene cluster encoding the stressosome components RsbR, RsbS and RsbT is not restricted to the bacilli but can be also found in bacteria as diverse as cyanobacteria, bacteroidetes, proteobacteria, and deinococci. Furthermore, sequence analyses predict that in contrast to *B. subtilis*, in these species the stressosome is functionally linked to complex two-component systems or proteins controlling the turnover of the second messenger molecule c-di-GMP, rather than controlling the activity of an alternative sigma factor. These observations suggest that the stressosome has been recruited to relay signals to different output modules and hence may control various biological processes.
To address these points experimentally, we investigated the stressosome of the pathogen marine γ-proteobacterium *Vibrio vulnificus*. We found that the *V. vulnificus* stressosome is expressed in a minimal medium upon entry of the cells into the stationary phase. Expression of the *V. vulnificus* stressosome requires the presence of iron. Interestingly, the *V. vulnificus* RsbR homolog, termed VvR hereafter, possesses a predicted N-terminal sensor globin domain that may function as an oxygen sensor. This is in contrast to *B. subtilis* where the molecular nature of the signal sensed by the stressosome remains unknown. Indeed, purified VvR showed an absorption spectrum typical for heme proteins. Furthermore, to gain insight into the structure of the *V. vulnificus stressosome,* we used cryo-electron microscopy in combination with single particle analysis and present a model for the *V. vulnificus* VvR:VvS complex in its oxidized state at 7.6 A resolution.

RSP41

Biological role and regulation of bioluminescence in the entomopathogenic bacterium *Photorhabdus luminescens*

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Entomopathogenic bacteria of the genus *Photorhabdus* live in symbiosis with the nematode *Heterorhabditis,* which infects insect larvae. After infection the bacteria are released in the haemolymph and kill the insect by producing a wide range of bioactive compounds.
Photorhabdus luminescens is named after its light production. So far it is the only known terrestrial bioluminescent bacterium. Like for other bioluminescent bacteria, the biological role of bioluminescence is still unclear. The most common theories are the protection of cells against oxidative stress[1], the stimulation of DNA repair[2] or scavenging excess reducing equivalents[3]. This study investigates if any of these theories might be true for *P. luminescens* by comparing the wild type strain with a mutant strain unable to produce light any more and a high luminescent mutant strain.
The regulation of bioluminescence has been investigated best for *Vibrio* species, showing that autoinduction by β-ketocaproyl homoserine lactone is playing a key role[4]. A potential candidate for autoinduction in *Photorhabdus luminescens* is the product of the gene *luxS* and thus, the effect of a *luxS* deletion on bioluminescence is also studied.

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RSP42

Regulation Of The Anthraquinone Biosynthesis In *P. luminescens* By A New Transcriptional Regulator

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Photorhabdus luminescens (*P. luminescens*) is a Gram-negative, entomopathogenic, bioluminescent bacterium, which lives in symbiosis with nematodes of the genus *Heterorhabdits*[1].
P. luminescens produces a broad range of bioactive compounds, which are indispensable for killing the insect prey and for the nematode´s development. Furthermore they are used as antibiotics against microbiological competitors as well as repellents against scavenging insects[1,2,3]. The variety of different natural products necessarily requires a sophisticated regulation system. Here we are especially interested in the regulation of the secondary metabolism by small metabolites.
In this context a new transcriptional regulator of the anthraquinone (AQ) biosynthesis was identified, which is mandatory for the production of AQs in *P. luminescens*. The binding sites of the regulator were elucidated by EMSA. In the following a coregulator binding to the predicted (blastp) ligand binding domain (WYL) should be identified. For that the binding of different potential ligands to the heterologously expressed regulator will be *in vitro* tested using surface plasmon resonance or AlphaScreen^{R}.
As no structural information for WYL domains is available yet, our aim is its structure elucidation using NMR analysis.

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RSP43

Crystallization of the oxygen-sensing transcriptional regulator Fnr of *Bacillus stearothermophilus*

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The *Bacillus subtilis* redox regulator Fnr controls genes of the anaerobic metabolism in response to low oxygen tension. The anaerobic expression of *narGHJI* encoding the nitrate reductase was shown to be Fnr-dependent. The Fnr binding site was located at promoter position -41.5 of the *narG* promoter. Unlike its *E. coli* counterpart *B. subtilis* Fnr utilizes three cysteine residues and the non-cysteine ligand aspartate 141 for the formation of the oxygen sensing $[4Fe\text{-}4S]^{2+}$ cluster (1, 2). Furthermore, Fnr of *B. stearothermophilus* was recombinantly produced and anaerobically purified. Ligation of the $[4Fe\text{-}4S]^{2+}$ cluster was detected by UV/Vis spectroscopy and DNA binding of the purified Fnr was analyzed using EMSA. The purified Fnr protein at a concentration of 10 mg/ml was used for various crystallization attempts in the presence and absence of DNA. Here, a 29 bp double stranded DNA fragment containing the Fnr binding sequence of the *narG* promoter was used. The final aim is the structural analysis of active Fnr bound to DNA.

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RSP44

Genetic and metabolic adaptation mechanisms in *Pseudomonas aeruginosa*

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The adaptability to environmental changes and the establishment of complex cellular phenotypes is essential for the survival of bacteria and is strictly dictated by transcriptional and translational regulation. In this study we analyzed cellular and phenotypic adaptations of the nosocomial pathogen *Pseudomonas aeruginosa* to changing environmental conditions as they occur during the course of an infection.
By combining global profiling techniques like RNA sequencing, ribosome profiling and novel proteomic analyses with phenotypic characterization and reporter studies, we could show that post-transcriptional regulation is a key determinant for adaptation processes to fluctuations in amino acid concentrations. We found that a biased codon composition and the competition among tRNAs isoacceptors for amino acid acetylation is responsible for an altered translation rate of proteins involved in quorum sensing (QS), motility and c-di-GMP signaling leading to an adaptive response. Thus, codon degeneracy not only expands the bacterial repertoire of responses to environmental perturbations but also lead to changes in complex bacterial behavior in the face of the availability of distinct amino acids. Intriguingly, we ascertained that *P. aeruginosa* can also actively influence the amino acid metabolism and hence translational efficiency in a QS-dependent manner irrespective of nutrient availability. The present study highlights novel post-transcriptional regulation mechanisms that shed light on the gene-specific genetic adaptation to the environment.

RSP45

Heterogeneity within *Staphylococcus aureus* populations: Is the SOS response involved?

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Introduction: Bacteria respond to stressful conditions by a multitude of adaptive mechanisms such as activation of alternative sigma factors, stringent response and the SOS response. The SOS response is induced upon DNA-double strand breaks which then leads to the activation of the RecA protein. RecA is not only important for DNA repair but also results in the auto cleavage of the transcriptional repressor LexA thus activating the SOS response genes. This involves the induction of error-prone polymerases which results in increased mutation frequency. Several antibiotics can induce the SOS response at sub-inhibitory concentrations which accelerates the emergence of antibiotic resistance due to higher mutation rates.
Materials and Methods: Site-specific and deletion mutants (*lexA*, *recA*) were constructed. The accumulation of phenotypic variants was monitored by plating passaged cultures on sheep blood agar. These were further confirmed by hemolysin complementation streak test and sequencing of the *agr* locus. Growth curves revealed differences in fitness characteristics of different strains.
Results: We find that in *Staphylococcus aureus,* non-hemolytic variants develop rapidly over time in response to sub-inhibitory concentrations of SOS inducing antibiotics. These variants were found to be defective in function of the quorum sensing system *agr*. Further, *agr* defective strains were shown to have a fitness advantage over the wild type. We created *lexA* and *recA* mutants in order to assess the role of the SOS response in the development of this phenotype. The non-cleavable *lexA* mutant was still capable of developing this phenotype over several subcultures. However, fewer variants accumulate in the *recA*- mutant background.
Discussion: In this study, exposure to SOS-response inducing antibiotics increased the selection of *agr*-defective variants with higher fitness attributes. Given the central role of the *agr* quorum sensing system in virulence, the observation that such *agr*-defective variants are easily selected in the population is of clinical relevance. The instances of bacterial infections comprising a high degree of heterogeneity point to the definite advantage for the organism in maintaining such diversity. Since a heterogeneous bacterial population may significantly alter the course of infection, this necessitates a review of current antimicrobial therapies in combating infection.

RSP46
The YycFG two-component regulatory system of *Staphylococcus aureus* is controlled by its auxiliary proteins YycH and YycI

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YycFG (WalRK/VicRK) represents the only essential two-component regulatory system in the Gram-positive pathogen *Staphylococcus aureus* and plays a major role in cell wall metabolism controlling genes involved in cell wall degradation during cell division. Furthermore, YycFG has repeatedly been reported to be involved in the intermediate resistance of *S. aureus* to vancomycin and daptomycin. Recent studies showed that high alkali salt concentrations and low temperatures were necessary to stimulate the activity of the kinase YycG, suggesting that YycG reacts to an altered membrane environment in the division septum. In *B. subtilis*, previous reports had suggested that the membrane-bound auxiliary proteins YycH and YycI act as regulators in a negative manner on the kinase YycG. Less is known about the regulation of the YycG autophosphorylation activity in *S. aureus*. To address this question, the full-length recombinant proteins of the YycFGHI operon have been expressed and tested under *in vitro* conditions using phospholipid-liposomes to investigate the regulatory function of the auxiliary proteins YycH and YycI on YycG autophosphorylation and phosphotransfer to the response regulator YycF. *In vivo*, overexpression and knock-down of either the membrane bound kinase YycG or the regulatory proteins YycH and YycI influenced growth behaviour, autolysis and the resistance to vancomycin.

RSP47
The cell wall stress response of *Aspergillus niger* involves the activity of at least two transcription factors: RlmA and MsnA

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Comprehensive understanding on how fungi adapt and survive cell wall stress conditions is still missing. Recently, we have shown the importance of the cell wall integrity pathway and its downstream targets RlmA (transcription factor) and AgsA (α-1,3 glucan synthase) for *Aspergillus niger* to survive sublethal concentrations of caspofungin, fenpropimorph and the antifungal protein AFP (Meyer et al 2007, Hagen et al. 2007) . In this study, we extended these transcriptomic and physiologic analyses to study the response of *A. niger* towards aureobasidin A (AbaA), an inhibitor of the sphingolipid biosynthesis and FK506, an inhibitor of the calcium-calcineurin signaling pathway. Upon AbaA treatment, 237 responsive genes were found which were mainly assigned to function in (i) lipid metabolism, (ii) cell wall remodelling, (iii) vesicle transport, (iv) nutrient transport and (vi) proteasomal degradation. Expression of 96 genes changed upon FK506 treatment, which are predicted to function in (i) ion homeostasis, (ii) calcium signaling, (iii) protein folding and maturation and (iv) vesicle trafficking. In silico analysis of all responsive genes and their promoter regions predicted that beside RlmA, another transcription factor, MsnA, might guard *A. niger* against these cell wall stressors. Analysis of the phenotype of *A. niger* when depleted for MsnA indeed confirmed that MsnA is important for *A. niger* to withstand cell wall stress.

RSP48
Structure and function of a LOV-photoreceptor from *Dinoroseobacter shibae*

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Light, oxygen, voltage (LOV) domains are widely distributed in plants, algae, fungi and bacteria and represent the photo-responsive domains of various blue-light photoreceptors. The photocycle of these photoreceptor proteins involves the blue-light triggered adduct formation between the C(4a) atom of a non-covalently bound flavin chromophore and the sulfur atom of a conserved cysteine. LOV proteins show considerable variation in the structure of their N- and C-termini as well as in the lifetime of the adduct state. Here, we report the photochemical, structural and functional characterization of a LOV protein from the photoheterotrophic marine α-proteobacterium *Dinoroseobacter shibae* named *Ds*LOV which exhibits an average adduct state lifetime of 10 s at 20 °C, and thus represents the fastest reverting bacterial LOV protein reported so far. Mutational analysis revealed a unique role of *Ds*LOV in controlling the induction of photopigment synthesis by *D. shibae* in the absence of blue light. The dark-state crystal structure of *Ds*LOV determined at 1.5 Å resolution reveals a conserved core domain with an extended N-terminal cap. The dimer interface in the crystal structure forms a unique network of hydrogen bonds involving residues of the N-terminus and the β-scaffold of the core domain.
Due to its unique photophysical, structural and regulatory properties *Ds*LOV may serve as an alternative model system for LOV protein-mediated light perception. Its regulatory function of activating photopigment synthesis in the dark represents another divergent mechanism how photosynthetic bacteria optimize photopigment formation in response to a specific light regime.

RSP49
Small RNAs in the photosynthetic gene cluster of *Rhodobacter sphaeroides*

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The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility and possesses different ways to generate energy for its growth. Under low oxygen conditions it forms intracytoplasmatic membranes harboring the photosynthetic complexes (PC) needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. There are two different gene clusters, the *puf* and the *puc* operon, both containing essential genes responsible for the development of the two light harvesting complexes (LHCI and LHCII) and the reaction center (RC). RNAseq and *Northern blot* analysis of transcripts derived from the *puf* operon (coding for the LHCI and the RC) revealed that besides normal mRNAs also certain small RNAs (sRNAs) were transcribed. Up to date two different *puf* operon associated sRNAs were identified. One abundant putative sRNA, RSspufX, was detected downstream of the *pufX* gene with transcription in the same direction as the *puf* genes. The putative sRNA was also enriched in an Hfq-immuno precipitation, indicating interaction to this RNA chaperone. Another, less abundant sRNA was detected antisense to the 5´region of the *pufL* gene (RSaspufL), extending into the *pufA-pufL* intercistronic region and possibly even further. RNAseq data also indicated that the abundance of the two *puf*-related sRNAs was strongly dependent on growth conditions. Additionally, in case of RSaspufL growth condition dependant processing was observed. Analysis of the possible function of both sRNAs indicated a clear function in pigment composition control in *R. sphaeroides* cells. An artificial increase in the amount of these sRNAs by plasmid driven over expression led to a reduced pigmentation and LHC amount in the cells. Taken together, for PC development different signals have to be integrated (e.g. redox state in the cell, light availability, oxygen tension). In addition to the already characterized sRNA PcrZ (photosynthesis control RNA Z; Mank et al., 2012) also other sRNAs seem to play a role in the fine tuning of the photosystem development.

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RSP50
Investigation of nitrogen regulation by a putative $P_{II\text{-}like}$ protein in *Staphylococcus aureus*

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P_{II} proteins are signal transduction proteins in bacteria, archaea, and plants which coordinates and regulates many aspects of nitrogen metabolism by interacting with enzymes, transcription factors, and membrane transport proteins. Signal perception by P_{II} proteins can occur at two levels. The primary mode of signal perception appears to be almost universal and involves the binding of the effector molecules 2-oxoglutarate (2-OG) and ATP/ADP. A secondary mode of signal perception, which is less conserved, involves covalent modifications of residue within the T-loop. The genome of *Staphylococcus aureus* NCTC8325 does not encode a homologue of a canonical P_{II} protein. However, the SAOUHSC_00452 gene, annotated as a hypothetical protein, encodes a protein, which has predicted structural similarity to canonical P_{II} and is therefore termed $P_{II\text{-}like}$ protein. In this study, we generated a deletion mutant of the $P_{II\text{-}like}$ encoding gene in *S. aureus* NCTC8325 and studied its function by comparative phenotypic analysis. Deletion of the putative $P_{II\text{-}like}$ gene resulted in severe impairment during the exponential phase of growth in nitrogen deficient and excess medias. The activity of nitrate reductase with anaerobically grown cells in the presence of $NaNO_3$ showed no difference between the wild type and $\Delta P_{II\text{-}like}$.Furthermore, $\Delta P_{II\text{-}like}$ showed higher glutamine synthetase (GS) activity compared the wild type strain. This result suggest that high GS activity might be the cause for poor growth in $\Delta P_{II\text{-}like}$ during the exponentially phase. We investigated the thermodynamics of binding of $P_{II\text{-}like}$ proteins to several molecules such as ATP, ADP, cyclic diadenosine monophosphate (c-di-AMP) using isothermal titration calorimetry (ITC). The bacterial second messenger c-di-AMP showed a significant binding signal to $P_{II\text{-}like}$. Also, $\Delta P_{II\text{-}like}$ exhibited an increased biofilm formation phenotype than the wild type strain. This result indicates a possible role of $P_{II\text{-}like}$ protein in the central metabolic process of cells that needs to be discovered.

RSP51
Truncated soluble mouse CD14 influences macrophage response to LPS stimulation

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Introduction: CD14 is a glycoprotein fixed to the cell membrane by a glycosylphosphatidylinositol anchor which plays an important role in the signaling cascade of lipopolysaccharide (LPS) detection. By binding LPS CD14 is essential for the initial step of signaling finally leading to the activation of transcription factor NFkB and thus to expression of proinflammatory cytokines. We want to investigate whether a truncated, soluble form of CD14 is able to influence this process. We suppose that the signaling cascade cannot be started without a membrane-bound form of CD14.
Materials and Methods: C-terminally truncated mouse CD14, as described by Tamura et al. [1] and codon optimized for yeast *Saccharomyces cerevisiae* was N-terminally merged with the transport signal of the yeast pheromone alpha-mating factor and transformed into probiotic yeast *Saccharomyces boulardii* and four other laboratory *Saccharomyces cerevisiae* strains, using the shuttle plasmid p426-Kana. Yeasts were inoculated in YPD medium and incubated for 48 hours at 30°C on a shaking incubator. Supernatants were sterile-filtered and concentrated. Presence and specificity of CD14 was confirmed by ELISA analysis and immunoblotting. To assess the biological activity of the recombinant protein, an assay using a LPS stimulated mouse macrophage cell line was developed, by means of which changes of the macrophage cytokine response induced by soluble murine CD14 can be investigated.
Results: All yeasts were able to synthesize recombinant CD14 in quantities ranging from 10 to 318 ng/ml and to release it to the extracellular space, where it could be detected and verified by ELISA and immunoblotting. First trials with the functional assay suggest that yeast supernatants, containing recombinant soluble CD14, are able to reduce the proinflammatory activity of the LPS stimulated macrophages.
Discussion
Due to deletion of the specific sequence of the GPI anchor, recombinant CD14 could be exclusively found in the culture supernatant. We suggest that the lack of CD14 fixation to the membrane leads to insufficient activation of toll-like receptor 4 which is important for signaling transfer. If the recombinant soluble CD14 still binds LPS, it might be possible that these LPS molecules do not lead to an activation of the LPS signaling cascade. Thus, LPS would be effectively neutralized, resulting in a significantly lowered release of proinflammatory cytokines. This effect might enable construction of optimized probiotics which will ultimately improve treatment of chronic inflammatory bowel diseases.

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RSP52
Two *cis*-asRNAs affect adaptation to Amadori products in *Bacillus subtilis*

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The *frlBONMD*-operon is instrumental in the degradation of Amadori products (fructosamines). Expression is controlled by the two negative transcriptional regulators, CodY and FrlR. Besides, newly identified antisense RNAs are involved: a transcript produced by the elongation of the *frlBONMD* transcript into the convergently orientated *frlR* noncoding strand a shorter one. The transcripts consist of the antisense *frlR* with the downstream *yurJ* (encoding an ATPase predicted to serve ABC amino-sugar transporters). As for the *frl*-mRNAs the *frlR* transcript and the asRNAs are targets of the major RNase Y, since these were significantly stabilized upon depletion of the latter. Promoter test analyses revealed that *frlR*-asRNA overexpression causes the derepression of FrlR-regulated promoters. Switching from one growth phase to another was faster on Amadori products in an as*frlR* positive strain compared to an as*frlR*-RNA negative mutant, emphasizing the role of as*frlR* for adaptation to Amadori products. The start of growth on Amadori products was furthermore significantly accelerated when antisense formation was impeded, which is possibly due to the decrease of *yurJ* transcription resulting in reduced import of detrimental amounts of Amadori products.

RSP53
The role of Fur in iron homeostasis in *Clostridium difficile*

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Clostridium difficile is known as a human pathogen causing diarrhoea among patients in hospitals and health care units in Germany. Diseases caused by *C. difficile* are referred to as *Clostridium difficile* associated disease (CDAD). Since the late 1970s *C. difficile* has become the most common cause of hospital-acquired infectious diarrhoea and has been recognised as a significant cause of morbidity and mortality. In contrast to its clinical importance, almost nothing is known about the gene regulatory networks employed by the bacterium during host colonisation and infection. Our group is particularly interested in elucidation of *C. difficile* iron homeostasis. For that purpose we use a combined genetic and molecular systems biology approach. BLAST searches displayed a gene homolog of *Bacillus subtilis fur* gene in *C. difficile.* In *B. subtilis* Fur acts as the global

regulator of ferric iron. However, the role of Fur in *C. difficile* remains completely unclear. The presence of multiple iron-uptake systems in *C. difficile* illustrates the importance of iron acquisition for clostridial growth and the ability of the bacterium to adapt to both iron-overloaded and iron-restricted environments. Subsequently, a *fur* mutant in *C. difficile* was generated using a group II intron based technology and first growth experiments in *C. difficile* minimal media (CDMM) displayed a similar growth profile compared to the wild type. Surveillance of different iron sources (i.e. hemin) in iron-limitation and iron-depletion provide a deeper understanding of the colonisation and pathogenesis of *C. difficile.* Moreover, a combined metabolomic, transcriptomic and proteomic approach of limited iron conditions and the *fur* mutant in comparison to the wild type will contribute to a better understanding of the role of iron in the infection cycle of *C. difficile.*

RSP54
Signal transduction and antibiotic stress response in *Streptomyces venezuelae*

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The bacteria belonging to the genus *Streptomyces* show a complex life cycle and produce a range of secondary metabolites making them the most important producers of clinically relevant antibiotics. Most *Streptomyces* species live in the soil, where they share their habitat with bacteria of the phylum Firmicutes, i.e. low G+C Gram-positive bacteria. While Firmicutes bacteria have long been associated with the production of antimicrobial peptides, it has only recently become clear that some Streptomycetes are also able to synthetize such compounds. Despite the resulting likely exposure of *Streptomyces* to peptide antibiotics, both self-produced and derived from competitors, resistance against this class of antibiotics has not been studied in these organisms to date. Therefore, we aimed to study the sensitivity of a number of different *Streptomyces* species against several peptide antibiotics. Our results show that *Streptomyces venezuelae* was overall the most resistant species with a particularly strong resistance against bacitracin. Our current work investigates the molecular basis for this high-level resistance of *S. venezuelae* through a random chemical mutagenesis approach followed by screening for bacitracin sensitive strains. Identified mutants will then be sequenced to identify the genetic changes leading to sensitivity. In a parallel approach, we are performing transcriptome analyses to study the stress-response of *S. venezuleae* to bacitracin. *S. venezuelae* is easy to grow in the laboratory and also is amenable to genetic manipulation, making it an ideal model to study the resistance mechanisms against peptide antibiotics in Actinobacteria.

RSP55
Iron dependent gene regulation in the marine microorganism *Dinoroseobacter shibae*

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The marine bacterium *Dinoroseobacter shibae* belongs to the *Roseobacter* clade which represents one of the major lineages of near surface waters in the global ocean. In well oxygenated seawater at natural pH the concentration of dissolved ferric iron is rather low leading to iron limiting conditions. Our major goal is the analysis of the fine tuned regulatory system of iron homeostasis in our model organism *D. shibae.* To overcome the burden of iron limitation bacteria developed different strategies. In many bacteria iron dependent gene regulation is mediated by the global ferric-uptake regulator, Fur. In the presence of iron the Fur protein binds directly to the DNA and represses several iron acquisition genes. Moreover, in alpha-proteobacteria iron dependent gene regulation is mediated by the iron responsive regulator, Irr, and the rhizobial iron regulator, RirA. Interestingly, *D. shibae* exhibits not only a gene encoding the global regulator Fur, but it also possesses genes encoding Irr and RirA protein homologs. Compared to the wild type strain the *fur* and *irr* mutant strains exhibit a growth deficient phenotype indicating an important impact of these proteins in iron dependent regulation. Using transcriptome analyses we will define the Fur and Irr regulons of *D. shibae*. First transcriptome and proteome analyses of the wild type strain comparing normal and iron limited growth conditions showed over 270 genes differently expressed and 109 proteins differing more than 2-fold. A potential hemin transport system encoded by the *hmuSTUV* operon was found upregulated under iron limitation. Moreover, addition of hemin to iron depleted media yield in an increased growth of the wild type strain and indicate the role of the potential hemin uptake system. In contrast, growth of the *fur* mutant strain under iron limitation cannot be rescued by the addition of hemin indicating a Fur dependent regulation of the hemin uptake system. Genomic analysis of *D. shibae* revealed no evidence for siderophore production. However, the *fhuA* gene encoding a potential Ton B-dependent siderophore receptor was found upregulated under iron limited growth conditions. To detect siderophore production in *D. shibae* we established the chrome azurol S assay and got first evidence for the production of siderophores [1]. Our aim is to identify the nature of the siderophore and identify the genes and proteins involved in the synthesis. This will give us the possibility to identify a new siderophore produced by *D. shibae*. Our results indicate a major impact of iron on the physiology of the marine bacterium *D. shibae.* Further studies will give us important insights in the iron regulation of *D. shibae* and may elucidate the environmental success of the *Roseobacter* clade.

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Secondary Metabolism

SMP01
Identification and heterologous expression of the gene encoding for the alternariol-*O*-methylthransferase of *Alternaia alternata*

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Black-moulds of the genus *Alternaria* contaminate many foodstuffs and agricultural products. Besides the economic damage these fungi can produce harmful secondary metabolites, the *Alternaria* toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME) and altenuene (ALT) have been described as cytotoxic, genotoxic and mutagenic *in vivo* and *in vitro*. The production of these mycotoxines is strongly influenced by different nitrogen and carbon sources [Brzonkalik 2011]. One of the postulated core enzymes in the biosynthesis of AOH is a polyketide synthase (PKS). In a draft genome sequence of *A. alternata* Saha et al. [2012] identified 10 putative PKS encoding genes with PksJ being responsible for the AOH formation.
The alternariol-*O*-methyltransferase (AOH-MT) catalyzes the subsequent reaction from AOH to AME by transferring a methyl group from S-(5′-Adenosyl)-L-methionine chloride (SAM) to AOH like shown in the figure below. The AOH-MT is not part of the PKS cluster nor in the vicinity of it so the aim of this work was to identify the DNA-Sequence of the gene encoding the AOH-MT from *A. alternata*. Therefore, the enzyme was purified from crude extract of *A. alternata* using hydrophobic interaction chromatography and sequenced by Edman degradation. Subsequently, primer for cloning were designed by comparison of the obtained sequences with the genome database of *Alternaria brassicicola*. The gene was cloned in a pET-11a vector and transformed into E. coli. After induction with IPTG (Isopropyl-β-D-thiogalactopyranosid) heterologous expression was verified via an activity assay and SDS-PAGE.

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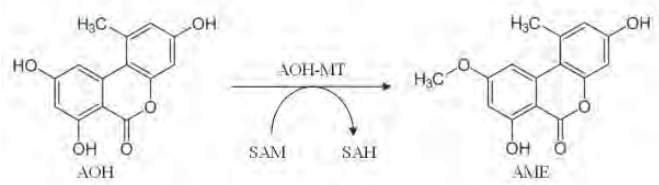
Figure 1



SMP02
Nonribosomal Secondary Metabolites from the Bee Pathogenic Bacterium *Paenibacillus larvae*

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Introduction: *Paenibacillus larvae* is the etiological agent of American Foulbrood (AFB) a world-wide distributed fatal disease of the honey bee brood. Despite the tremendous impact of AFB, the underlying molecular mechanisms remain widely elusive. This lack of knowledge makes AFB difficult to fight and it is therefore imperative that biological pathways characteristic for *P. larvae* are evaluated for points of interference. The sequencing of the *P. larvae* genome provided evidence that this bacterium harbors putative functional nonribosomal peptide synthetases and polyketide synthases.[2] Therefore, *P. larvae*might produce nonribosomal peptides and polyketides. Such biosynthesis products have been shown to display a wide-range of biological activities such as antibacterial, antifungal or cytotoxic activity.

Materials and Methods:

- Mass spectrometry
- Gas chromatography
- NMR spectroscopy
- Antibacterial and antifungal assays
- Bee larval assays
- Construction of gene inactivation strains

Results: By means of gene inactivation experiments in combination with mass spectrometry we were able to identify 3 nonribosomal secondary metabolites of *P. larvae*. Isolation of these metabolites and subsequent NMR spectroscopy as well as mass spectrometric analyses led to the structures of these compounds.

One of these secondary metabolites is the novel polyketide/ nonribosomal peptide paenilamicin with antifungal and antibacterial activity. By the help of bee larval co-infection assays we could show that this compound is responsible for fighting ecological niche competitors.

Besides the paenilamicin we elucidated a nonribosomal synthesized tripeptide[3] and a siderophore.

Conclusions: Our work on *P. larvae* shed light on most of the nonribosomal synthesized secondary metabolites produced by this bacterium. The findings are of high significance not only because they give explorations about the etiopathogenesis of AFB (e. g. host-pathogen interactions), but also because of the discovery of new bioactive compounds that could turn into drug development programs.

The structure elucidation of paenilamicin provides insights into a fascinating biosynthetic machinery.[4]

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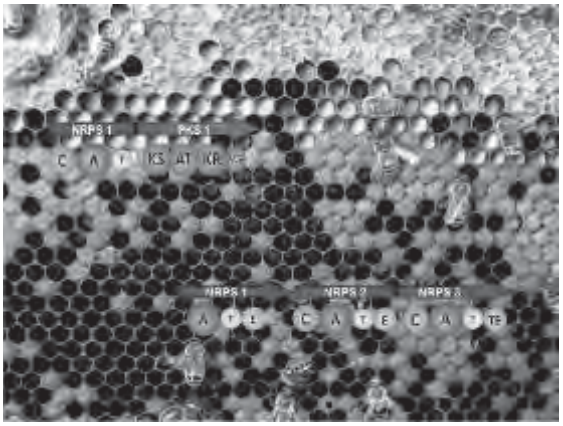
Figure 1



SMP03
The structure of the methylglyoxal synthase MgsA of *Bacillus subtilis*

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Methylglyoxal is a highly toxic compound and it is formed as a side-product of several metabolic pathways e.g. the methylglyoxal bypass of the glycolosis. In *Bacillus subtilis* methylglyoxal is generated by the conversion of dihydroxyaceton phosphate. This reaction is catalyzed by the methylglyoxal synthase MgsA. As a unique function of Crh in *B. subtilis*, it regulates the flux through the harmful methylglyoxal bypass of the glycolysis. Non-phosphorylated Crh inhibits the enzymatic activity of the key enzyme MgsA by direct protein-protein interaction. Phosphorylation and dephosphorylation of Crh depend on the metabolic state of the cell. In the presence of preferred substrates such as glucose, the FBP level increases within the cell and activates the kinase function of HPrK/P. This results in the formation of Crh~P and subsequently in the release of MgsA from inhibition and thus to activation of the methylglyoxal bypass.

To shed further light into regulation of MgsA by Crh we have solved the structure of MgsA by X-ray crystallography. The active form of MgsA comprises of 6 molecules. This hexamer is arranged as a Trimer of Dimers revealing two distinct interaction surfaces between single MgsA molecules. The helix at the C-terminus of MgsA forms a rather stable bond characterized by salt bridges. In contrast a weak interaction is exerted by hydrogen bonds of the helix located in the middle part of MgsA. This is leading to the suggestion that Crh breaks apart the MgsA complex into 3 dimers by disrupting the hydrogen bonds upon binding. Currently we are working on the structure of the MgsA-Crh complex to proof our assumption.

SMP04
Spirocyclic drimanes from the marine fungus *Stachybotrys* sp. strain MF347

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The fungal genus *Stachybotrys* (class: Sordariomycetes, order: Hypocreales) comprises approximately 100 species. Members of *Stachybotrys* spp. are distributed worldwide and are commonly isolated from soil and various decaying plant substrates. *S. chartarum* is reported to be involved in animal and human toxicoses, which are associated with “sick building syndrome” in wet buildings. Marine isolates of *Stachybotrys* spp. have been gained from various marine environments as the rhizosphere of mangroves, soil and mud of the intertidal zone, intertidal pools, brackish waters, marine sediments and sponges, marine algae, and sea fans. In order to find new natural products from marine microorganisms exhibiting, e.g., antibacterial and cytotoxic activities, *Stachybotrys* sp. strain MF347 from a marine driftwood sample was cultured and the secondary metabolites were investigated. A novel

spirocyclic drimane coupled by two drimane fragment building blocks, stachyin B, and a new drimane, stachyin A, were identified in mycelia and culture broth of *Stachybotrys* sp. MF347. Their structures were established by spectroscopic means. This is the first example of spirocyclic drimane coupled by a spirodihydrobenzofuranlactam unit and a spirodihydroisobenzofuran unit; and the connecting position being N-C instead of an N and N connecting unit. Strain MF347 produced also the known spirocyclic drimanes stachybocin A and stachybocin B featured by two sesquiterpene-spirobenzofuran structural units connected by a lysine residue; the known spirocyclic drimanes chartarlactam O; chartarlactam K; F1839A; stachybotrylactam; stachybotramide; and 2α-acetoxystachybotrylactam acetate; as well as ilicicolin B, a known sesquiterpene. The relative configuration of two known spirobenzofuranlactams was determined. All compounds were subjected to biological activity tests. The spirocyclic drimane stachyin B, stachybocin A and stachybocin B, as well as the sesquiterpene ilicicolin B, exhibited antibacterial activity against the clinically relevant methicillin-resistant *Staphylococcus aureus* (MRSA).

SMP05
Contributions to the elucidation of pentachloropseudilin biosynthesis in Actinoplanes sp. ATCC 33002

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Halogenases have recently been of great interest because of their potential in regioselective and specific halogenation in chemical and especially pharmaceutical synthesis. Thus, the biosynthesis of pentachloropseudilin, a highly chlorinated broad-spectrum antibiotic from *Actinoplanes* sp. ATCC 33002 [1], is of special interest.
Its 26.4 kb sized biosynthetic gene cluster was recently obtained from a cosmide library [2]. Comparison with the NCBI database revealed at least 14 ORFs proposed to participate in the synthesis, transport and regulation. The biosynthesis was suggested to start with proline that is oxidised to pyrrole-2-carboxylic acid, which is then used as starter unit for a polyketide synthase that establishes the phenyl ring. Despite the presence of five chlorine atoms, only three genes of $FADH_2$-dependent halogenases could be found [3]. The corresponding proteins of two of them, *pcpI* and *pcpK* (former called *halA* and *halB*) show homology to some not yet very well studied phenylpyrrole halogenases.
For PcpI, we constructed a deletion mutant that did not only show a different phenotype but also a complete loss of pentachloropseudilin production. Additionally, we could express the corresponding gene *pcpI* as a fusion protein with the maltose binding protein. From this construct, partially purified protein could be obtained by amylose affinity and size-exclusion chromatography. Enzyme activity assays were performed with different phenylpyrrole derivatives as substrates using crude extract and partially purified protein.

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SMP06
Biological Activities of Leaf Extracts from Plants of the Genus *Rhododendron*

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Medicinal plants are used as traditional treatments of numerous human disorders including infectious diseases caused by microorganisms. Due to the increasing resistance of many pathogens to the commonly used antimicrobial agents, there is an increasing interest to identify novel antimicrobial compounds. Plants of the genus *Rhododendron* belong to the woody representatives of the family of *Ericaceae*. There are more than 1,000 *Rhododendron* species distributed particularly in the Northern hemisphere. The City of Bremen harbors the Rhododendron-Park in which approximately 600 different species of *Rhododendron* and hybrids are grown. This enables research with about two third of all known *Rhododendron* species.
The bioactivity of leaf extracts from the 120 *Rhododendron* species have been tested against 25 different Gram-positive and Gram-negative bacteria. Crude extracts were prepared by re-suspending 2 g of leaf powder in 10 ml of 80% methanol. The antimicrobial assay was performed by the agar diffusion method.
The extracts of 17 *Rhododendron* species showed the highest inhibitory activities against Gram-positive bacteria (radius of growth inhibition 0.6-1.2 cm). However, most leaf extracts of these species did not significantly inhibited the growth of the Gram-negative organism (radius of growth inhibition of 0.1-0.4 cm or less). Interestingly, all of the active *Rhododendron* species are belonged to the subgenus *Rhododendron*. Moreover, at the level of section, all except two were assigned to *Rhododendron*. The two others belonged to section *Pogonanthum*. Therefor, the subgenus *Rhododendron* might have a specific secondary metabolic compounds can serve as antibacterial agent.

SMP07
Chlorination of indole derivatives by flavin-dependent halogenases

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There are many natural halogenated compounds produced by living organisms with a broad range of biological activities. In many cases, the activity of these compunds depends on the presence of a halogen. An enzyme system that consists of two components, a flavin-dependent halogenase and a non-specific flavin reductase, catalyse the incorporation of chloride and bromide atoms. PrnA, ThdH and PyrH are members of the enzyme family that catalyses the regioselective halogenation of free tryptophan at the positions 7, 6 and 5, respectively [1-3]. These halogenases are well characterized and the 3-D structure of two of them, PrnA and PyrH, was elucidated [4,5].
Using these enzymes we attempt the regioselective halogenation of different substrates *in vitro*. A variety of substituted indoles, including indole 3-acetic acid (IAA), the most widespread auxin, indole 3-acetonitrile and tryptamine were tested. HPLC analysis showed the formation of new products, corresponding to the expected halogenated derivatives. The obtained products with IAA as substrate for PrnA, ThdH and PyrH show clearly different elution times in HPLC, strongly suggesting differences in the positions of chlorine incorporation. The enzymatic products will be purified and their structure will be elucidated. Furthermore, the biological activity of the compounds will be investigated.
These results show the potentiality of flavin-dependent halogenases to be used *in vitro* and also *in vivo* by plants and bacteria for the production of novel halogenated indole derivatives.

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SMP08
Identification and characterization of a tryptophan 4-halogenase from *Pisum sativum*

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[2]*John Innes Centre, Department of Biological Chemistry, Norwich, United Kingdom*
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Natural products with wide-ranging biological activities are produced by many organisms. The biological activity and/or the bioavailability of these compounds often depend on the pattern of substituents in these molecules. One modification that normally has a large activity-increasing effect is halogenation (chlorination and bromination) by flavin-dependent halogenases. Flavin-dependent tryptophan halogenases, apart from their high substrate specificity, show an extremely high regioselectivity. Tryptophan halogenases with regioselectivities for the 5-, 6-, and 7-position, respectively, have recently been isolated and characterized in some detail. No tryptophan 4-halogenase had yet been detected, although it was known that 4-chloroindole-3-acetic acid is produced from 4-chlorotryptophan by peas (*Pisum sativum*). By obtaining mRNA from three tissue sets of *Pisum sativum* (mature plant leaves, young seeds and mature seeds) followed by transcriptome sequencing and analysis, we were able to detect a candidate gene with high homology to known halogenase enzymes. The deduced

protein sequence contains the glycine motif (GxGxxG) as well as a slightly modified tryptophan motif (WxWxIP), which are the two highly conserved sequence motives of flavin-dependent halogenases. Comparison of the amino acid sequence with known flavin-dependent halogenases shows that this enzyme aligns with the tryptophan halogenases known from bacteria. [1, 2, 3]

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SMP09

Biosynthesis of the Lipodepsipeptide Antibiotic Empedopeptin

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Empedopeptin, a potent antibiotic lipodepsipeptide was first isolated in 1984 from *Empedobacter haloabium*.[a] The octapeptide contains several unusual hydroxylated or D-configured amino acids. It is linked to a 3-OH-tetradecanoic acid and builds a cyclic lactone. Empedopeptin inhibits bacterial growth by blocking the cell wall biosynthesis: It binds primarily to lipid II at the outside of the cytoplasmic membrane in a Ca^{2+}-dependent manner in a 2:1 stoichiometry.[b] With regard to its spectrum and potency, empedopeptin is comparable with vancomycin. The challenge with the biosafety-level 2 organism *E. haloabium* is, apart from the sophisticated cultivation and extraction procedure, to increase its very low production rate of empedopeptin (2.6 mg/L). In order to overcome these obstacles, it is necessary to understand the biosynthesis of this antibiotic. Since empedopeptin derivatives with multiple hydroxylated proline units show an enhanced activity, we are particularly interested in the β-hydroxylation process of the proline residues within the context of the non-ribosomal peptide synthetase (NRPS) machinery. Consequently, we sequenced the genome (6.9Mb) of *E. haloabium* and discovered the corresponding gene cluster including eligible candidate genes responsible for the hydroxylation. Using a BAC-library[c] and a heterologous host system, we are planning to not only characterize the involved genes but also to develop tools for enhanced genetic modification, in order to generate derivatives with enhanced antimicrobial activity and to increase the production of empedopeptin.

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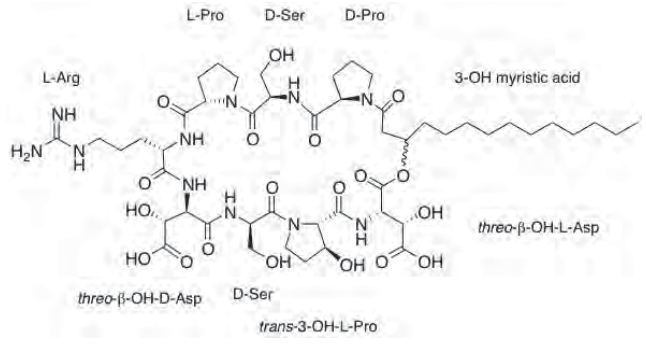
Figure 1



SMP10

Towards exploring the chemoecology of *Planctomycetes* - phototrophs interactions

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Planctomycetes are environmentally important, ubiquitous bacteria. They comprise a complex cell biology, are major players in global carbon- and nitrogen cycles and produce novel secondary metabolites that might be interesting for drug development. We study this phylum employing novel methods in the fields of cell biology, ecology and biotechnology, aiming for a holistic understanding of the unique planctomycetal biology.
Planctomycetes are slow growing organisms, which attach to surfaces such as algae or cyanobacteria and surprisingly dominate biofilms. We determined that limnic and marine *Planctomycetes* utilize compounds of phototrophic origin such as for example algal polysaccharides, employing high throughput phenotypic MicroArrays. This led to the hypothesis that *Planctomycetes* form biofilms on the surface of phototrophs where they feed for example on capsule material of Cyanobacteria or on seaweed surfaces. While slow growing, they likely produce small molecules to defend their ecological niche from other faster growing heterotrophs. This is in good accordance with our recent discovery of many secondary metabolite related gene clusters among the already sequenced planctomycetal genomes. We suspect that phototrophic compounds serve as trigger signals for the secretion of small molecules from *Planctomycetes*. Since the phylum *Planctomycetes* differs in so many ways from other bacteria, it will be interesting to see whether secondary metabolites from these organisms are different from those produced by the "usual suspects" such as Streptomycetes or Myxobacteria. In order to investigate the chemoecology of *Planctomycetes* - phototrophs interactions, we follow en ecomimetic approach. We developed a so-called chemically defined maintenance medium, allowing *Planctomycetes* to survive, but not to grow or divide. Adding carbon and energy sources derived from phototrophs, we could induce alterations in the production of secondary metabolites as detected by HPLC. To further characterize such metabolites we developed a protocol for planctomycetal cultivation in computer -controlled bioreactors. In addition, we constructed genetic tools to allow heterologous expression of identified gene clusters putatively encoding for secondary metabolite biosynthesis in our model organism *Planctomyces limnophilus*. Combining these techniques with transcriptome profiling, we work towards the identification of genes responsible for the production of specific secondary metabolites. This will allow to construct mutants and to reveal the molecular background of *Planctomycetes* - phototrophs interactions.

SMP11

Production of bioactive compounds via inducible promoter exchange in *Xenorhabdus doucetiae*

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Xenorhabdus doucetiae is a Gram negative, entomopathogenic bacterium. It is symbiotically associated with the nematode *Steinernema diaprepesi* and together they can infect and kill insect larvae [1]. Due to its symbiosis *X. doucetiae* produces a variety of bioactive compounds, that protect the insect cadaver against competitors, are involved in killing the insect and might be involved in maintaining the bacteria-nematode symbiosis. Under lab conditions usually only some of these compounds are produced as their production is regulated by factors from the insect and/or the nematode. Here we present an efficient method to produce compounds derived from NRPS gene clusters, that were identified in the genome of *X. doucetiae* DSM 17909 via antiSMASH [2] analysis by exchanging the native promoter against the P_{BAD}-araC promoter. With this method we obtained a significant overproduction for the already well known natural products from *Xenorhabdus* and *Photorhabdus* like xenoamicins [3], GameXPeptides, xenorhabdines, under standard cultivation conditions plus arabinose as inducer. This promising approach also allows to study the influence of the individual compounds during pathogenesis and/or symbiosis as we have shown for the highly toxic xenorhabdins that act as virulence factors against different organisms including insects. Thus it allows us to activate so called „silent" gene clusters in the genome and detect novel compounds.

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INTRACELLULAR TRANSPORT AND SECRETION

TSP01
Plasmid pLS20-mediated conjugation is driven by a RAP/Phr regulated master repressor in *Bacillus subtilis*

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The process of DNA transfer through a direct cell-to-cell contact is known as conjugation and represents the major reason for the spreading of antibiotic resistance genes among bacteria. Conjugation of plasmid pLS20 from *Bacillus subtilis* is limited to a time window between early and late exponential growth. Genetic evidence has suggested that pLS20-encoded protein Rco_{LS20} represses expression of a large conjugation operon, while Rap protein Rap_{LS20} relieves repression. We provide biochemical evidence that Rco_{LS20} binds to a specific inverted repeat sequence upstream of the conjugation operon, which we show to be more than 33 kbp long, and to at least one other promoter within pLS20. Rco_{LS20} is thus a true repressor protein, and forms a dimer in solution, as does Rap_{LS20}. The latter directly binds to Rco_{LS20}, in a 1:1 stoichiometry, which prevents DNA binding of Rco_{LS20}. Rap_{LS20} binds to the helix-turn-helix containing domain of Rco_{LS20}, likely obstructing DNA-binding. The activity of Rap_{LS20} in turn is counteracted by the addition of the Phr_{LS20} peptide, which directly binds to its cognate Rap protein. Thus, the Rap_{LS20} protein acts directly as an antirepressor during regulation of plasmid conjugation by turning on conjugation, and is counteracted by the Phr_{LS20} peptide, which by analogy to known Rap/Phr systems is secreted and taken back up into the cells, mediating cell density-driven regulation.
Additionally, we were interested in the assembly and composition of the pLS20 encoded type IV secretion system (T4SS) and performed a quantitative subcellular localization and interaction screen with putative T4S proteins of pLS20. We found that the T4SS mediating the transfer of pLS20 is composed of at least 9 more proteins in addition to the conserved components VirB1, VirB4, VirB11, VirD2 and VirD4. Based on our data, we propose a preliminary model for the assembly of the pLS20 encoded T4SS.

TSP02
Rubber Oxygenase (RoxA) and Latex Clearing Protein (Lcp) Cleave Rubber to Different Products and Use Different Cleavage Mechanisms

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Two types of enzyme for oxidative cleavage of poly(*cis*-1,4-isoprene) are known. One is rubber oxygenase (RoxA) that is secreted by *Xanthomonas* sp. 35Y and a few other Gram-negative rubber-degrading bacteria during growth on polyisoprene. RoxA was studied in the past (1) and the recently solved structure showed a structural relationship to bacterial cytochrome *c* peroxidases (2). The other enzyme is latex-clearing-protein (Lcp) that is secreted by rubber degrading actinomycetes but Lcp has not yet been purified. Here, we expressed Lcp of *Streptomyces* sp. in a Δ*roxA* background of *Xanthomonas* sp. 35Y and purified native Lcp. The specific activities of Lcp and RoxA were determined as 0.70 U/mg and 0.48 U/mg, respectively. Lcp differed from RoxA in the absence of (covalently bound) heme groups and other characteristics. Notably, Lcp degraded polyisoprene via endo-cleavage to tetra-(C_{20}) and higher oligo-isoprenoids with aldehyde and keto end groups whereas RoxA employed an exo-cleavage type mechanism to give the main end product 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD). RoxA was able to cleave isolated Lcp-derived oligo-isoprenoid molecules to ODTD. Inhibitor studies, spectroscopic investigations and metal analysis gave no indication for the presence of transition metals or co-factors in Lcp. Our results suggest that Lcp could be a member of the growing group of co-factor-independent oxygenases (3) and differs in the cleavage mechanism from heme-dependent RoxA.
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TSP03
Plasma membrane localized KDEL receptors ensure endocytic entry and retrograde transport of a viral A/B toxin in yeast

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A/B toxins such as Cholera toxin and the yeast K28 toxin possess an H/KDEL amino acid motif at the C-terminus of their cell binding B-subunits which is recognized and bound by KDEL receptors (KDELRs) of their target cells. The main function of KDELRs is to retrieve resident ER proteins that escaped from the secretory pathway back to the ER. Until now, it was believed that the initial toxin interaction with KDELRs occurs within the Golgi, i.e. after receptor-mediated endocytosis and endosomal trafficking. However, we recently demonstrated that the yeast KDEL receptor Erd2p is not only present in the ER and Golgi but also in the plasma membrane (PM). We demonstrate that PM localized Erd2p is capable to bind Kar2p/BiP from outside the cell, leading to its endocytosis and retrograde transport to the ER where it restores the growth defect of a *kar2*ts mutant. In addition to its essential physiological role in the uptake of secreted HDEL-bearing proteins from the PM, we show that Erd2p is crucial for A/B toxin binding and endocytosis. Consequently, we used K28 as model cargo to mechanistically dissect the internalization of A/B toxins and to understand the general basis of receptor endocytosis in yeast and higher eukaryotic cells. *In vivo* topology of Erd2p matches the postulated topology of KDELRs in mammalian and plant cells. Interestingly, three potential endocytic motifs within each KDELR ensure recognition and internalization of HDEL-cargo from the PM. By using biochemical analysis, rational receptor mutagenesis and toxin sensitivity assays, toxin/receptor internalization was shown to be regulated by multiple mechanisms, amongst which (i) (mono)ubiquitylation of Erd2p via Ubc4p (E2) and Rsp5p (E3), (ii) the involvement of AP-2 complex components as well as (iii) the Sla1p/Pan1p complex were identified as being key elements in KDELR endocytosis.
Supported by the Deutsche Forschungsgemeinschaft (SPP1365, SFB1027)

TSP04
Influence of the intramembrane cleaving protease RasP on protein secretion in *Bacillus subtilis*

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B. subtilis RasP belongs to the S2P-family of intramembrane cleaving proteases (i-CliPs) that cut the transmembrane part of their substrate proteins in the plane of the membrane. RasP is involved in 'regulated intramembrane proteolysis' of the anti-sigma factors RsiW and RsiV, and of the cell division protein FtsL [1]. In higher eukaryotic cells, different families of i-CliPs act as signal peptide peptidases (SPPs) that remove signal peptides of secretory proteins residing in the membrane after being processed by signal peptidase. For bacteria, a prevailing concept assigns signal peptide peptidase function to the membrane-bound self-compartmentalized serine protease SppA, which is not related to i-CliPs. However, the S2Ps RseP of *Escherichia coli* and *B. subtilis* RasP have recently been shown to exert SPP activity. *B. subtilis* mutants with a defect in RasP activity display a pleiotropic phenotype, one of which is a secretion defect of α-amylase (AmyQ). In order to estimate whether the secretion defect is caused by the absence of signal peptide peptidase activity, we analyzed the secretion of AmyQ and of SacB (levansucrase) in *B. subtilis*. To that purpose, GFP was fused to the N-terminus of preAmyQ and of preSacB as a marker for signal peptide degradation. In summary, our data suggest that RasP is involved in membrane clearance rather than acting specifically as a signal peptide peptidase.

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TSP05
Thiol-switch control in A/B toxin cell entry and compartmental trafficking

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Disulfide bond reduction is a key step of trafficking of some A/B toxins, including Cholera, Shiga Toxin and ricin. Using yeast killer toxin K28 as a model protein, we are investigating this process. Killer toxin K28 is produced by virus-infected *S. cerevisiae* strains and consists of two subunits (α and β) connected by a single disulfide bond. It is taken up by sensitive cells by receptor-mediated endocytosis and travels the secretory pathway backwards via early endosomes, Golgi and ER to finally translocate into the cytosol. After reductive cleavage of the connecting disulfide bond in the cytosol, cytotoxic K28α is released and enters the nucleus to kill the host cell by blocking DNA synthesis. Three other cysteine residues in the β subunit are also involved in this reaction, suggesting thiol-switch control of the compartmental trafficking.

TSP06
KDEL receptor trafficking and dynamics in yeast and mammalian cells

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Resident proteins of the endoplasmic reticulum (ER) possess a c-terminal tetrapeptide motif (KDEL) that prevents their secretion. Binding and retrieval of KDEL-carrying ER residents is facilitated by a six transmembrane KDEL-receptor (KDELR), Erd2p in yeast. Despite its role in recycling soluble ER proteins back to the secretory pathway, we could recently show that KDELRs are also present in the plasma membrane. Interestingly, cell surface localized KDELRs not only bind and internalize KDEL-carrying proteins that escaped from the secretory pathway, they are also parasitized by certain A/B protein toxins for host cell entry. We demonstrate that all three mammalian KDELRs and Erd2p homologues functionally complement a yeast *erd2* deletion, fully restoring toxin sensitivity in the former resistant *erd2* knock-out. By using live-cell imaging with fluorescently labeled variants of human KDELRs and A/B toxins such as K28 and ricin (RTA), our studies aim at getting deeper insight into KDEL-receptor localization and -trafficking as well as in spatiotemporal receptor/ligand distribution in subcompartments of yeast and human cells.

Kindly supported by the Deutsche Forschungsgemeinschaft (SPP1365, SFB1027)

TSP07
ER-exit of a yeast viral A/B toxin: SECrets of K28

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K28 is a virus encoded A/B protein toxin secreted by the yeast *Saccharomyces cerevisiae* that enters susceptible target cells by receptor-mediated endocytosis. After retrograde transport from early endosomes through the secretory pathway, the α/β heterodimeric toxin reaches the cytosol where the cytotoxic α-subunit dissociates from β, subsequently enters the nucleus and causes cell death by blocking DNA synthesis and arresting cells at the G1/S boundary of the cell cycle. To elucidate the molecular mechanism(s) of ER-to-cytosol toxin transport in yeast as well as in mammalian cells, the major focus of the present study is to identify cellular components (including the nature of the ER translocation channel) involved in this process. The requirement of proteasomal activity and ubiquitination to drive ER export, and the identification of cellular K28 interaction partners of both, the α/β toxin as well as K28α are being analysed in toxicity tests and various biochemical assays. K28 is the first example of a protein that exits the ER in a heterodimeric conformation, although retrotranslocation itself critically depends on the unfolding activity of Pdi1p probably assisting its targeting to the export channel. We conclude that it is the isomerase and unfolding activity of Pdi1p rather than its oxidase activity that is required to ensure translocation competence of the α/β heterodimeric toxin.

TSP08
"What goes out first": Substrate orientation during type 1 secretion in *Escherichia coli*

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Type 1 secretion systems (T1SS) are important for substrate and toxin secretion in Gram-negative bacteria e.g. *Escherichia coli*. The paradigm of T1SS is hemolysin A (HlyA) secretion system in *E. coli* that consist of the inner membrane ABC transporter hemolysin B, the periplasm spanning membrane fusion protein hemolysin D and the outer membrane factor TolC (*1*). These proteins assemble to one complex translocator that secretes the 110 kDa toxin HlyA in an unfolded manner in one step from the cytoplasm directly into the extracellular space (*2, 3*). Characteristic for T1SS is the C-terminally located secretion signal within the substrate protein which is not cleaved during translocation. It is essential and proven to be already sufficient for secretion (*4*). This raises the question whether the N- or C-terminus of HlyA is transported first (*5*). To address this question, we express fusion proteins of the *Candida antarctica* lipase b (CalB) with the secretion signal of HlyA that should block the translocator due to the fast folding of CalB. By using specific fluorophore labeled antibodies and the usage of confocal laser scanning microscopy the secretion signal was observed on the surface of the cell. This result shows that the secretion signal is needed for secretion and is recognized and transported first through the T1SS.

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TSP09
Type IVB Secretion System (T4BSS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria

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Introduction: *Rickettsiella* bacteria (*Gammaproteobacteria: Legionellales*) are intracellular pathogens of arthropods that multiply inside replicative vacuoles within host cells. As in the related vertebrate pathogens *Legionella* and *Coxiella*, delivery of bacterial proteins across the vacuole membrane to the host cell's cytosol is believed to be of key importance for successful infection and pathogenesis.
Materials and Methods: Comparative genomic analysis within the order *Legionellales* has been employed to identify type IVB secretion systems (T4BSS) and possible T4BSS substrates.
Results: Genomic analysis of *Rickettsiella* and related bacteria has revealed the presence of a complete set of gene clusters presumably encoding a T4BSS in two *Rickettsiella* strains of the pathotypes `*R. melolonthae´* and `*R. armadillidii´*, i.e. infecting an insect, the European cockchafer, and a crustacean, the pill bug, respectively. Hypothetical *Rickettsiella* T4BSS key components show high similarity to orthologs in the Dot/Icm systems of the related vertebrate pathogens *Legionella pneumophila* and *Coxiella burnetii*, and T4BSS gene cluster organization is moderately well conserved in these bacteria.
In *Legionella* and *Coxiella*, involvement of Dot/Icm systems and their substrates into infection and pathogenesis has been demonstrated previously. *In silico* identification of putative T4BSS substrates across the *Rickettsiella* genome has revealed a plethora of effector candidates. Apart from a conserved family of ankyrin-like proteins, putative T4BSS substrate sets are found highly divergent in these phylogenetically related pathogens and comprise a high number of orphan genes.
Discussion: A T4BSS appears operational in *Rickettsiella* bacteria. The identified putative T4BSS substrates are highly interesting targets for the study of *Rickettsiella* pathogenicity. The observed very high degree of divergence from effector sets in related bacteria is in line with expectations

from the specific adaptation to very different hosts. The systematic experimental validation of identified effector candidates is currently under way.

TSP10
A systematic proteomic analysis of *Listeria monocytogenes* house-keeping protein secretion systems

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Listeria monocytogenes is a facultative intracellular firmicute bacterium causing serious infections in humans due to consumption of contaminated food. The majority of its virulence factors required for host cell invasion and intracellular growth are released into to the medium or attached to the bacterial envelope. *L. monocytogenes* is supposed to encode at least eight different protein secretion pathways. Great efforts have been made to predict secretory proteins and their secretion routes based on sequence information, however, experimental evidence for these predictions is lacking in most cases. We have constructed mutant strains in in several putative secretion systems and in the three main housekeeping protein secretion systems of *L. monocytogenes*, which are the SecA-dependent transport, the two YidC-like membrane insertases SpoIIIJ and YqjG, as well as the twin-arginine translocation pathway. The effect of these mutations on viability, virulence and protein secretion was analysed by phenotypical assays, infection experiments and proteomics, respectively. Sec-dependent protein secretion and membrane insertion of proteins via SpoIIIJ and YqjG were clearly essential for viability of *L. monocytogenes* and depletion of SecA or YidC activity affected bulk protein secretion. In contrast, the Tat-pathway was dispensable for secretion, viability and virulence. We show that secretion of many virulence factors and of enzymes synthesizing and degrading the cell wall depends on the SecA route. Analysis of secretion of substrates typically dependent on the accessory SecA2 ATPase in wild type and azide resistant *secA* mutants of *L. monocytogenes* furthermore revealed that SecA2-dependent protein secretion also requires the ATPase activity of the house-keeping SecA protein. Taken together, the work presented on this poster represents the first experimental study comprehensively describing the contribution of the different protein secretion pathways to growth and physiology of the important human pathogen *L. monocytogenes*.

TSP11
Excretion of cytoplasmic proteins in staphylococci

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Many microorganisms and also eukaryotic cells excrete typical cytoplasmic proteins. As none of the classical secretion systems appears to be involved, this type of secretion has been referred to as "non-classical protein secretion". So far it is neither known by which mechanism nor why cytoplasmic proteins are excreted. Our research is concentrated on why, how, where and when staphylococci excrete cytoplasmic proteins. Our preliminary results have shown that in Staphylococcus aureus more than 20 typical cytoplasmic proteins were excreted and that the excretion of cytoplasmic proteins already starts in the exponential phase and appears to be more pronounced in the clinical isolates than in the non-pathogenic staphylococcal species. We could show that there is no correlation between the quantity of cytoplasmic proteins in the cytoplasm and their release to the extracellular environment. We assume that there exists a selection procedure in the excretion of cytoplasmic proteins. We want to address the question why microorganisms excrete cytoplasmic proteins, which is a loss of resources and energy. Is the excretion of such proteins an accident or is it intentional?

TSP12
Functional analysis of potential endocytotic motifs within cellular KDEL receptors in yeast

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KDEL receptors (KDELRs) are responsible for the retrieval of soluble ER-resident proteins and have important signalling functions in Golgi structure maintenance. Structural analysis of the yeast KDELR Erd2p revealed a 6 transmembrane domain receptor with N- and C-termini facing the cytosol. A minor fraction of KDELR colocalizes at the plasma membrane thereby ensuring uptake of the A/B toxin K28 and its retrograde transport to the ER. The primary KDELR structure reveals three potential endocytotic motifs: A C-terminal lysine cluster could be responsible for ubiquitin-dependent receptor endocytosis. Our results indicate that Erd2p is ubiquitylated *in vivo* and, consequently, signal intensity of ubiquitylated Erd2p was markedly reduced in a lysine-free receptor variant. In addition, yeast mutants with defects in protein ubiquitylation and endocytosis also showed a significant decrease in ubiquitylated KDELR, whereas higher molecular weight Erd2p-Ub signals increased in a yeast mutant defective in protein deubiquitylation. Furthermore, we confirmed the expression and plasma membrane localization of a receptor variant with simultaneous defects in the endocytotic motifs NPF and YNEM. Interestingly, the three mammalian KDELRs (Erd21-23) fully complement Erd2p function in yeast and restore K28 sensitivity. By using an adapted RAS-recruitment system and western analysis, plasmamembrane localization and *in vivo* ubiquitylation of mammalian KDELRs was confirmed in yeast.

Biology of Conventional and Non-Conventional Yeasts

YEP01
Yeast as a tool to identify molecular targets of aging modulators

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The identification of compounds that affect lifespan (aging modulators) and the characterization of their molecular targets is an important aspect of aging research. Such studies lead to the discovery of new aging pathways and establish the basis for treatments that might delay cellular aging or shorten lifespan of cancer cells. Yeasts as simple organisms have contributed in large part to the understanding of molecular fundamentals of aging. Here we present first results in identifying and validating new small molecule aging modulators, as well as their mode-of-action using yeast as a tool. Novel aging modulators were identified by high-throughput screening (HTS) in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* using natural compound libraries. Several technologies like expression profile analyses, haploinsufficiency approaches and yeast three hybrid assays were used in order to determine their targets and to get first insight into their mode-of-action. Using yeast as an experimental system we are able to get more information about pathways that are affected by aging modulators and how cellular aging might be controlled.

YEP02
Evolution of the AMP-activated protein kinase controlled gene regulatory network in yeast

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Yeasts are unicellular eukaryotic organisms and since early in human history they are used in making bread and alcoholic beverages. Moreover, "the yeast" meaning *Saccharomyces cerevisiae* (baker's yeast) has become an important model system in molecular biology, because many essential cellular processes are conserved between yeast and man. About 23 per cent of the human genes have counterparts in yeast. In trying to understand how gene regulatory networks evolve we compare the response to energy limitation between *S. cerevisiae* with its distant cousin *Kluyveromyces lactis* (milk yeast). These two yeast species have diverged over more than 150 Mio. years and both have evolved sophisticated strategies to gain selective advantage on various nutrient sources. In both cases, the master kinase Snf1, a yeast orthologue of mammalian AMP-activated protein kinase (AMPK), acts as an energy sensor of the cell. By phosphorylation of down-stream effectors, such as transcription factors, enzymes and signaling molecules, Snf1 mediates metabolic adaptation to limiting conditions. To analyze evolution of the regulatory networks we focus on the Snf1 regulated transcription factors Sip4 and Cat8, belonging to the big class of fungal specific zinc cluster transcriptional activators. Our genome-wide expression studies by RNA sequencing comparing wild-type and *snf1*D, *cat8*D and

*sip4*D mutants shifted to energy-limiting conditions yielded remarkable differences between the two species. First, the numbers of differentially expressed genes is significantly higher in *S. cerevisiae* than in *K. lactis* [29 %, 3,7% and 2,2 % of ~6300 genes in *snf1*D, *cat8*D and *sip4*D mutants of *S. cerevisiae*, respectively, compared to 13%, ~0.6 % and ~0,4 % of ~5600 genes in *K. lactis snf1*D, *sip4*D and *cat8*D mutants]. Furthermore, we find differences in the expression of *MIG* genes, of key enzymes involved in glyoxylate cycle or gluconeogenesis and the carnitine shuttle between the two yeasts. The latter plays an important role in transporting acetyl-CoA across peroxisomal and mitochondrial membranes from yeast to man. Our results indicate that the two yeasts have diverged in the regulation of central metabolic processes. It is likely that both species have evolved different strategies to gain selective advantage on various nutrient sources. The molecular basis for this divergences remains to be identified.

YEP03
New Integration Platforms for Gene Expression in *Yarrowia lipolytica*

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For the construction of stable production stains of the industrial interesting yeast *Yarrowia lipolytica* different integration platforms are used, e.g. pBR322, zeta or rDNA. These platforms were reliably used for strain construction, but show pitfalls like strain dependency (pBR322), heterologous integration events (zeta) or interference with genetic elements (rDNA). We developed reliable integration platforms for single and reusable integration into specific sites of the host genome, which can be adapted rapidly to the expression of the protein of interest. This platform was used to integrate a model secretion cassette under the control of the strong and constitutive *TEF1*-Promoter, containing a fusion protein consisting of a *Y. lipolytica* lipase for activity measurement, the FMN-binding fluorescent protein as a reporter, a Twin-Strep-tag for protein purification and a factor X protease site for complete removal of the N-terminal fusion partner.
This system was succussfully used for the investigation of protein secretion, for the overexpression and localization of several enzymes and transporter of *Y. lipolytica,* and for the heterologous production of the highest known lycopene amounts reported for eukaryotic organisms.

YEP04
Oxidative stress responses of refractive ascomycetes: genetic manipulation of the rock-inhabiting model fungus *Knufia petricola* A95

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Rock-inhabiting microcolonial fungi (MCF) are able to colonise barren surfaces in almost every environment and are unequalled among eukaryotes in their ability to withstand extreme environmental conditions. Pigments, like melanin and carotenoids, have been proven to contribute to this unique robustness. An excellent model system to investigate the involvement of pigments in stress response and DNA repair is the MCF Knufia petricola strain A95. This non-pathogenic fungus possesses all characteristic features of MCF, including meristematic growth, melaninised cell-walls and extensive secondary metabolite production. K. petricola is easy to handle, physiologically characterised and genetically tractable. Recently, The Black Yeast Project of the Broad Institute (Boston, MA, USA) released the annotated genome of A95 and related fungi. This study is focusing on MCF responses to oxidative stress - one of the most significant environmental challenges encountered by MCF. The exact role of pigments and especially the interplay between carotenoids and melanin in the oxidative stress response is studied in wild type cells, existing melanin mutants and specially constructed pigment knock-out mutants. Oxidative stress which is mostly represented as reactive oxygen species (ROS) in the cell are extremely cytotoxic since they interact directly with DNA/RNA, proteins and lipids. The first line of the cellular defence system comprises superoxide dismutases (SOD), catalases (CAT) and peroxidases which enzymatically inactivate ROS. Another line of defence is the repair of damaged DNA by an intricate network of DNA repair mechanisms. Comparative gene expression analyses are used to identify genes which are especially regulated under oxidative stress conditions to help elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

ZOONOSES

ZOP01
Studies for eradication of and re-contamination with multidrug-resistant pathogens in two housing systems of a pig-producing farm

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We tested the rate of recontamination of resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-Spectrum-ß-Lactamase bearing *Enterobacteriaceae* (ESBL) in pigs, dust, air and water on a pig-producing farm before and after total decontamination of the stable with drug resistant bacteria. The goal of the study was to address whether a complete exchange of pigs and a total decontamination of the former stable and the construction of an additional new stable lead to a permanent decontamination of the pig farm. The study was planned as a two-step approach. In the first step samples of pigs, humans, dust, air and water were obtained in the original stable (before decontamination). Then all sampled pigs were slaughtered, followed by a professional decontamination (DESTEC GmbH) of the stable and construction of a new nearly identical stable. In the second step, incoming pigs (previously identified as MRSA and ESBL negative) as well as humans, dust, air and water were immediately sampled in May with monthly repeats for 3 months. In total 1242 samples were obtained (426 pig, 12 human, 368 dust, 138 air, 298 water samples). MRSA were detected in 40.3% of the pig samples before compared to 42.6%/41%/20% (May/June/July) after decontamination. MRSA was isolated in 25% of the dust samples before decontamination and in 5.6%/21.8%/14.5% (3 months repeats) afterwards. Regarding the air samples all were MRSA positive before decontamination unlike 33.3% (May) (38.5% in June/July) positive samples after decontamination. The contamination rates with MRSA of the water (samples taken in every compartment) were documented with 18.9% before and with 5.4% (May) respectively 2.6% (June/July) after decontamination. ESBL-producing bacteria were only detected in anal swabs of pigs. Whereas 37.1% of pigs were ESBL positive before decontamination but no pig was ESBL positive after decontamination. Overall, differences of the contamination rates between the decontaminated and the new stable were not significant. A resistance-free environment could not be created and a reduced prevalence of MRSA in pigs by professional decontamination and new-construction of the stables was not achieved. However, the MRSA prevalence in stable environment (dust, air, water) could be reduced by strict disinfection measures and hygiene management. The contamination level of ESBL in pigs could be reduced initially but was not stable over time.
The transmission pathways for new- or possibly re-contamination need to be identified.

ZOP02
Diagnosis of tick-borne relapsing fever in south Iran

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Tick-borne relapsing fever (TBRF) is a known endemic disease in Iran, with more than1400 confirmed cases during 1997-2006 from 19 provinces. Most infections in the country are attributed to *Borrelia persica* transmitted via infective bites of *Ornithodourous tholozani* soft ticks. Three other *Borrelia* species including *B. microti*, *B. latyschevii,* and *B. baltazardi,* have also been described in Iran. There has been no recent report on occurrence of human infection with *B. latyschevii* or *B. baltazardi*, however, the epidemiological evidence for human infection with *B. microti* in South Iran is strong as infections are mostly from localities in which *O. erraticus* ticks, the known soft tick vector for this species, predominate. Molecular characterization of an isolate of *B. microti* originated from *O. erraticus* soft ticks revealed its highest homology with *B. duttonii* (99.84%) and B. *recurrentis* (99.67%). The present study was aimed to identify the causing agent of relapsing fever in human blood samples from South Iran. Blood samples were collected from 25 feverish people residing in rural areas of Jask County, Hormozgan Province. All blood samples examined

microscopically and the DNAs extracted from the sera were screened for presence of *Borrelia* DNA by a Real-time TagMan PCR assay designed based on *B. duttonii* flagellin gene. Also, species-specific and genus-specific PCRs targeting *glpQ* and IGS genes, respectively were performed on DNA samples. In all assays DNAs from in-vivo cultured *B. microti* and *B. persica* spirochetes were included as positive controls. Real-time PCR detected *Borrelia* DNA in 5 samples, of which two were microscopically positive. PCR assays amplified *glpQ* and *IGS* genes in the same 3 samples including microscopically positive ones. This study identified *B. microti* as the causing agent of *B. microti* in South Iran. Further studies including multispacer sequence typing is required for phylogenetic analysis of the identified agent.

ZOP03

Molecular detection of Crimean-Congo hemorrhagic fever virus on hard ticks (Ixodidea) collected from Tarom/Zanjan Province of Iran

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Question: Why the CCHF is important in Iran?
Crimaen congo hemorrhagic fever is a zoonotic viral disease with a high mortality rate in humans .The disease is caused by Crimean-congo hemorrhagic fever virus(CCHFV).This virus is transmitted through the bite of an infected tick,or by direct contact with infected patient or the products of infected live stock.This viral disease is asymptomatic in infected live stock,but cause Serious threat to human.Among animals,Sheep as the most important vertebrate hosts for CCHFV.This virus belongs to the Family *Bunyaviridea* and the genus *Nairovirus.*The most important reserviors of CCHFV are ticks and the virus has been isolated from at least 31 different tick species.Since 2000 data has shown the disease to be prevalent in 23 out of 30 provinces of Iran.
Materials and Methods: Ticks was separated from the body of animals and transported to laboratory of medical entomology and were identified with local identification key.In this study,RT-PCR has been used for detection of the CCHF virus genome.
Result: This study was conducted to determine the infection rate of CCHF virus in thicks in Tarom,in the province of Zanjan.
Among the whole ticks collected from different region in Tarom a total of 100 ticks were selected and they were investigated for detection of CCHFV using RT-PCR method .The virus was found in 7 % of ticks.The infected species belonged to *Hyalomma dromedarii*, *H.marginatum* ,*H.asiaticum*, *Rhipicephalus bursa* and *Rh.sanguineus.*
Conclusions: Due to presence of virus in different region, We recommend the use of acaricides and repellent to prevent disease transmission among humans. Greta care should be taken by the people who are working in slaughter houses. Vector control programs should be applied for reducing population density of potential tick vectors in this county.

ZOP04

Development of a lateral flow immunoassay for the rapid detection of *Coxiella burnetii*

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The main reservoirs of the Q fever pathogen *Coxiella burnetii* are ruminants like goats, sheep and cattle. These animal species are also the major reasons for human disease when getting infected through inhalation of contaminated aerosols, ingestion of raw milk or contact with infected sheep, especially during lambing. Therefore fast and specific identification of this pathogen in veterinarian and environmental samples is important. Suspected materials from affected animals/flocks were normally screened by molecular methods performed in specialized laboratories. A rapid pre-screening of suspected materials in the field and/or in the diagnostic laboratory will allow a much faster identification and reduce the risk for the laboratory personal when handling materials.
The antigenic virulent phase I of *Coxiella burnetii* consists of a lipopolysaccharide complex, in which two unusual and unique sugars occur: virenose and streptose. Following production of a virenose-specific monoclonal antibody (Palkovicova et al. 2009) a lateral flow assay (immunochromatographic rapid test) was developed and produced. The limit of detection was determined by a serial dilution of a heat-inactivated reference strain (RSA493, Nine Mile). Samples migrate via capillary forces through test cassette and test result is observed within 20 min after sample application. A control line was included to ensure the validity of Results: The assays were visually read out by naked eye. It was possible to detect 1,250 genome equivalents of *Coxiella burnetii* per microliter. The specificity was tested further with some selected members of the families *Rickettsiaceae, Chlamydiaceae and Enterobacteriaceae.* All showed negative results with our new lateral flow immunoassay. Some generic testing with abortion materials from goats, sheep and cattle showed no cross reactions and positive samples were identified well.
The here presented new lateral-flow assay will allow a specific, sensitive and fast detection of *Coxiella burnetii* from different biological and environmental matrices and is a valuable addition in the diagnostic process in laboratory settings. Furthermore this rapid assay might be also useful in a bioterroristic/ biocriminal situation.

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ZOP05

Echinococcus multilocularis: an emerging zoonotic parasite from northeastern, Iran

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Introduction: *Echinococcus multilocularis*, the agent of alveolar echinococcosis, is a potentially fatal zoonotic disease in the northern hemisphere. The parasite is perpetuated in nature through a sylvatic cycle involves wild carnivores as definitive hosts and small mammals as intermediate hosts. Human can be infected through direct contact with definitive hosts or by ingestion of contaminated materials. The current study was carried out to investigate the presence of *E. multilocularis* infection in definitive and intermediate hosts in northeastern of Iran.
Materials and Methods: During 2009- 2011 seventy seven fecal samples from dogs and the intestine of 16 wild carnivores from northeastern, Iran were examined using the intestinal scraping technique(IST) and sedimentation and counting technique(SCT) followed by flotation/ sieving and multiplex PCR. For identifying intermediate hosts 85 small mammals were examined using multiplex PCR.
Results: The IST and SCT techniques detected *E. multilocularis* adult worms in 5 of 10 jackals and one wolf. Multiplex PCR was able to identified *E. multilocularis*, *E. granulosus* and *Taenia* spp. in 19, 24, and 28 fecal samples, respectively. In total, *E. multilocularis* infection was detected in the faces of all wild carnivores including nine jackals, three foxes, one wolf, one hyena, and five dogs. In small mammals, *E. multilocularis* infection was identified in 30 of 85 investigated liver samples, including 23 *Microtus transcaspicus*, three *Ochotona rufescens*, two *Mus musculus*, one *Crocidura gmelini*, and one *Apodemus witherbyi* by multiplex PCR.
Conclusion and Discussion: The current study confirms the existence of definitive and intermediate hosts and establishment of the cestode in the studied area. Therefore, it shows that *E. multilocularis* is an important source of human alveolar echinococcosis in the region. Thus, it is recommended to increase the local awareness, and comprehensive educational programs should be established in the area and control and prevention programs should be implemented by health centers in the region.

ZOP06

Role of a putative hemolysin encoded by *V. parahaemolyticus_VN-0028_959* of *Vibrio parahaemolyticus* patient isolate VN-0028

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Introduction: The Gram negative bacterium *Vibrio parahaemolyticus* is the main cause of foodborne illnesses worldwide and causes, among others, gastroenteritis and watery diarrhea.
The shotgun sequenced and partially annotated patient isolate *V. parahaemolyticus* VN-0028 is TDH-related hemolysin (*trh*) positive. Genome-wide analysis showed at least 7 additional hemolysin genes. BLAST analysis revealed 86 % identity of the amino acid sequence between VllY, a known hemolysin of *Vibrio vulnificus*, and the protein encoded by *Vp _VN-0028_959*. Therefore, the function and physiology of this so far undescribed putative hemolysin in *V. parahaemolyticus* should be investigated.
Materials and Methods: The up- and downstream region of the gene encoding the putative hemolysin Vp_VN-0028_959 was cloned into suicide vector pRE112, which contains a λ*pir*-dependent origin of replication and provokes sucrose sensitivity. After transformation into an *E. coli* donor, mating of donor and recipient strains was done by conjugation. Concerning the locus of the gene in the *V. parahaemolyticus* VN-0028 genome, 34 bp from the end of the gene *Vp_VN-0028_959* will remain in the genome. Similarily, a *trh* deletion mutant and a double deletion mutant *V. parahaemolyticus* VN-0028Δ959Δtrh were constructed.
In the following, growth behavior of the mutants was analyzed. Hemolytic activity of the corresponding mutants was determined in a quantitative hemolytic assay. Therefore, wild type and mutant strains were harvested in the stationary growth phase and incubated with a final concentration of 2 % different mammalian erythrocytes for 4 hours at 37 °C. Subsequently, absorbance was measured at 540 nm. Hemolysis was calculated regarding spontaneous and maximal lysis of the erythrocytes.
Additionally, *Vp_VN-0028_959* was cloned into the high copy expression vector pUC19 and transformed into the non-hemolytic *E. coli* MDS42. The heterologous overexpression should give further insights into mode of action and putative hemolytic activity of this protein.
Results: Examination of the growth behavior of *V. parahaemolyticus* VN-0028Δ959 compared to the wild type did not show any differences. Moreover, quantitative determination of the relative hemolytic activity of the mutant compared to the wild type revealed a trend of decrease of 50 ± 8.1 % with sheep erythrocytes and a significant decrease of 37 ± 3.5 % with human erythrocytes.
Discussion: Deletion of the putative hemolysin Vp_VN-0028_959 affects the hemolytic activity of the patient isolate *V. parahaemolyticus* VN-0028. The loss of this gene leads to reduced hemolysis displayed by quantitative measurement of the strain's hemolytic activity. While the protein encoded by *Vp_VN-0028_959* contributes to a hemolytic phenotype, it is not the only biologically active hemolysin in *V. parahaemolyticus* VN-0028, because a remaining hemolysis is still measurable after its deletion.

ZOP07

Source attribution of foodborne ESBL-*E. coli* using data from the RESET consortium

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Extended-spectrum beta-lactamases (ESBL) producing *E. coli* are resistant against a wide spectrum of ß-lactam antibiotics, including 3rd- and 4th-generation cephalosporins. In addition, such bacteria are often resistant to other antimicrobial classes. Furthermore, the underlying genetic information can be potentially transferred inter- and intra-species which may contribute to the public health risk.
There is an ongoing discussion about the origin of ESBL-producing *E. coli* in humans and the role of animal food sources. Within the German RESET project (www.reset-verbund.de) several studies investigated the prevalence of ESBL-producing *E. coli* in animal and human populations and characterized the isolates using phenotypic and genotypic techniques. Based on these study results, we estimated the possible contribution of different animal sources (broiler, fattening pigs, and cattle) to the colonization of the general population with ESBL-producing *E. coli*. In addition, we considered in one approach hospital acquired cases as 'source' to investigate the correspondence with ESBL-subtypes found in the community. Our model approach is based on Tine Hald's *Salmonella* source attribution model using microbial subtyping data. Information on ESBL-genes, phylogenetic groups and the antibiotic resistance pattern were incorporated to define the subtypes considered in the model.
Our results show that - on the basis of ESBL-genes and phylogenetic groups - several subtypes found in human cases colonized with ESBL-producing *E. coli* can be explained by animal food sources. If only cattle, pigs and broilers are considered as sources, around half of the human findings might be explained by these sources. If information on antimicrobial resistance pattern is included for subtype definition, the number of findings in the human community, which can be explained by any of the investigated animal sources decreased considerably. This is due to the huge variation of subtypes found in each of the populations. As expected, quite a proportion of all subtypes found in the community match with those found in hospital acquired cases, if included as 'source' to the model. This indicates that these human populations share a considerable number of subtypes and that these animal sources may explain only a part of the findings of ESBL-genes in humans.
This approach can be further developed to assess the role of the different reservoirs for exposure of humans with ESBL-producing *E. coli* or ESBL-genes.

ZOP08

Molecular Assay on Crimean Congo Haemorrhagic Fever Virus in Ticks (Ixodidae) Collected from Kermanshah Province, Western Iran

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Introduction: Crimean - Congo Hemorrhagic Fever (CCHF) is a feverous and hemorrhagic disease. Causal agent of the viral CCHF is resulted from arbovirus group, Bunyaviridae family and Nairovirus genus. The viral reservoir in the nature nature is essentially ticks. However small vertebrates and a wide range of the domestic and wild animals are regarded as the reservoir.
Materials and Methods: This study was conducted to determine the infection rate of CCHF virus in the ticks in Sarpol-e-Zahab county, Kermanshah province, west of Iran . It has more than 330 Km common border with Iraq. Among the whole ticks collected from 8 villages, a total number of 122ticks were selected and they were investigated for detection of CCHF virus using RT-PCR method.
Results: The virus of CCHF was found in 4.09% of the ticks. Results indicate that genus of *Hyalomma* is the major virus carrier in the study area.
Discussion: As no study has been conducted on the infection rate of domestic animals and ticks with CCHF virus in Sarpol-e- Zahab city, and according to the fact that the city has an Ashayeri (nomadic) context and occupation of most of the people is husbandry, this study can provide evidence of epidemiology of disease status for choosing the appropriate plans for disease control.

ZOP09

Real time interaction analysis of Shiga toxins with microdomain-associated toxin receptors using surface acoustic wave biosensors

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Introduction: Plasma membrane lipids are laterally organized in highly dynamic microdomains of the liquid ordered phase known as lipid rafts [1]. Certain pathogens and toxins have evolved strategies to bind to lipid raft-associated glycosphingolipids (GSLs) and to unfold their lethal effect after internalization. Shiga toxins (Stxs) of pathogenic enterohemorrhagic *Escherichia coli* (EHEC) bind to the GSLs globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer), which are particularly expressed by microvascular endothelial cells of human kidney and brain [2]. Adhesion of GSL-binding antibodies and Stx to microdomain-associated receptor GSLs is not well understood. Here, we show that real time interaction analysis is a

powerful tool to investigate non-covalent interaction of GSL-specific antibodies and Stxs with GSLs.
Materials and Methods: Liposomes were prepared in a multi-step process with varying content of phospholipids, cholesterol and Stx receptors Gb3Cer or Gb4Cer. Lipid mixtures were employed for the production of multilamellar vesicles, which were sized to small unilamellar vesicles (SUVs) by extrusion. Liposomes were captured onto 11-mercaptoundecanoic acid self-assembled monolayers (SAMs) in the presence of divalent cations. The formation of stable artificial membranes with lipid domains has been finally probed byatomicforcemicroscopy (AFM) under fluid conditions. Real time interaction measurements were performed with a surface acoustic wave (SAW) biosensor (sam5, SAW Instruments).
Results: In this study we experimentally addressed label-free Stx receptor analysis using biosensor-based lipid bilayers with Stx GSL-receptors embedded in a specific lipid microenvironment. Repeated extrusion of lipid mixtures led to SUVs of an average size of 120 nm as revealed by dynamic light scattering experiments. The formation of stable artificial bilayer membranes on the SAW chip surface was found to be strongly influenced by the lipid composition as well as by the amount of magnesium ions used as a mediator, which induced spreading and burst of SUVs after adsorption. Preliminary data obtained by AFM-analysis suggest the formation of lipid raft like microdomains within the adsorbed artificial membranes coated chips. These optimized experimental conditions enabled to monitor association and dissociation kinetics of antibodies or Stxs to GSLs by real time interaction analysis. Data indicate binding of Gb3Cer- and Gb4Cer-specific antibodies as well as Stxs to GSL-spiked model membranes.
Conclusions: Further investigations may help to better understand receptor-mediated interaction of Stx with human and animal target cells aimed at the development of novel therapeutic measures to combat life-threatening EHEC-infections.

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ZOP10
The Role of EHEC Autotransporters in Biofilm Formation

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Enterohemorrhagic *Escherichia coli* (EHEC) can colonize the human intestine after ingestion of contaminated food products. During an infection, EHEC expresses several virulence factors that may result in severe clinical outcomes such as hemorrhagic colitis (HC) and the hemolytic-uremic syndrome (HUS). Previous studies demonstrated that autotransporters can significantly contribute to the virulence of Gram-negative pathogens[1].
Bacterial populations which are attached to a surface and enclosed in a self-produced polymeric matrix are known as biofilms. Biofilm formation is known to result in enhanced resistance to antibiotics and host immune defenses and is therefore contributing to persistence of bacterial infections.
In this study, we analyzed the capability of clinical isolates of HUS-associated enterohemorrhagic *E. coli* (HUSEC)[2] strains to form biofilms *in vitro*. Out of 42 HUSEC strains, 10 strains produced biofilms under nutrient-poor environmental conditions. In addition, we investigated the expression of the autotransporter **C**alcium-binding **a**ntigen 43 **h**omologue (Cah) and EHEC autotransporter A (EhaA) via Western Blot. Both proteins belong to the group of self-associating autotransporters (SAATs)[3]. Members of this class of proteins were shown to contribute to the formation of bacterial aggregates and biofilms. 81 % of HUSEC encode for the *ehaA* gene and 31 % for the *cah* gene but not all *ehaA* or *cah* positive EHEC strains do actually express these proteins.
Further studies will help to elucidate the roles of expressed EHEC autotransporters in formation of biofilms. Additional analysis of function and regulation of *ehaA* and *cah* will clarify if certain alleles of these genes are in fact important EHEC virulence factors.

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ZOP11
Lysogenic conversion of *Staphylococcus aureus* ST398 strains by Panton-Valentine leukocidin (PVL) encoding phages

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Methicillin-resistant *Staphylococcus* (*S.*) *aureus* (MRSA) are significant human pathogens. Livestock are often carriers of MRSA, in particular of the sequence type ST398 which is found worldwide along the food chain. Persons with direct contact to animals have high risk of colonization and possible subsequent infection with these livestock-associated MRSA (LA-MRSA). Strains of ST398 usually lack most of the known virulence genes. However, they are often multi-resistant to a variety of antibiotics. Many virulence factors of *S. aureus*, such as Panton-Valentine leukocidin (PVL) and enterotoxin A are encoded by temperate phages. These toxin genes can be spread in bacterial populations by phages released from toxigenic bacteria that may infect other strains. Therefore, the question arises whether *S. aureus* ST398 can acquire new virulence genes by phage mediated transfer (lysogenic conversion).
In the course of this study, two PVL encoding phages (P240 and P1105) were isolated from MRSA strains of human origin and further characterized. Both phages belong to the family *Siphoviridae*, but differ by their morphology (P240 has an elongated, P1105 a hexagonal head). A total of 26 ST398 strains of different *spa* types were lysed by the two phages. 81 ST398 strains were selected for lysogenization experiments, of which several strains were lysogenized by the phage. Moreover, upon induction with mitomycin C, the phages were released again from their bacterial host. For molecular analysis, both phage genomes were sequenced. The obtained sequences revealed a close relationship of P240 to other known PVL encoding phages. In contrast, phage P1105 harbours both *pvl* genes and a gene for enterotoxin A. This combination of virulence genes has so far only been described for a phage identified in an Indian isolate.
In this study it could be shown that a phage mediated transfer of toxin genes into *S. aureus* ST398 strains is possible.

ZOP12
A novel approach in detection and structural characterization of Shiga toxin receptors in crude lipid extracts of toxin-sensitive cells

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Introduction: Shiga toxins (Stxs) are the major virulence factors released by certain pathogenic Stx-producing *Escherichia coli* (STEC), notably by enterohemorrhagic *E. coli* (EHEC), which are implicated in life-threatening complications such as the potentially fatal hemolytic-uremic syndrome. The glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer/CD77) and globotetraosylceramide (Gb4Cer) act as high- and low-affinity receptors for Stxs, respectively [1]. Stx GSL receptors are minor constituents of the plasma membrane and, hence, are hardly detectable in crude lipid extracts of Stx-sensitive cells without laborious purification steps. Here we developed a straightforward approach for detection and structural characterization of Stx GSL receptors that is based on pretreatment of crude cellular lipid extracts with phospholipase C (PLC) for enzymatic removal of phospholipids.
Materials and Methods: Gb3Cer- and Gb4Cer-harboring toxin-sensitive human monocytic THP-1 cells [2, 3] were used as a model cell line. Extraction of total lipids, subsequent separation of Stx GSL receptors by thin-layer chromatography (TLC), and detection with anti-GSL specific antibodies were followed by structural elucidation of different Stx GSL lipoforms using direct infrared matrix-assisted laser desorption/ionization mass spectrometry (IR-MALDI MS) [4].
Results: Our novel approach includes the following steps: (1) extraction of total lipids, (2) disintegration of phospholipids by pretreatment with PLC, (3) separation of Stx GSL receptors by TLC and immunodetection with specific anti-GSL antibodies, and (4) structural characterization of Stx GSL receptors on the TLC plate implementing IR-MALDI MS. We demonstrate here that the results of mass spectrometry analysis of Stx GSL receptors in PLC-pretreated crude lipid extracts of THP-1 cells are comparable concerning sensitivity and accuracy to those obtained using highly purified reference GSL preparations [4]. In addition, we show feasibility of simultaneous detection of both Gb3Cer and Gb4Cer on one and the same TLC plate using a mix of anti-GSL specific antibodies combined with subsequent direct IR-MALDI-MS analysis. Collectively, sample enzyme

pretreatment resulted in enormously enhanced detection efficiency of GSLs and allowed for their structural assignment by mass spectrometry.
Conclusion: Our novel technique is suitable for rapid small-scale sample preparation and structural characterization of Stx GSL receptors without labor-intensive purification steps.

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ZOP13
Parallel detection of six zoonotic pathogens using on-chip PCR assays

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Introduction: In recent years, there has been a trend to transform laboratory methods into miniaturized scale, especially those that are used for routine diagnosis in veterinary and human medicine. Various miniaturized approaches have already been published for PCR assays. Major advantaged of such lab-on-a-chip systems are compact design enabling portability of the systems and reduced reagent requirement. It is usually necessary to optimize the time/temperature profile of the PCR assays to perform parallel detection of several pathogens in a microfluidic chip. This study provides data on stationary and continuous flow on-chip PCR assays for the detection of six highly pathogenic zoonotic bacteria species that are relevant in veterinary and human medicine and play a major role as biological warfare agents. The aim of the study was to establish and optimize parallel detection of six pathogens for two different microfluidic chip designs.
Materials and Methods: Chips for stationary on-chip PCR include cavities to be filled with PCR mixture. Temperature management was carried out similar to standard thermocycler instruments. Continuous flow PCR-chips include meandering microchannels, where liquid is guided over two adjustable heating zones with constant temperature on an instrument. Initial DNA concentrations between 10^6 GE/reaction and 10^4 GE/reaction were used. In addition to purified template-DNA, also bacterial powder prepared from suspensions by vacuum centrifugation was tested without need for sample preparation. All on-chip PCR experiments were compared with standard laboratory thermocyclers.
Results: All PCR assays could be performed successfully with both on-chip PCR methods allowing for detection of all selected species. Similar protocol settings for all selected assays were established with 45 PCR cycles of 15 s at 95 °C and 45 s at 60 °C for stationary on-chip PCR and a profile consisting of 20 s at 95 °C and 60 s at 60 °c for continuous flow PCR. For some assays the stationary chip achieved considerably better results than flow PCR-chips. Direct detection of spore containing powder samples was successful for both chip technologies.
Conclusion: On-chip PCR is a useful tool for specific detection of zoonotic bacteria, although for some applications further optimization might be needed to improve the detection limit. The results can help to design a more complex chip allowing for fast analysis of one sample with different assays in parallel. As they are used in the study, both PCR-chip technologies are well suitable for species identification direct from “white powder” samples.

ZOP14
The Distinct Roles of Matrixmetalloproteinases-2 and -9 in Mediating Murine *Campylobacter Jejuni* Induced Enterocolitis

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Introduction: Matrixmetalloproteinases (MMPs) comprize a tighly controlled heterogenous family of matrix-degrading endopeptidases which are physiologically involved in tissue development, differentiation, proliferation and regeneration. A dysbalance between activators and inhibitors of MMP expression, however, results in diseases such as arthritis, atherosclerosis, or cancer. Furthermore, the gelatinases A and B (MMP-2- and MMP-9, respectively) are upregulated in human inflammatory bowel diseases. In this study we for the first time investigated the impact of gelatinases A and B in experimental *Campylobacter (C.) jejuni* induced enteritis.
Methodology and Principal Findings: Given that conventional mice display a physiological colonization resistance against *C. jejuni* due to their intestinal microbiota composition, we generated gnotobiotic MMP-2-/- and MMP-9-/- mice following broad-spectrum antibiotic treatment to assure stable colonization upon peroral infection with *C. jejuni* strain 81-176. *C. jejuni* colonized the intestines of gnotobiotic mice irrespective of the genotype at comparable loads. *C. jejuni* infected MMP-2-/-, but not MMP-9-/- mice displayed lower colonic apoptotic cell numbers at day 14 post infection (p.i.). Both, MMP-2-/- and MMP-9-/- infected mice, however, exhibited less distinct pro-inflammatory immune responses in the colon as compared to wildtype controls as indicated by lower numbers of colonic T lymphocytes, macrophages and neutrophils and concomitant lower IL-1b and TNF-a mRNA expression levels. Furthermore, gnotobiotic IL-10-/- mice suffering from severe *C. jejuni*-induced enterocolitis within one week p.i. were perorally treated with the selective gelatinase inhibitor RO28-2653 from d1 until d6 p.i. (75 mg/kg body weight/day, once daily). Remarkably, RO28-2653 treatment of *C. jejuni* infected gnotobiotic IL-10-/- mice ameliorated ulcerative enterocolitis as indicated by significantly less clinical pathology (i.e. bloody diarrhea), lower numbers of Casp3+ apoptotic cells, but higher number of Ki67+ proliferating cells in the colonic mucosa at day 7 p.i. as compared to placebo control mice. Furthermore RO28-2653 treatment resulted in less distinct influx of T and B cells as well as macrophages into the colonic mucosa of *C. jejuni* infected mice as compared to placebo treated controls. Taken together, MMP-2 and MMP-9 are both essentially involved in *C. jejuni* induced immunopathology.
Conclusion and Significance: Selective gelatinase blockage might be a promising treatment option of (e.g. *C. jejuni* induced) intestinal inflammation in humans.

ZOP15
Seroprevalence of *Coxiella burnetii* among forest workers

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Introduction: Q fever is a widespread zoonosis caused by the obligate intracellular and highly pathogenic bacterium *Coxiella burnetii* (*C. burnetii*). Up to now little is known about the prevalence of this infectious disease. Therefore a study was performed in a population potentially being at high risk for getting Q fever: a group of forest workers as well as a control group was analysed for antibodies against *C. burnetii*. Furthermore possible risk factors were determined..
Materials and Methods: A cross-sectional study with a total of 605 participants (forest workers and control group) of the North Rhine-Westphalian “Landesbetrieb Wald und Holz” was conducted. Risk factors were ascertained by a specific questionnaire. *C. burnetii* specific tests were done: first we screened the sera for IgM and IgG antibodies against phase II antigen using an Enzyme Linked Immunosorbent Assay (ELISA). Positive samples were confirmed by indirect immunofluorescence test (IFT) to determine exact antibody titers.
Results: Altogether 605 sera were tested whereof 33 showed specific antibodies against *C. burnetii* (5,5%). Looking at the group of forest workers we could show that 6,0% showed positive serology. In comparison only 4,2% in the control group (no forest workers) showed positivity for antibodies against *C. burnetii*. Specific risk factors for Q fever were identified by a questionnaire.
Discussion and Conclusion: To conclude: in this study we could show that the prevalence for *C. burnetii* in forest workers was higher than in the control group. For Q fever the results for the persons at risk (forest workers) confirmed the expectations while it was surprising that even the control group presents a relative high prevalence. In the future the risk factors have to be analysed in more detail. So far we have to point out that knowledge on the distribution of Q fever in Germany is still poor. Further investigations are necessary to get a more detailed insight into this infectious disease. Strategies and methods have to be improved to support the public health system to detect possible outbreaks and to protect the population from this illness.

ZOP16

Ticks (Acari: Ixodidae) of Livestock and Their Seasonal Activities, Meshkin-Shahr, Ardabil Province, Iran

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Introduction: Ticks as the obligate hematophagous arthropods transmit wide range of pathogens by parasitizing different groups of vertebrates across the world. Tick-borne pathogens in addition to animals encompass the human and lead to over 100,000 of clinical cases in the world annually. Several studies have been conducted in order to determine the tick fauna in different regions of Iran, their seasonal activity, host distribution, infestation rates and their infection with pathogens. Ardabil Province has been located between two different climates, wet-forest climate (Caspian sea region, Guilan Province) and subtropical-mountainous areas (Azarbaijan region), the identification of the ticks and their host in these regions is of considerable importance.
Materials and Methods: More than 10% of the 220 villages of Meshkin-Shahr County were selected randomly from different areas of the county. The sample collections were conducted from May 2012 – May 2013. The animal dwellings were visited and the whole body of sheep, cows, goats and dogs were examined for their probable infestation. The collected samples were counted and transferred to the laboratory in labeled glass vials. Samples were identified at the level of species according to the standard morphological key.
Results: In this study totally 569 host including 40 cows, 450 sheep, 70 goats and 9 dogs were examined for infestation and among them 255 were infested which showed a 44% of infestation among examined livestock. Totally, nine species (*Dermacentor marginatus, Dermacentor niveus, Haemaphysalis erinacei*, *Haemaphysalis punctata, Hyalomma anatolicum*, *Hyalomma asiaticum*, *Hyalomma marginatum, Rhipicephalus bursa* and *Rhipicephalus sanguineous*) were identified in this study analyzing the species composition showed that the most diversity has been observed among the genus *Hyalomma* by 3 species. Two species have been identified from the other three genera (*Rhipicephalus*, *Dermacentor* and *Haemaphysalis*).
Discussion: The studied region could be assumed as a representative for the western-north part of Iran according to the geographical, cultural and socio-economic properties. The results of this study and other studies of the region showed the probability of the establishment and development of the burden of several tick-borne diseases. Some of the tick-borne diseases have been studied but more studies are needed especially for determination more details of the relations between different cycles of the diseases.

ZOP17

Identification of the anti-apoptotic region of the *Coxiella burnetii* effector AnkG

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The obligate intracellular bacterium *Coxiella burnetii* is the causative agent of the zoonotic disease Q-fever. Q-fever is often a mild flu-like illness, but can develop into an atypical pneumonia or hepatitis. Furthermore, the infection can lead to chronic infection which is typically characterized by bacterial endocarditis and is potentially fatal. *C. burnetii* pathogenesis depends on a functional type IV secretion system (T4SS), used to translocate bacterial proteins into the host cell in order to manipulate host cell pathways. To date over 100 effector proteins have been identified, however their functions mainly remains elusive. We have demonstrated that the T4SS effector AnkG inhibits host cell apoptosis and it is believed that this activity is essential for the establishment of a persistent infection. However, the mode of action of AnkG is still not fully understood.
Here, we compared the activity of AnkG encoded by different *C. burnetii* strains. The differences between the AnkGs from *C. burnetii* Nine Mile, Dugway and G isolate are amino acid exchanges at position 11 and 72. Although there are only two amino acids exchanged, we observed a difference in anti-apoptotic activity. Thus, *C. burnetii* Dugway displayed a significantly increased anti-apoptotic activity compared to *C. burnetii* Nine Mile. *C. burnetii* Dugway contains an Isoleucine at aa 11 whereas *C. burnetii* Nine Mile contains an Leucine at this position. To investigate the role of amino acid 11 in inhibition of apoptosis, we constructed different AnkG mutants exchanging Isoleucine to either Glutamic Acid ($AnkG_{I11E}$) or to Threonine ($AnkG_{I11T}$). Our results demonstrate that neither the expression of GFP-$AnkG_{I11E}$ nor the expression of GFP-$AnkG_{I11T}$ interferes with staurosporine-induced apoptosis, while the expression of GFP-AnkG and -$AnkG_{I11L}$ inhibited staurosporine-induced apoptosis. Thus, AnkG requires the amino acids isoleucine or leucine, but not glutamic acid or threonine at position 11 for anti-apoptotic activity. This indicates that the region around amino acid 11 is the active anti-apoptotic domain of AnkG, which fits with previous observations that the first 69 amino acid of AnkG are necessary and sufficient for anti-apoptotic activity. Furthermore, our data suggests that not the amino acid sequence but rather the polarity of the amino acid at position 11 is important for the anti-apoptotic function of this *C. burnetii* effector protein.

ZOP18

Establishment of chicken enterocyte cell lines as primary infection target for Salmonella and Campylobacter infection studies

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The two most important zoonotic pathogens causing gastroenteritis are Campylobacter- and Salmonella spec. provoking together about 24% of food-borne diseases in the US. [1] The primary infection target is represented by the avian (chicken) enterocyte. It seems reasonable that by reduction of the pathogen load in chicken, the main source of infection, the risk for consumers to develop illness could be reduced [2]. To come away from time and money consuming field studies we aimed to implement an *in vitro* testing platform enabling the interference in the zoonotic infection process. Hereby cells were isolated from the intestine of embryonic chicken derived from pathogen free eggs. Cells were immortalized with a combination of genetic transformation and growth factor dependent propagation. Upon sophisticated cloning steps 224 immortal clones of the embryonic chicken could be established representing at least 6 different morphotypes. In further steps these cell lines have than been characterized for their chicken origin and epithelial properties.
An assay for infections studies with the zoonotic pathogens Salmonella and Campylobacter based on this newly developed cell lines was established. This system might represent a powerful tool to identify infection inhibitors *in vitro* and therefore could be used to reduce animal trials in poultry feed additive research. Furthermore, this assay might be extended for other pathogens.

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ZOP19

Anthrax in human and animal in Bangladesh: A risk assessment study

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Introduction: Anthrax is an emerging zoonotic disease in Bangladesh. About twelve outbreaks of anthrax occurred in Bangladesh. Due to peri-domestic nature of human and large number of population over a small land the chance of transmission of anthrax from animal source to human is high. However the present study was undertaken to assess the risk involved in transmission and occurrence of this disease.
Materials and Methods: The study was conducted in 5 district of Bangladesh where anthrax prevalence is high. The areas were investigated and samples were collected from soil, animal and human in order to explore the transmission dynamics of anthrax. The effects of seasons, climate, vaccination, habitant, grazing pattern, localities, health and hygienic aspect on prevalence of anthrax were also investigated.
Results: The study revealed that unhygienic condition, lack of proper knowledge on abattoir management, flood and climate change, schedule vaccination, pasture management, little or no veterinary public health service and few wild animals and birds are responsible for occurrence and outbreak of anthrax in animal and human.

Conclusion and Discussion: The study assessed the risk for anthrax in Bangladesh which provides inevitable help in taking necessary steps to prevent and control of anthrax in Bangladesh.

ZOP20

A survey of Crimean Congo Hemorrhagic Fever (CCHF) virus genome using RT-PCR approach among Ixodid ticks collected from the area of Khuzestan province

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Introduction : Crimean- Congo Hemorrhagic Fever (CCHF), also known as Asian Ebola, is a viral zoonotic tick-born disease that is asymptomatic in infected animal, but a serious threat to humans. The causative agent of CCHF is the virus of genus *Nairovirus* and family *Bunyaviridae*. Numerous genera of ticks serve both as vector and reservoir for CCHF virus. CCHFV is transmitted to humans by the bite of infected tick and by direct contact with blood or tissue from infected humans and livestock. In addition to zoonotic transmission, CCHFV can be spread from person to person and is one of the rare hemorrhagic fever viruses able to cause nosocomial outbreaks in hospitals. The disease is one of the most widely distributed viral hemorrhagic fevers occurring in Africa, the Middle East, Asia, and some parts of Europe. The history of CCHF in Iran shows that the disease has been detected in Iran since 1970.
Materials and Methods: resident ticks in the livestock have been collected from different regions of the province, and identified with local identification key. Finally, RT-PCR has been used for detection of the CCHFV genome.
Results: A total of 102 hard ticks were collected. The genome of CCHFV was detected in 6.8% of ticks resident in livestock involved in this survey. The infected species belonged to *Rh.sanguineus, Hy.sp,* and *Hy.marginatum*. *Rh.sanguineus* was the predominant tick species and accounted for 86.2% of the ticks. The ratio of female was more than male ticks.
Conclusion: Although CCHF is the most common infection in Iran, no comprehensive data is so far available on the CCHF in the province of Khuzestan in southwest in Iran. This study investigates CCHF in this region, and confirms the circulation of the virus in this region. so people in close contact with livestock and health care workers should be alarmed.

ZOP21

Identification of Risk factors associated with MRSA infections in companion animals

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Infections with methicillin-resistant *S. aureus* (MRSA) are a burden in both, the field of human and veterinary medicine. Especially companion animals such as dogs, cats and horses suffered increasingly from MRSA infections within recent years. Since MRSA were frequently associated with a multidrug-resistant phenotype, these infections led to serious difficulties with respect to antibiotic treatment possibilities. To implement targeted intervention strategies for limiting the number of MRSA infections, it is crucial to gain more knowledge about putative risk factors. Therefore, we conducted a case-control study including 194 companion animals with MRSA (n=100) or MSSA (n=94) infections. The isolates were obtained from 155 different veterinary practices and originated from various infected body sites.
All *S. aureus* were initially isolated from routine specimens received for diagnostic purposes and identified by use of the Vitek2 system (bioMérieux). Genotypic confirmation was carried out by detection of the *nuc* gene and the methicillin-resistance encoding *mec*A gene. Veterinarians who received an MRSA or MSSA positive result from the routine diagnostic lab were invited to take part in this study by replying to a questionnaire. Information for each case (MRSA) and control (MSSA) included parameter of the host signalment and clinical history. Furthermore, veterinarians provided information concerning their individual veterinary practice. Firstly, putative risk factors were investigated using univariable logistic regression (likelihood ratio test) including all parameters from patients and veterinary settings. All factors with $p<0.2$ were integrated in the final multivariable model and risk factors were identified using manual stepwise backward elimination (multinomial logistic regression).
Patients with antibiotic treatment prior sampling and surgical site infections were more likely to suffer from MRSA infections in comparison to MSSA infections. Further, animals that had been treated in practices or clinics with more than ten employees were at a higher risk to be infected with MRSA. These results indicate an association of MRSA infection with the size of the veterinary setting as well as with surgical site infections, underlining the importance of MRSA as nosocomial pathogen in companion animals.

ZOP22

Plasmids of EHEC O104:H4: Host range and transfer frequencies

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Introduction: The Enterohemorrhagic *Escherichia coli* strain O104:H4 (EHEC O104:H4) caused the largest recorded outbreak of EHEC related infections in Germany. This epidemic was unusual with respect to patient group (mainly adults) and severity of the disease (~23% developed hemolytic-uremic syndrome, HUS) (1). The subsequent genome analysis revealed that the unusual course of the disease is associated with an untypical gene content: EHEC O104:H4 is very closely related to the enteroaggregative *E. coli* (EAEC) strain 55989 and thus appears to be a hybrid that shares virulence factors from both *E. coli* pathotypes. Before 2011, *E. coli* O104:H4 was only once isolated from a HUS patient. This strain in the National Consulting Laboratory for HUS differs from the epidemic strain not only in chromosomal markers, but also in plasmid content (2). In order to learn more about the horizontal gene transfer events that resulted in the highly virulent 2011 EHEC O104:H4 isolate, we tested the *in vivo* stability of the ESBL (extendedspectrumbeta-lactamase) encoding plasmid pTY1 and compared host range and transfer frequencies with the broad host range plasmid RP4.
Results: We were not able to obtain pTY1 free *E. coli* clones by using classical curing methods. Both, host range and transfer frequencies of pTY1 appear to be generally limited when compared to broad host range plasmid RP4. No co-transfer of chromosomal markers by pTY1 was observed under standard laboratory conditions.
Discussion: The EHEC O104:H4 plasmid pTY1 is stable and resistant to classical curing methods. This property of the plasmid is background independent, as we failed to cure the plasmid from an *E. coli* K-12 background as well, indicating a very efficient plasmid-encoded addiction system. PTY1 is readily transferable by conjugation under standard laboratory conditions, including from and into other gram negative pathogens. Even though a conversion of *E. coli* K-12 into an *Hfr* strain was not observed under standard laboratory conditions, it will be very interesting to repeat these experiments under detrimental environmental conditions (e.g. antibiotic stress). Co-transfer of chromosomal markers by a conjugative plasmid could constitute an alternative and eventually more efficient mechanism to introduce new virulence genes into pathogens, including *stx* encoding prophages.

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ZOP23
Molecular detection of Crimean-Congo hemorrhagic fever virus on hard ticks (Ixodidea) collected from Tarom/Zanjan Province of Iran

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Introduction: Crimaen congo hemorrhagic fever is a zoonotic viral disease with a high mortality rate in humans .The disease is caused by Crimean-congo hemorrhagic fever virus(CCHFV).This virus is transmitted through the bite of an infected tick,or by direct contact with infected patient or the products of infected live stock.This viral disease is asymptomatic in infected live stock,but cause Serious threat to human.Among animals,Sheep as the most important vertebrate hosts for CCHFV.This virus belongs to the Family *Bunyaviridea* and the genus *Nairovirus.*The most important reserviors of CCHFV are ticks and the virus has been isolated from at least 31 different tick species.Since 2000 data has shown the disease to be prevalent in 23 out of 30 provinces of Iran.
Materials and Methods: Ticks was separated from the body of animals and transported to laboratory of medical entomology and were identified with local identification key.In this study,RT-PCR has been used for detection of the CCHF virus genome.
Results: This study was conducted to determine the infection rate of CCHF virus in thicks in Tarom,in the province of Zanjan.
Among the whole ticks collected from different region in Tarom a total of 100 ticks were selected and they were investigated for detection of CCHFV using RT-PCR method .The virus was found in 7 % of ticks.The infected species belonged to *Hyalomma dromedarii*, *H.marginatum* ,*H.asiaticum*, *Rhipicephalus bursa* and *Rh.sanguineus.*
Conclusions: Due to presence of virus in different region, We recommend the use of acaricides and repellent to prevent disease transmission among humans. Greta care should be taken by the people who are working in slaughter houses. Vector control programs should be applied for reducing population density of potential tick vectors in this county.

INVITED SPEAKERS

INV01
"VAAM Forschungspreis"
Antibacterial strategies and bacterial defenses

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Throughout evolution bacteria have been confronted with antibiotics. Microbes themselves are arguably the most ancient source, but with the emergence of higher organisms antimicrobial molecules have also played a role in host-microbe interactions. In Earth's more recent history, mankind discovered these antibiotics. The industrial production on the scale of tons saved many lives but also led to the large-scale anthropogenic discharge of antibiotics into the environment. Since bacteria have struggled to live with antibiotics for billions of years, the microbial world is adapting fast to the present-day ubiquity of antibacterial agents - with far-reaching consequences for human society. In the face of multi-resistant pathogens it is high time to revive development of effective antibacterial strategies.
Among the novel antibacterial strategies studied in our lab are natural compounds, small molecules, and antimicrobial peptides. Investigating how these exert their antibacterial activity, we aim to identify clinically unexploited targets or chemical entities that hit clinically proven targets but circumvent existing resistance mechanisms. At the same time we are interested in elucidating intrinsic bacterial defense mechanisms that might get in the way of a clinical application. To illustrate our system-based approach to studying antibiotic action and defense mechanisms [1], recent works on a very small cationic antimicrobial peptide will be highlighted [2].
Complementing the long-established antibiotic approach, we investigate technical plasmas - ionized gases - as a further antibacterial strategy [3]. The utility of plasmas for sterilization processes is obvious and implemented in industrial processes. What's new is that first clinical trials with cold atmospheric-pressure plasmas have shown plasma treatment to be beneficial to wound healing by wound disinfection and simultaneous stimulation of eukaryotic cell proliferation. By characterizing the effects of plasma components and synergistic effects between them, we aim to shed light on the molecular mechanisms underlying disinfection [4].
Bacteria are true survivalists and any advantage we gained in the fight against bacterial pathogens by using antibiotics seems to be of temporary nature. However, antibiotic strategies have been successful through billions of years of bacterial evolution and we hope to learn from the 'expert antibiotic users', the microbes themselves, about how to utilize these precious resources more effectively and sustainably.

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INV02
Pathogenicity mechanisms of *Candida albicans* and *Aspergillus fumigatus*

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The estimated number of fungal species world wide is between 3 and 5 million, yet only a few species are known to cause disease in humans. However, these fungi infect billions of people every year and kill at least as many people as tuberculosis or malaria. Surprisingly, fungi still remain largely under-appreciated as pathogens of humans (1). The yeast *Candida albicans* and the filamentous fungus *Aspergillus fumigatus* are by far the most important "hidden killers".
C. albicans can be both, a harmless commensal of mucosal surfaces in healthy individuals and an aggressive pathogenic yeast in susceptible hosts. In the commensal phase, the fungus attaches to host surfaces, co-exists with the microbiota, acquires nutrients accessible on mucosal surfaces, adapts to local conditions and replicates without causing inflammation. These conditions change dramatically during the transition to a pathogenic growth style. The transition includes invasion into and damage of epithelial cells and requires nutrient acquisition directly from host cells or molecules. Invasion and damage in turn cause inflammation and recruitment of phagocytic cells. To counteract killing by phagocytes, *C. albicans* has developed distinct survival strategies. These pathogenicity mechanisms enable *C. albicans* not only to cause superficial infections in millions of people every year, but also to cause life-threatening nosocomial systemic infections in immunocompromised patients.
The filamentous fungus *A. fumigatus* is the most important air-borne fungal pathogen causing 90 % of all systemic *Aspergillus* infections. Conidia of this saprobic fungus can be isolated nearly everywhere. The most severe disease caused by *A. fumigatus* is invasive aspergillosis (IA), which occurs almost exclusively in immunocompromised patients. A lack of reliable diagnostic tools and effective treatment options, results in high mortality rates (between 30 and 90 %) despite therapy . Because of their abundance in air, several hundred *A. fumigatus* conidia are inhaled daily. In immunosuppressed patients, the lung is the primary site of infection. In immunocompetent individuals, phagocytic cells normally prevent the disease. However, there is a correlation between the degree of immunosuppression and the risk for IA. Consequently, important risk factors include neutropenia, T cell depletion, CD34-selected stem cell products, corticosteroid therapy, and cytomegalovirus infections.
Despite the different pathogenesis of infections caused by *C. albicans* and *A. fumigatus*, there are several common traits when the host response is considered. (i) The pathogens must be able to overcome epithelial barriers. (ii) Both fungi possess physiological characteristics and virulence determinants and capabilities for immune evasion. (iii) Both diseases, *i.e.*, invasive candidiasis and IA, are mainly found in patients with an impaired immune system either due to weakened activities of immune effector cells or defects in epithelial barriers. (iv) For both pathogens, innate immunity represents the major defence system.
Currently we aim at identifying and characterising both, *A. fumigatus*- and *C. albicans*-specific pathogenic determinants and common principles of fungal pathogenesis. For example, we identified fungal-specific zinc-acquisition systems and a *Candida*-specific factor with virulence and avirulence properties (2, 3) and investigated the role of the secondary metabolites for infection and *Aspergillus*-specific adhesion and immune evasion-strategies (4, 5).

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INV03

Keeping membrane lipids in balance: deciphering the metabolic flux of fatty acids in yeast

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Introduction: How does a cell regulate the content of its subcellular membranes? Fatty acids are the major structural constituents of membrane forming phospholipids but they also serve essential functions as energy substrates in the form of triacylglycerols or as signaling molecules. Phospholipids and triacylglycerols share the same precursors, i.e. phosphatidic acid and diacylglycerol, which thus requires a tight regulation of the partitioning of fatty acids between phospholipid synthesis in growing cells or their deposition into storage lipids, in preparation for starvation when nutritional supply ceases. Major metabolic diseases such as obesity and diabetes type II are believed to result from fatty acid induced lipotoxic effects due to deregulation of their metabolic partitioning.
Materials and Methods: Using yeast as an experimental system we apply a wide spectrum of biochemical, genetic and molecular biological tools to understand the metabolic flux of fatty acids at the cellular level.
Results: Dys-regulated de novo synthesis of fatty acids triggers a mechanism that redirects excess fatty acids exclusively into storage lipids and retains phospholipid levels unaltered, despite changes in their acyl-chain composition. Degradation of triacylglycerols, on the other hand, plays an important role to provide lipid precursors to drive cell cycle progression.
Conclusion and Discussion: Cell growth and division relies on a finely tuned balance between synthesis of fatty acids and their channeling into either phospholipids or triacylglycerols. Cells are able to tolerate fatty acid overload only if triacylglycerol synthesis is intact, underscoring their essential cellular and physiological function.

INV04

Diversity and regulation of amino acid transporters in Saccharomyces cerevisiae

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Yeast Saccharomyces cerevisiae, the model eukaryotic cell, possesses about 20 distinct amino acid permeases present at the plasma membrane. The intracellular compartments of yeast cells including the vacuole and mitochondria contain a wide variety of amino acid permeases as well. These yeast membrane transporters provide powerful systems to explore the molecular mechanisms governing the regulation of membrane transporters in general. These control mechanisms act at gene transcription, protein intracellular traffic and intrinsic activity levels. Ubiquitin emerged in 1995 as a key molecule controlling the intracellular traffic of yeast permeases, a concept extrapolated to other categories of membrane proteins in many species including human. During evolution, specific amino acid permeases lost their transport activity and evolved into sensors of external amino acids. Many questions about the role, tridimensional structure, lateral distribution in the membrane, regulation and potential sensing function of amino acid permeases are at the center of many ongoing studies and offer exciting perspectives for the future.

INV05

Structural studies of autotransport and immune evasion in gram-negative bacteria

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A key feature of all pathogenic micro-organisms is the ability to evade the alternative pathway of complement, as this system is always active and, indeed, is the only defence mechanism available in a naïve host. They typically do this by recruiting various host molecules, including the complement regulator factor H, vitronectin, as well as binding immunoglobulins in a non-immune fashion.

In recent work, we have studied how the Lyme borreliosis pathogen *Borrelia burgdorferi* and other microbes evade complement. We showed that they all recruit factor H via its C-terminal domain (FH20) in a manner analogous to, but not identical with, how glycosylaminoglycans bind FH20, thus providing the structural connection between disparate diseases, from atypical haemolytic uremic syndrome to borreliosis. The mode leads to FH binding C3b and down-regulation of complement activation. We have solved the structure of the key *Borrelia burgdorferi* regulatory protein, outer surface protein E (OspE) both alone and complexed to FH19-20.

We have also shown how chimeric trimeric autotransporters adhesins (TAAs) require a uniform barrel domain to pass through the membrane, but can use divergent passenger domains. This indicates that the signal for transport is contained within the barrel, and suggests that the function of the BAM complex may be insertion of the barrel, not transport of the passenger domain. Finally, we have also crystallised a complex of the TAA *E. coli* immunoglobulin binding protein D with the Fc part of immunoglobulin G, as there are no structures of trimeric autotransporters adhesins with their cognate ligands. I will present our most recent data on this as well.

INV06

Modulation of host cell functions by bartonella type IV secretion effectors

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Abstract has not been submitted.

INV07

Type IV secretion systems in *Helicobacter pylori*

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Introduction: *Helicobacter pylori* represents a highly successful bacterial pathogen infecting humans worldwide. Stomach infection by *H. pylori* occurs usually in childhood and can induce gastric pathologies, including chronic gastritis, peptic ulceration, MALT lymphoma or even gastric adenocarcinoma. Certain *H. pylori* strains have the ability to inject a special protein, the bacterial oncoprotein CagA, directly into different target cells, such as epithelial cells or leukocytes. CagA is encoded by the cytotoxin-associated gene (*cag*) pathogenicity island, which a type IV secretion system (*cag*-T4SS).
Materials & methods: We have applied a set of different methods to study the structure and function of the *cag*-T4SS of *H. pylori* in detail. These include genetic methods, such as the specific knockout of all *cag*-PAI genes individually, protein-protein interaction studies by yeast–two-hybrid and protein pulldown assays, as well as confocal laser scanning and electron-microscopy studies.
Results: The *cag*-T4SS forms a pilus-like structure on the bacterial surface. The complex secretion machinery consists of about 30 proteins, some of which, including CagI, CagL, CagY and CagA, are surface located and bind the host cell receptor β1 integrin, which is an essential step for the delivery of CagA across the host cell membrane. CagA becomes phosphorylated by tyrosine kinases of the Src and Abl families and interferes with the

activation or inactivation of multiple intracellular signalling pathways. CagA-dependent signalling capabilities of the T4SS include the induction of membrane dynamics, disruption of cell-cell junctions and actin-cytoskeletal rearrangements, as well as pro-inflammatory, cell cycle-related and anti-apoptotic transcriptional responses. Recently, the crystal structure of some *cag*-T4SS proteins has been reported. The *cag*-T4SS is one of four different T4SSs that may be present in *H. pylori*.

Conclusion/Discussion: The binding of the *cag*-T4SS to β1 Integrin receptor changed our view about the mechanism of the *cag*-T4SS as a genuine needle–like injection apparatus. Besides β1 integrin we have recently identified an additional cell surface receptor, also necessary for the function of the *cag*-T4SS. Thus, we are still far away from understanding the function of this complex secretion machinery in detail.

INV08

Biological diversity of bacterial type IV secretion systems

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Introduction: Type IV secretion systems (T4SSs) are widely-distributed among many Gram-negative and -positive bacterial species. T4SSs are composed of a conjugation channel and, in Gram-negative bacteria, a conjugative pilus whose function is to initiate donor-recipient cell contacts. In Gram-positive bacteria, T4SSs do not elaborate pili and the mechanism for establishment of mating pairs is not known. This talk will compare T4SS architectures of Gram-negative and –positive bacteria, with emphasis on the distinct surface features required for establishment of mating junctions. In Enterococcus faecalis, pCF10 encodes a highly efficient conjugation system upon pheromone induction of the prgQ transfer operon. This operon codes for three cell-wall-anchored proteins - PrgA, PrgB (aggregation substance), and PrgC - and a T4SS through which the plasmid is delivered to recipient cells. Here, we defined contributions of the surface proteins to plasmid transfer, biofilm formation and virulence using the Caenorhobditis elegans infection model.

Results: Deletions of prgB or of the entire prgA-C cassette diminished plasmid transfer by ~1-2 orders of magnitude, whereas deletions of prgA or prgC did not appreciably affect transfer. However, the Prg factors contributed significantly to biofilm development as shown by the capacity of pCF10-carrying cells to form robust biofilms with 4-8 h following pheromone induction compared with plasmid-free cells or cells carrying a prgA-C mutant plasmid. We further determined that PrgB-mediated aggregation requires extracellular DNA (eDNA) and that pCF10-mediated attachment to abiotic surfaces and biofilm formation requires PrgA, PrgB, and eDNA, but not PrgC. Previous work has established the importance of PrgB for infection using a mammalian model, and here we further showed that PrgA and PrgC - but not PrgB - are essential for killing of C. elegans.

Conclusion/Discussion: PrgA, PrgB, and PrgC appear to coordinate their activities, possibly as a functional complex, at the cell surface to mediate attachment to abiotic and biotic surfaces. Our findings support a model whereby pCF10 has retained the prg surface gene cassette over evolutionary time for pheromone-mediated establishment of biofilm communities. In natural settings, biofilm communities composed of plasmid-less and plasmid-bearing enterococci and other species are optimal environments for pheromone signal exchange, mating pair formation, and genetic exchange.

INV09

Metabolic engineering of yeasts to optimize the production of biofuels and other bio-based chemicals

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Industrial biotechnology will significantly contribute to the replacement of petrochemicals by biobased chemicals. New bioprocesses will be more economical as well as more ecological compared to their petrochemical counterparts. In my lab, we use different yeast species as suitable platform hosts for the production of biofuels and biochemicals and improve their properties by genetic and metabolic engineering.

We have constructed a novel industrial *Saccharomyces cerevisiae* strain (derived from Ethanol Red, Lesaffre, which is commonly used in many 1st generation ethanol plants worldwide [1]) able to ferment efficiently the pentose (xylose, arabinose) and hexose (glucose) sugars present in lignocellulosic biomass hydrolysates. The new strain exhibits superior properties also for 2nd generation cellulosic ethanol production. I will present detailed analyses of strain construction, sugar consumption and ethanol production with this strain, in different kinds of media and hydrolysates. The new strain should close the gap in the availability of yeast strains for the production of 2nd generation cellulosic ethanol.

Moreover, we have engineered a yeast strain for the production of isobutanol [2]. Isobutanol is a superior biofuel compared to ethanol. For this, we have overexpressed enzymes involved in valine degradation to isobutanol. Moreover, we have replaced the mitochondrial valine biosynthesis pathway by an optimised cytosolic pathway. The final strain produced nearly 1g/L isobutanol in an industrial background.

All pentose fermenting yeast strains prefer their natural substrate glucose over xylose and consume the two sugars sequentially in mixed sugar fermentations. This is due to the preference of the sugar uptake systems for glucose. It significantly increases fermentation times. Using an elaborate screening system we were able to engineer specific xylose transporters from *S. cerevisiae* hexose transporters [3]. These transporter versions should prove valuable for fast glucose-xylose cofermentations.

The non-conventional yeast *Pichia ciferrii* is known to secrete the sphingoid long-chain base phytosphingosine in a tetraacetylated form (TAPS). Sphingolipids are important ingredients in cosmetic applications as they play important roles in human skin. Extensive metabolic engineering of precursor availability and the sphingolipid biosynthesis pathway resulted in a final recombinant *P. ciferrii* strain producing up to 2 g/L TAPS, which should be applicable for industrial TAPS production (reviewed in [4]).

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INV10

Engineering by design: systems biology based development of microbial production strains

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Abstract has not been submitted.

INV11

Pathway engineering in *Corynebacterium glutamicum* for the production of amino acids and other value-added products

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Corynebacterium glutamicum is a facultative anaerobic, Gram-positive organism that grows on a variety of sugars, alcohols and organic acids as single or combined carbon and energy sources. The organism is generally regarded as safe (GRAS status) and is the workhorse for large scale production of amino acids, such as L-glutamate and L-lysine. The central carbon metabolism, the physiology and the regulation of relevant pathways in *C. glutamicum* have been analyzed in detail and genetic tools as well as systems biology approaches have been developed and employed. Recent studies also explored the usefulness of this organism for the production of further amino acids (e.g., L-valine) and of other commodity chemicals, such as organic acids (e.g., pyruvate, lactate, succinate, 2-ketoisovalerate), polymer precursors (e.g., diaminopentane) or biofuels (e.g., isobutanol). Attractive metabolic engineering targets in *C. glutamicum* are the central metabolic pathways for improving the respective precursor(s) supply and the enzymatic routes for broadening the substrate spectrum. Since pyruvate is a key metabolite of the central metabolism and since several biotechnologically relevant products derive from pyruvate, a set of *C. glutamicum* strains were engineered with either inactivated or attenuated pyruvate dehydrogenase complex activity in combination with alterations in the pathways leading to desired products. The growth and production abilities of these novel strains were analyzed and tailored production processes have been implemented. In addition, the substrate spectrum of *C. glutamicum* was extended to (raw) starch and based on this carbon source, L-lysine and pyruvate production processes have been established.

INV12
White Biotechnology for Black Gold: BASF-Wintershall Initiatives for Enhanced Oil Recovery

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The production of crude oil is no longer conceivable without modern and highly specialized technologies. Wintershall, a subsidiary of BASF, is Germany's largest crude oil and natural gas producer and has the strategic goal to strengthen its technology position in the field of such advanced technologies, which are also called "enhanced oil recovery" (EOR) approaches. In that context, petroleum engineers from Wintershall Holding GmbH and researchers from BASF SE's Industrial Biotechnology platform have initiated several innovation projects in the past years. The two most advanced projects will be presented:
Schizophyllan is a biopolymer with high potential for an application in polymer flooding. It is produced by the filamentous fungus *Schizophyllum commune* and shows excellent viscosifying efficiency and high tolerance towards harsh reservoir conditions such as high temperature, salinity and shear. These unique properties allow for application in reservoirs that could so far not be polymer-flooded. Moreover, as Schizophyllan is bio-based and biodegradable, it is an environmentally friendly alternative to the commonly used synthetic polymers. In years of comprehensive research, an efficient fermentation and purification process has been developed and scaled-up. Since end of 2012 the biopolymer is produced in large scale at BASF in Ludwigshafen, transported to Northern Germany and injected into the Wintershall oil field Bockstedt in a two-year-lasting field trial. First conclusions from the field trial will be discussed.
Another joint project aims at the realization of Microbial Enhanced Oil Recovery (MEOR). The goal of the project is the development of a nutrient solution that triggers growth of advantageous endogenous microbes and thus production of metabolites that can lead to enhanced oil recovery. Work packages of our research include sampling, identifying microbes in the oil samples, cultivation of various microbes, screening for advantageous metabolites (e.g. acids, gases, solvents, biosurfactants, biopolymers), identifying a suitable nutrient solution and evaluating enhanced oil recovery effects in model systems like microfluidics and sandpack columns. Recently, we obtained technical proof of concept that the developed nutrient feeding strategy indeed mobilizes additional oil in lab model systems. These data are an important step towards a field trial.

INV13
"Colonization resistance: a ménage à trois with the host?"

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The bacterial microbiota of the human large bowel is a complex ecosystem consisting of more than a hundred, mostly anaerobic species, with each microbe utilizing a different 'winning strategy' for nutrient acquisition and utilization. Intestinal inflammation is accompanied by a severe disruption the microbiota composition characterized by an expansion of facultative anaerobic Enterobacteriaceae. Recent studies show that the local inflammatory response creates a unique nutritional environment that supports a bloom of Enterobacteriaceae because these bacteria are able to utilize inflammation-derived nutrients. The elucidation of how Enterobacteriaceae alter the bacterial community structure during inflammation is beginning to provide insights into mechanisms that dictate the balance between the host and its microbiota.

INV14
Ecosystems biology: from integrated omics to control strategies for mixed microbial communities

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Introduction: Mixed microbial communities are complex and dynamic systems. Integrated omics, combining metagenomics, metatranscriptomics, metaproteomics and metabolomics, is currently gaining momentum. This approach offers the potential to provide enhanced understanding of community structure, function, evolution and dynamics *in situ* as well as offering the potential to discover novel biological functions within the framework of Eco-Systems Biology.
Materials and methods: An integrative workflow comprising wet- and dry-lab methodologies has been developed to enable systematic measurements of microbial communities over space and time, and the integration and analysis of the resulting multi-omic data. Two distinct approaches have been developed allowing the deconvolution of integrated omic data either at the population- or community-level.
Results: By resolving multi-omics data at the population-level, we have uncovered patterns which suggest that in our model microbial community (lipid accumulating microbial consortia from a biological wastewater treatment tank) the ecological dominance of a microbial generalist is linked to finely tuned resource usage as reflected in the distinctive gene expression profiles of this population. Community-wide analysis of reconstructed metabolic networks has resulted in the identification of "keystone genes", analogous to keystone species in species interaction networks, which likely play essential roles in governing community structure and function.
Conclusion/Discussion: Integrated omics will likely become the future standard for the large-scale characterization of microbial consortia within an Eco-Systems Biology framework. In particular, by integrating information from genome to metabolome, integrated omics allows deconvoluton of structure-function relationships by identifying key members and functionalities. For example, identified keystone species and/or genes likely represent driver nodes which may be exploited in view of future control strategies. However, to test emerging hypotheses and formulate predictive models which support such endeavours, an iterative discovery-driven planning approach is required. This should ultimately allow the manipulation of microbial communities and steer them towards desired outcomes.

INV15
Response of Candida albicans to the infection environment

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Abstract has not been submitted.

INV16
C-di-GMP signaling and *Escherichia coli* biofilm architecture

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Bacterial macrocolonies represent biofilms that can produce intricate three-dimensional structures. Macrocolony morphology depends on an extracellular matrix of adhesins, amyloid fibres (e.g. curli) and exopolysaccharides (e.g. cellulose). In *E. coli*, synthesis of curli fibres and cellulose is under control of the stationary phase sigma factor RpoS and the second messenger c-di-GMP. The latter is produced by diguanylate cyclases (DGC, with GGDEF domains) and is degraded by specific phosphodiesterases (PDE, with EAL or HD-GYP domains). Many bacterial species possess multiple GGDEF/EAL domain proteins (29 in *E. coli* K-12). As shown by fluorescence microscopy of cryosections and scanning EM, *E. coli* macrocolony biofilms exhibit a stratified architecture that develops along gradients of nutrients. In essence, small starved cells literally encased in a nanocomposite of curli fibres and cellulose are found in the top layer of macrocolonies, whereas the outer edges and the lower layer (adjacent to the nutrient-providing agar) consist of vegetatively growing cells with entangled flagella. The intricate spatial arrangement of curli fibres and cellulose in the top layer generates the tissue-like cohesiveness and elasticity that allows the buckling up and folding of macrocolonies into their complex 3D-structure (1). The production of matrix components follows the expression of the key regulator CsgD, which is promoted by a spatially controlled regulatory network of several directly interacting DGCs, PDEs and the transcription factor MlrA (2). Massive matrix production only in the top layer as well as short-range cellular heterogeneity of matrix synthesis in the transition zone between the two strata are essential for macrocolony integrity and morphogenesis.
Overall, these studies reveal the microanatomy and physiological heterogeneity of a bacterial biofilm and relate these to the underlying regulatory networks prominently featuring RpoS and c-di-GMP.

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INV17
Physiological Genomics of Yeast Response and Resistance to Stress

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Introduction: The survival of living cells depends on their ability to sense alterations in the environment and to appropriately respond to the new stressing situations by remodeling genomic expression. In the postgenomic era, genome-wide approaches have become obligatory research tools to allow an integrative view on how cells interact with a stressing environment. After two decades of post-genomic research, *Saccharomyces cerevisiae* is a robust experimental model for an understanding, at the genome scale, of the physiological mechanisms behind yeast response and resistance to stress, with impact on the fields of Toxicogenomics and Industrial Biotechnology [1,2,4,5].
Materials and Methods: Genome-wide expression analyses, chemogenomics, bioinformatics, and molecular and cellular biology and biochemistry studies were used to unveil genome-wide adaptive response programs and resistance determinants to chemical stresses in yeast [1-5],
Results: Insights into genome-wide adaptive response programs, underlying signaling pathways and mechanisms of resistance to specific stresses were obtained, in particular to the herbicide 2,4-D (or 2,4-dichlorophenoxyacetic acid) [1,2,4] and to acetic acid, a food preservative and an inhibitory *compounds in lignocellulosic* biomass *hydrolysates* [1,2, 5]. Progress on the understanding of the physiological role and involvement in chemical stress defense of multidrug resistance transporters and their complex transcription regulatory networks was obtained, as well as on the the extrapolation of the gathered knowledge in yeast to more complex and less accessible eukaryotes [2].
Conclusion/Discussion: The impact of the knowledge gathered through the exploitation of a Physiological Genomics strategy is highlighted and discussed.

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INV18
Molecular Mechanisms of Fungal Pathogen - Host Interactions & Stress Response

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Abstract has not been submitted.

INV19
Do we have to face superbugs in food and food-producing animals?

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Introduction: Antimicrobial resistance is considered as one of the important public health issues. The emergence and global spread of multidrug resistant (MDR) microorganisms in humans, both in the hospital environment and in the community occurs worldwide. Human infections with these bacteria, sometimes referred as "superbugs", are very difficult to treat, and this has big consequences for mortality and morbidity and a huge economic and social impact. The question is, do we have to face "superbugs" in the food chain as well?
Material and Methods: Within the food chain, we investigate livestock and foods. To answer the question a review of the scientific literature, collection of data from different experts and revision of own data obtained from different national and international research projects (SafefoodERA-ESBLs, Med-Vet-Net, RESET, EFFORT, EMIDA-MRSA) were used. Especially focus was given to the presence and spread of zoonotic pathogens (i.e. Salmonella, Enterohaemorragic Escherichia coli, Staphylococcus aureus), or commensals (i.e. E. coli) resistant to clinically important antimicrobials as reported by the WHO.
Results: The term "superbug" can refer to different types of bacteria: bacteria that represent a threat to the general population, bacteria that represent a threat for specific groups of the population (i.e. immune depressed, elderly people, children), bacteria that have the potential to become a major health problem, or bacteria that just become "famous" due to public attention. During the last years more and more reports for some of the microorganisms mentioned above at different steps of the food chain could be found. Among them we found: carbapenemase producers in livestock animals and foods, spread of ESBLs/AmpC Enterobacteria, spread of Salmonella Kentucky ST198, and livestock associated MRSA. The transmission of these bacteria to humans can happen by consuming colonised or contaminated foods or by direct contact with the livestock.
Conclusion: Multidrug Resistant bacteria considered as "superbugs" are already present in some steps of the food chain. However, in many cases the magnitude of the problem still remains to be investigated in more detail. Globalisation allows the trade of animals, foods, and implicitly, of MDR bacteria or resistance mechanisms. Consequently ongoing initiatives to monitor and control antimicrobial resistance at this stage are necessary to avoid increasing health problems in humans and animals.

INV20
Viruses in water and food, new techniques and emerging pathogens

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Symptomatic and asymptomatic individuals excrete a wide diversity of viruses in urine or feces that are collected in urban wastewater. Even in areas with implemented sanitation programs, wastewater represents the main vehicle for the dissemination of viral pathogens through the environment. Despite regulations intended to assure food and water safety, many viral foodborne and waterborne outbreaks occur each year in developed countries. The pathogens associated with environmental transmission routes, including water and food, encompass a wide diversity of bacteria, protozoa and viruses such as Norovirus (NoV), hepatitis A virus (HAV), hepatitis E virus (HEV) and adenoviruses. Thus, the use of indicators is essential for investigating water quality, food safety and industrial microbiology. Some concerns have been stated regarding current regulations, mainly based on bacteriologic parameters. Various authors have concluded that these indicators could fail to predict the risk for water and food-borne pathogens including viruses.
Studies in our laboratory leaded to the proposal of Human adenoviruses (HAdV) as indicators of human fecal contamination and human polyomavirus (JCPyV) as a human-specific parameter, complementary to the use of standard bacterial fecal indicators for the microbiological control of water and food. Specific animal polyomaviruses and adenoviruses have also been identified: porcine adenoviruses (PAdV), bovine and ovine polyomaviruses (OPyV and BPyV) and are used as microbial source tracking (MST) tools.
Advances in concentration methods for viruses in water and molecular assays provide sensitive and quantitative analytical tools for the study of viruses in water and to assess the efficiency of virus removal in water treatment plants. Data on dissemination and stability of classical and emerging viruses will be discussed (Adenovirus, Norovirus, Hepatitis E virus, Merkel Cell Polyomavirus). The applicability of high throughput sequencing methods will be also discussed. New viruses have been recently described in urban sewage and river water. A metagenomic study has identified members of 26 different families in urban sewage being with more than the 90% of the sequences detected, potentially belonging to novel viruses that have not been described previously.

INV21
Viruses in water and food, new techniques and emerging pathogens

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Introduction: In the last 10 years, Extended-Spectrum Beta-Lactamase-producing Enterobacteria (ESBL-E) have become one of the main challenges, largely because of the current CTX-M pandemic. At the turn of the century CTX-M enzymes were hardly found and now it is found extensively all over the world. The highest prevalences of intestinal ESBL-E

carriage are reported from developing countries but also in Western Europe carriage of ESBL-E in the community is as high as 10%. The sources of ESBL-E are not entirely clear. International travel has been identified as a risk factor. Also Food Production Animals (FPA) may play a significant role in this. This study aimed to quantify the importance of retail chicken meat as a source of ESBL-E in humans.
Methods: One hundred forty-five ESBL-E isolates from retail chicken meat, human rectal carriers, and blood cultures were analyzed using multilocus sequence typing, phylotyping, ESBL genes, plasmid replicons, virulence genes, amplified fragment length polymorphism (AFLP), and pulsed-field gel electrophoresis (PFGE).
Results: Three source groups overlapped substantially when their genetic composition was compared. A combined analysis using all variables yielded the highest resolution (Wilks lambda [Λ]: 0.08). Still, a prediction model based on the combined data classified 40% of the human isolates as chicken meat isolates. AFLP and PFGE showed that the isolates from humans and chicken meat could not be segregated and identified one perfect match between humans and chicken meat. The study also provides arguments that strains originating from human infections are a non-random sample from the intestinal flora and are therefore not suitable to study the transmission of ESBL-E from food sources.
Conclusions: We found significant genetic similarities among ESBL-EC isolates from chicken meat and humans according to mobile resistance elements, virulence genes, and genomic backbone. Therefore, chicken meat is a likely contributor to the recent emergence of ESBL-E in human infections in the study region. This raises serious food safety questions regarding the abundant presence of ESBL-E in chicken meat.

INV22
Hypoxia and response to infection
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Abstract has not been submitted.

INV23
Hypoxia induction during Shigella infection
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Enteric pathogens must survive in the anaerobic environment of the large intestine, and many of them use shared mechanisms to sense lack of oxygen and to enhance their virulence (e.g. Type Three Secretion Systems, T3SSs). Shigella spp. are the leading cause of bacillary dysentery, and are able to invade non-epithelial cells in the gastrointestinal (GI) track. During the infectious process, Shigella has to resist to neutrophils bactericidal activity, as they represent the most abundant immune cell population recruited during Shigella invasion.
Exploiting in vivo models (rabbit, guinea pig) and in vitro approaches (epithelial cells culture), we have shown that Shigella infection is associated with hypoxia induction within the colonic mucosa. We observed that neutrophils recruitment and activation is the leading cause of hypoxia. Additionally, we have shown that neutrophils viability is maintained under anoxic conditions, which promote their bactericidal functions upon infiltration in infected tissues.
We characterized the adaptation of Shigella to low oxygen conditions in order to successfully colonize the colonic mucosa. We demonstrated that Shigella virulence is modulated by oxygen abundance, through the regulation of the T3SS function (Marteyn et al, 2010, Nature). We also identified a novel cell-division protein, named ZapE, which is essential for Shigella growth under low oxygen conditions (Marteyn et al, 2014, mBio).
Altogether, we demonstrate that Shigella and the innate immune system adapt to low oxygen environments. We aim at deciphering respective benefits of such physiological conditions on bacteria virulence and host response efficiency.

INV24
Insights into an ancient anaerobe *Methanococcus maripaludis*
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The methanogenic archaea are an ancient lineage of strict anaerobes that obtain their energy for growth from methanogenesis. They likely evolved greater than 3 Ga prior to the introduction of O_2 into the earth's atmosphere. Because their exposure to O_2 was limited by their strictly anaerobic lifestyle, they might still possess pathways common before O_2 became abundant in the biosphere. The unusual S metabolism of methanococci is a candidate for such an ancient pathway. Unlike many aerobes, methanococci use sulfide or elemental sulfur as sole sulfur sources. Cysteine is biosynthesized by a tRNA-dependent pathway, and many of the canonical enzymes for Fe-S cluster and methionine biosynthesis are absent. How sulfur is incorporated in methanococci remains unknown. To gain insight into these and other processes, a Tn-seq experiment was performed in *Methanococcus*. About 89,000 unique transposon insertions were mapped to the genome, which allowed for the classification of 526 genes or about 30% of the genome as possibly essential or strongly advantageous for growth in rich medium. Many of these genes were homologous to eukaryotic genes that encode fundamental processes in replication, transcription and translation, providing direct evidence for their importance in Archaea. Presumably, these represent processes that evolved in Archaea prior to the divergence of eukaryotes. Some genes classified as possibly essential were unique to the archaeal or methanococcal lineages, such as the gene encoding the DNA polymerase PolD. Unlike the crenarchaeotes, PolD appears to be the replicative DNA polymerase in methanococci and other euryarchaeotes. Thus, major changes in DNA replication are likely to have occurred during the evolution of the modern lineages of Archaea. Similarly, 121 hypothetical ORFs were classified as possibly essential and are likely to play fundamental roles in methanococcal information processing or metabolism that are not established outside this group.

INV25
The gut microbiota of termites: evolutionary origin and functional adaptations
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Termites degrade lignocellulose with the help of their intestinal microbiota. The general role of the microbial symbionts in the digestive process is slowly emerging, but the specific functions of individual populations and their evolutionary origin are still in the dark. Deep sequencing of the hindgut community revealed strong differences among the major host groups, and dramatic changes in the abundance of particular taxa coincide with major events in termite evolution. The acquisition of cellulolytic protists by an ancestral cockroach gave rise to large populations of bacterial symbionts, which specifically colonize the gut flagellates in all evolutionary lower termites. They were recruited from the gut microbiota and serve to complement deficits in the nitrogen metabolism of their flagellate hosts. After the loss of flagellates in higher termites, the wood particles became available for bacterial colonization, providing new niches for fiber-digesting populations. There is strong evidence for a core of termite-specific bacterial lineages. Many of these lineages are present also in cockroaches and may be cospeciating with termites since the early Cretaceous, whereas others appear to be independently acquired from the environment by habitat selection.

INDUSTRIELLE MIKROBIOLOGIE (VAAM)

IMV01
Cell factories: Engineering yeast for terpene production
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The yeast *Saccharomyces cerevisiae* is a robust, well-established industrial production organism, it exhibits very good growth characteristics, a broad substrate spectrum and a high acid- and osmotolerance. Additionally, *S. cerevisiae* is genome-sequenced, genetically and physiologically well characterized, and tools for genetic optimization are established. These features make yeast particularly suitable for the biotechnological production of bulk and fine chemicals. Lipophilc Terpenes - e.g. with a size of C30 or higher - are often of high commercial relevance but not easily accessible in large scales due to limited natural resources for extraction. We addressed the fermentative production of this class of compounds by constructing strains of *Saccharomyces cerevisiae* for the biotechnological production of squalene and squalene deriviatives.. We engineered yeast strains, which contain a deregulated terpene biosynthetic pathway and deletions of genes responsible for the formation of triacylglycerols and sterylesters. While the deregulation

of the pathway induces a strong increase in terpene productivity, the gene deletions lead to an abolishment of the formation of the neutral lipids triacylglycerols and sterylesters, which facilitates the downstream processing of lipophilic terpene compounds. The high productivity observed makes *Saccharomyces cerevisiae* a perfect host for the biotechnological production of valuable lipophilic terpenes.

IMV02

Pseudomonas exotoxin A fusion proteins for tumor therapy

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Pseudomonas exotoxin A (PE) is a protein toxin produced by Pseudomonas aeruginosa. It binds to the surface of eukaryotic cells, internalizes, enters the cytoplasm, and there inactivates translation elongation factor 2. Irreversible inactivation of eEF2 subsequently stalls protein synthesis and leads to cell death. The cell binding domain of PE can be recombinantly replaced with antibody modules that specifically bind to tumor cells. This generates immunotoxins (cytolytic fusion proteins) which specifically bind to and kill tumor cells. First generation PE-derived immunotoxins that were developed in Ira Pastans lab at the NCI already show promising activities in clinical trials in selected applications. However, anti-drug immune responses which are primarily directed at the bacterial toxin, has been limiting their general applicability. This hurdle shall be overcome by a new generation of PE-derived immunotoxins which are 'humanized' by eliminating B- and T-cell epitopes of PE.

IMV03

"ESETEC": Teaching an old workhorse new tricks - bioprocess innovations with E.coli

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Brief talk summary: The talk will focus and describe the use of Wacker's E. coli-based secretion technology (Brand name:
ESETEC®) for the expression of novel classes of therapeutic proteins, the so called next generation biopharmaceuticals (e.g. antibody fragments, scaffolds). The brand ESETEC® contains a family of genetically modified E. coli strains which are capable to secrete the desired target proteins directly to the culture medium. In addition to the strain, we developed over the last years a comprehensive toolbox of expression elements to handle difficult to express proteins as well as challenging new molecules. We will present the technology itself and some details on scientific results and selected case studies.

A few bulleted points Wacker's secretory system enables
- expression of correctly folded proteins directly to the culture medium
- high fermentation titers
- easy recovery of these proteins from the culture medium
- increased process efficiency and overall yield
- reduced cost of goods by lowering the amount of DSP steps

IMV04

Autodisplay - a new dimension in surface display for whole cell biocatalysis and bioanalytics

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The display of a peptide or protein with a distinct function at the cell surface bears considerable advantages for many biotechnological applications. The molecule displayed at the cell surface is freely accessible to the substrate or binding partner for activity or binding studies, eliminating the need for membrane crossing. Proteins have proven to be more stable when connected to a matrix rather than as free molecules. Displaying proteins on the cell surface also makes preparing or purifying the protein unnecessary in many instances. Whole cells displaying the molecule of interest can be used in reactions or analytical assays and can then be easily removed by centrifugation. The Autodisplay system has been developed on the basis of the natural secretion mechanism of the AIDA-I autotransporter protein. It offers the expression of more than 10^5 recombinant molecules per single cell, permits the multimerization of subunits from monomeric genes at the cell surface and the functional display of proteins carrying prosthetic groups. Currently, Autodisplay Biotech GmbH has set a focus on three application fields: whole cell biocatalysts, functionalising solid surfaces and screening systems. Autodisplay's technologies add significant value to processes in a broad range of industries such as the pharmaceutical, diagnostics, cosmetics, chemical, food and biotechnology industries.

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07745 Jena (DE)
Tel. +49 3641 31 16-316/-317
Fax +49 3641 31 16-243
www.conventus.de
dghm-vaam@conventus.de

Redaktion:
Dr. Christine Schreiber
Redaktion BIOspektrum
Springer Spektrum | Springer-Verlag GmbH
Tiergartenstraße 17
69121 Heidelberg (DE)
Tel.: +49 (0)6221 - 487 8043
Fax: +49 (0)6221 - 487 68043
christine.schreiber@springer.com
biospektrum@springer.com

Verlag:
Springer Spektrum | Springer-Verlag GmbH
Tiergartenstraße 17
69121 Heidelberg (DE)
Tel.: +49 (0)6221 - 487 8043
Fax: +49 (0)6221 - 487 68043
www.springer-spektrum.de

Geschäftsführer:
Derk Haank, Martin Mos, Peter Hendriks

Anzeigen:
top-ad Bernd Beutel
Schlossergäßchen 10
69469 Weinheim (DE)
Tel.: +49 (0)6201-290 92 0
Fax: +49 (0)6201-290 92 20
info@top-ad-online.de

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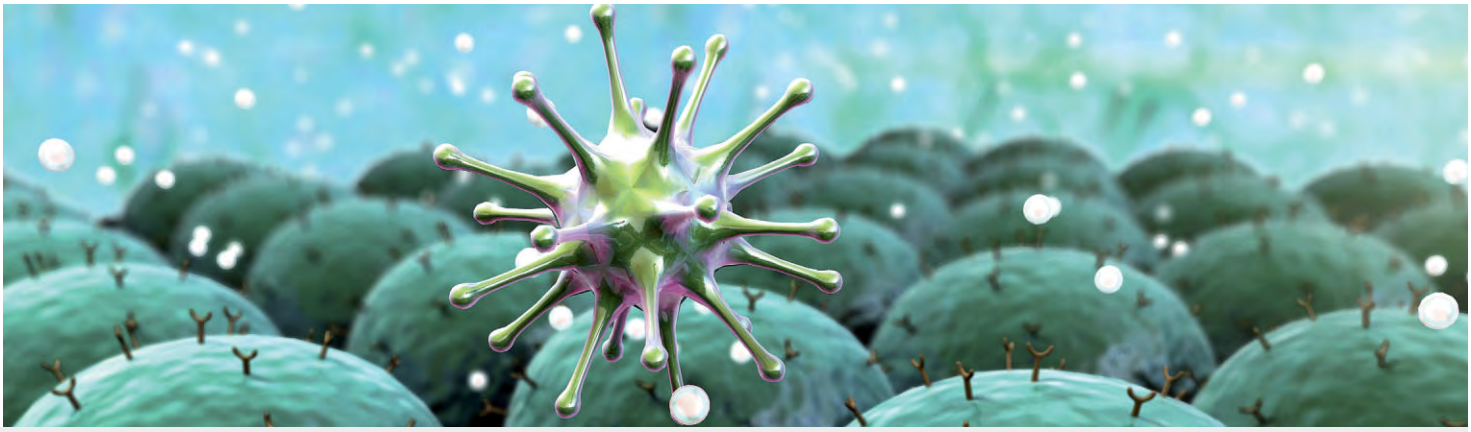
Druck:
Stürtz GmbH, Würzburg



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