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Pyoluteorin production by *Pseudomonas fluorescens* strain CHA0 is involved in the suppression of *Pythium* damping-off of cress but not of cucumber

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Abstract. *Pseudomonas fluorescens* strain CHA0 is an effective biocontrol agent of various soilborne pathogens. It controls damping-off or root rot caused by *Pythium ultimum* on cucumber, wheat and cress. Strain CHA0 synthesizes several antibiotic metabolites such as hydrogen cyanide, 2,4-diacetylphloroglucinol, and pyoluteorin. The role of pyoluteorin in the suppression of damping-off was investigated. Two Tn*5* mutants (CHA660 and CHA661) of strain CHA0 were isolated which had lost the capacity to produce pyoluteorin but still produced 2,4-diacteylphloroglucinol and HCN. These mutants still inhibited *P. ultimum* on malt agar (which favours the production of 2,4-diacetylphloroglucinol) but had partially lost the ability to inhibit this pathogen on King's B agar (which favours the production of pyoluteorin). The two pyoluteorin-negative mutants showed a reduced capacity to suppress damping-off of cress caused by *P. ultimum* but were as effective in the protection of cucumber against this pathogen as the wild-type strain.

These results indicate that, depending on the plant, pyoluteorin production plays a role in the suppression of damping-off by strain CHA0 without being a major mechanism in disease suppression. We suggest that the contribution of pyoluteorin to the biocontrol activity of strain CHA0 is determined by the quantity of this antibiotic produced in the rhizosphere, which might depend on the root exudates of the host plant.

Introduction

Fluorescent pseudomonads produce a wide array of antibiotic metabolites, some of which are involved in the biocontrol of soilborne pathogens. An important role in disease suppression has been demonstrated for phenazine-1-carboxylic acid, hydrogen cyanide and 2,4-diacetylphloroglucinol [Keel et al., 1990; Keel et al., 1991; Keel et al., 1992; Thomashow and Weller, 1988; Thomashow et al., 1990; Voisard et al., 1989]. For oomycin A there is also evidence that this compound is involved in biocontrol [Gutterson, 1990; Howie and Suslow, 1991; James and Gutterson, 1986]. Pyrrolnitrin and pyoluteorin are further metabolites of *Pseudomonas* strains for which a role in disease suppression has been suggested [Homma and Suzui, 1989; Howell and Stipanovic, 1979; Howell and Stipanovic, 1980; Jayaswal et al., 1992; Maurhofer et al., 1992].

Pseudomonas fluorescens strain CHA0, which was isolated from a soil suppressive to black root rot of tobacco, is an effective biocontrol agent of various soilborne diseases in greenhouse experiments [Défago et al., 1990] and reduces take-all severity in the field [Wüthrich and Défago, 1991]. This strain produces the antifungal metabolites hydrogen cyanide, pyoluteorin (Plt), 2,4-diacetylphloroglucinol (Phl), and monoacetylphloroglucinol (mPhl) a precursor of Phl (Fenton et al., Fourth International Symposium on *Pseudomonas*, Vancouver 1993, Book of abstracts, page 49). Phl and HCN contribute to the suppression of black root rot of tobacco [Keel et al., 1990; Keel et al., 1992; Voisard et al., 1989]. Phl is also involved in the suppression of take-all of wheat [Keel et al., 1991]. Plt is highly toxic to *Pythium ultimum in vitro* [Défago et al., 1990, Howell and Stipanovic, 1980; Maurhofer et al., 1992] and also to some plants [Maurhofer et al., 1992; Ohmori et al., 1978]. Strain CHA0(pME3090), a derivative of strain CHA0 overproducing Plt and Phl shows an improved capacity to suppress *P. ultimum* induced damping-off of cucumber, compared with the wild-type strain [Maurhofer et al., 1992]. This improved disease suppressive capacity is suggested to be due to enhanced production of Plt, since Plt is much more toxic to *P. ultimum* than is Phl. Furthermore, treatment of cotton seeds with Plt suppresses damping-off caused by *P. ultimum* [Howell and Stipanovic, 1980]. These findings suggest that Plt might contribute to the suppression of damping-off by fluorescent pseudomonads. However, Plt-negative Tn*5*-induced mutants of *P. fluorescens* strain Pf-5 do not have a reduced capacity to protect cotton and cucumber from *P. ultimum*, indicating that Plt production by strain Pf-5 does not play a role in the suppression of damping-off of these plants [Kraus and Loper, 1991; Kraus and Loper, 1992].

The purpose of this study was to determine whether Plt production by *P. fluorescens* CHA0 affects the suppression of *P. ultimum* infecting two different host plants. To this end, the protective effects of the wild-type strain CHA0 was compared with those of Tn*5*-induced, Plt-deficient mutants.

Materials and methods

Microorganisms and culture conditions. P. fluorescens (Trevisan) Migula strain CHA0 [Stutz et al., 1986] and its derivatives were cultivated routinely on nutrient agar [NA; Stanisich and Holloway, 1972] at 27 °C. Cultures of Tn*5* insertion mutants were amended with 25 μg kanamycin (km) × ml^{-1} medium. For disease suppression assays, bacteria were grown and added to soil as described earlier [Keel et al., 1989]. *P. ultimum* Trow strain 67-1 (obtained from Allelix Agriculture, Mississauga, Canada) was cultivated on malt agar (15 g of Oxoid malt extract, 17 g of Difco agar, and 1 L of distilled water) at 20 °C for 7 d. For disease suppression assays,

a 0.6-cm plug of a *P. ultimum* culture was placed in a sterilized 300-ml Erlenmeyer flask containing 25 g of autoclaved millet seeds (1.2 mm in diameter, Biofarm, Kleindietwil, Switzerland) and 12 ml sterilized double-distilled water. After a 2-wk-incubation at 20 °C, the mycelium-covered millet was reduced to small pieces with a sterile spatula and mixed into the soil.

Transposon mutagenesis and screening for Plt-negative mutants. Transposon mutagenesis using pLG221 was carried out according to the methods described by Voisard et al. [1988, 1989]. The recipient strain CHA0 was cultivated in King's medium B [King et al., 1954] at 35 °C for 26 h. The donor strain *Escherichia coli* W3110 [Bachmann, 1972] containing the Tn*5* suicide plasmid pLG221 [Boulnois et al., 1985] was cultivated in nutrient-yeast extract broth (NYB) [Stanisich and Holloway, 1972] at 37 °C for 18 h. Equal volumes of donor and recipient were combined, washed once with saline, spread on NA plates and incubated at 30 °C for 4 h. The bacteria were removed from the agar plates with saline, diluted five times, plated on media selective for putative Tn*5* mutants (NA containing 25 and 20 μg × ml^{-1} km and chloramphenicol, respectively) and incubated at 27 °C for 48 h. Km resistant mutants were screened for Plt production. Each mutant was grown in 8 ml King's medium B at 24 °C for 4 d and the cultures were then extracted with the same volume of ethyl acetate after acidification to pH 2 with 1.2 M HCl. The ethyl acetate phase was evaporated *in vacuo* and the extracts were dissolved in methanol and analyzed by HPLC as described before [Maurhofer et al., 1992].

Characterization of the Tn5 insertion mutants. For quantitative assays of antibiotic production, 10 ml NYB was inoculated with a single colony of each Tn*5*-induced *P. fluorescens* mutant and was shaken at 27 °C for 16 h. From these overnight cultures 200 μl was plated on malt agar or King's B agar and incubated at 18 °C for 4 d. Antibiotics were extracted from the agar and analyzed by HPLC as described earlier [Maurhofer et al., 1992].

For quantitative cyanide determination, *P. fluorescens* strains were grown with shaking in 10 ml Castric medium [Castric, 1977] placed in a hermetically sealed 20-ml flask at 28 °C. After 24 h cyanide in the medium was determined following the method of Gewitz et al. [1976].

Pyoverdine-dependent fluorescence was tested by growing *P. fluorescens* strains on succinate minimal medium [Meyer and Abdallah, 1978] with or without the addition of 20 μM EDDHA at 24 °C for 24 h. Auxotrophic defects were also tested on succinate minimal medium.

Extracellular protease production was detected on skim milk agar plates which contained 1.5% (w/v) skim milk powder (Oxoid), 0.4% blood base agar (Oxoid), 0.05% yeast extract (Difco) and 1.35% (w/v) bacteriological agar (Oxoid). Plates were incubated at 27 °C for 24 h. Formation of clear halos around the colonies indicated extracellular protease activity.

For growth assessment the mutants were grown on NA, malt agar, King's B agar and in liquid culture, and NYB at 27 °C for 16 h. Bacteria were removed from the agar plates with sterile distilled water. Serial dilutions of the resultant suspensions and of the liquid cultures were plated on King's B agar. Colonies were counted after a 48-h incubation at 27 °C.

Inhibition of P. ultimum in vitro. Bacteria were grown on King's B agar at 27 °C for 2 d. Single colonies were picked with a sterile toothpick and streaked on malt- or King's B agar plates along a square of 6 cm side length. After incubating the agar plates at 27 °C for 2 d (malt agar) or directly after inoculation with the bacteria (King's B agar), a 0.6-cm plug of a culture of *P. ultimum* was placed in the center of the plates. Malt agar plates were incubated at 27 °C for 2 d and King's B agar plates for 3 d. Inhibition was scored by measuring the distance between the fungal mycelium and the bacterial colony.

Suppression of P. ultimum *under gnotobiotic conditions.* Disease suppression by *P. fluorescens* strain CHA0 and its Plt-negative derivatives was tested in a sterile flask containing an artificial soil consisting of pure vermiculite as clay mineral mixed with quartz sand of different sizes [Keel et al., 1989]. The matrix potential of the artificial soil was –4.0 MPa. *P. fluorescens* (10^7 cfu $\times$ g^{-1} soil) and millet covered with *P. ultimum* (0.5 mg $\times$ g^{-1} soil for cucumber, 0.83 mg $\times$ g^{-1} soil for cress) were added to the soil and cucumber seedlings (*Cucumis sativus*, L. cv. 'Chinesische Schlange', Altorfer Samen, Zürich, Switzerland) were planted or cress seeds (*Lepidium sativum* L., cv. 'Gartenkresse einfach', Altorfer Samen) sown and moistened with modified Knop solution [Keel et al., 1989] as described previously in detail [Maurhofer et al., 1992]. Flasks were placed in randomized blocks in a growth chamber with 70% relative humidity and 16 h of light (160 µmol $\times$ m^{-2} $\times$ sec^{-1}) 22 °C and a 8-h-dark period at 18 °C (cucumber) or 15 °C (cress). After 1 wk (cress) or 2 wk (cucumber) the plants were removed from the flasks by flushing with non-sterile tap water. After washing the roots free from adhering soil, plants were briefly blotted dry with paper tissues and assessed for root and total fresh weight. As an indication of pre-emergence damping-off (cress) or post-emergence damping-off (cucumber) the percentage of emergence (cress) or the percentage of survival of seedlings (cucumber) was evaluated. Cress bioassay: The experiment was repeated four times. One experiment consisted of eight replications per treatment and one flask with 75 seeds per replication. Cucumber bioassay: the experiment was repeated three times. One experiment consisted of three replications per treatment and one flask with three seedlings per replication.

Bacterial root colonization. After weighing the whole plants, then the

roots, bacterial root colonization was determined as described earlier [Keel et al., 1989].

Data analysis. Biocontrol of *P. ultimum*: Experiments were first analyzed separately using the Duncan multiple range test after a significant F test ($P = 0.05$). Then all experiments were analyzed together by the Student t test. Each value presented in Table 3 is the mean of the means of the individual experiments (data were not pooled). Each mean was compared with each other mean using the Student t test, considering one independent experiment as a repetition. The base ten-logarithmic transformation was applied to individual estimations of root colonization prior to statistical analysis.

Inhibition of *P. ultimum* and antibiotic and cyanide assays: Each value presented in Tables 1 and 2 is the mean of three independent experiments. Each mean was compared with each other mean using the Student t test, considering one independent experiment as a repetition.

Results

Isolation of Tn5 insertion mutants of strain CHA0. After mating between the recipient strain CHA0 and the donor *E. coli* strain W3110/pLG221 putative Tn*5* mutants of strain CHA0 were obtained at a frequency of 4×10^{-6} per donor. Out of 1500 Tn*5* mutants screened six did not produce Plt in King's B liquid cultures. Four of these mutants were pleiotropic also lacking extracellular protease, HCN, and Phl, whereas two were defective in the production of Plt but still produced Phl, HCN and extracellular protease.

Characterization of Plt-negative mutants. The wild-type strain CHA0 and the two Plt-negative mutants CHA660 and CHA661 were tested for several phenotypic characteristics. Strains CHA660 and CHA661 were prototrophic and showed no difference in fluorescent pigment and growth on NA, malt agar, King's B agar and in liquid culture, and NYB compared to the wild-type strain (data not shown). On malt agar strain CHA0 produced mPhl, Phl and some Plt (Table 1). The two mutants did not produce Plt but produced the same levels of mPhl and Phl as the wild-type strain (Table 1). On King's B agar strain CHA0 produced Plt and a small amount of Phl (Table 1). Strains CHA660 and CHA661 produced both Phl and mPhl, but no Plt. On this medium the Phl production of the two mutants was about 12 times higher than that of the wild-type strain (Table 1). In Castric liquid medium strain CHA0 and the two mutants produced similar amounts of HCN (Table 1). Strains CHA660 and CHA661 showed no difference in the production of extracellular protease compared to the wild-type strain (data not shown).

Table 1. Production of monoacetylphloroglucinol (mPhl), 2,4-diacetylphloroglucinol (Phl), pyoluteorin (Plt) and hydrogen cyanide by *Pseudomonas fluorescens* strain CHA0 and its derivatives CHA660 and CHA661

Bacteria[x]	Malt agar[yz]			King's B agar[yz]			Castric medium[yz]
	mPhl	Phl	Plt	mPhl	Phl	Plt	HCN
CHA0	3.1 a	31.0 a	1.2 a	0.0 a	0.4 a	34.6 a	38.7 a
CHA660	4.9 a	41.7 a	0.0 b	0.9 b	6.0 b	0.0 b	44.4 a
CHA661	3.9 a	38.8 a	0.0 b	1.5 b	5.9 b	0.0 b	39.6 a

[x] CHA0 = wild-type strain of *P. fluorescens*, CHA660 and CHA661 = pyoluteorin-negative Tn*5* insertion mutants of strain CHA0. For antibiotic production the bacteria were grown on agar plates at 18 °C for 4 d. For cyanide production the bacteria were grown in the Castric liquid medium at 28 °C for 24 h.
[y] Antibiotic or HCN production in culture medium (μM).
[z] Means within the same column followed by the same letter are not significantly different at $P = 0.05$ according to the Student *t*-test, comparing each mean with each other mean considering one independent experiment as a repetition. Antibiotics: each value is the mean of eight independent experiments with one replicate and one extraction per replicate. HCN: each value is the mean of three independent experiments with three replicates and the measurement of HCN in one bacterial culture per replicate.

Inhibition of P. ultimum in vitro. Strains CHA660 and CHA661 were significantly less inhibitory of *P. ultimum* on King's B agar than the wild-type strain (Table 2). The inhibition of *P. ultimum* on malt agar was similar for all strains tested (Table 2).

Influence of Plt production on the suppression of P. ultimum *in artificial soil under gnotobiotic conditions.* In the presence of *P. ultimum* the fresh weights of cucumber and cress plants were drastically reduced (Table 3). Emergence of cress seedlings was only 12% compared to that of untreated control seedlings and 82% of the cucumber plants were dead (Table 3). The presence of strain CHA0 provided a good protection against the disease (Table 3), confirming earlier results [Maurhofer et al., 1992]. The fresh weight of protected plants was 13 times higher compared to plants grown in the presence of *P. ultimum* alone and reached 80% (cress) and 60% (cucumber) of the fresh weight of untreated control plants. Emergence of cress seedlings protected by strain CHA0 was only 15% lower than that of untreated control seedlings and 89% of the cucumber plants survived (Table 3). Strains CHA660 and CHA661 showed a reduced capacity to protect cress against *P. ultimum* in terms of plant fresh weight and of seedling emergence, but protected cucumber as efficiently in terms of plant fresh weight and of plant survival as did the wild-type strain (Table 3). Cress plants protected by the Plt-negative mutants reached only 60% (CHA660) or 73% (CHA661) of the fresh weights of plants protected by the wild-type strain. Emergence of cress seedlings protected by the Plt-negative strains

Table 2. Inhibition of *Pythium ultimum* by *Pseudomonas fluorescens* strain CHA0 and its pyoluteorin-negative derivatives CHA660 and CHA661 on agar plates

Bacteria[x]	Inhibition of *P. ultimum*[yz]	
	Malt agar	King's B agar
CHA0	1.4 a	8.0 a
CHA660	1.3 a	4.8 b
CHA661	1.4 a	4.2 b

[x] CHA0 = wild-type strain of *P. fluorescens,* CHA660 and CHA661 = pyoluteorin-negative Tn*5* insertion mutants of strain CHA0. The agar plates were inoculated with *P. ultimum* and bacteria as described in Materials and methods.
[y] Inhibition of *P. ultimum* is expressed as the distance (mm) between fungal mycelium and bacterial colony.
[z] Means within the same column followed by the same letter are not significantly different at $P = 0.05$ according to the Student *t*-test, comparing each mean with each other mean considering one independent experiment as a repetition. Each value is the mean of three independent experiments with three replicates and one agar plate per replicate.

was 39% (CHA660) and 32% (CHA661) lower than that of seedlings protected by the wild-type (Table 3). In the absence of the pathogen neither of the strains tested had an influence on the total plant weight of cress or cucumber (Table 3). Strain CHA0, however, (slightly but significantly) reduced cress root weight.

All the strains colonized cress roots in the presence or absence of *P. ultimum* at 7.3 to 7.7 log cfu $\times$ g^{-1} fresh root (Table 3). Cucumber roots were colonized at 7.8 to 8.2 log cfu $\times$ g^{-1} fresh root in the absence and at 9 to 9.4 log cfu $\times$ g^{-1} roots in the presence of the pathogen (Table 3).

Separate analysis of each independent experiment showed that in all the individual experiments (i) the treatment effect was highly significant (analysis of variance, $P < 0.001$), (ii) strains CHA660 and CHA661 gave significant (Duncan multiple range test, $P = 0.05$) less protection of cress against *P. ultimum* in terms of fresh weight and in terms of seedling emergence compared to strain CHA0 and (iii) strains CHA660 and CHA661 protected cucumber as efficiently against *P. ultimum* as did the wild-type strain.

Discussion

In this study two Tn*5* mutants, defective in the production of Plt (CHA660 and CHA661) were characterized. The gene which was mutated in strains CHA660 and CHA661 by transposon insertion, is not known. The mutation could either have occurred in a gene required for Plt synthesis or in a regulatory gene mediating Plt production. Although the two mutants did not

Table 3. Protection of cucumber and cress against *Pythium ultimum* by *Pseudomonas fluorescens* strain CHA0 and its pyoluteorin-negative derivatives CHA660 and CHA661 under gnotobiotic conditions

Host	Microorganisms added[v]		Root fresh weight[wx] (mg)	Plant fresh weight[wx] (mg)	Emergence of seedlings or plant survival (%)[y]	Fluorescent pseudomonads[y] (log cfu $\times$ g^{-1})[z]
	Bacteria	*P. ultimum*				
Cress	none	–	1107 a	3026 a	100 a	0.0 a
	CHA0	–	989 b	2914 a	100 a	7.3 b
	CHA660	–	1173 ab	3019 a	100 a	7.3 b
	CHA661	–	1146 a	3131 a	99 a	7.3 b
	none	+	71 e	179 d	5 d	0.0 a
	CHA0	+	809 c	2346 b	85 b	7.5 b
	CHA660	+	401 d	1407 c	52 c	7.7 b
	CHA661	+	502 d	1715 c	58 c	7.6 b
Cucumber	none	–	273 a	881 a	100 a	0.0 a
	CHA0	–	244 a	839 a	100 a	7.8 b
	CHA660	–	222 a	756 ab	100 a	8.2 bc
	CHA661	–	219 a	769 ab	100 a	8.2 bc
	none	+	24 b	38 c	18 c	0.0 a
	CHA0	+	107 a	499 ab	89 b	9.0 c
	CHA660	+	107 a	489 b	81 b	9.0 c
	CHA661	+	106 a	488 b	93 b	9.4 c

[v] CHA0 = wild-type strain of *P. fluorescens*, CHA660 and CHA661 = Plt-negative Tn*5* insertion mutants of strain CHA0. Bacteria and *P. ultimum* were added as described in Materials and methods.

[w] Fresh weight per living plant for cucumber and fresh weight from all plants emerged from 75 seeds (in one flask) for cress.

[x] Means for the same host plant within the same column followed by the same letter are not significantly different at $P = 0.05$ according to the Student *t*-test, comparing each mean with each other mean considering one independent experiment as a repetition. Cress: each value is the mean of four independent experiments with eight replicates per experiment and one flask with plants grown from 75 seeds per replicate. Cucumber: each value is the mean of three independent experiments with three replicates and one flask with three plants per replicate.

[y] Cress: emerged seedlings in percentage of the untreated control (100% = 71 seedlings); cucumber: percentage of plant survival (100% = nine plants).

[z] The base ten-logarithmic transformation was applied to individual estimations of root colonization (colony-forming units $\times$ g^{-1} fresh root) prior to statistical analysis.

differ from the wild-type strain CHA0 in growth on different media, fluorescence, prototrophy, the production of HCN and protease as well as the production of Phl on malt agar except that they overproduced Phl on King's B agar (Table 1) it can not be excluded that other phenotypes than Plt production are also affected by the Tn*5* insertion.

Plt production appeared to be responsible, in part, for the inhibition of *P. ultimum* on King's B agar (Table 2). The Plt-negative strains, however, still showed a certain inhibition of the fungus on King's B agar. We suggest that this inhibition was rather due to HCN production than to Phl production since the amount of Phl produced by the Plt-negative mutants on this medium (Table 1) was to low to inhibit *P. ultimum*. In fact, previous results showed that 200 μM of Phl are necessary to inhibit this fungus [Maurhofer et al., 1992]. On malt agar, the Plt-negative mutants inhibited *P. ultimum* to the same extent as the wild-type, probably because only small quantities of Plt were produced by strain CHA0 on this medium (Tables 1 and 2). Previous studies have shown that overproduction of Plt in strain CHA0 leads to improved inhibition of *P. ultimum* on King's B agar [Maurhofer et al., 1992]. Other studies have given similar results with *P. fluorescens* strain Pf-5. Strain Pf-5 produces Plt and suppresses *Pythium*-induced damping-off of cotton [Howell and Stipanovic, 1980] and cucumber [Kraus and Loper, 1991]. Eight Tn*5* insertion mutants of Pf-5 which are defective in the production of Plt were not inhibitory of *P. ultimum* on 523 agar whereas three mutants overproducing Plt caused increased inhibition compared to the wild-type [Kraus and Loper, 1992].

Strains CHA660 and CHA661 had a reduced capacity to control damping-off of cress but still provided significant protection compared to the disease control (Table 3). Plt production might therefore contribute to the protection of cress by strain CHA0 but is not the only mechanism.

In contrast to the experiments with cress, the Plt-negative mutants protected cucumber as efficiently against *P. ultimum* as did the wild-type strain (Table 3). Thus, in strain CHA0, Plt production does not seem to be in-volved in the protection of this plant against *P. ultimum*. Other mechanisms, such as the production of antifungal metabolites other than Plt or competition for nutrients, might be responsible for suppression of *P. ultimum* on cucumber by strain CHA0. Our findings agree with Kraus and Loper [1992], who found that Plt production in strain Pf-5 was not required for the suppression of damping-off of cucumber. Enhanced Plt production, however, is suggested to be responsible for the improved protection of cucumber against *P. ultimum* by strain CHA0(pME3090), an antibiotic overproducing derivative of strain CHA0 [Maurhofer et al., 1992]. We therefore propose that in the suppression of *P. ultimum* on cucumber, Plt can also be effective, but that in the rhizosphere of this plant the level of Plt produced by the wild-type may not be high enough to be of importance whereas it may be sufficient in the case of the recombinant strain CHA0(pME3090). In contrast, in the study of Kraus and Loper, Plt over-

producing (Plt^{++}) mutants of strain Pf-5 were not more disease suppressive compared to the wild-type [Kraus and Loper, 1992]. These authors suggest that Plt^{++} mutants may overproduce the antibiotic in culture media but not in the spermosphere of cucumber. These differences in the results found with strain CHA0(pME3090) and the Plt^{++} mutants of strain Pf-5 might also be due to the different soils and conditions used in the biocontrol tests.

The fact that Plt-negative mutants showed a reduced disease suppressive capacity in the rhizosphere of cress but not in that of cucumber, might be related to differences in exudates released by cress and cucumber roots. We suggest that strain CHA0 produces higher amounts of Plt in the rhizosphere of cress compared to that of cucumber, amounts which probably are sufficient to be effective against the pathogen. Our observations that strain CHA0 was slightly toxic to the growth of cress roots, but not of cucumber roots (Table 3) supports this. We suggest that this toxicity is due to Plt production since the Plt-negative strains have no influence on the growth of cress. Synthetic Plt is about as toxic to cress as to cucumber [Maurhofer et al., 1992], so that the fact of strain CHA0 being slightly toxic to cress but not to cucumber is probably due to a high Plt production in the rhizosphere of cress.

In conclusion, depending on the plant, Plt production might play a role in the suppression of damping-off by strain CHA0 but it is not a major mechanism in disease suppression. We suggest that the contribution of Plt to the biocontol activity of strain CHA0 is determined by the level of this antibiotic produced in the rhizosphere, which might be dependent on the root exudates of the host plant.

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