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Enhanced production of indole-3-acetic acid by a genetically modified strain of *Pseudomonas fluorescens* CHA0 affects root growth of cucumber, but does not improve protection of the plant against *Pythium* root rot

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Abstract

The biocontrol strain CHA0 of *Pseudomonas fluorescens* produces small amounts of indole-3-acetic acid via the tryptophan side chain oxidase and the tryptophan transaminase pathways. A recombinant plasmid (pME3468) expressing the tryptophan monooxygenase pathway was introduced into strain CHA0; this resulted in elevated synthesis of indole-3-acetic acid in vitro, especially after addition of L-tryptophan. In natural soil, strain CHA0/pME3468 increased fresh root weight of cucumber by 17–36%, compared to the effect of strain CHA0; root colonization was about 10^6 cells per g of root. However, both strains gave similar protection of cucumber against *Pythium ultimum*. In autoclaved soil, at 6×10^7 cells per g of root, strain CHA0 stimulated growth of roots and shoots, whereas strain CHA0/pME3468 caused root stunting and strong reduction of plant weight. These results are in agreement with the known effects of exogenous indole-3-acetic acid on plant roots and suggest that in the system examined, indole-3-acetic acid does not contribute to the biocontrol properties of strain CHA0.

Keywords: *Pseudomonas fluorescens*; Biocontrol; Indole-3-acetic acid; Auxin; Root growth; *Pythium ultimum*

1. Introduction

Many plant-associated bacteria synthesize the plant hormone and growth regulator indole-3-acetic acid (IAA) [1–4]. Several metabolic pathways lead from L-tryptophan to IAA. The indole-3-acetamide pathway is catalyzed by tryptophan monooxygenase and indoleacetamide hydrolase, the products of the *iaaM* and *iaaH* genes, respectively, e.g. in *Pseudomonas syringae* subsp. *savastanoi* [5]. The indole-3-pyruvate pathway is initiated by transamination of tryptophan; indole-3-pyruvate is decarboxylated, and subsequent oxidation of indole-3-acetaldehyde results in IAA, e.g. in *Enterobacter* and *Azospirillum* spp. [6,7]. In the tryptophan side chain oxidase pathway of *Pseudomonas fluorescens*, indole-3-acetaldehyde is also a key intermediate [8,9]. Additional

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IAA pathways, one of which may be independent of L-tryptophan, have been reviewed elsewhere [1,4].

Do phytohormones produced by root-associated bacteria enhance plant growth? There is evidence that part of the plant growth-promoting effect of *Azospirillum brasilense* may be due to IAA production [1,4]. Moreover, application of tryptophan to the rhizosphere can stimulate vegetative growth of corn and this effect has been ascribed to the conversion of tryptophan to IAA by rhizosphere bacteria [10]. However, no totally IAA-deficient mutants have yet been isolated in *A. brasilense* or in other root-associated bacteria, as there are multiple IAA pathways in these organisms [1,4,9]. For this reason, it is difficult to relate plant-beneficial effects directly to bacterial IAA production.

By contrast, plant-deleterious effects related to high IAA concentrations of bacterial origin are well documented [11]. Olive and oleander knot caused by *P. syringae* subsp. *savastanoi* [12], crown galls developing through infection by *Agrobacterium tumefaciens* [13], and galls induced by *Erwinia herbicola* pv. *gypsophilae* [14] are all manifestations of local overproduction of IAA (and cytokinins).

P. fluorescens strain CHA0 is a root-colonizing bacterium whose plant-beneficial effects essentially depend on the suppression of root-pathogenic fungi [15,16]. Strain CHA0 produces IAA in vitro via the tryptophan side chain oxidase and the indolepyruvate pathways [9], but in quantities that are two orders of magnitude lower than those synthesized by plant-beneficial bacteria, such as *A. brasilense* SpM7918 [17] and *P. fluorescens – putida* B10 [18]. We therefore asked whether, by increasing IAA production, we could improve the plant growth-promoting effects of strain CHA0. To this end, we introduced the *iaaMH* genes of *P. syringae* subsp. *savastanoi* into strain CHA0. The CHA0 recombinant strains expressing these genes produced elevated levels of IAA via the indoleacetamide pathway, which is not present naturally in the wild-type strain CHA0 [9].

2. Materials and methods

2.1. Bacterial strains and growth conditions

Escherichia coli strain DH5α [19] and *P. fluorescens* strain CHA0 [15,16] were grown on nutrient

Table 1
Plasmids used in this study

Plasmid	Vector	Characteristics[a]	Reference
pCP3	pBR328	Containing *iaaM* and *iaaH* from pIAA1 downstream of the vector's Cm^R promoter; Ap^R	[5]
pME6000	pBBR1MCS	Broad-host-range cloning vector having about 18 copies in *P. fluorescens*; Tc^R	[21]
pME3468	pME6000	Carrying the 4.0-kb *Ssp*I–*Nco*I fragment from pCP3 containing *iaaM* and *iaaH* downstream of the Cm^R promoter from pCP3; Tc^R	This study, Fig. 1
pME3469	pME6000	Carrying the 1.3-kb *Pst*I–*Bam*HI fragment from pSAV401 and the 9.5-kb *Spe*I–*Bam*HI fragment from pME3468; containing *iaaM* and *iaaH* behind the *iaa* promoter[b]; Tc^R	This study, Fig. 1
pME3470	pME6000	Cm^R promoter-less derivative of pME3468, deleted for the 0.7-kb *Eco*RI–*Eco*RI fragment; Tc^R	This study, Fig. 1
pSAV401	pUC118	Carrying the 1.3-kb *Pst*I–*Bam*HI fragment containing the 5′-portion of *iaaM* from pIAA2;Ap^R	Scott Soby, personal communication; [22]

[a] Ap^R, ampicillin resistance; Cm^R, chloramphenicol resistance; Rif^R, rifampicin resistance; Tc^R, tetracycline resistance.
[b] Note that the *iaaMH* genes, including their promoter, are the same on pIAA1 and pIAA2 [23].

agar (NA) and in nutrient yeast broth (NYB) as previously described [9]. *P. syringae* subsp. *savastanoi* strain 229 was isolated from *Nerium oleander* (R. Grimm, Swiss Federal Research Station, Wädenswil, Switzerland) and grown on solid medium B [12]. *P. fluorescens* CHA0 and its rifampicin-resistant derivative CHA0-Rif [20] were cultivated on NA and in NYB containing rifampicin (100 μg ml^{-1}) when appropriate. Plasmids used in this study are listed in Table 1. NA and NYB were supplemented with tetracycline at 25 μg ml^{-1} for *E. coli* and 125 μg ml^{-1} for *P. fluorescens* whenever the strains harbored the vector pME6000 [21] or a derivative thereof. Bacterial growth was monitored as optical density at 600 nm (OD_{600}). One OD_{600} unit equals 5×10^8 cfu ml^{-1} for *P. fluorescens* strain CHA0 grown in minimal medium OS [24]. For quantitative determination of IAA in vitro, *Pseudomonas* strains were grown at 30°C with shaking (180 rpm) in 100 ml Erlenmeyer flasks containing 20 ml of minimal medium OS without antibiotics. When specified, the medium containing glucose (25 mM) and ammonium sulfate (7.6 mM) was supplemented with 1 mM L-tryptophan (Trp). Triton X-100 (0.05%, w/v) was routinely added to the medium to reduce clumping of the cells. Cultures were started with 1:100 inocula (grown in the same media) and incubated until an OD_{600} value of about 3.5 was reached in stationary phase.

2.2. DNA manipulations and cloning procedures

Small-scale preparations of plasmid DNA from *E. coli* were obtained by the CTAB (hexadecyltrimethylammonium bromide) method [25] and from *P. fluorescens* by the alkaline lysis method [19]. Large-scale preparations were performed using Qiagen-Tips (Qiagen). Restriction-enzyme digestions, ligations and agarose-gel electrophoresis were performed using standard procedures [19]. Restriction fragments were purified from agarose gels using the Geneclean II Kit (BIO 101). Transformation of *E. coli* DH5α in cloning experiments was carried out by the classical $CaCl_2$ procedure [19]. Recombinant plasmids were mobilized from *E. coli* to *P. fluorescens* with the helper plasmid pME497 [26]; selection was on NA with tetracycline (125 μg ml^{-1}), rifampicin (100 μg ml^{-1}) and chloramphenicol (100 μg ml^{-1}).

2.3. Extraction and analysis of IAA and indoleacetamide

An aliquot (5 ml normally, 15 ml for detection of low IAA concentrations) of the culture medium to be analyzed was acidified to pH 2.5 with HCl, centrifuged, and applied to a Silica-C-18 cartridge (Sep Pak, 3 ml; Waters) equilibrated with 10 mM *ortho*-phosphoric acid (H_3PO_4). The cartridge was washed with 2 ml 10 mM H_3PO_4 and eluted with 2 ml 80% (v/v) acetonitrile in 10 mM H_3PO_4. Prior to HPLC analysis, the sample (200 μl) was diluted with an equal volume of 10 mM H_3PO_4. An aliquot of 100 μl was injected into an HPLC system (Hewlett-Packard 1050 series LC system) equipped with a diode array detector (DAD) and a fluorescence detector (FLD). The sample was chromatographed on a Nucleosil 120 reversed-phase C-18 column (length 25 cm, inner diameter 4 mm, particle diameter 5 μm) protected by a 20 mm guard column of identical material (Bischoff). A binary gradient consisting of solvent A (10 mM H_3PO_4) and solvent B (95% (v/v) acetonitrile in 10 mM H_3PO_4) was used as follows: 0–7 min with 14–19% B, 6 min with 19–45% B, 4 min with 45–60% B, 0.5 min with 60–80% B and 4.5 min with 80% B. Elution was carried out at 40°C at a flow rate of 1.2 ml min^{-1}. Compounds were identified by their retention time and UV spectra. Indole-3-acetamide and IAA were eluted at 8.1 and 12.5 min, respectively; they were quantified at 280 nm and by fluorescence using a detector set at 229 nm (excitation) and 345 nm (emission) with a 295-nm cut-off filter and a pulsed Xenon lamp (110 Hz, 2.5 W). IAA was quantified by DAD between 114 and 7 μM and by FLD between 7 and 0.18 μM. The detection limit for IAA and indole-3-acetamide tested by FLD was 5 nM.

2.4. Plant assays

For plant assays, 1-l Erlenmeyer flasks were filled partially with 300 g of a sandy loam soil from Eschikon (Switzerland) [20] and autoclaved twice with an interval of 2 days or left untreated. For disease-suppression assays, 2 mg of a millet seed inoculum of *Pythium ultimum* strain 67-1 [27] was added per gram of soil. Bacterial inocula were prepared as follows. Bacteria were cultivated in Luria–Bertani broth

[19] for 24 h and washed twice with sterile H_2O. The suspensions obtained were added to the soil with a sterile syringe (10 ml par flask) 6 h after introduction of *P. ultimum*. Final bacterial concentration was about 10^7 cfu per g of soil. Two days later, five sterile-grown, 3-day-old cucumber seedlings (*Cucumis sativus* cv. Chinesische Schlange) were planted per flask as previously described [27]. The flasks were placed in a growth chamber with 80% relative humidity and incubated at 22°C with light for 16 h, followed by an 8-h dark period at 18°C. The plants were harvested after 10 days. Statistical analysis was carried out as described [27].

3. Results

3.1. Construction of IAA-overproducing derivatives of *P. fluorescens* CHA0

The IAA biosynthetic genes *iaaMH* from *P. syringae* subsp. *savastanoi* were cloned from plasmid pCP3, a derivative of the natural pIAA1 plasmid, into the multiple-copy-number vector pME6000, resulting in pME3468 (Fig. 1). In this construct, the *iaaMH* genes are expressed from the constitutive chloramphenicol resistance promoter carried by the pCP3 vector moiety. In a second construct, pME3469 (Fig. 1), the *iaaMH* genes were cloned downstream of the native *iaa* promoter [22,23]. In

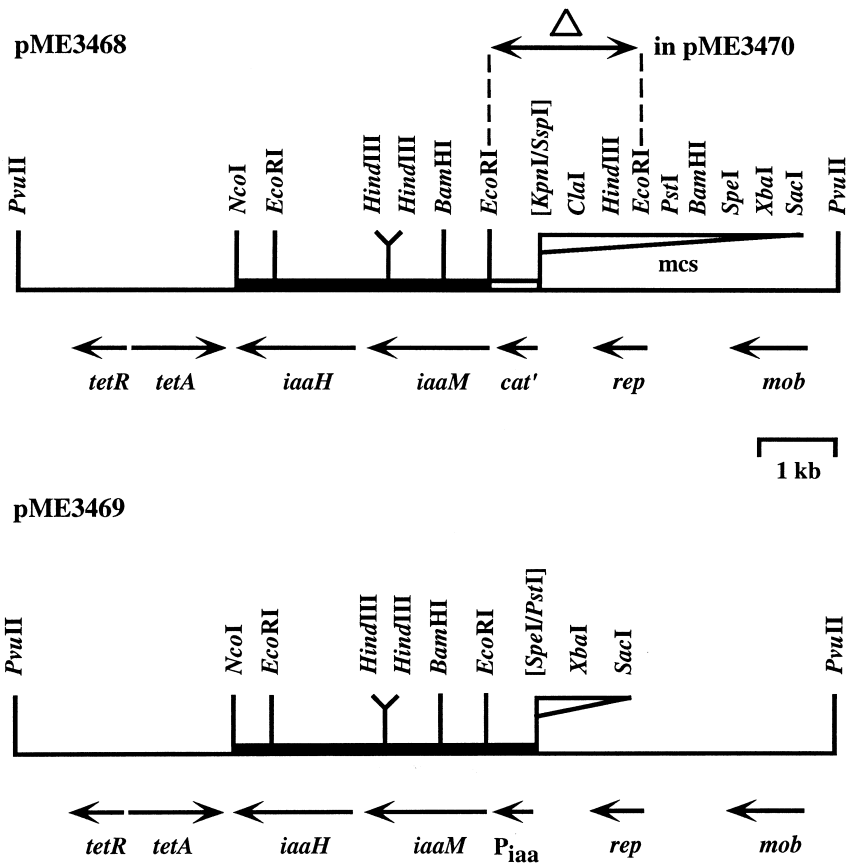

Fig. 1. Restriction maps of *iaaMH* recombinant plasmids. Black bar, *P. syringae* subsp. *savastanoi* insert with *iaaMH* genes [5] and, in the case of pME3469, the 406-bp *iaa* promoter region (P_{iaa}) [22,23]; white bar, segment originating from pBR328 carrying a chloramphenicol resistance fragment with its promoter (*cat'*) [12]; ——, vector pME6000 with tetracycline resistance genes (*tetAR*), replication region (*rep*) and a site for mobilization (*mob*) by IncP plasmids [21]; mcs, multiple cloning site; △, deletion.

Table 2
IAA content in culture supernatants of *Pseudomonas fluorescens* strains

Strain/plasmid	Addition of 1 mM Trp	IAA (μM)
CHA0-Rif	−	< 0.004
CHA0-Rif	+	< 0.004
CHA0-Rif/pME6000	−	< 0.004
CHA0-Rif/pME6000	+	< 0.004
CHA0-Rif/pME3468	−	172 ± 8
CHA0-Rif/pME3468	+	875 ± 8
CHA0-Rif/pME3469	−	208 ± 10
CHA0-Rif/pME3469	+	519 ± 8
CHA0-Rif/pME3470	−	36 ± 1
CHA0-Rif/pME3470	+	125 ± 2

Bacteria were grown in minimal medium OS with or without addition of 1 mM L-tryptophan. Cultures were harvested in early stationary phase (OD_{600} 3.1–3.5) after 42 h of incubation. Results are the means of three replicate cultures (± S.D.). IAA was chemically stable in minimal medium OS without added bacteria. However, IAA may be modified or degraded slowly by *P. fluorescens* in stationary phase (data not shown).

a third construct, pME3470 (Fig. 1), the chloramphenicol promoter of pME3468 was removed by deletion of a 0.7-kb *Eco*RI fragment. This plasmid was constructed to achieve intermediate IAA production, which was due to expression of the *iaaMH* genes from a minor unknown promoter, perhaps located on the vector pME6000.

The *iaaMH* recombinant plasmids were transferred to *P. fluorescens* CHA0 by mobilization, and the transconjugants were tested for IAA production in minimal medium OS with and without addition of L-tryptophan. IAA was not detected in culture supernatants of the wild-type strain CHA0 (data not shown) or its rifampicin-resistant derivative strain CHA0-Rif grown to early stationary phase (Table 2). However, in agreement with earlier results [9], strain CHA0 produced small amounts of IAA in minimal medium when grown to late stationary phase for 72 h. IAA concentrations were 42 nM and 310 nM in media without and with 1 mM L-tryptophan, respectively. The *iaaMH* overexpressing plasmids pME3468 and pME3469, in strain CHA0-Rif, gave strongly (> 1000-fold) elevated IAA levels, especially in medium containing tryptophan, whereas the vector plasmid pME6000 had no effect (Table 2). The construct lacking a strong promoter upstream of the *iaaMH* genes, pME3470, led to the production of lower IAA levels (Table 2). Indoleacetamide, the intermediate in the conversion of tryptophan to IAA via the *iaaMH* pathway, was below the detection limit (5 nM) in the culture supernatants of all strains tested. In terms of IAA production, the recombinant strain CHA0-Rif/pME3468 and the wild-type strain of *P. syringae* subsp. *savastanoi* 229 were similar: the IAA concentrations were 188 and 253 μM, respectively, after incubation in minimal medium with 1 mM tryptophan for 72 h.

3.2. Effects of IAA-overproducing derivatives of *P. fluorescens* CHA0 on cucumber

The IAA overproducers CHA0-Rif/pME3468, CHA0-Rif/pME3469, and CHA0-Rif/pME3470

Table 3
Effect of *P. fluorescens* CHA0-Rif and its IAA-overproducing derivatives on the growth of cucumber in natural soil from Eschikon

Bacterial strain added*	Emerged plants (%)**	Shoot fresh weight (g)**	Root fresh weight (g)**	Root length (mm)**	Log_{10} cfu per g of roots**	Colonies with plasmid (%)**
None	91 a	0.86 ab	0.25 ab, yz	103 a		
CHA0-Rif	99 a	0.86 ab	0.24 ab, yz	102 a	6.95 a	
CHA0-Rif/pME6000	97 a	0.83 a	0.23 b, z	99 a	6.71 a	97 a
CHA0-Rif/pME3468	98 a	0.84 ab	0.28 a, x	99 a	6.74 a	95 a
CHA0-Rif/pME3469	95 a	0.89 b	0.29 a, x	103 a	6.35 a	63 b
CHA0-Rif/pME3470	100 a	0.87 ab	0.26 a, xy	103 a	6.60 a	97 a

*Initial counts of added bacteria were $0.6–1.5 \times 10^7$ cfu g^{-1} of soil. See Section 2.4 for more details on the set-up of the plant assay.
**Data represent the means from four independent repetitions of the same experimental set-up, with five replicates per treatment and experiment (one replicate corresponds to one flask containing five plants). Each mean was compared with each other mean using the Student's *t*-test, considering one independent experiment as a repetition. Values in the same column with different letters are significantly different at $P = 0.05$ (letters a and b) and at $P = 0.15$ (letters x, y and z).

Table 4
Suppression of *Pythium* damping-off and root rot of cucumber by *P. fluorescens* CHA0 and an IAA-overproducing derivative in natural soil from Eschikon

Bacterial strain added*	*P. ultimum* added*	Surviving plants (%)**	Shoot fresh weight per flask (g)**	Root fresh weight per flask (g)**	Log_{10} CFU per g of roots**	Colonies with plasmid (%)**
None	−	88 bc	4.00 a	1.79 b		
CHA0-Rif	−	91 ab	4.01 a	1.87 b	6.08 a	
CHA0-Rif/pME3468	−	100 a	4.39 a	2.55 a	5.62 a	96 a
None	+	29 d	1.02 c	0.37 d		
CHA0-Rif	+	68 c	2.42 b	1.19 c	6.20 a	
CHA0-Rif/pME3468	+	66 c	2.63 b	1.17 bc	5.76 a	94 a

*Initial counts of added bacteria were $0.4–1.1 \times 10^7$ cfu g^{-1} of soil. See Section 2.4 for more details on the set-up of the plant assay.
**Data are means from two independent experiments with five replicates per treatment and experiment (one replicate corresponds to one flask containing five plants). Individual data from the two experiments were pooled for statistical analysis. Values in the same column with different letters are significantly different at $P = 0.05$ (Student's *t*-test).

were compared with the parental strains CHA0-Rif and CHA0-Rif/pME6000 for their effects on cucumber growing in a natural sandy loam soil from Switzerland (Table 3). The constructs pME3468 and pME3469 enhanced root fresh weight significantly by 17%, compared with the controls, but had no significant effect on shoot fresh weights (Table 3). As a result, these constructs increased the overall plant fresh weight by about 5%, but this effect was not statistically significant. The construct pME3470 did not give significant changes (Table 3). Note that all strains tested colonized the roots to the same extent, at about 4×10^6 cfu per g of root. Plasmid pME3468 appeared to be more stable than plasmid pME3469 (Table 3) and for this reason was preferred in subsequent experiments.

The natural soil used in the experiment shown in Table 3 may contain some pathogens, as indicated by a plant emergence of $< 100\%$. However, the level of plant disease was low and hence the parental strain CHA0-Rif has little opportunity to deploy its plant-beneficial potential based on disease suppression. Therefore, the experiment was repeated with natural soil to which the root pathogen *P. ultimum* had been added (Table 4). As expected from our previous work [27], strain CHA0-Rif suppressed *Pythium* root rot: the emergence of plants, root weight and shoot weight were all improved significantly, relative to the infected control without bacteria added (Table 4). The IAA overproducer CHA0-Rif/pME3468 was indistinguishable from the parental strain CHA0-Rif in terms of disease suppression (Table 4). In soil not infested with *P. ultimum*, the IAA overproducer (colonizing with 4×10^5 cfu per g) again increased root fresh weight, in this experiment significantly by 36% (Table 4).

The IAA overproducer CHA0-Rif/pME3468 and the parent CHA0-Rif were also tested with cucumber

Table 5
Effect of an IAA-overproducing derivative of *P. fluorescens* CHA0-Rif on the growth of cucumber in autoclaved soil from Eschikon

Bacterial strain added*	Root length (mm)**	Shoot fresh weight (g)**	Root fresh weight (g)**	Log_{10} cfu per g of root**
None	77 b	0.62 b	0.23 b	
CHA0-Rif	137 a	0.77 a	0.29 a	7.80 a
CHA0-Rif/pME6000	147 a	0.71 ab	0.28 a	7.76 a
CHA0-Rif/pME3468	23 c	0.38 c	0.23 ab	7.81 a

*Initial counts of added bacteria were about 4×10^6 cfu g^{-1} of soil. See Section 2.4 for more details on the set-up of the plant assay.
**Data represent the means from three independent repetitions of the same experimental set-up, with five replicates per treatment and experiment (one replicate corresponds to one flask containing five plants). Each mean was compared with each other mean using the Student's *t*-test, considering one independent experiment as a repetition. Values in the same column with different letters are significantly different at $P = 0.05$.

in autoclaved soil (Table 5). Under these conditions, strain CHA0-Rif showed a marked stimulation of plant growth (Table 5). This phenomenon probably results from the ability of the introduced bacteria to degrade plant-toxic compounds in the autoclaved substrate. Cucumber appears to be sensitive to autoclaved soil whereas wheat is not (our unpublished observations). By comparison with strain CHA0-Rif, the recombinant CHA0-Rif/pME3468 was clearly deleterious to the plant, in that the latter strain caused strong root stunting and a significant reduction of shoot fresh weight (Table 5). It is important to point out that in autoclaved soil, root colonization by *P. fluorescens* was about 6×10^7 cfu per g of root (Table 5), i.e. about 60× higher than colonization in natural soil. Thus, it is likely that enhanced colonization by strain CHA0-Rif/pME3468 in autoclaved soil created relatively high, local IAA concentrations in the rhizosphere, resulting in a deleterious effect on the plant.

4. Discussion

In natural soil, the IAA-overproducing recombinant strains CHA0-Rif/pME3468 and CHA0-Rif/pME3469 stimulated root growth of cucumber by about 20%, in comparison with plants colonized by the parental strain CHA0-Rif (Tables 3 and 4). This stimulation probably approaches the greatest positive effects that rhizobacterial IAA production can exert on root development, although, in this study, no comparison could be made with an IAA-negative mutant of strain CHA0. (Such a mutant would be difficult to isolate, given that the strain has at least two IAA pathways and more than one enzyme transaminates tryptophan [9].) We also examined the IAA recombinant strains in natural soil planted with corn or wheat and could not observe significant effects on root growth (data not shown). Previously, Dubeikovsky et al. [28] have noted that an IAA-overproducing strain of *P. fluorescens* BSP53a stimulates root development of blackcurrant, but inhibits that of sour cherry under gnotobiotic conditions.

Defense of cucumber against *Pythium* root rot was not influenced by the capacity of strain CHA0 to produce high concentrations of IAA (Table 4). It has been argued that stimulation of ethylene biosynthesis by IAA-like auxins could be accompanied by an increase in cyanide levels, because cyanide is a co-product during ethylene synthesis from 1-amino-cyclopropane-1-carboxylic acid [29]. Cyanide can play a role in plant defense against pathogens [15]. However, in the plant-pathogen system studied here, no support could be obtained for IAA strengthening defense.

In autoclaved soil, the IAA overproducer CHA0-Rif/pME3468 had a dramatic deleterious effect on cucumber in that roots were much shorter and thicker and shoot weight was reduced (Table 5). Clearly, this negative impact stems from enhanced IAA concentrations due to a higher level of bacterial root colonization, which was about 60-fold above the level seen in natural soil. A deleterious effect, i.e. severe root stunting, was also observed for strain CHA0-Rif/pME3468 colonizing wheat in autoclaved soil (data not shown). Thus, the known deleterious consequences of an IAA overdose in the rhizosphere [11,18,30] are confirmed by our experiments.

In conclusion, the concentration range in which exogenous IAA can have a positive effect on root growth and development is relatively narrow; for corn, stimulating concentrations are in the nanomolar range [31]. Higher (micromolar) concentrations of exogenous IAA applied to roots have clear-cut negative effects [11,31], and this fact limits the potential to improve the plant growth-enhancing effect of certain rhizobacteria by genetically manipulating their IAA production [28,31]. In terms of biocontrol efficacy, IAA appears not to have the same importance as other secondary metabolites produced by fluorescent pseudomonads, such as 2,4-diacetylphloroglucinol, pyoluteorin, hydrogen cyanide, and phenazines [15,16]. Thus, at least in the plant–pathogen system examined here, plant yields and biocontrol efficacy are not improved significantly by elevating IAA levels in strain CHA0.

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References

[1] Costacurta, A. and Vanderleyden, J. (1995) Synthesis of phytohormones by plant-associated bacteria. Crit. Rev. Microbiol. 21, 1–18.
[2] Fett, W.F., Osman, S.F. and Dunn, M.F. (1987) Auxin production by plant-pathogenic pseudomonads and xanthomonads. Appl. Environ. Microbiol. 53, 1839–1845.
[3] Glickmann, E., Gardan, L., Jacquet, S., Hussain, S., Elasri, M., Petit, A. and Dessaux, Y. (1998) Auxin production is a common feature of most pathovars of *Pseudomonas syringae*. Mol. Plant-Microbe Interact. 11, 156–162.
[4] Patten, C.L. and Glick, B.R. (1996) Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42, 207–220.
[5] Yamada, T., Curtis, J.P., Brooks, B. and Kosuge, T. (1985) Nucleotide sequences of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc. Natl. Acad. Sci. USA 82, 6522–6526.
[6] Costacurta, A., Keijers, V. and Vanderleyden, J. (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. Mol. Gen. Genet. 243, 463–472.
[7] Koga, J. (1995) Structure and function of indolepyruvate decarboxylase, a key enzyme in indole-3-acetic acid biosynthesis. Biochim. Biophys. Acta 1249, 1–13.
[8] Narumiya, S., Takai, K., Tokuyama, T., Noda, Y., Ushiro, H. and Osamu (1979) A new metabolic pathway of tryptophan initiated by tryptophan side chain oxidase. J. Biol. Chem. 254, 7007–7015.
[9] Oberhänsli, T., Défago, G. and Haas, D. (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol strain CHA0 of *Pseudomonas fluorescens*: role of tryptophan side chain oxidase. J. Gen. Microbiol. 137, 2273–2279.
[10] Sarwar, M. and Frankenberger, W.T. (1994) Influence of L-tryptophan and auxins applied to the rhizosphere on the vegetative growth of *Zea mays* L. Plant Soil 160, 97–104.
[11] Kawaguchi, M. and Syono, K. (1996) The excessive production of indole-3-acetic acid and its significance in studies of the biosynthesis of this regulator of plant growth and development. Plant Cell Physiol. 37, 1043–1048.
[12] Comai, L. and Kosuge, T. (1982) Cloning and characterization of *iaaM*, a virulence determinant of *Pseudomonas savastanoi*. J. Bacteriol. 149, 40–46.
[13] Liu, S.T., Perry, K.L., Schardl, C.L. and Kado, C.I. (1982) *Agrobacterium* Ti plasmid indoleacetic acid gene is required for crown gall oncogenesis. Proc. Natl. Acad. Sci. USA 79, 2812–2816.
[14] Manulis, S., Haviv-Chesner, A., Brandl, M.T., Lindow, S.E. and Barash, I. (1998) Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophilae*. Mol. Plant-Microbe Interact. 11, 634–642.
[15] Voisard, C., Bull, C.T., Keel, C., Laville, J., Maurhofer, M., Schnider, U., Défago, G. and Haas, D. (1994) Biocontrol of root diseases by *Pseudomonas fluorescens* CHA0: current concepts and experimental approaches. In: Molecular Ecology of Rhizosphere Microorganisms (O'Gara, F., Dowling, D.N. and Boesten, B., Eds.), pp. 67–89. VCH, Weinheim.
[16] Keel, C. and Défago, G. (1997) Interactions between beneficial soil bacteria and root pathogens: mechanisms and ecological impact. In: Multitrophic Interactions in Terrestrial Systems (Gange, A.C. and Broen, V.K., Eds.), pp. 27–46. Blackwell Science, Oxford.
[17] Prinsen, E., Costacurta, A., Michiels, K., Vanderleyden, J. and Van Onckelen, H. (1993) *Azospirillum brasilense* indole-3-acetic acid biosynthesis: evidence for a non-tryptophan dependent pathway. Mol. Plant-Microbe Interact. 6, 609–615.
[18] Loper, J.E. and Schroth, M.N. (1986) Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology 76, 386–389.
[19] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[20] Natsch, A., Keel, C., Pfirter, H.A., Haas, D. and Défago, G. (1994) Contribution of the global regulator gene *gacA* to persistence and dissemination of *Pseudomonas fluorescens* biocontrol strain CHA0 introduced into soil microcosms. Appl. Environ. Microbiol. 60, 2552–2560.
[21] Maurhofer, M., Reimmann, C., Schmidli-Sacherer, S., Heeb, S., Haas, D. and Défago, G. (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88, 678–684.
[22] Gaffney, T.D., Da Costa e Silva, O., Yamada, T. and Kosuge, T. (1990) Indoleacetic acid operon of *Pseudomonas syringae* subsp. *savastanoi*: transcription analysis and promoter identification. J. Bacteriol. 172, 5593–5601.
[23] Palm, C.J., Gaffney, T. and Kosuge, T. (1989) Cotranscription of genes encoding indoleacetic acid production in *Pseudomonas syringae* subsp. *savastanoi*. J. Bacteriol. 171, 1002–1009.
[24] Ornston, L. and Stanier, R.Y. (1966) The conversion of catechol and procatechuate to β-ketoadipate by *Pseudomonas putida*. J. Biol. Chem. 241, 3776–3786.
[25] Del Sal, G., Manfioletti, G. and Schneider, C. (1988) A one-tube plasmid DNA mini-preparation suitable for sequencing. Nucleic Acids Res. 16, 9878.
[26] Voisard, C., Rella, M. and Haas, D. (1988) Conjugative transfer of plasmid RP1 to soil isolates of *Pseudomonas fluorescens* is facilitated by certain large RP1 deletions. FEMS Microbiol. Lett. 55, 9–14.
[27] Maurhofer, M., Keel, C., Haas, D. and Défago, G. (1995) Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. Plant Pathol. 44, 40–50.
[28] Dubeikowsky, A.N., Mordukhova, E.A., Kochetkov, V.V., Polikarpova, F.Y. and Boronin, A.M. (1993) Growth promotion of blackcurrant softwood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased

amount of indole-3-acetic acid. Soil Biol. Biochem. 25, 1277–1281.
[29] Grossmann, K. (1996) A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms. Physiol. Plantarum 97, 772–775.
[30] Xie, H., Pasternak, J.J. and Glick, B.R. (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr. Microbiol. 32, 67–71.
[31] Pilet, P.E. and Saugy, M. (1987) Effect on root growth of endogenous and applied IAA and ABA. Plant Physiol. 83, 33–38.