

SUPPLEMENTARY INFORMATION

The marine bacterium *Phaeobacter inhibens* secures external ammonium by rapid buildup of intracellular nitrogen stocks

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SUPPLEMENTARY RESULTS AND DISCUSSION

Carbon and energy metabolism of *P. inhibens* DSM 17395. The sole organic substrate available in excess during the entire incubation time is glucose. Glucose catabolism proceeds via the Entner Doudoroff (ED) pathway connected to the lower branch of the Embden Meyerhof Parnas (EMP) pathway to ultimately feed via pyruvate dehydrogenase into the TCA cycle^{1,2}. Most detected enzyme components (e.g. enolase and succinate dehydrogenase) and metabolites (2-phosphoglycerate) of this central metabolic route displayed only subtle abundance changes during active growth, which was also observed for protein components of the aerobic respiratory chain and ATP generation (Supplementary Table S3). During stationary growth phase (≥ 60 h of incubation), however, some of the TCA cycle enzymes (e.g. isocitrate dehydrogenase) and all detected metabolites (e.g. fumarate, succinate) were reduced in their abundances. On the contrary, the protein components of pyruvate dehydrogenase increased in abundance during stationary growth phase as well as those involved in the turnover/mobilization of PHB, including phasin PhaP and depolymerase PhaZ. The PHB monomer 3-hydroxybutanoate was detected across the complete incubation time, at first with very low abundance (≤ 20 h), which peaked during early stationary growth phase (50–60 h) to then decrease strongly during late stationary growth phase (Supplementary Table S4). This agrees with the onset and abundance profile of PHB as determined by flow cytometry (Fig. 1).

Stationary growth phase response of *P. inhibens* DSM 17395. According to Fig. 1, *P. inhibens* transits into stationary growth phase upon nitrogen limitation to then experience nitrogen starvation. The stationary phase response of *P. inhibens* deduced from the differential proteomic/metabolomic analyses corresponds essentially with those reported previously for other Gram-negative bacteria³. These responses include preparedness to acquire alternative nutrient sources (including recycling of proteins by Clp proteases), alterations in housekeeping protein pools, induction of stress response and adaptation to carbon excess.

A considerable number of periplasmic solute-binding proteins of differing specificities showed similarly increased abundances during stationary and exponential growth phases as compared to early linear growth (Supplementary Table S3). These profiles may be interpreted as a means to access a variety of alternative nutrients, in particular nitrogen, released from non-growing or lysing cells. A further nitrogen source could be tapped by recycling surplus, futile intracellular proteins via the Clp proteases (ClpP, 2.7-fold increased abundance in stationary phase) as previously shown for *Bacillus subtilis*⁴.

To avoid unproductive use of resources and energy, cellular and metabolic processes for maintenance, anabolism and genetic information processing are generally tuned down during stationary growth phase. Correspondingly, proteins involved in synthesis of amino acids (e.g. CarB of Arg biosynthesis, –11.5-fold), purines (e.g. PurH, –5.5-fold) and pyrimidines (e.g. PyrG, –3.8-fold), as well as for transcription

(e.g. RpoA, -1.9-fold) and translation (e.g. PheS, -2.9-fold; Ef-G, -5.3-fold) displayed decreased abundances during stationary growth phase of *P. inhibens*. Such comprehensive changes of the overall cellular physiology in response to nutritional starvation are controlled in Gram-negative bacteria by the stringent response involving synergistic action of the alarmone (p)ppGpp and the transcriptional factor DskA on RNA polymerase⁵⁻⁷. Identification of DskA as well as the SpoT protein governing the cellular (p)ppGpp level suggests stringent response to also occur in *P. inhibens*. Another protein for transcriptional tuning during stationary growth phase in *E. coli* is the alternative sigma factor⁸ σ^{70} , which is however not encoded in the genome of *P. inhibens*¹.

Several proteins displaying increased abundances in stationary growth phase of *P. inhibens* could be attributed to general stress response. They include Dsp-like PGA1_262p00250 (4.5-fold) and SodB (2.1-fold) which could protect against oxidative stress^{9,10} arising from iron-induced OH-radical formation¹¹ promoted by the low pH conditions (~5.5) of stationary *P. inhibens* cultures. Other identified proteins of general stress response are GroL (4.8-fold), the small heat shock protein IbpA (2.3-fold) and a putative universal stress protein (PGA1_c07120; 3.3-fold).

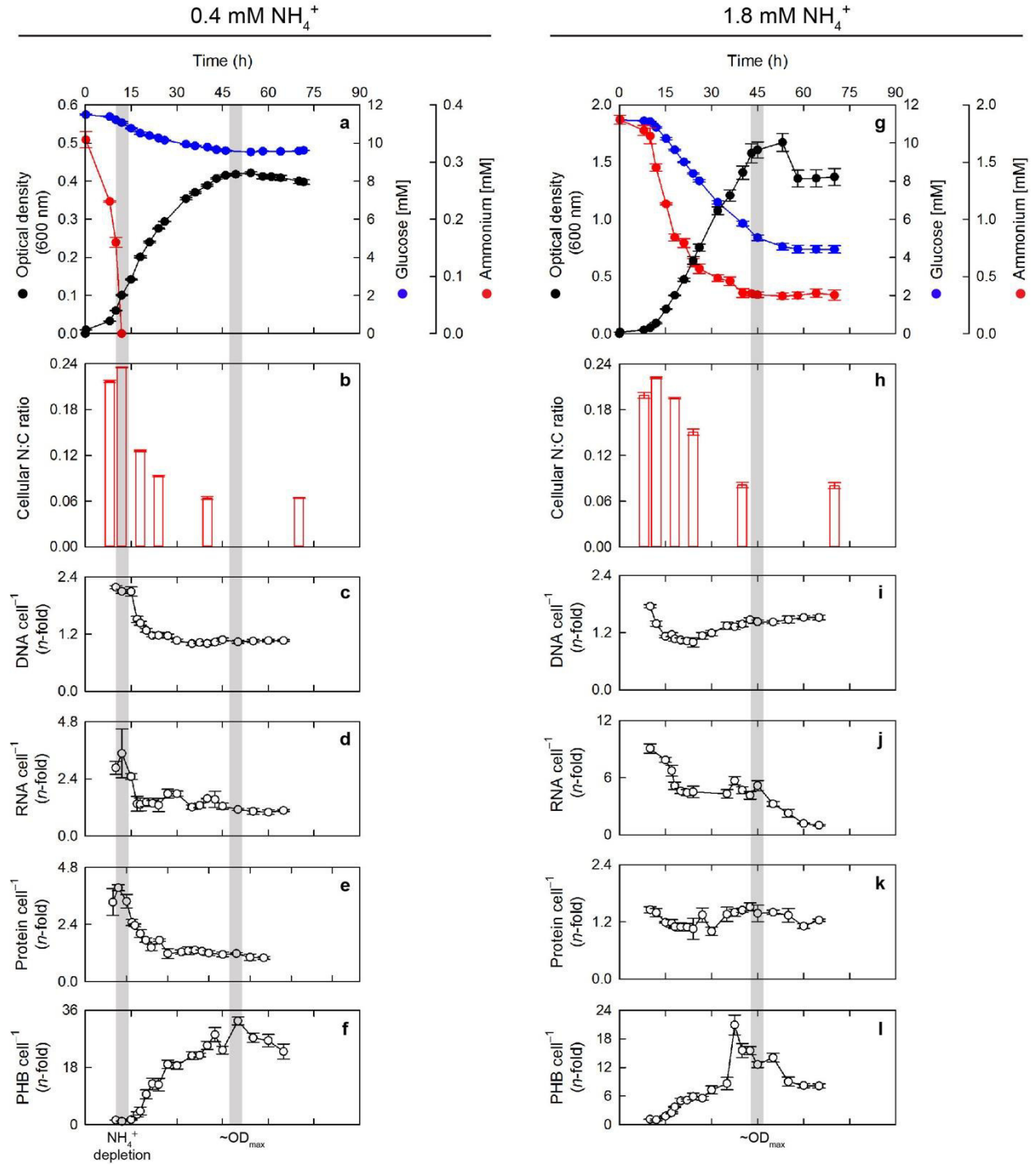


Figure S1. Substrate consumption and cellular characteristics of *Phaeobacter inhibens* DSM 17395 during growth with 0.4 mM NH_4^+ or 1.8 mM NH_4^+ . a, g, Growth and substrate consumption profiles. b, h, Cellular (molar) N:C ratios. Cellular contents of c, i, DNA, d, j, RNA, e, k, protein and f, l, PHB, as determined by flow cytometry. The *n*-fold change relates each data point to the lowest measured value of the respective data set. Displayed data is based on 3–4 biological replicates.

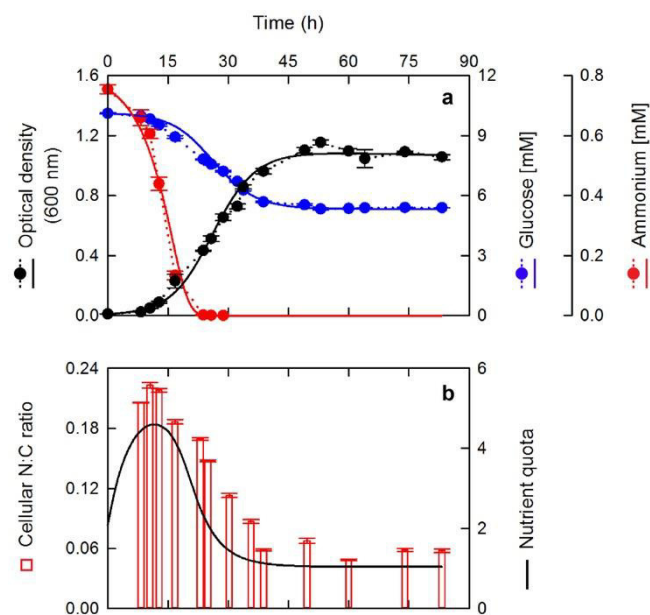


Figure S2. Fitting the cell quota model to growth and substrate consumption of *Phaeobacter inhibens* DSM 17395 (see Fig. 1). **a**, Experimental values (dashed lines) and model fit (solid lines) for optical density and consumption of glucose and NH_4^+ . **b**, Experimentally determined N:C ratios of biomass and modeled nutrient quota. The Droop model relates the internal nutrient concentrations ("cell quota") to growth rate. It extends from the classical one-compartment Monod model (external substrate) to a two-compartment model considering external and internal substrates and handling limiting and excess substrate conditions at the same time¹². For determined model parameters see Supplementary Table S2.

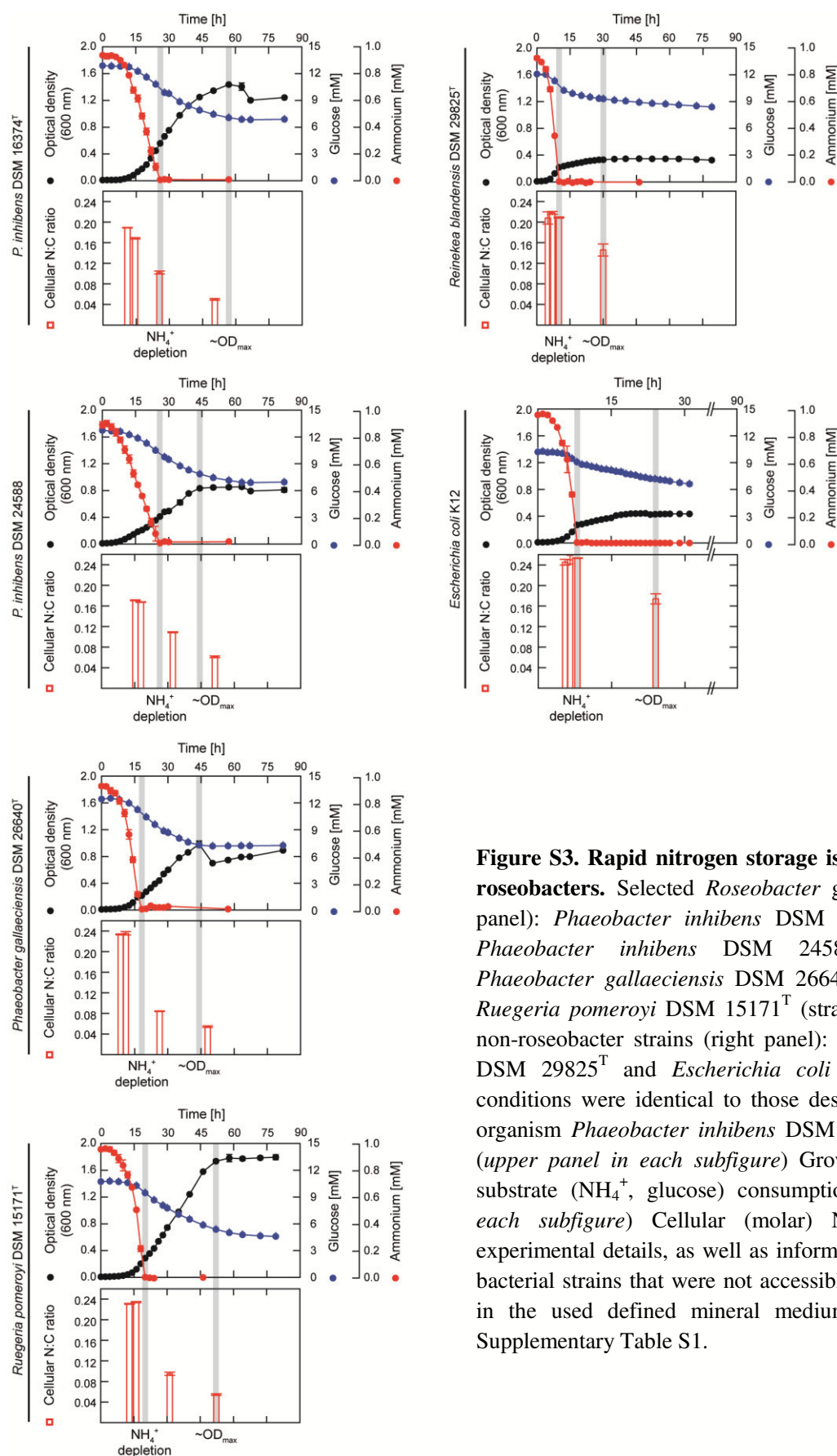


Figure S3. Rapid nitrogen storage is prevalent in other roseobacters. Selected *Roseobacter* group members (left panel): *Phaeobacter inhibens* DSM 16374^T (strain T5), *Phaeobacter inhibens* DSM 24588^T (strain 2.10), *Phaeobacter gallaeciensis* DSM 26640^T (strain BS 107), *Ruegeria pomeroyi* DSM 15171^T (strain DSS-3). Selected non-roseobacter strains (right panel): *Reinekea blandensis* DSM 29825^T and *Escherichia coli* K12. Experimental conditions were identical to those described for the study organism *Phaeobacter inhibens* DSM 17395 (see Fig. 1). (upper panel in each subfigure) Growth (by OD₆₀₀) and substrate (NH₄⁺, glucose) consumption. (lower panel in each subfigure) Cellular (molar) N:P ratios. Further experimental details, as well as information on other tested bacterial strains that were not accessible for growth studies in the used defined mineral medium, are compiled in Supplementary Table S1.

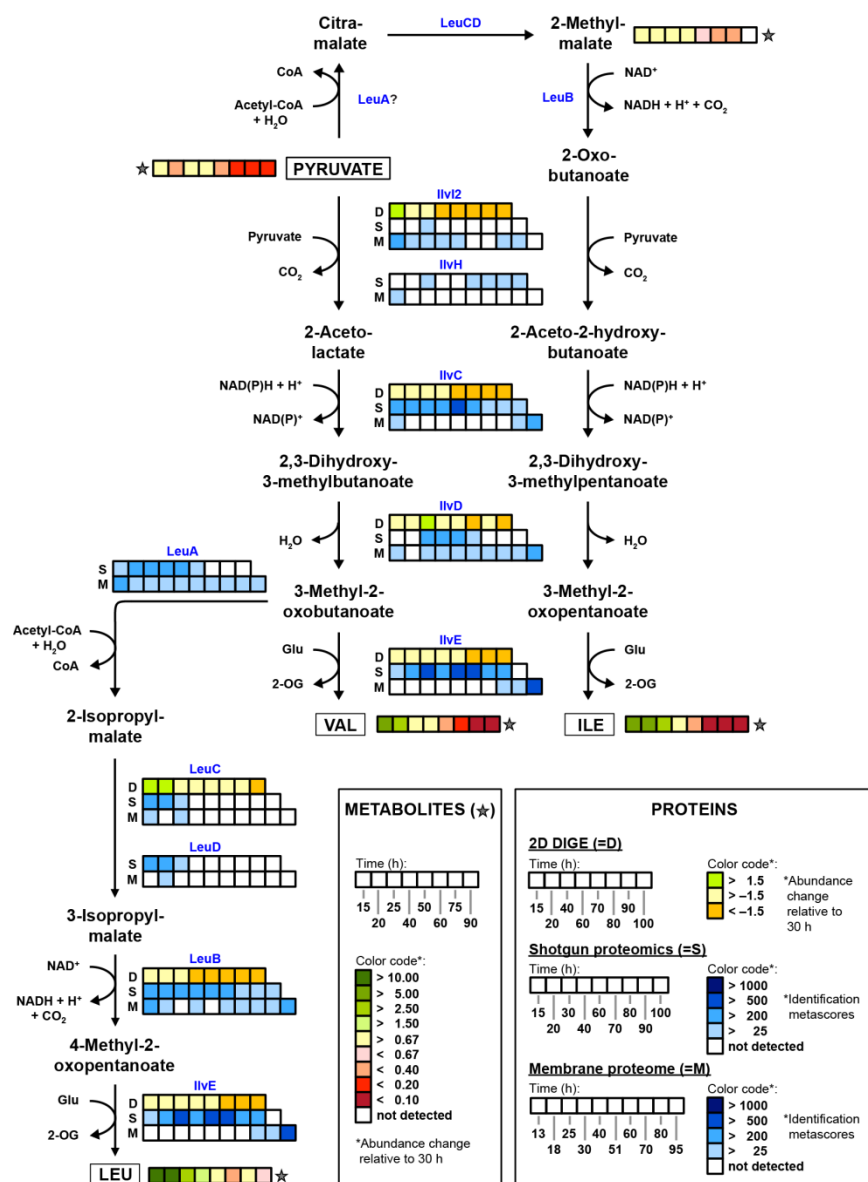


Figure S4. Time-dependent profiles of enzymes and metabolites involved in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine ("pyruvate family") in *Phaeobacter inhibens* DSM 17395. Harvesting time points for analysis of the soluble proteins (2D DIGE or shotgun) and of intracellular metabolites are as indicated in the inserts; in case of 2D DIGE and metabolites, the 30 h incubation time point served as reference state. Fold changes in protein (2D DIGE) or metabolite abundances or for meta-scores of protein identification (shotgun, membrane proteins) are indicated with a color code. Enzyme names (all identified, blue; alphabetic order): IlvC, acetohydroxy acid isomerase-reductase; IlvD, dihydroxy acid dehydratase; IlvE, valine amino-transferase; IlvI2 and IlvH, subunits of acetolactate synthase; LeuA, α -isopropylmalate synthase; LeuB, β -isopropylmalate dehydrogenase; LeuCD, α -isopropylmalate isomerase. Detailed proteomic and metabolomic datasets are compiled in Supplementary Tables S1 and S2.

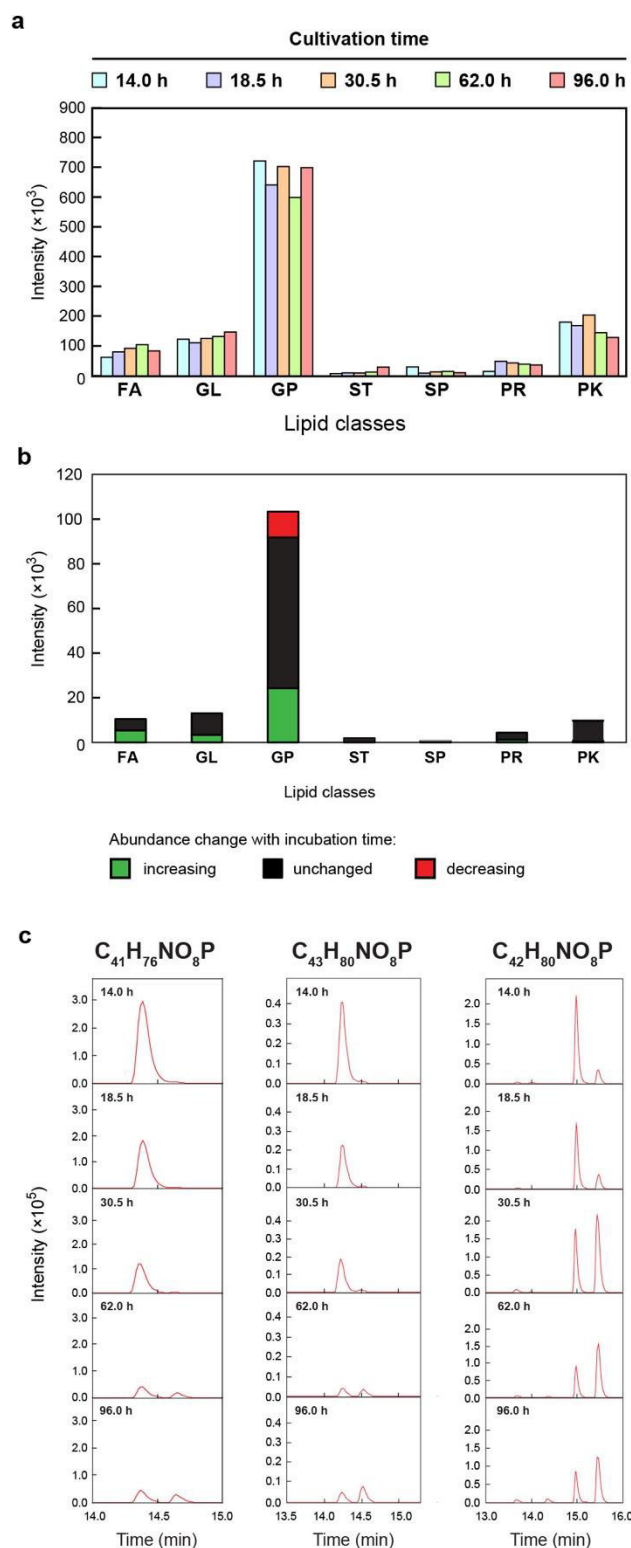
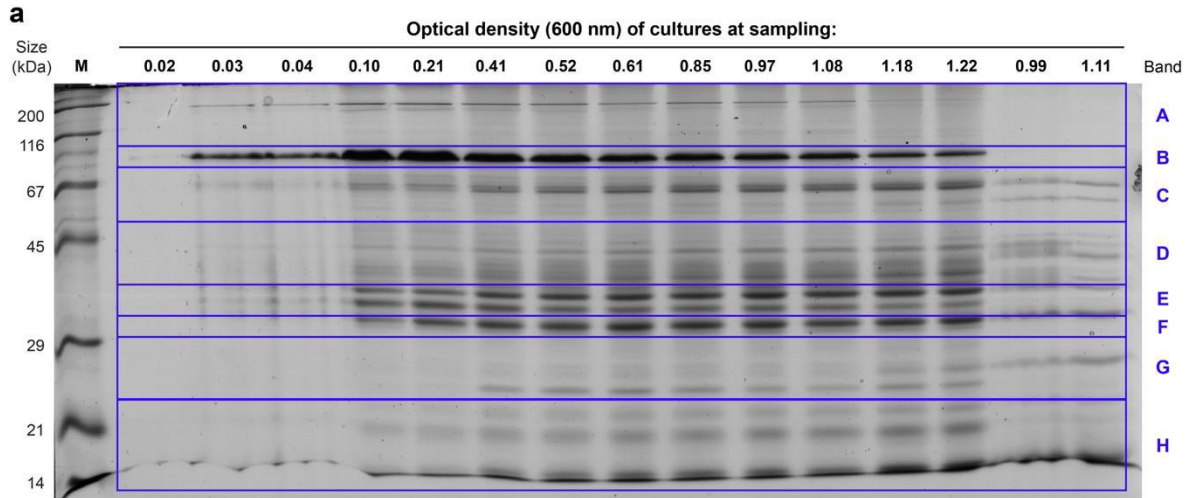


Figure S5. Analysis of the lipidome of *Phaeobacter inhibens* DSM 17395 during growth with 0.8 mM ammonium (see Fig. 1). **a**, Detected lipids were categorized into different classes: FA, fatty acids; GL, glycerol lipids; GP, glycerol phospholipids; ST, sterols; SP, sphingolipids; PR, prenols; PK, polyketides. The lipidome appears to be stable across growth, since major changes in lipid classes were not detected. Glycerol phospholipids (PL) represented the dominant lipid group. **b**, Non-parametrical statistical analysis revealed a certain modulation of single lipid species (R^2 0.633; Q^2 0.487). Pearson correlation analysis was the basis for categorization of lipids into three classes during growth: increasing, decreasing and constant lipid species. A linear modulation behavior was assumed and the correlation coefficient-based intervals for categorization were set to -1 to -0.5 (increasing), -0.5 to 0.5 (constant) and 0.5 to 1.0 (decreasing). The y-axis displays MS-detected intensities of lipids that were normalized to the sample weight. **c**, Exemplary chromatograms displaying different identified N-containing glycerol phospholipids that decrease in abundance during growth. Among cellular lipids, N-containing glycerol PL represented only a minor fraction (i.e. their intensity was ~ 10 -fold lower than the most abundant PL). Sum formulas represent phosphatidylcholine (PC) or phosphatidylethanolamine (PE) species with different degrees of saturation. Their abundance decline may suggest them to serve as transitory nitrogen sink during early growth or reflect rearrangement of the lipidome in response to emerging nitrogen limitation. Data based on three biological replicates. Displayed data in subfigures **a** and **b** are based on three biological replicates per sampling time.



b

Optical density (600 nm) of cultures at sampling:

Accession (PGA1_)	Functional prediction	Band	0.02	0.03	0.04	0.10	0.21	0.41	0.52	0.61	0.85	0.97	1.08	1.18	1.22	0.99	1.11
RTX-like proteins																	
262p00640	Hemolysin-type calcium-binding protein repeat protein	D						39		148	108				66		61
262p01050	Hemolysin-type calcium-binding protein repeat protein	D															95
262p01140	Hemolysin-type calcium-binding protein repeat protein	A						36		115	89						
65p00350	Hemolysin-type calcium-binding protein repeat protein	B	192	202	269	311	377	257	258	250	354	223	211	231	190	107	66
c16980	Hemolysin-type calcium-binding protein repeat protein	C					174	214	219		155			129	120		
c17480	Hemolysin-type calcium-binding protein repeat protein	C					62	133									
c18150	Hemolysin-type calcium-binding protein repeat protein	C					46	109									
c26140	Hemolysin-type calcium-binding protein repeat protein	A	88		709		1722	1682			1886			1539	1651		106
Proteases																	
262p00920	Putative peptidase, M23 family	G						236	387			401			496	578	104
c25160	Putative peptidase, M23 family	F										73			95		
c32810	Peptidase, M16 family	C													89	99	
c32820	Peptidase, M16 family	C										92			226	263	107

Identification score n.a. n.d. >25 >200 >500 >1000

c

Time (h)	OD (600 nm)	CDW ^a [mg l ⁻¹]	Total exoproteins ^b [μg l ⁻¹]	RTX protein (PGA1_65p00350) ^c (%)	RTX protein (PGA1_65p00350) ^d [μg l ⁻¹]	Total exoproteins ^e [μg (mg CDW) ⁻¹]	RTX protein (PGA1_65p00350) ^e [μg (mg CDW) ⁻¹]	TDA ^f [Abs _{398nm}]	TDA ^g [Abs _{398nm} (μg CDW) ⁻¹]
5.08	0.023	9.3	31.4 ± 3.2	33.1 ± 6.8	10.4 ± 2.4	3.36 ± 0.43	1.11 ± 0.27	0.000 ± 0.000	0.000 ± 0.000
7.50	0.026	10.4	39.7 ± 5.9	59.1 ± 3.9	23.5 ± 3.8	3.80 ± 0.59	2.25 ± 0.38	0.000 ± 0.000	0.000 ± 0.000
9.50	0.044	17.8	58.1 ± 15.3	58.7 ± 3.1	34.1 ± 9.2	3.26 ± 0.88	1.92 ± 0.53	0.000 ± 0.000	0.000 ± 0.000
11.83	0.097	38.9	173.8 ± 39.5	49.1 ± 3.8	85.3 ± 20.4	4.47 ± 1.02	2.19 ± 0.53	0.030 ± 0.002	0.776 ± 0.053
15.58	0.214	57.0	900.2 ± 43.5	46.1 ± 3.3	414.8 ± 35.7	15.80 ± 0.83	7.28 ± 0.64	0.118 ± 0.009	2.079 ± 0.163
22.42	0.414	110.3	2015.3 ± 130.5	30.5 ± 2.5	614.3 ± 64.0	18.27 ± 1.20	5.57 ± 0.58	0.218 ± 0.010	1.980 ± 0.093
24.33	0.519	138.2	2346.9 ± 139.5	26.6 ± 1.2	623.7 ± 46.9	16.98 ± 1.01	4.51 ± 0.34	0.241 ± 0.015	1.746 ± 0.107
25.92	0.611	162.7	2485.2 ± 225.1	22.3 ± 4.8	554.1 ± 128.5	15.28 ± 1.38	3.41 ± 0.79	0.242 ± 0.021	1.490 ± 0.132
31.33	0.845	225.0	3689.2 ± 147.2	19.4 ± 1.3	716.2 ± 55.5	16.40 ± 0.66	3.18 ± 0.25	0.248 ± 0.016	1.103 ± 0.071
34.83	0.969	258.1	3821.6 ± 248.5	16.7 ± 1.2	640.0 ± 63.3	14.81 ± 1.00	2.48 ± 0.25	0.254 ± 0.004	0.984 ± 0.024
38.58	1.078	287.0	3385.5 ± 76.5	18.7 ± 1.8	633.1 ± 61.1	11.80 ± 0.27	2.21 ± 0.21	0.253 ± 0.001	0.882 ± 0.004
48.83	1.180	314.2	3898.0 ± 243.7	11.2 ± 1.7	436.8 ± 72.0	12.41 ± 0.94	1.39 ± 0.24	0.235 ± 0.004	0.749 ± 0.035
59.83	1.224	325.8	1003.9 ± 160.1	5.7 ± 2.8	57.2 ± 29.6	3.08 ± 0.49	0.18 ± 0.09	0.210 ± 0.023	0.643 ± 0.071
73.83	0.988	263.1	81.4 ± 14.8	0.0 ± 0.0	0.0 ± 0.0	0.31 ± 0.06	0.00 ± 0.00	0.149 ± 0.014	0.567 ± 0.054
83.08	1.108	295.0	88.4 ± 11.1	0.1 ± 0.1	0.1 ± 0.1	0.30 ± 0.04	0.00 ± 0.00	0.163 ± 0.017	0.552 ± 0.057

^a Cellular dry weight (CDW) calculated from optical density (OD) values using the strong linear correlations between OD and CDW (OD ≤ 0.1: y = 401.02x with R² = 0.999; OD > 0.1: y = 266.27x with R² = 0.958).

^b Total exoprotein concentration determined in cell-free culture supernatant following precipitation of proteins with trichloroacetic acid (TCA).

^c Calculated share (%) of the abundant RTX protein PGA1_65p00350 (band B in subfigure a) from the total visualisable exoproteome (i.e. SDS PAGE-resolvable and cCBB-stainable). Values determined by software-based density analysis of gel bands from four separate SDS PAGE gels with equal protein loads per lane.

^d Estimated concentration of the RTX protein PGA1_65p00350 in cell-free culture supernatant. Values were calculated from the % share of the RTX protein from the visualisable (by SDS PAGE) total exoproteome^c and with the total exoprotein concentration in cell-free culture supernatant^b.

^e Total exoprotein^b or RTX protein^d concentration in cell-free culture supernatant divided by the CDW formed^a.

^f Antibiotic tropodithietic acid (TDA) formation was measured indirectly by the absorbance at 398 nm.

^g Absorbance at 398 nm divided by the CDW formed^a.

Figure S6. High-resolution profiles of secreted potential RTX proteins and proteases during growth of *Phaeobacter inhibens* DSM 17395 with 0.8 mM ammonium (see Fig. 1). **a**, Exoproteome samples were obtained from cell-free culture supernatant and resolved by SDS PAGE (equal protein loads per lane) and proteins were identified by nanoLC-ESI-MS/MS analysis. **b**, Identified potential RTX proteins and proteases; n.a., not analyzed; n.d., not detected. Further proteins identified at 13, 30, 50 and 80 h of incubation are provided in Supplementary Table S3. **c**, Results of the exoproteome analysis and calculation of values for the abundant potential RTX protein PGA1_65p00350 and antibiotic TDA were related to CDW (data basis for Fig. 5). Displayed data is based on 3–4 biological replicates.

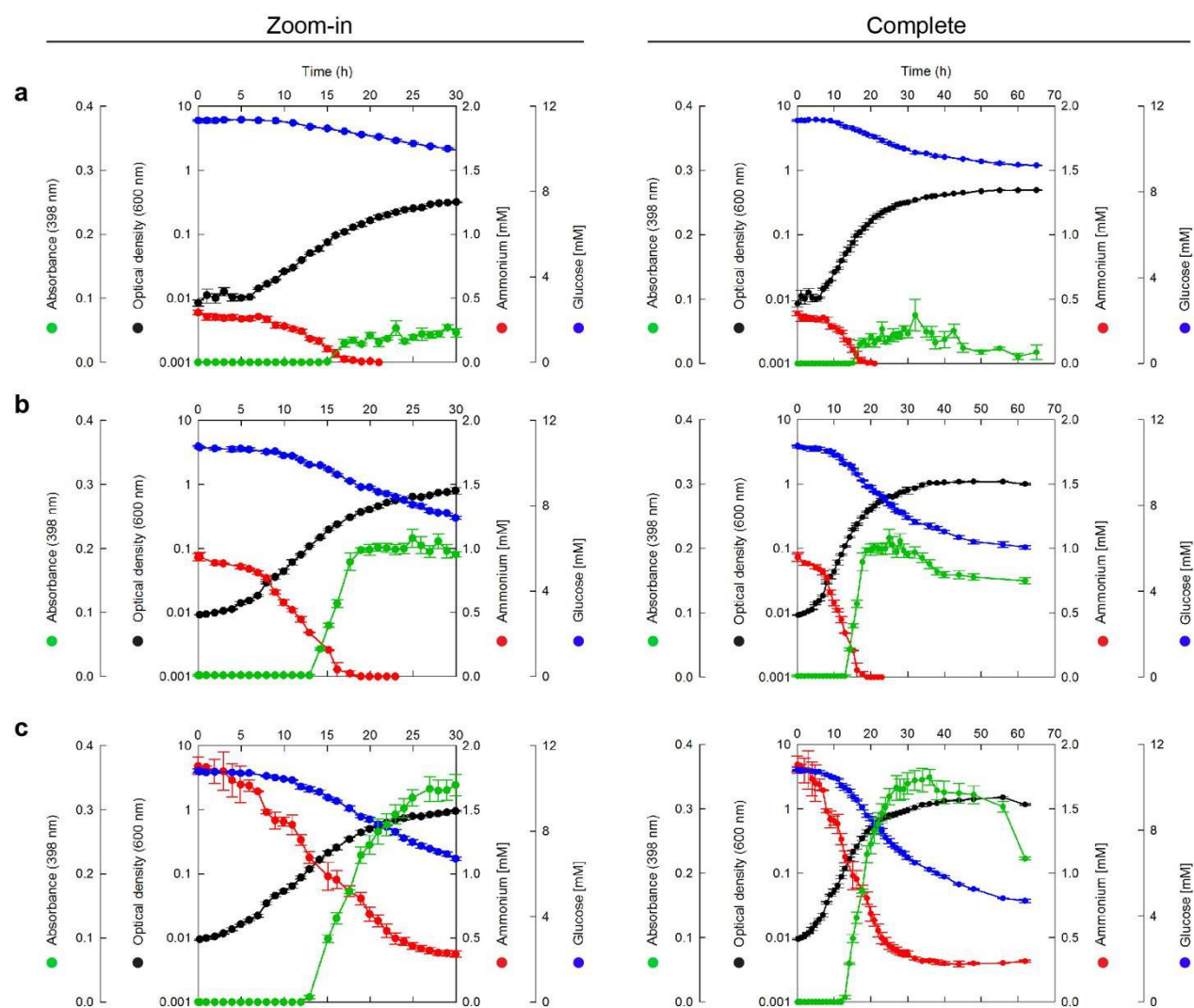


Figure S7. High-resolution growth curves of *Phaeobacter inhibens* DSM 17395 with glucose (11 mM) and different concentrations of NH_4^+ : a, 0.4 mM, b, 0.8 mM or c, 1.8 mM. Absorption of cell-free culture supernatant at 398 nm is used as proxy for tropodithietic acid (TDA) formed by *P. inhibens* DSM 17395. Displayed data is based on 3–4 biological replicates.

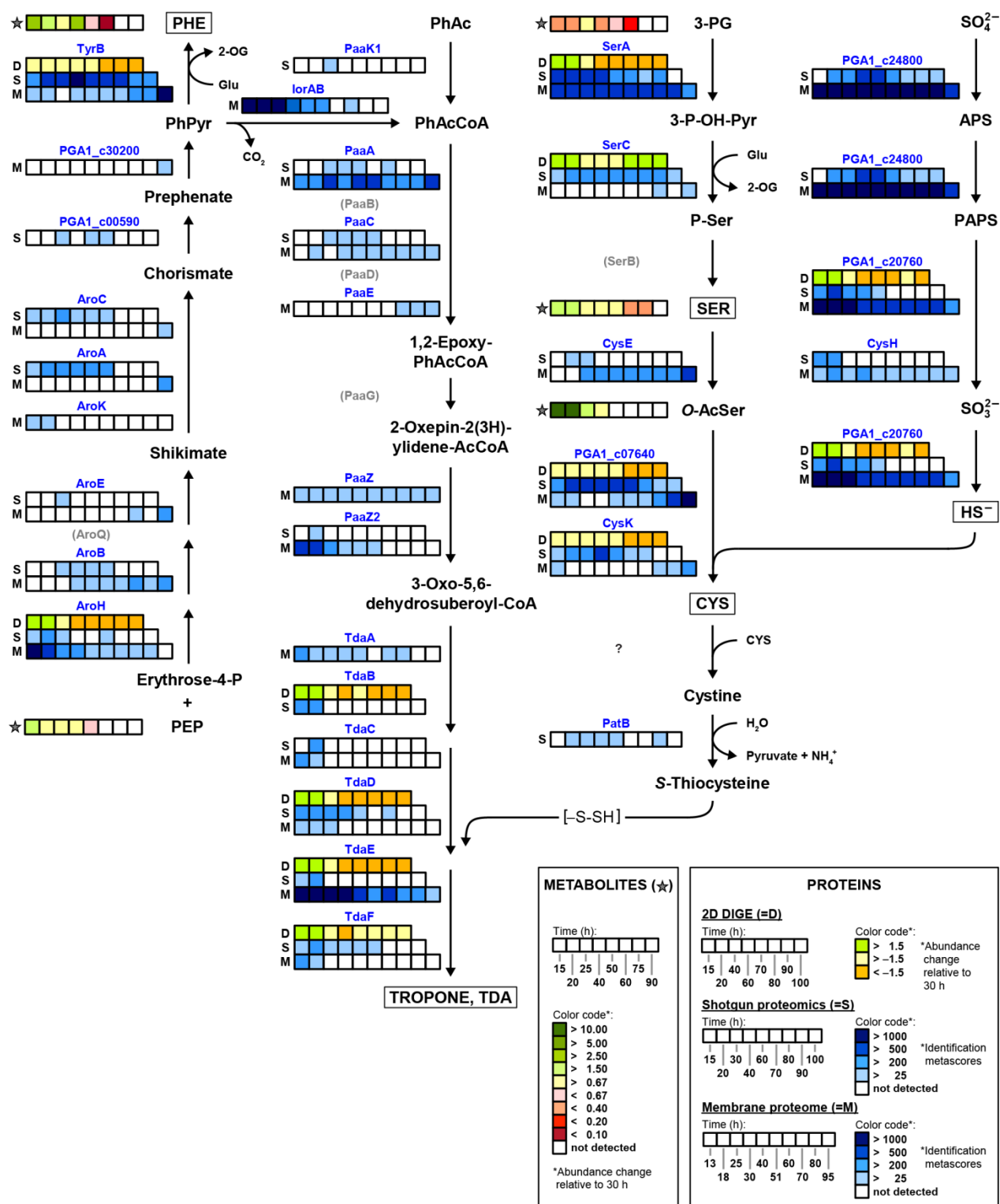


Figure S8. Interconnection of amino acid anabolism (Phe, Ser, Cys), sulfur assimilation and biosynthesis of antibiotic tropodithietic acid (TDA) in *Phaeobacter inhibens* DSM 17395 as revealed by integrated proteomic and metabolomic analysis. Harvesting time points for analysis of the soluble proteins (2D DIGE or shotgun) and of intracellular metabolites are as indicated in the inserts; in case of 2D DIGE and metabolites, the 30 h incubation time point served as reference state. Fold changes in protein (2D DIGE) or metabolite

abundances or for metascopes of protein identification (shotgun, membrane proteins) are indicated with a color code. Compound names (alphabetic order): APS, adenosine-5'-phosphosulfate; Cys, cysteine; epoxy-PhAc-CoA, *ring*-1,2-epoxy-phenylacetyl-CoA; *O*-AcSer, *O*-acetylserine; 2-oxepin-2(3*H*)-ylidene-AcCoA, (Z)-2-(oxepin-2(3*H*)-ylidene)-acetyl-CoA; erythrose-4-P, erythrose-4-phosphate; 2-OG, 2-oxoglutarate; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; PEP, phosphoenolpyruvate; 3-PG, 3-phosphoglycerate; PhAc, phenylacetate; PhAc-CoA, phenylacetyl-CoA; PhPyr, phenylpyruvate; 3-P-OH-Pyr, 3-phosphohydroxypyruvate; 3-P-Ser, 3-phosphoserine; TDA, tropodithietic acid. Enzyme names (identified highlighted in blue; predicted in grey; alphabetic order): AroA, 3-phosphoshikimate-1-carboxyvinyltransferase; AroB, 3-hydroxylate synthase; AroC, chorismate synthase; AroE, shikimate dehydrogenase; AroH, phospho-2-dehydro-3-deoxyheptanoate aldolase; AroK, shikimate kinase; AroQ, 3-dehydroxylate dehydratase; CysE, serine acetyltransferase; CysH, phosphoadenosine phosphosulfate reductase; CysK, cysteine synthase; IorAB, indolepyruvate oxidoreductase; PaaA–E,G,Z, phenylacetic acid degradation proteins; PaaK1, phenylacetate-CoA ligase; PaaZ2, oxepin-CoA hydrolase/3-oxo-5,6-dehydrosuberil-CoA semialdehyde dehydrogenase (NADP⁺); PatB, cystathionine beta-lyase; PGA1_c00590, chorismate mutase; PGA1_c07640, *O*-acetylserine sulfhydrylase; PGA1_c20760, putative nitrite/sulfite reductase; PGA1_c24800, putative bifunctional SAT/APS kinase; PGA1_c30200, prephenate dehydratase; SerA, 3-phosphoglycerate dehydrogenase; SerB, phosphoserine phosphatase; SerC, phosphoserine aminotransferase; TdaA, transcriptional regulator, LysR family; TdaB; putative beta etherase; TdaC, conserved hypothetical protein, similar to prephenate dehydratase; TdaD, thioesterase superfamily protein; TdaE, acyl-CoA dehydrogenase; TdaF, putative flavoprotein, HFCD family; TyrB, aromatic-amino-acid aminotransferase. Detailed proteomic and metabolomic datasets are compiled in Supplementary Tables S3 and S4.

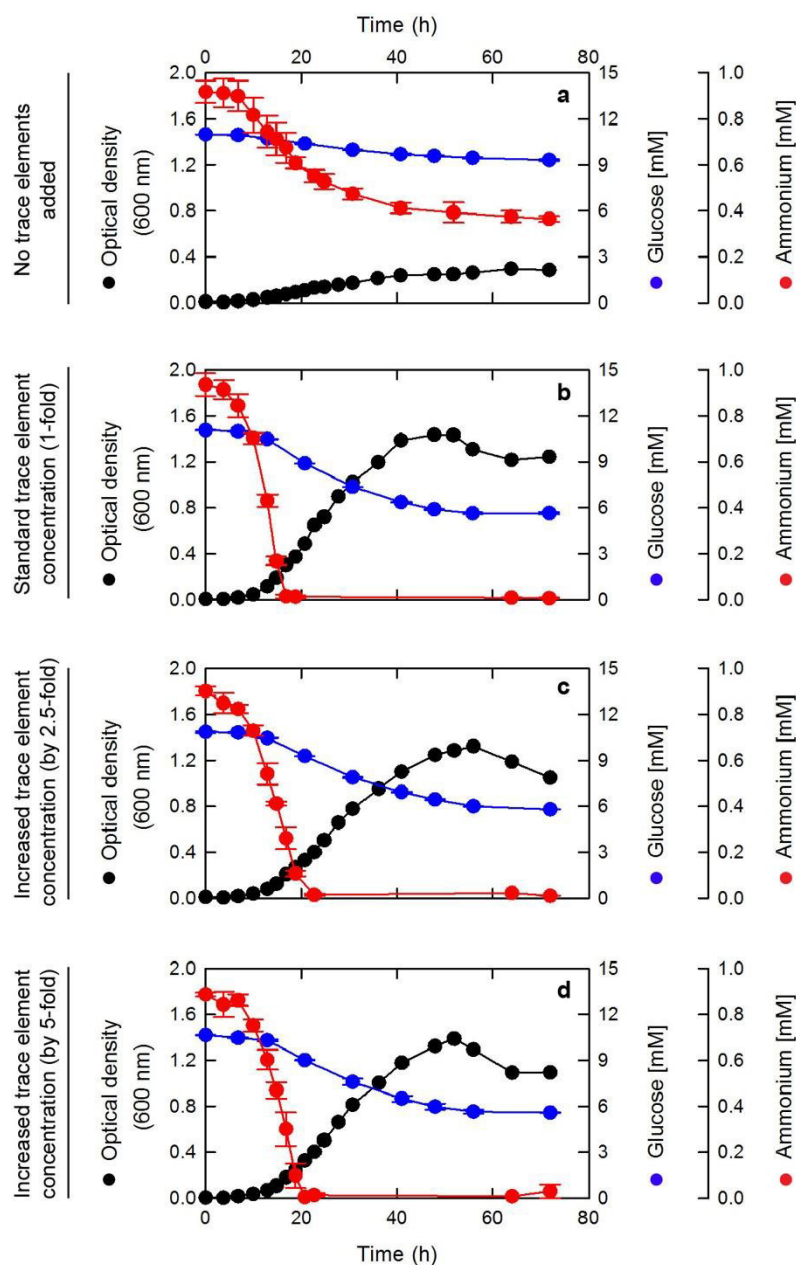


Figure S9. Effect of trace elements on growth (OD₆₀₀) and substrate (NH₄⁺, glucose) consumption in *Phaeobacter inhibens* DSM 17395. **a.** No trace elements added. **b.** Standard concentration of trace elements added in the present study. Addition of **c** 2.5-fold and **d** 5.0-fold increased trace element concentrations. The supplied trace element mixture was composed as previously described¹. Increased trace element concentrations did not affect the observed uncoupling of NH₄⁺ depletion from bulk growth (Fig. 1). Thus, cryptic limitations by micronutrients can be excluded to affect the observed nutritional strategy of rapid nitrogen storage in *P. inhibens* DSM 17395. Additional supplementation of the mineral medium with ten vitamins¹³ did not affect growth of *P. inhibens* (i.e. prototroph with respect to vitamins)¹⁴.

Table S1. Selected bacterial strains from major bacterioplankton groups tested for growth in the defined mineral medium used for *Phaeobacter inhibens* DSM 17395 in this study. The representatives of roseobacters were chosen based on their co-occurrence with *Emiliania huxleyi* blooms¹⁵. Other strains were chosen based on literature information that included detailed documentation of isolation source, reported ability to utilize glucose and representing the type strain of the species. The abundant Sar11 representative “Candidatus *Pelagibacter ubique*” could not be included, since the strain requires amino acids (organic nitrogen) as essential components in the mineral medium¹⁶. Each lyophilized strain (obtained from DSMZ; Braunschweig, Germany) was first cultivated in MB medium to generate glycerol stock cultures. Revival of stock cultures was performed using a serial dilution (up to 10⁻⁵) in MB medium. This was followed by three successive passages of MB-grown cells (mostly from the 10⁻⁴ dilution) of each strain (transfer always at the respective ~0.5OD_{max}) in (i) the same mineral medium used for *P. inhibens* DSM 17395 (see Methods section) and (ii) one that was additionally supplemented with ten vitamins¹³. All strains showed good growth with MB medium, but only five strains grew sufficiently well in the mineral medium to perform comparative growth studies, including profiling of cellular N:C ratios (Fig. 2; Supplementary Fig. S3).

Tested bacterial strains	Source of isolation			This study ^a	
	Geographical region	Isolated from	Reference	MB medium	Mineral medium ^b
ROSEOBACTERS					
<i>Phaeobacter inhibens</i> DSM 16374 ^T	North Sea, Germany	Surface water of tidal mud flat	17	+	+
<i>Phaeobacter inhibens</i> DSM 24588	Pacific Ocean, Australia	Surface of green macroalga <i>Ulva lactuca</i>	18	+	+
<i>Phaeobacter gallaeciensis</i> DSM 26640 ^T	Atlantic Ocean, Spain	Seawater from larval cultures of scallop <i>Pecten maximus</i>	19	+	+
<i>Ruegeria pomeroyi</i> DSM 15171 ^T	Atlantic Ocean, GA, USA	Seawater of coastal area	20	+	+ ^c
<i>Leisingera methylohalidivorans</i> DSM 14336 ^T	Pacific Ocean, CA, USA	Seawater from tidal pool	21	+	–
<i>Dinoroseobacter shibae</i> DSM 16493 ^T	North Sea, Germany	Surface of marine dinoflagellate <i>Prorocentrum lima</i>	22,23	+	(+/-)
<i>Roseobacter denitrificans</i> DSM 7001 ^T	Pacific Ocean, Japan	Surface of high-tidal seaweeds	24	+	(+/-)
ALPHAPROTEOBACTERIA (NON-ROSEOBACTERS)					
<i>Pseudovibrio ascidiaceicola</i> DSM 16392 ^T	Pacific Ocean, Japan	Sea squirt <i>Polycitor proliferus</i>	25	+	(+/-)
FLAVOBACTERIA (BACTERIODETES)					
<i>Maribacter forsetii</i> DSM 18668 ^T	North Sea, Germany	Surface seawater	26	+	(+/-)
<i>Formosa algae</i> DSM 16476 ^T	Pacific Ocean, Russia	From brown alga <i>Fucus evanescens</i>	27	+	(+/-) ^d
GAMMAPROTEOBACTERIA					
<i>Reinekea blandensis</i> DSM 29825 ^T	Blanes Bay, Mediterranean Sea, Spain	Surface seawater	28	+	(+/-)
<i>Microbulbifer epialgicus</i> DSM 18641 ^T	Pacific Ocean, Palau	From marine algae at seashore	29	+	–

^a +, Good and reproducible growth; (+/-), insufficient growth (low ODs and/or irreproducible) owing to inadequate medium (potentially limited by micro-nutrients other than the supplied trace elements and vitamins); –, no growth observed.

^b The defined mineral medium contained 11 mM glucose, ~1 mM NH₄⁺ and trace elements¹.

^c Additional vitamin supplementation required for growth.

^d Growth observed only in the first passage.

Table S2. Model parameters resulting from fit to experimental data (see Methods for details).

Parameter	Value
ρ_{max}^a	1.263
ρ_s^a	4.864e-1
ρ_{max}^g	6.180
ρ_s^g	3.550
α	8.499e-1
β	4.385
m	4.835e-4
μ_{max}^A	2.118e-1
Q_{min}	1.044

			Harvesting times (see Fig. 1) and applied proteomic approaches																														
			2D DIGE										Shotgun							Membrane proteins							Exoproteins						
			Fold change in protein abundance (as compared to 30 h)										Identification metascors							Identification metascors							Identification metascors						
			15 h 20 h 40 h 60 h 70 h 80 h 90 h 100 h										15 h 20 h 30 h 40 h 60 h 70 h 80 h 90 h 100 h							13 h 18 h 25 h 30 h 40 h 51 h 60 h 70 h 80 h 95 h							13 h 30 h 50 h 80 h						
Accession (PGA1_)	Gene	Functional prediction																															
EXTRACELLULAR PROTEINS																																	
RTX-/Hemolysin-type proteins																																	
262p00640		Hemolysin-type calcium-binding protein repeat protein																				224											
262p01050		Hemolysin-type calcium-binding protein repeat protein																				350 295											
262p01140		Hemolysin-type calcium-binding protein repeat protein																				317											
5p00350		Hemolysin-type calcium-binding protein																				412 203 196 175											
c16980		Hypothetical protein																				255 183 156 143											
c17480		Hemolysin-type calcium-binding repeat-containing protein																				51 36	515 258 145										
c18150		Hypothetical protein																				84											
c26140		Hypothetical protein																				107 66 66 141 80 67 124 129 82 28	2057 1478 1343 464										
Proteases/peptidases																																	
262p00920		Putative protease, M23 family	2.6 2.8	1.2 1.2	1.7 2.7	2.3 3.3															121 294	91 194 159	65 32 58 84 71 127 322 449 326 417	883 1219 1267 1080									
c08290		Putative peptidase M75											340 204											217 298 375 107									
c21610		Putative serralysin	1.0 1.1	1.4 -1.5	-2.0 -2.4	-1.8 -2.4															81	74	548 890 309										
c25160		Putative peptidase, M23 family																				117 202 345 496 540 490 486 350 241	79	101 101 57 50 161	453 419 133								
c32810		Peptidase, M16 family																				178 327 479 493 472 515 562 457 352	116 86 146 278 255 342 315 281	548 890 309									
c32820		Peptidase, M16 family																											453 419 133				
Periplasmic solute-binding proteins																																	
c02710	ugpB	sn-Glycerol-3-phosphate-binding periplasmic protein	7.6 4.6	-1.1 -1.1	1.4 1.8	3.4 2.0															416 333	120 189	59 111 222 139 237	1500 1637 1847 1216									
c07420		Extracellular solute-binding protein	3.3 2.4	-1.2 1.1	1.5 2.3	3.9 2.6															69											130 377 673 343	
c07860	aglE	alpha-Glucosides-binding periplasmic protein	7.3 5.5	2.3 1.2	1.4 1.8	10.7 2.6															401 284	348											940 1177 918 498
c11570		Putative nopaline-binding periplasmic protein	2.5 1.9	-1.7 1.1	1.2 1.6	2.3 1.9															96											355 394 579 403	
c12890		Periplasmic solute-binding protein	-1.1 -1.4	-2.4 1.3	1.2 1.7	2.5 2.1															88	133 143 217 228 148 78											385 702 819 799
c12960	dctP2	TRAP transporter, periplasmic solute-binding protein	3.1 1.5	-1.7 -1.3	-1.1 1.2	2.5 1.6																									722 642 747 820		
c15890		Extracellular ligand-binding receptor	6.3 4.1	-2.2 -1.2	-1.2 1.2	2.4 1.4															393 383 132	101 148 160 187 125	49	621 799 933 726									
c27930		Putative sugar ABC transporter, periplasmic binding protein	2.1 1.6	-1.5 1.0	-1.0 1.5	2.3 1.8															130 208	191 98 143											832 1165 1160 1067
c31410		Putative receptor family ligand-binding protein	2.3 2.3	-1.0 1.1	1.1 1.4	2.6 2.0																									76 91 76	618 1045 1035 1324	
c33920	dctP8	TRAP transporter, periplasmic solute-binding protein	2.7 2.0	-1.4 1.4	1.6 1.9	2.9 2.6															108											926 1154 1121 1178	
c34050	thiB	Thiamine-binding periplasmic protein	3.5 2.7	-2.2 -1.5	-1.1 1.4	2.7 1.5															241 242 82	136	182 147 163	1076 1272 1183 1055									
c01980	bztA	Glutamate/glutamine/aspartate/asparagine-binding protein	2.3 2.2	-1.0 1.6	2.4 4.3	5.8 5.6															659 697 588 528 625 850 700 652 575	425 203 316 524 496 624 976 1143 1086 1235	1452 1737 1817 1469										
c16270		Putative periplasmic dipeptide transport protein	-2.1 -2.5	-2.2 2.2	2.4 2.6	2.6 2.9															339 360 260 102 283 260 200 237 125	35 93 60	1156 1321 1720 1868										
c20180	dctP5	TRAP transporter, periplasmic solute-binding protein	-1.1 1.1	-1.5 -1.0	1.4 2.0	2.3 2.4															560 749 662 523 641 692 708 478 396	73 29	229 323 229 668	662 1241 1131 1124									
c20680	dctP6	TRAP transporter, periplasmic solute-binding protein	3.5 3.2	1.2 1.4	2.1 2.8	4.4 3.3															201 267	138 135 206 201 127	181 311 614 519 569 602	612 1265 944 378									
c31310		Periplasmic solute-binding protein, PotD-like	1.9 1.3	-1.9 1.8	2.3 3.3	4.8 3.1															290 242 240 102 481 568 504 369 193	86 160 251 394 775 1065 722 1068	1008 126										

Table S3 continued

			Harvesting times (see Fig. 1) and applied proteomic approaches																																														
Accession (PGA1_)	Gene	Functional prediction	2D DIGE										Shotgun										Membrane proteins										Exoproteins																
			Fold change in protein abundance (as compared to 30 h)										Identification metascores										Identification metascores										Identification metascores																
			15 h	20 h	40 h	60 h	70 h	80 h	90 h	100 h	15 h	20 h	30 h	40 h	60 h	70 h	80 h	90 h	100 h	13 h	18 h	25 h	30 h	40 h	51 h	60 h	70 h	80 h	95 h	13 h	30 h	50 h	80 h																
Peptide uptake																																																	
262p00310		Putative peptide/glutathione transport system permease protein																					60																										
262p00330		Oligopeptide ABC transporter, ATP-binding protein																																															
262p01030		Putative peptide/glutathione transport system permease protein																					67				33		52	30	61	32	37																
262p01040		Periplasmic solute-binding protein of putative peptide/glutathione transport system										89								99												33			390	488	620	433											
262p01700		Periplasmic solute-binding protein of putative peptide/glutathione transport system	1,5	1,4	1,2	-1,1	-1,8	-2,7	-2,4	-3,3										62								73	58	141	59	102			112	100													
262p01710		Putative peptide/glutathione transport system permease protein																							179	207	272	313	343	335	272	134	118																
262p01720		Putative peptide/glutathione transport system permease protein																						92	247	316	230	99	272	185	83	67	146																
262p01730		Putative peptide/glutathione ABC transporter ATP-binding protein																													31																		
262p01740		Putative peptide/glutathione ABC transporter ATP-binding protein																													50	122	127	134															
c04420	gsiD1	Putative glutathione transport system permease protein																						155	184	87	111	116	154	226	262	213	255																
c04430		Putative peptide permease protein																						91	98	29	135	69	31	101	94	121	176																
c04440		Putative extracellular solute-binding protein																													54	32	49			142	103												
c04450	gsiA1	Glutathione import ATP-binding protein																						109	118	336	213	433	383	332	384	246	145																
c16270		Putative periplasmic dipeptide transport protein	-2,1	-2,5	-2,2	2,2	2,4	2,6	2,6	2,9		339	360	260	102	283	260	200	237	125									35	93	60				1156	1321	1720	1868											
c16280		Putative dipeptide transport system permease protein																																															
c16290	dppC2	Dipeptide transport system permease protein																																															
c16300	dppD2	Dipeptide transport ATP-binding protein																	291	196	94																												
c16310		Oligopeptide /dipeptide transport ATP-binding protein																																															
c28410		ABC transporter, ATP-binding protein																																															
c28430		Putative D,D-dipeptide transport system permease protein																																															
c28450	dppA	Putative periplasmic dipeptide transport protein																																			416	568	539	499									
c30150		Oligopeptide/dipeptide ABC transporter, ATP-binding protein																		97																													
c30160		ABC transporter permease protein																																															
c30170		Transporter permease protein																																															
c30180		ABC transporter extracellular solute-binding protein																																															
Phospholipid synthesis																																																	
c03520	plsC1	1-Acyl-sn-glycerol-3-phosphate acyltransferase																																															
c03860	plsB	Glycerol-3-phosphate acyltransferase, PlsB-like																																															
c11250	plsX	Fatty acid/phospholipid synthesis protein																																															
c03010	plsY	Glycerol-3-phosphate acyltransferase																																															
c15560		Acyltransferase domain-containing protein																																															
c14460		Putative phosphatidylserine decarboxylase																																															
c29610	pmtA	Phosphatidylethanolamine N-methyltransferase																																															
c16100		Putative cardiolipin synthetase																																															
c02890	pgsA	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase																																															
c02890	pgsA	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase																																															
Energy metabolism																																																	
c06250	ctaC	Cytochrome c oxidase subunit 2																																															
c06710	ctaD	Cytochrome c oxidase subunit 1-b																																															
c10370	nuoA	NADH-quinone oxidoreductase subunit A																									</																						

Table S3 continued

			Harvesting times (see Fig. 1) and applied proteomic approaches																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
Accession (PGA1_)	Gene	Functional prediction	2D DIGE							Shotgun										Membrane proteins										Exoproteins																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
			Fold change in protein abundance (as compared to 30 h)							Identification metascores										Identification metascores										Identification metascores																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
			15 h	20 h	40 h	60 h	70 h	80 h	90 h	100 h	15 h	20 h	30 h	40 h	60 h	70 h	80 h	90 h	100 h	13 h	18 h	25 h	30 h	40 h	51 h	60 h	70 h	80 h	95 h	13 h	30 h	50 h	80 h																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
c32000		Cytochrome <i>c</i>	-1,1	-1,0	1,1	-1,1	-1,2	-2,1	-2,1	-2,3																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									

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			Harvesting times (see Fig. 1) and applied proteomic approaches																															
			2D DIGE	Shotgun													Membrane proteins										Exoproteins							
			Fold change in protein abundance (as compared to 30 h)	Identification metascopes													Identification metascopes										Identification metascopes							
			15 h	20 h	40 h	60 h	70 h	80 h	90 h	100 h	15 h	20 h	30 h	40 h	60 h	70 h	80 h	90 h	100 h	13 h	18 h	25 h	30 h	40 h	51 h	60 h	70 h	80 h	95 h	13 h	30 h	50 h	80 h	
Accession (PGA1_)	Gene	Functional prediction																																
Pyruvate dehydrogenase complex																																		
c17550	<i>pdhA</i>	Pyruvate dehydrogenase E1 component alpha subunit	-1.6	-1.4	-1.1	1.5	1.3	-1.0	-1.0	-1.1	79	215	245	188	422	341													55	63	445			
c17560	<i>pdhB</i>	Pyruvate dehydrogenase E1 component beta subunit	-1.6	-1.4	-1.2	1.4	1.6	1.9	1.7	1.8	381	518	606	654	816	735	733	480	477		270	204	200	262	379	438	631	692	980	1604				
c17570	<i>pdhC</i>	Dihydrolipoylysine-residue acetyltransferase component of pyruvate dehydrogenase complex									311	509	591	750	707	694	766	425	537		77	112	30					109	292	559				
c17390	<i>lpdA2</i>	Dihydrolipoyl dehydrogenase	-2.5	-2.0	-1.0	2.6	2.5	2.2	2.2	2.3	181	533	596	801	1010	752	541	356	170		922	918	961	784	632	699	763	1014	1046	1669	180	82	336	393
TCA cycle																																		
c16970	<i>glitA1</i>	Citrate synthase	1.1	1.1	1.1	-1.6	-1.7	-2.7	-3.4	-4.8	284	308	210	205	331	139					300	312	369	457	479	384	425	498	351	597				
c18830	<i>acnA</i>	Aconitate hydratase	1.0	-1.1	-1.1	-1.3	-1.8	-3.6	-2.8	-4.6	369	616	781	1031	813	602	258	311	168		48					59	230	213	502	677	707		420	
c28340	<i>icd</i>	Isocitrate dehydrogenase [NADP] complex	1.3	1.3	-1.1	-1.8	-2.0	-4.5	-3.5	-5.2	710	1005	1030	1184	1037	775	405	204	165					38				55	171	874	593		188	
c03590	<i>sucB</i>	2-Oxoglutarate dehydrogenase E1 component									340	420	620	781	934	829	592	375	229		885	630	545	584	555	606	640	803	700	1494				
c03600	<i>sucA</i>	Succinyl-CoA ligase [ADP-forming] alpha subunit									82	184	179	179	482	170					336	270	434	553	759	634	431	744	539	1707				
c03550	<i>lpd2</i>	Dihydrolipoyl dehydrogenase 2	-2.9	-2.1	-1.8	2.0	2.0	1.4	1.3	1.5											38								782			167	404	
c03610	<i>sucD</i>	Succinyl-CoA ligase [ADP-forming] beta subunit	-1.6	-1.3	-1.4	-1.1	1.0	1.1	-1.0	1.0	415	462	488	739	535	229	174	117			108	83	68	74	78	94	168	146	265	618	207	79	182	
c03630	<i>sucC</i>	Succinate dehydrogenase cytochrome <i>b</i> ₅₅₈ subunit	-1.0	1.0	-1.2	-1.4	-1.6	-2.5	-2.2	-3.5	295	455	440	438	545	496	335	150	105		214	126	133	114	206	194	233	327	440	1113	226	108	433	
c03740	<i>sdhC</i>	Succinate dehydrogenase hydrophobic membrane anchor subunit									477	610	831	812	842	800	551	231	144		59	30	89	59	63		134	58	66	65				
c03750	<i>sdhD</i>	Succinate dehydrogenase flavoprotein subunit	1.1	-1.0	1.1	-1.8	-2.6	-3.6	-4.8	-5.1											65	29	476	857	834	965	903	965						

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Fold change: < -5.0 < -1.5 > |1.5| > 1.5 Metascore: < 200 < 500 > 500 > 1000

Table S4. Compiled intracellular metabolome data for *Phaeobacter inhibens* DSM 17395 during growth with 0.8 mM ammonium (see

Metabolites	<i>n</i> -Fold change in metabolite abundance (relative to 30 h)							
	15 h	20 h	25 h	40 h	50 h	60 h	75 h	90 h
Metabolites with nitrogen								
Amino acids and derivatives								
Alanine	3.34 ± 0.54	2.44 ± 0.44	1.10 ± 0.21	1.11 ± 0.23	0.92 ± 0.19	0.47 ± 0.17	0.02 ± 0.01	0.02 ± 0
Aspartate	5.78 ± 0.91	5.78 ± 0.79	0.55 ± 0.06	1.15 ± 0.14	1.65 ± 0.29	0.50 ± 0.15	n.d.	n.d.
β-Alanine	3.35 ± 0.53	3.00 ± 0.34	1.57 ± 0.19	0.97 ± 0.10	0.44 ± 0.07	0.15 ± 0.05	n.d.	n.d.
Glutamate	1.89 ± 0.20	1.84 ± 0.13	0.85 ± 0.09	1.21 ± 0.05	0.95 ± 0.08	0.33 ± 0.08	0.01 ± 0	0.00 ± 0
Glycine	1.63 ± 0.09	1.05 ± 0.06	1.32 ± 0.11	1.07 ± 0.07	0.37 ± 0.06	0.09 ± 0.03	0.00 ± 0	0.00 ± 0
Homoserine	1.70 ± 0.22	1.33 ± 0.14	0.91 ± 0.07	0.84 ± 0.06	0.94 ± 0.14	0.95 ± 0.09	1.25 ± 0.10	1.61 ± 0.15
Isoleucine	6.79 ± 1.35	9.81 ± 2.22	1.75 ± 0.42	1.39 ± 0.31	0.61 ± 0.11	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.02
Leucine	14.8 ± 4.8	19.7 ± 7.2	3.50 ± 1.74	2.00 ± 1.09	0.74 ± 0.25	0.26 ± 0.10	0.91 ± 0.74	0.47 ± 0.15
Methionine	6.71 ± 0.77	3.96 ± 0.41	1.85 ± 0.20	1.46 ± 0.16	0.63 ± 0.10	0.52 ± 0.15	n.d.	n.d.
<i>N</i> -Acetyl-glutamate	1.48 ± 0.47	1.63 ± 0.44	1.06 ± 0.21	1.43 ± 0.25	0.77 ± 0.18	0.38 ± 0.09	n.d.	n.d.
<i>N,N</i> -Dimethylglycine	0.40 ± 0.06	0.44 ± 0.06	0.76 ± 0.11	1.38 ± 0.20	2.00 ± 0.28	0.63 ± 0.17	0.14 ± 0.04	n.d.
<i>O</i> -Acetyl-serine	14.3 ± 2.6	30.9 ± 3.2	1.67 ± 0.27	1.04 ± 0.09	n.d.	n.d.	n.d.	n.d.
Phenylalanine	2.62 ± 0.48	2.05 ± 0.17	1.33 ± 0.12	4.36 ± 0.35	0.48 ± 0.04	0.07 ± 0.02	n.d.	n.d.
Proline	3.28 ± 0.63	3.34 ± 0.76	1.26 ± 0.20	1.00 ± 0.25	0.53 ± 0.09	0.31 ± 0.07	0.02 ± 0	0.04 ± 0.01
Pyroglutamate	2.40 ± 0.29	2.14 ± 0.26	1.41 ± 0.15	1.08 ± 0.12	0.61 ± 0.10	0.25 ± 0.05	0.01 ± 0	0.01 ± 0
Serine	2.44 ± 0.53	2.35 ± 0.32	2.06 ± 0.19	1.47 ± 0.28	1.21 ± 0.17	0.39 ± 0.07	0.24 ± 0.14	n.d.
2-Methyl-serine	1.09 ± 0.11	1.02 ± 0.11	1.20 ± 0.13	0.97 ± 0.14	1.19 ± 0.15	1.19 ± 0.21	n.d.	n.d.
Threonine	3.76 ± 0.26	2.92 ± 0.19	1.27 ± 0.09	1.28 ± 0.09	0.61 ± 0.06	0.33 ± 0.07	n.d.	n.d.
Valine	7.33 ± 0.76	4.37 ± 0.67	1.46 ± 0.12	0.86 ± 0.05	0.32 ± 0.02	0.13 ± 0.04	0.03 ± 0	0.06 ± 0
Biogenic amines and dipeptides								
Glycyl-glycine	18.7 ± 2.3	10.8 ± 2.0	0.84 ± 0.17	3.17 ± 0.27	1.15 ± 0.24	0.20 ± 0.06	n.d.	n.d.
Putrescine	11.4 ± 1.7	13.4 ± 1.0	1.61 ± 0.19	1.84 ± 0.11	0.58 ± 0.10	0.75 ± 0.18	n.d.	n.d.
Nucleotides								
Cytosine	17.2 ± 5.8	12.8 ± 6.0	0.92 ± 0.18	1.29 ± 0.25	13.3 ± 5.9	n.d.	n.d.	n.d.
Thymine	3.57 ± 0.90	4.45 ± 1.15	1.33 ± 0.33	1.17 ± 0.33	0.73 ± 0.23	0.24 ± 0.06	n.d.	n.d.
Uracil	3.67 ± 1.22	3.23 ± 1.15	1.09 ± 0.38	1.02 ± 0.39	0.55 ± 0.25	0.28 ± 0.11	0.37	n.d.
Amino alcohols								
Diethanolamine	5.50 ± 1.40	6.77 ± 1.14	1.70 ± 0.29	1.12 ± 0.16	0.99 ± 0.16	0.61 ± 0.15	n.d.	n.d.
Ethanolamine	1.31 ± 0.15	0.76 ± 0.09	0.81 ± 0.22	0.74 ± 0.09	0.70 ± 0.11	0.39 ± 0.08	0.05 ± 0.01	0.07 ± 0.02
Carboxylates (and derivatives)								
3-Aminoisobutanoate	4.20 ± 0.53	4.73 ± 0.46	1.49 ± 0.16	0.83 ± 0.09	0.54 ± 0.06	0.43 ± 0.08	n.d.	n.d.
4-Aminobutanoate	9.28 ± 1.82	7.23 ± 0.99	1.37 ± 0.20	1.23 ± 0.17	n.d.	n.d.	n.d.	n.d.
5-Aminopentanoate	1.61 ± 0.20	1.54 ± 0.20	0.76 ± 0.09	3.38 ± 0.42	1.31 ± 0.21	0.98 ± 0.35	n.d.	n.d.
Iminodiacetate	4.30 ± 0.73	2.03 ± 1.12	2.67 ± 0.30	0.40 ± 0.04	0.21 ± 0.03	0.06	n.d.	n.d.
Nicotinate	1.39 ± 0.13	1.39 ± 0.18	1.13 ± 0.15	5.60 ± 0.81	2.84 ± 0.33	0.77 ± 0.11	n.d.	n.d.
Nicotinamide	2.52 ± 0.86	2.59 ± 0.90	0.77 ± 0.10	0.63 ± 0.09	2.18 ± 0.70	1.36 ± 0.42	n.d.	n.d.
Sugars								
<i>N</i> -Acetylglucosamine	1.74 ± 0.17	1.63 ± 0.19	2.65 ± 0.62	1.07 ± 0.08	0.75 ± 0.05	n.d.	n.d.	n.d.
Metabolites without nitrogen								
Glycolysis (ED and EMP pathway)								
Glucose	0.99 ± 0.06	0.88 ± 0.06	1.48 ± 0.12	0.56 ± 0.03	0.30 ± 0.02	0.20 ± 0.02	0.06 ± 0.01	0.04 ± 0
Hexose-6-phosphate	n.d.	0.11 ± 0.04	0.74 ± 0.26	0.54 ± 0.18	n.d.	n.d.	n.d.	n.d.
Glucono-1,5-lactone	0.40 ± 0.08	0.37 ± 0.04	2.06 ± 0.30	0.26 ± 0.03	0.19 ± 0.02	0.18 ± 0.02	0.02 ± 0	n.d.
2-Keto-3-deoxy-gluconate (KDG)	0.93 ± 0.10	0.52 ± 0.06	2.05 ± 0.17	1.44 ± 0.14	0.30 ± 0.05	0.16 ± 0.03	n.d.	n.d.
Dihydroxyacetone-phosphate	0.85 ± 0.15	n.d.	1.08 ± 0.05	0.69 ± 0.04	0.41 ± 0.09	n.d.	n.d.	n.d.
1,3-Dihydroxyacetone	1.02 ± 0.14	0.93 ± 0.15	1.52 ± 0.21	0.84 ± 0.11	0.65 ± 0.10	0.54 ± 0.07	n.d.	n.d.
Fructose	0.88 ± 0.14	1.40 ± 0.15	1.49 ± 0.10	0.64 ± 0.14	0.35 ± 0.02	0.32 ± 0.03	0.12 ± 0.01	n.d.
Fructose-1-phosphate	n.d.	0.16 ± 0.06	0.76 ± 0.27	0.50 ± 0.16	n.d.	n.d.	n.d.	n.d.
Fructose-6-phosphate	n.d.	0.86	0.95 ± 0.12	0.55 ± 0.07	n.d.	n.d.	n.d.	n.d.
3-Phosphoglycerate	0.29 ± 0.03	0.20 ± 0.03	0.71 ± 0.13	0.34 ± 0.03	0.53 ± 0.14	0.19 ± 0.09	n.d.	n.d.
2-Phosphoglycerate	1.19 ± 0.13	0.99 ± 0.07	1.11 ± 0.09	0.82 ± 0.05	0.81 ± 0.09	n.d.	n.d.	n.d.
Phosphoenolpyruvate	1.50 ± 0.13	1.26 ± 0.09	1.32 ± 0.12	0.84 ± 0.05	0.62 ± 0.07	n.d.	n.d.	n.d.
Pyruvate	0.81 ± 0.15	0.39 ± 0.04	0.89 ± 0.13	0.94 ± 0.09	0.39 ± 0.07	0.14 ± 0.02	0.12 ± 0.02	0.18 ± 0.02
TCA cycle								
Citrate	0.90 ± 0.29	0.31 ± 0.10	0.93 ± 0.16	0.31 ± 0.05	0.43 ± 0.11	0.52 ± 0.18	0.41 ± 0.14	n.d.
2-Oxoglutarate	0.99 ± 0.13	0.68 ± 0.04	1.53 ± 0.10	1.35 ± 0.10	0.48 ± 0.05	0.31 ± 0.03	n.d.	n.d.
Succinate	2.08 ± 0.27	2.41 ± 0.42	2.30 ± 0.39	1.43 ± 0.23	0.77 ± 0.14	0.29 ± 0.06	0.02 ± 0.01	0.09 ± 0.02
Fumarate	2.38 ± 0.35	2.36 ± 0.43	1.63 ± 0.22	1.43 ± 0.25	0.88 ± 0.14	0.30 ± 0.05	0.12 ± 0.02	0.14 ± 0.02
Malate	2.19 ± 0.28	1.28 ± 0.14	2.13 ± 0.20	1.75 ± 0.21	0.47 ± 0.06	0.12 ± 0.02	n.d.	n.d.
Phospholipids								
Glycerol-3-phosphate	0.65 ± 0.08	0.21 ± 0.02	0.81 ± 0.07	0.69 ± 0.06	0.38 ± 0.04	0.11 ± 0.02	n.d.	n.d.
Glycerophosphoglycerol	0.35 ± 0.16	0.16 ± 0.04	0.39 ± 0.09	1.30 ± 0.28	0.82 ± 0.43	n.d.	n.d.	n.d.
Octanoate (C _{8:0})	1.04 ± 0.34	0.53 ± 0.19	0.69 ± 0.24	0.58 ± 0.20	0.71 ± 0.23	0.74 ± 0.25	0.70 ± 0.24	0.83 ± 0.30
3-Hydroxydecanoate (C _{10:0, 3-OH})	1.22 ± 0.15	0.86 ± 0.12	0.93 ± 0.14	0.82 ± 0.11	0.84 ± 0.15	0.65 ± 0.12	0.46 ± 0.07	0.56 ± 0.09
Dodecanoate (C _{12:0})	1.33 ± 0.17	1.08 ± 0.16	1.01 ± 0.14	1.10 ± 0.28	0.97 ± 0.13	1.00 ± 0.14	1.07 ± 0.14	1.45 ± 0.18
Tetradecanoate (C _{14:0})	1.34 ± 0.12	1.39 ± 0.12	1.29 ± 0.16	1.07 ± 0.20	0.71 ± 0.10	0.71 ± 0.08	0.63 ± 0.08	1.02 ± 0.07
Hexadecanoate (C _{16:0})	1.30 ± 0.27	1.62 ± 0.33	1.48 ± 0.37	1.04 ± 0.22	0.54 ± 0.21	0.66 ± 0.24	0.74 ± 0.14	1.31 ± 0.26
Octadecanoate (C _{18:0})	0.87 ± 0.20	1.00 ± 0.20	1.30 ± 0.31	1.03 ± 0.16	0.50 ± 0.28	0.39 ± 0.16	0.53 ± 0.11	1.13 ± 0.30
Octadecenoate (C _{18:1})	1.27 ± 0.21	0.95 ± 0.20	1.00 ± 0.20	0.72 ± 0.12	0.75 ± 0.16	0.71 ± 0.18	n.d.	n.d.

Table S4 continued

Metabolites	<i>n</i> -Fold change in metabolite abundance (relative to 30 h)							
	15 h	20 h	25 h	40 h	50 h	60 h	75 h	90 h
Carboxylates								
2-Hydroxybutanoate	1.39 ± 0.12	1.17 ± 0.07	1.09 ± 0.11	0.95 ± 0.07	0.82 ± 0.10	0.70 ± 0.07	0.80 ± 0.10	1.03 ± 0.10
3-Hydroxybutanoate	0.16 ± 0.02	0.18 ± 0.03	0.89 ± 0.08	1.08 ± 0.10	3.06 ± 0.31	3.77 ± 0.41	0.12 ± 0.02	0.07 ± 0.01
4-Oxobutanoate	1.43 ± 0.20	1.29 ± 0.17	1.18 ± 0.16	0.99 ± 0.14	0.68 ± 0.09	0.51 ± 0.08	n.d.	n.d.
Benzoate	1.24 ± 0.25	1.03 ± 0.21	1.22 ± 0.26	0.85 ± 0.16	0.97 ± 0.17	0.61 ± 0.11	0.75 ± 0.12	0.94 ± 0.14
Erythronate	1.02 ± 0.10	1.35 ± 0.15	1.40 ± 0.12	0.62 ± 0.05	0.38 ± 0.04	0.13 ± 0.01	n.d.	n.d.
Glycerate	0.66 ± 0.10	0.61 ± 0.10	0.70 ± 0.11	0.52 ± 0.07	2.01 ± 0.38	2.51 ± 0.54	0.34 ± 0.04	0.48 ± 0.07
2-Hydroxyglutarate	0.66 ± 0.08	0.45 ± 0.04	1.50 ± 0.17	1.41 ± 0.14	0.38 ± 0.05	0.33 ± 0.08	n.d.	n.d.
Lactate	0.89 ± 0.22	0.63 ± 0.15	1.00 ± 0.31	1.15 ± 0.35	0.41 ± 0.13	0.50 ± 0.21	0.33 ± 0.10	0.47 ± 0.10
Malonate	1.07 ± 0.18	1.32 ± 0.20	1.24 ± 0.20	1.12 ± 0.19	0.73 ± 0.11	0.60 ± 0.09	0.76 ± 0.10	0.96 ± 0.13
2-Methylmalate	0.89 ± 0.11	0.88 ± 0.12	0.75 ± 0.09	0.81 ± 0.10	0.57 ± 0.07	0.30 ± 0.03	0.21 ± 0.03	n.d.
Succinate methylester	2.07 ± 0.23	2.19 ± 0.34	1.27 ± 0.19	1.04 ± 0.17	1.07 ± 0.13	1.03 ± 0.13	1.31 ± 0.13	1.69 ± 0.17
Tartrate	4.05 ± 0.49	13.7 ± 1.3	4.61 ± 0.53	1.27 ± 0.10	0.17 ± 0.02	0.05 ± 0	n.d.	n.d.
Threonate	2.76 ± 0.42	5.08 ± 0.55	1.82 ± 0.16	0.97 ± 0.08	0.30 ± 0.02	n.d.	n.d.	n.d.
Toluate	1.62 ± 0.13	1.63 ± 0.17	1.20 ± 0.12	0.93 ± 0.09	0.83 ± 0.08	0.48 ± 0.06	0.35 ± 0.06	0.57 ± 0.06
Sugars								
1,6-Anhydroglucose	2.25 ± 0.24	1.98 ± 0.23	2.22 ± 0.31	1.06 ± 0.12	0.19 ± 0.03	n.d.	n.d.	0.61 ± 0.13
6-Deoxymannose	7.36 ± 0.38	11.4 ± 1.0	1.31 ± 0.08	1.51 ± 0.13	0.71 ± 0.05	0.68 ± 0.10	0.47 ± 0.03	0.74 ± 0.09
Arabinosate	0.63 ± 0.04	0.75 ± 0.04	1.60 ± 0.09	0.78 ± 0.04	0.33 ± 0.02	0.20 ± 0.01	n.d.	n.d.
Erythrose/threose	0.60 ± 0.12	0.70 ± 0.16	0.92 ± 0.21	0.88 ± 0.17	0.59 ± 0.12	0.53 ± 0.08	0.53 ± 0.10	0.66 ± 0.12
Galactarate	1.05 ± 0.26	2.19 ± 0.53	1.81 ± 0.27	0.96 ± 0.08	0.55 ± 0.09	n.d.	n.d.	n.d.
Gluconate	0.41 ± 0.09	0.47 ± 0.11	1.53 ± 0.18	0.48 ± 0.03	0.20 ± 0.07	0.10 ± 0.04	0.01 ± 0	n.d.
Glucosheptonate-1,4-lactone	0.89 ± 0.06	0.91 ± 0.15	1.22 ± 0.09	0.98 ± 0.08	0.49 ± 0.08	n.d.	n.d.	n.d.
Gluconate-1,4-lactone	1.19 ± 0.40	0.64 ± 0.24	2.90 ± 0.90	0.50 ± 0.17	0.55 ± 0.17	1.40 ± 0.52	0.45 ± 0.17	0.24 ± 0.07
Glucuronate-3,6-lactone	1.68 ± 0.33	6.12 ± 1.56	2.03 ± 0.18	1.01 ± 0.08	0.08 ± 0.02	0.08	n.d.	n.d.
Ribose/xylose	1.02 ± 0.09	0.93 ± 0.08	1.00 ± 0.08	0.87 ± 0.06	0.80 ± 0.06	0.68 ± 0.05	0.54 ± 0.07	0.36 ± 0.06
Ribose-/xylose-5-phosphate	1.10 ± 0.26	0.64 ± 0.14	0.94 ± 0.17	0.94 ± 0.15	0.63 ± 0.29	0.45	n.d.	n.d.
Unclassified								
Borate	0.73 ± 0.22	3.68 ± 0.56	1.30 ± 0.23	2.60 ± 0.41	0.66 ± 0.34	0.05 ± 0.03	0.16 ± 0.04	0.38 ± 0.09
Diphosphate	1.40 ± 0.64	1.27 ± 0.54	1.49 ± 0.51	1.25 ± 0.52	0.44 ± 0.18	0.19 ± 0.06	0.22 ± 0.06	0.34 ± 0.10
Phosphate	1.22 ± 0.26	0.91 ± 0.17	1.09 ± 0.29	0.93 ± 0.17	0.69 ± 0.21	0.41 ± 0.09	0.16 ± 0.04	0.24 ± 0.05
Phosphate monomethyl ester	1.35 ± 0.25	1.14 ± 0.24	1.02 ± 0.19	0.81 ± 0.14	0.78 ± 0.14	0.85 ± 0.19	n.d.	n.d.
Propane-1,2-diol	1.95 ± 0.39	1.74 ± 0.24	1.11 ± 0.18	1.08 ± 0.20	0.98 ± 0.17	0.86 ± 0.14	1.00 ± 0.14	1.78 ± 0.28
Fold change: (n.d., not detected)								
	> 10.00	> 5.00	> 2.50	0.67 – 1.50	< 0.67	< 0.40	< 0.20	< 0.10