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Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots

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Abstract

We determined the actual (no tryptophan, Trp, addition) and potential (Trp 0.1 g l^{-1} added) production of indolic and phenolic compounds produced in different culture media by *Paenibacillus polymyxa* strains which had been isolated from different proximity to wheat roots. The production of indole-3-acetic acid (IAA) and related indolic metabolites by all strains tested was greatly enhanced upon Trp addition, suggesting that Trp is important as IAA precursor. A gradual decrease in potential IAA and an increase in potential indole-3-ethanol and indole-3-lactic acid production by strains isolated from non-rhizosphere soil (NRS) over rhizosphere soil (RS) to the rhizoplane (RP) were obtained. The NRS strain showed also a higher (potential) production of oxidized compounds such as indole-3-carboxylic and benzoic acid besides IAA. The determined metabolic differences indicate together with previous results on genetic and physiological parameters of *P. polymyxa* populations that plant roots select different *P. polymyxa* subpopulations. Our results suggest that the selected genotypes are differentially adapted to the decreasing oxygen pressure from NRS to RP. Possible effects of the probably microhabitat-specific metabolic profiles of the *P. polymyxa* isolates for plant growth are discussed.

Keywords: Auxin; Indole-3-acetic acid; Indole-3-ethanol; Tryptophan; *Paenibacillus polymyxa*; Plant growth promotion

1. Introduction

Paenibacillus polymyxa (formerly *Bacillus polymyxa* [1]) is a Gram-positive spore-forming dinitrogen-fixing bacterium commonly found in soil and in the rhizosphere. *P. polymyxa* strains have been isolated from the rhizosphere of different Gramineae, forage plants [2–4] and from within lodgepole pine roots [5].

The association between plants and *P. polymyxa* seems to be specific and to involve co-adaptation

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Abbreviations: AnA, anthranilic acid; BA, benzoic acid; cfu, colony-forming units; IAA, indole-3-acetic acid; IAld, indole-3-aldehyde; IAM, indole-3-acetamide; ICA, indole-3-carboxylic acid; Ind, indole; NRS, non-rhizosphere soil; PGP, plant growth promotion; RP, rhizoplane; RS, rhizosphere soil; TOL, indole-3-ethanol (tryptophol); Trp, L-tryptophan; YE, yeast extract

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processes [6]. *P. polymyxa* strains isolated from the wheat rhizoplane (RP), rhizosphere soil (RS) and non-rhizosphere soil (NRS) were different with respect to various phenological, physiological and genetic microbial parameters [7]. The strains could be clustered into either RP, RS or NRS groups. The highest intraspecies diversity was obtained for the NRS isolates. The selective pressure of the ecological niches wheat RP, RS and NRS seems to create specifically adapted genotypes of *P. polymyxa*. This is probably due to the different properties of root surface, deposited root-derived material and bulk soil. The gradually decreasing availability of energy-rich C-sources from RP to NRS may have particular importance in this regard [8]. The highest selective pressure in the rhizosphere gradient seems to be at the root surface [4].

P. polymyxa has a potential to promote plant growth [6,9–11]. Plant growth promotion (PGP), however, has not always been obtained after inoculation with *P. polymyxa* [12,13]. Reasons for this may be a cultivar specificity for the establishment of a productive association [9], too high nutrient availability of the site [12] and a threshold for inoculation density [14] besides other possible factors. Similar explanations have been proposed for the fact that PGP has not been achieved consistently in inoculation experiments with other plant growth-promoting rhizobacteria (e.g., *Azospirillum* spp.) [15,16].

There are many mechanisms by which plant-associated rhizobacteria can promote plant growth [8,15–17]. The contribution of associative N_2 fixation to PGP appears to be negligible in temperate climates [16,18]. Soil microbial production of phytohormones, particularly of auxin-related compounds such as indole-3-acetic acid (IAA), is regarded as an important source of PGP [15,16,19,20]. IAA production could be demonstrated for different *Bacillus* species [14,21]. A heat-labile fraction from *Bacillus* spp. culture filtrates contained IAA and was shown to evoke PGP, but IAA production was not quantified [14].

Soil microbial production of indole-3-ethanol (TOL) also seems to be of particular importance in PGP [19,21]: PGP by *B. cereus* was proposed to be due to its production of TOL, but not to the production of IAA which is phytotoxic at high concentrations [20,21]. TOL is easily taken up by plants, serves as an IAA storage form, and can be converted to the active phytohormone, IAA, by plant TOL-oxidase and O_2 [22]. Soil microbial conversion of the auxin precursor L-tryptophan (Trp) seems to be under environmental control. The metabolite production shifts from IAA to TOL immediately after environmental disturbance such as drying/rewetting [23] or freezing/thawing cycles [Lebuhn and Hartmann, in preparation]. The nutrient release caused by such (stress) events can induce microbial activity. Colonizers or *r*-strategists are considered to be favoured at such conditions [24]. High TOL production may therefore be typical for microbial colonizers and/or for activated metabolism at O_2-deficiency which can result from increased microbial activity in soil microhabitats [23,25,26].

We intended to study if subpopulations of soil microbes selected by rhizoplane, rhizosphere and non-rhizosphere conditions have different profiles in Trp metabolism. For this purpose we determined the production of auxin and various other indolic and phenolic compounds by the above-mentioned *P. polymyxa* RP, RS and NRS isolates under different culture conditions. The study should also give an idea on the potential ability of the isolates to affect plant growth by production of these compounds in the rhizosphere.

2. Materials and methods

2.1. Bacterial strains and growth conditions

A large collection of *P. polymyxa* strains had been isolated from the rhizoplane (RP), from rhizosphere soil (RS), and from non-rhizosphere soil (NRS) of 18-day-old spring wheat (*Triticum aestivum* L., cv. Fidel) by immunotrapping. The strains had been characterized by various phenotypic, physiological, serological and genetic features [4,7]. We tested RP strains PMD216 and PMD230, RS strains PMD112 and PMD128, and NRS strain PMD66 as representatives of the different *P. polymyxa* RP, RS and NRS groups [7].

The strains were first cultivated on amidon agar plates [27] for sporulation and proliferation. Comparison of Trp metabolism of the strains was carried out using a M9 medium [28] containing additionally yeast extract (YE) 0.1 g l^{-1} (M9-LY). To investigate effects of YE on Trp metabolism, strain PMD112 was tested as representative additionally in media M9-NY (no YE added) and M9-HY (YE 1 g l^{-1} added).

The strains were precultivated in these M9 media at 30°C and 133 rpm for 12–24 h, depending on the growth rate. After adaptation, aliquots giving an optical density at 436 nm (OD_{436}) of 0.05 were transferred to respective fresh media containing either no Trp or Trp 0.1 g l^{-1}. For the OD measurements, Trp was added at the start of incubation. For the determination of the potential production of indolic and phenolic compounds (see Section 2.2) in the late logarithmic/stationary phase, 5 ml Trp (1 g l^{-1}) were added to 45 ml of the cultures 4 h after inoculation, giving a final concentration of Trp 0.1 g l^{-1}. The cultures (50 ml, Erlenmeyer flasks) were incubated aerobically in the dark at 30°C and 133 rpm for 28 h in total, monitoring the growth curves at OD_{436} throughout the experiments. Until the extraction step, the experiments were performed under sterile conditions.

To determine the colony-forming units (cfu), 100 μl of the logarithmically growing cultures were taken (after 4 h) and plated on amidon agar. Colonies were counted from appropriate dilutions after 2 days of incubation at 30°C. The cfu present in 1 litre of culture medium at OD_{436} of 1 was calculated.

2.2. *Extraction and determination of microbially produced metabolites, actual contents and potential production rates*

The cell suspensions were transferred to sterile 50 ml polypropylene centrifugation vials (Becton-Dickinson, Falcon 2070) and centrifuged at 4500 × *g* and 4°C for 15 min (Heraeus-Sepatech Omnifuge 2,0 RS, swing-out-rotor 3360). The supernatants were filtered at partial vacuum (Sartorius 11106-25-N 0.45 μm cellulose acetate filters, Sartorius SM 16315 glass filtration equipment). Filtrate pH values were adjusted with H_3PO_4 conc. to pH 2.0–2.5.

The protocol of the following steps — solid phase extraction (SPE), HPLC-chromatography at different mobile phase pH values (pH 4.33, pH 2.13), and simultaneous measurement of UV absorption (233 or 280 nm and 316 nm) and fluorescence (excitation/emission: 280/360 nm, 315/405 nm, 340/408 nm) — is described in detail in Refs. [29,30]. The method is highly sensitive, has a high detection capacity, and allows the unambiguous identification and quantification of a broad spectrum of indolic and phenolic compounds. Even substances which are unstable in aqueous solution such as indole-3-pyruvate and indole-3-methanol could be determined months after the SPE extraction as constituents of the ethanolic eluate [29,30].

'Actual contents' are the concentrations of compounds present in the cultures without Trp addition after incubation (28 h). They are corrected for the respective concentrations in the media (see Section 2.3). Thus they represent the cumulative actual production by the strains during 28 h of incubation.

'Potential production rates' describe the production of the investigated compounds during the incubation with Trp 0.1 g l^{-1} added (24 h, see Section 2.1). They are corrected for the respective contents in the media and for abiotic production of compounds due to Trp decay during the incubation (see Section 2.3). Thus they represent the cumulative potential production by the strains in the late logarithmic/stationary phase during 24 h of incubation (plus the negligible actual production during 4 h of incubation without Trp in the lag/early logarithmic phase).

The individual compounds were identified and quantified with the help of the samples 'standard mix', 'medium background' and (the spontaneous) 'Trp decay' (see Section 2.3). The data were calculated for the activity of 10^{10} cfu present in 1 litre of the culture media at OD_{436} of 1 (see Section 2.1) during the incubation period. This standardization enabled us to compare the specific production by living cells, correcting for the presence of dead cells in the culture media measured by the OD_{436} determination.

2.3. *Calibration standards*

Medium background: We detected no indolic and phenolic compounds in sterile M9-NY medium and traces of indole-3-aldehyde (IAld), indole (Ind) and anthranilic acid (AnA) in the media with low (M9-LY) and high (M9-HY) yeast extract concentration. In medium M9-HY, the concentrations of indole-3-acetamide (IAM, traces), indole-3-ethanol (TOL, 0.01 mg l^{-1}), indole-3-acetic acid (IAA, 0.02 mg l^{-1}) and indole-3-carboxylic acid (ICA, 0.05 mg l^{-1}) were higher than in medium M9-LY. Benzoic acid (BA) and Trp were present in medium M9-HY in concentrations up to 0.14 and 9.25 mg l^{-1}, respectively. The signal integrals monitored for the sterile extracted media were subtracted from corresponding integrals monitored for samples from bacterial cultures (without Trp addition) and for standard mix samples.

Trp decay: Trp (0.1 g l^{-1}) was incubated for 24 h in the different media under sterile conditions, acidified and extracted just like the bacterial cultures and the samples standard mix. Peak integrals detected in bacterial culture samples (with Trp added) were corrected for the corresponding peak integrals detected in these samples.

Standard mix: Appropriate amounts of standard compounds were dissolved in the different sterile media, and immediately acidified and extracted just like the bacterial cultures (see Section 2.2). With the help of these samples, the compounds present in the culture samples were identified and quantified. A detailed description of the identification and quantification procedures is presented in [29,30].

Differences between two values were regarded as significant, if the standard deviations did not overlap. Statistical analyses were not carried out.

3. Results and discussion

3.1. Actual contents of indolic and phenolic compounds in sterile M9 media

We detected relatively high amounts of benzoic acid (BA, 0.14 mg l^{-1}) and tryptophan (Trp, 9.25 mg l^{-1}) besides traces of other indolic and phenolic compounds particularly in medium M9-HY (see Section 2.3). The compounds obviously originate from lysed yeast cells and/or from proteolysis. If media with biological components (e.g., YE) are used to determine the production of metabolites such as indole-3-acetic acid (IAA) or salicylic acid (SA), it has to be considered that precursors such as Trp and BA can be present.

3.2. Bacterial growth in different M9 media, and influence and production of tryptophan

All *P. polymyxa* strains grew logarithmically in the M9 media. There were no major differences between the strains in growth in medium M9-LY and in colony formation on amidon agar, with values for living cells present in the cultures (see Section 2.1) in the range of 4.4–6.7 × 10^{10} cfu (L OD_{436})$^{-1}$.

Growth rates were maximal in M9-LY medium after 4–4.5 h. The strains entered the stationary phase in this medium after ca. 14 h. Strain PMD112 entered the stationary phase in medium M9-NY after 22 h and in medium M9-HY after 8 h.

When Trp was added, the early logarithmic growth of the five strains was slightly reduced in medium M9-LY, and the late logarithmic growth was slightly enhanced. With Trp added, the early logarithmic growth of strain PMD112 was slightly decreased in medium M9-NY and slightly increased in medium M9-HY. In contrast, the addition of Trp increased its late logarithmic growth in medium M9-NY, and decreased it in medium M9-HY (not shown).

Without Trp supply, strain PMD112 released 64.7 ± 17.1 mg Trp (10^{10} cfu 28 h)$^{-1}$ in medium M9-NY. All strains depleted Trp from medium M9-LY with values ranging from 49.0 ± 48.3 mg

Table 1
Excretion of auxin metabolites by *P. polymyxa* strains in M9-LY medium not supplemented with L-tryptophan (actual production)

Strain	PMD66 (NRS)	PMD112 (RS)	PMD128 (RS)	PMD216 (RP)	PMD230 (RP)
IAA[a]	0.8 ± 0.3	0.7 ± 0.2	1.5 ± 0.7	4.1 ± 0.7	0.8 ± 0.0
TOL[a]	0.4 ± 0.1	0.5 ± 0.3	0.5 ± 0.5	0.9 ± 0.2	0.3 ± 0.2
ILA[a]	4.2 ± 1.0	2.2 ± 1.6	2.3 ± 2.0	1.1 ± 0.8	6.5 ± 5.8
ICA[a]	1.0 ± 0.6	3.8 ± 3.0	2.3 ± 1.4	3.9 ± 1.7	2.4 ± 2.0

[a] All data in mg (10^{10} cfu 28 h)$^{-1}$. Values are means of 4 measurements ± SD at different chromatographic and spectralanalytic conditions. For abbreviations, see list of abbreviations.

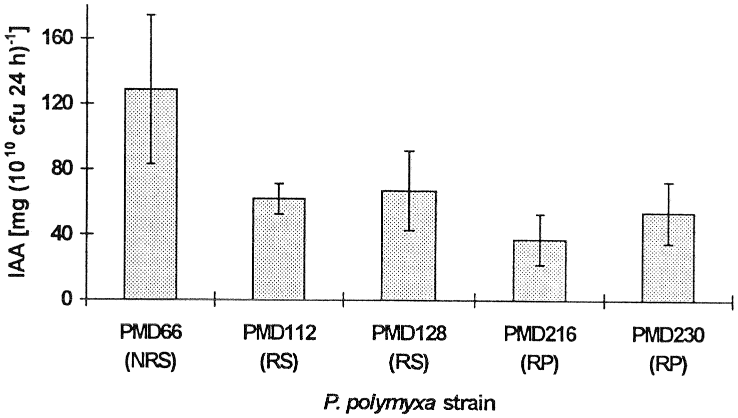

Fig. 1. Potential production of indole-3-acetic acid by *Paenibacillus polymyxa* strains in M9-LY medium supplemented with L-tryptophan (0.1 g l^{-1}).

Trp (10^{10} cfu 28 h)$^{-1}$ for strain PMD216 up to 483.0 ± 89.4 mg Trp (10^{10} cfu 28 h)$^{-1}$ for strain PMD112. From medium M9-HY, strain PMD112 depleted 634.5 ± 137.9 mg Trp (10^{10} cfu 28 h)$^{-1}$. Trp depletion is probably due to Trp uptake by the cells, since the production of various intracellularly produced Trp metabolites was enhanced upon Trp addition (see following sections).

The initial growth delay by Trp at low nutrient conditions (media M9-NY and M9-LY) may be due to toxic effects of the relatively high Trp concentrations. Adaptation during the logarithmic phase may explain the propagated late logarithmic growth by Trp under low nutrient conditions. High nutrient supply initially seems to counteract Trp toxicity. Earlier entry of the culture into the stationary phase, however, may have been caused by accelerated cell death due to increased nutritional limitations and the enhanced Trp uptake at high nutrient supply when Trp was added.

Studies on *Azospirillum brasilense* also suggested toxic effects of Trp addition [31]. The authors proposed that auxin metabolism may be a way of Trp detoxification. The data on metabolite pool sizes indicate that *A. brasilense* and *P. polymyxa* have almost no capacity for Trp degradation over the aromatic and quinoline pathways, as compared to *A. lipoferum* [32] (see Section 3.4). Tryptophanase seems to be inducible in both *Azospirillum* species but not in *P. polymyxa* [32] (see Section 3.4). These deficiencies could be reasons for toxic Trp effects in *P. polymyxa*, since auxin metabolism was intense in all three species [32] (see Section 3.3).

Trp serves as auxin precursor and is readily con-

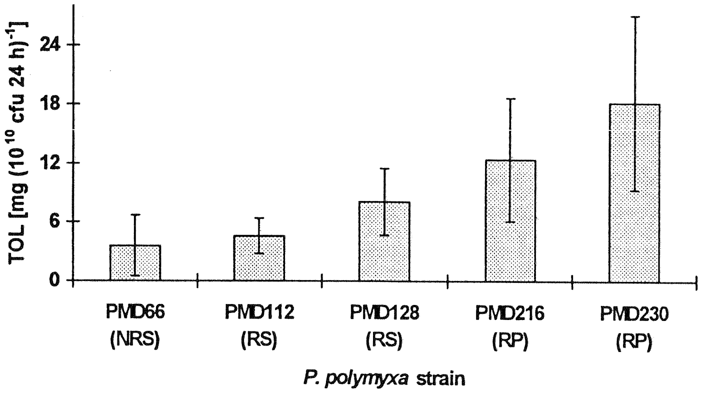

Fig. 2. Potential production of indole-3-ethanol by *Paenibacillus polymyxa* strains in M9-LY medium supplemented with L-tryptophan (0.1 g l^{-1}).

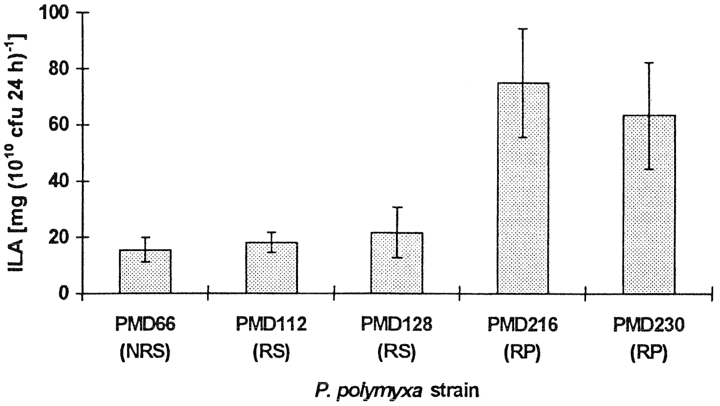

Fig. 3. Potential production of indole-3-lactic acid by *Paenibacillus polymyxa* strains in M9-LY medium supplemented with L-tryptophan (0.1 g l^{-1}).

verted by soil microbiota to various compounds including IAA [19,23,29,32]. Microbes releasing Trp such as *P. polymyxa* and azospirilla [32] could indirectly contribute to plant growth promotion if they excrete Trp also in the rhizosphere.

3.3. Actual and potential production of auxin metabolites by P. polymyxa strains

All tested *P. polymyxa* strains released the auxin metabolites IAA, TOL, ILA and ICA in non-Trp-supplemented M9-LY medium (actual production) (Table 1). Actual IAld excretion could not be shown for all strains. With the exception of the IAA intermediate IAM, the production of auxin metabolites was increased upon Trp addition (potential production) (Tables 1 and 2, Figs. 1–3). This indicates that Trp is a precursor of the auxin metabolites in *P. polymyxa*.

The addition of yeast extract increased the excretion of IAA and IAld by strain PMD112, but not of ILA, ICA, IAM and TOL (without Trp supply) (Table 2) and also not of benzoic acid (see Section 3.4). The nutrient status seems therefore to be one factor which can influence the production pattern of the different Trp metabolites.

The patterns of the specific potential production of IAA, TOL, ILA and ICA depended on the place of isolation of the strains. The potency for IAA (and ICA, not shown) production increased from RP to NRS isolates, and the potency for ILA and TOL production was enhanced with increasing proximity of the isolation place to the rhizoplane, from NRS to RP isolates (Figs. 1–3). These gradients were not observed without Trp addition, indicating that the metabolic differences are induced above a Trp threshold concentration.

TOL, ILA (and IAld) are preferentially produced from Trp by soil microbes under microaerobic/anoxic conditions, whereas oxidase-mediated IAA

Table 2
Actual and potential production of auxin metabolites by *P. polymyxa* strain PMD112 in different M9 media

Medium	Variant	IAA	TOL	IAM	ILA	IAld	ICA
M9-NY	−Trp[a]	1.3±0.3	traces	0.6±0.0	5.2±3.9	n.d.	18.9±9.5
M9-LY	−Trp[a]	0.7±0.2	0.5±0.3	0.9±0.1	2.2±1.6	0.1±0.1	7.2±4.4
M9-HY	−Trp[a]	4.6±1.0	1.2±0.5	−1.4±1.1	4.3±3.6	1.1±0.4	3.0±1.8
M9-NY	+Trp[b]	44.6±10.9	14.4±1.0	17.7±0.8	39.3±8.1	15.9±4.6	53.0±22.6
M9-LY	+Trp[b]	62.0±9.4	4.6±1.8	−1.1±1.1	18.2±3.7	31.1±14.3	15.8±5.1
M9-HY	+Trp[b]	91.7±17.0	4.6±1.7	−1.7±2.7	18.6±3.9	85.1±13.0	30.0±12.7

[a] Data in mg (10^{10} cfu 28 h)$^{-1}$. −Trp = actual release (no Trp addition).

[b] Data in mg (10^{10} cfu 24 h)$^{-1}$; +Trp = potential production (with Trp 0.1 g l^{-1} added).

Means of 4 measurements ± SD at different chromatographic and spectralanalytic conditions.

For media composition and abbreviations, see Section 2.1 and list of abbreviations.

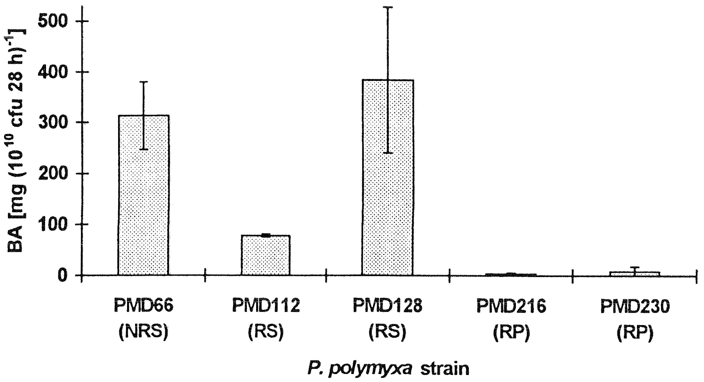

Fig. 4. Release of benzoic acid by *Paenibacillus polymyxa* strains in M9-LY medium not supplemented with L-tryptophan.

(and ICA) production is reduced [Lebuhn and Hartmann, in preparation]. We assume, therefore, that the decreasing oxygen pressure towards the rhizoplane [33] favours on the one hand RP ecotypes adapted by higher potential dehydrogenase expression and/or activity to RP conditions, and on the other hand NRS ecotypes adapted by higher potential oxidase expression and/or activity to NRS conditions. RS ecotypes seem to be intermediary in this regard. Previous results suggesting that the plant root exerts selective pressure on *P. polymyxa* populations in soil [7] support this hypothesis. High potential TOL, ILA (and IAld) production may be typical for colonizer metabolism, since potential soil microbial production of the reduced auxin metabolites is greatly increased under *r*-typical conditions [23, Lebuhn and Hartmann, in preparation]. In contrast, high potency for IAA, ICA (and BA, see Section 3.4) production may be a typical sustainer metabolism profile. This would argue for colonizer metabolism (high *r*) of microbes at the densely crowded root surface (*K*-conditions), suggesting that the *r*-/*K*-concept may not be valid in soil microbial ecology in terms of spatial resolution.

RP genotypes selected by the plant root are also characterized by higher chitinase production and soil-borne phytopathogen inhibition than NRS genotypes [34] besides higher potential TOL, ILA (and IAld) production. Hence several PGP activities seem to be more expressed in strains adapted to RP conditions. This argues for synergistic co-evolution between roots and associated microorganisms.

The production of the auxin catabolites, IAld and ICA, was about as intense as IAA production by the *P. polymyxa* strains (Tables 1 and 2). Potential ICA production was highest for NRS and potential IAld production for RP strains (not shown). Since ICA is an IAld-oxidase-mediated oxidation product of IAld [19], this supports our assumption that NRS conditions favour microbial ecotypes being better adapted to a higher oxygen pressure.

Some strains released and some took up IAM. Strain PMD112 released IAM in medium M9-NY and took up IAM in medium M9-HY (Table 2). The higher uptake of the IAA precursor IAM may be one reason for the more intense IAA production in medium M9-HY (Table 2). The detection of ILA, TOL in all and of IAM in at least some *P. polymyxa* cultures indicates the occurrence of the indole-3-pyruvic acid and the IAM pathways of auxin biosynthesis in *P. polymyxa* [19,35]. In addition, two further compounds showing high fluorescence at 280/360 nm were detected in all *P. polymyxa* cultures, and their production was increased upon Trp addition (not shown). Since they were not identical to any known auxin metabolite, they might be involved in a third Trp-dependent auxin biosynthesis pathway [33,35]. There was no evidence for the occurrence of the tryptamine, indole-glucosinolate and Trp-side-chain-oxidase pathways [19,26] in *P. polymyxa*. Indole-3-acrylic acid, indole-3-butyric acid and 5-hydroxylated indoles were also not detected in the cultures.

On the basis of the given culture conditions, IAA production of the *P. polymyxa* strains was about as intense as that of *A. brasilense* and *A. lipoferum* strains [32] which are highly potent IAA producers [15]. *P. polymyxa* might hence promote plant growth in the rhizosphere by IAA production which is regarded as an important PGP activity of plant-associated microbes [8,14–16,19,21,32,35]. IAA, however, can cause phytotoxic effects at high concentrations [20,21,35]. Indeed, lateral root growth of wheat was reduced at high inoculation density of *P. polymyxa* CF43 (10^{10} cfu l^{-1}) in the spermosphere model [T. Heulin, unpublished results]. This might be due to IAA overproduction by this strain at the rhizoplane.

TOL and ILA production by the *P. polymyxa* strains was even higher than that of *Azospirillum* cultures [32]. The *P. polymyxa* RP strains showed a higher ratio of potential production of TOL (and ILA) to IAA, as compared to the ratios for RS and even more to NRS strains. They showed also a higher potential auxin catabolism to IAld. This metabolic profile of *P. polymyxa* RP strains could: (1) avoid phytotoxic IAA concentrations near the rhizoplane which can be raised particularly in this compartment because the concentrations of the auxin precursor Trp are significantly higher in RS than in NRS soil [M. Lebuhn, unpublished results]; (2) promote plant growth by the action of TOL which is a plant IAA storage compound [22]; (3) diminish too high auxin concentrations near the plant root by effective auxin catabolism; (4) counteract exogenous phytotoxic IAA levels by ILA production. ILA was proposed to act as IAA antimetabolite in the plant [36], but new results refute this theory [37].

We hypothesize that at least some effects of too high inoculation density constraining plant growth [14–16] may be due to induced IAA overproduction in the rhizosphere by the introduced microorganisms. We propose that RP-associated microbes should be screened for high TOL production for inoculation purposes. Inoculation with such strains might result in more stable plant growth promotion.

3.4. *Actual and potential production of other indolics and phenolics by P. polymyxa strains*

Only traces of indole, kynurenine, kynurenic acid and anthranilic acid were detected in the culture supernatants. They were not significantly enhanced when Trp was added. If these compounds are not readily converted by subsequent metabolism, tryptophanase and regulative enzymes of the kynurenine, quinoline and aromatic pathways probably are only weakly expressed in *P. polymyxa*, and/or are not inducible by Trp. Salicylic acid was detected only in trace amounts in M9-HY cultures of RP strains.

P. polymyxa NRS strain PMD66 and RS strains PMD112 and PMD128 excreted more benzoic acid (BA) in M9-LY medium than RP strains PMD216 and PMD230 (Fig. 4). Strain PMD112 released more BA in medium M9-NY (234.0 ± 34.9 mg (10^{10} cfu 28 h)$^{-1}$) than in medium M9-LY (78.1 ± 3.2 mg (10^{10} cfu 28 h)$^{-1}$), and took up BA from medium M9-HY (7.6 ± 4.0 mg (10^{10} cfu 28 h)$^{-1}$). BA production was not increased upon Trp addition, indicating that BA production by *P. polymyxa* is not Trp-dependent. Probably it is more related to the metabolism of phenylalanine.

The more oxidative conditions in non rhizosphere soil (cf. Section 3.3) seem to favour *P. polymyxa* strains producing higher amounts of BA. Release of this antimicrobial metabolite may increase the competition power of NRS ecotypes in soil. In contrast, high microbial BA production at the rhizoplane could impede plant growth, since BA can be phytotoxic [8]. Microbial BA release at the rhizoplane might also induce plant (systemic) resistance mechanisms [38].

Our results indicate that the specific conditions (e.g., of oxygen) in the rhizosphere gradient may select different subpopulations of *P. polymyxa* with specific production profiles of phytoeffective indolic and phenolic compounds. However, this is a preliminary interpretation, since we could only study a limited number of RP, RS and NRS strains. Further physiological studies with other strains and also with other root-associated microbial species are needed to prove and generalize this finding. We assume that soil microbial production of secondary metabolites which can promote or constrain plant growth is finely tuned, controlled by environmental conditions and influenced by root activities. A selection at the subspecies level may be part of the interdependence of plant roots and the population structure and function of the root-associated soil microflora.

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