Disease Control and Pest Management

Influence of Enhanced Antibiotic Production
in *Pseudomonas fluorescens* Strain CHA0
on its Disease Suppressive Capacity

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ABSTRACT

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Pseudomonas fluorescens strain CHA0 suppresses various plant diseases caused by soilborne fungi and produces several antibiotic metabolites, two of which have been identified as pyoluteorin (Plt) and 2,4-diacetyl-phloroglucinol (Phl). A cosmid (pME3090) carrying a 22-kb insert of strain CHA0 DNA enhanced, in a CHA0 background, the production of Plt in vitro. The production of Phl was also increased but to a smaller extent. In a gnotobiotic system, the recombinant strain protected cucumber plants against disease caused by *Pythium ultimum* more than did wild type CHA0. In the absence of the pathogen, strains CHA0 and CHA0/pME3090 had no influence on cucumber. Both strains protected cress and sweet corn from *P. ultimum*. However, in the presence of *P. ultimum*, fresh weights of cress and sweet corn plants protected by strain CHA0/pME3090 were lower compared to those of plants protected by strain CHA0. Strain CHA0/pME3090 (but not strain CHA0) strongly reduced the growth of cress and sweet corn in the absence of the pathogen. In vitro, Plt was much more toxic to the growth of *P. ultimum* than was Phl. Sweet corn, cress, and cucumber were all inhibited by Plt and Phl; cucumber, however, was less sensitive to Phl than were cress and sweet corn. These results suggest that enhanced pyoluteorin production might be responsible for the increased capacity of strain CHA0/pME3090 to suppress damping-off of cucumber and that the strain's deleterious effect on cress and sweet corn might be due to the phytotoxic properties of both antibiotics. We conclude that, depending on the host-pathogen system, enhanced antibiotic production by *P. fluorescens* may result in improved disease suppression or, in contrast, in a toxic effect on the plant.

Fluorescent pseudomonads suppress a variety of diseases caused by soilborne pathogens (1,5,17,24,31). One of the mechanisms involved in disease suppression is the production of antifungal metabolites by *Pseudomonas* strains as demonstrated for phenazine-1-carboxylic acid, hydrogen cyanide, and 2,4-diacetylphloroglucinol (13,15,28-30). Oomycin, pyrrolnitrin, and pyoluteorin are additional metabolites of *Pseudomonas* for which a role in disease

suppression has been suggested (9-12). One approach to improve the biocontrol capacity of the *Pseudomonas* strains might be to enhance their antibiotic production. Gutterson and collaborators have constructed a derivative of *Pseudomonas fluorescens* (Trevisan) Migula strain Hv37a that overproduces oomycin A in vitro and shows improved protection of cotton seedlings against *Pythium ultimum* Trow (6,7).

P. fluorescens strain CHA0 is an effective biocontrol agent of take-all disease in the field and of various other diseases in greenhouse experiments (4,32). This strain synthesizes 2,4-di-

acetylphloroglucinol (Phl) and hydrogen cyanide; both have been shown to contribute to the suppression of black root rot of tobacco (8,15,30); Phl also is involved in the suppression of take-all of wheat (13). Furthermore, strain CHA0 produces pyoluteorin (Plt), a substance highly toxic to *P. ultimum* in vitro (4,10). The purpose of this study was to determine whether increased production of Plt by strain CHA0 could improve suppression of damping-off, caused by *P. ultimum*. Preliminary results have been presented (8).

MATERIALS AND METHODS

Microorganisms and culture conditions. *P. fluorescens* strain CHA0 (27) and a derivative carrying cosmid pME3090 were cultivated in media described previously (14,30). Cultures of strain CHA0/pME3090 contained 125 μg of tetracycline per milliliter of medium. For disease suppression tests bacteria were grown and added to soil as described earlier (14). *P. ultimum* strain 67-1 (obtained from Allelix Agriculture, Mississauga, Canada) was cultivated on malt agar plates at 20 C for 7 days. For the disease suppression assay 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 of sterilized double-distilled water. After incubating the flask at 20 C for 2 wk, the mycelium-covered millet was reduced to small pieces, and mixed with a sterile spatula and added to the soil.

Mobilization of the cosmids. Recombinant cosmids representing a genomic library of *P. fluorescens* strain CHA0 were mobilized from *Escherichia coli* strain HB101 to strain CHA0 by triparental mating, using the helper plasmid pME497 as described earlier (30). The resulting transconjugants were screened for enhanced inhibition of *P. ultimum* as described below.

Screening for enhanced inhibition of *P. ultimum* in vitro. Bacterial colonies were grown on King's B (16) agar at 27 C for 2 days. Then four colonies were picked with sterile toothpicks and inoculated onto a malt or King's B agar plate 1 cm from the edge. After incubating the agar plates at 18 C for 2 days, a 0.6-cm plug of a culture of *P. ultimum* was placed in the center of the plates. King's B agar plates were incubated for an additional 4 days and malt agar plates for 3 more days at 18 C. Inhibition was scored by measuring the distance between the fungal mycelium and the bacterial colony.

Southern hybridization of the CHA0 genome. Total DNA of strain CHA0 was digested by *Hind*III, separated on a 0.7% agarose gel (70 V, 12 h), transferred onto a nitrocellulose membrane, and hybridized with a ^{35}S-labeled pME3090 probe, according to the procedures of Maniatis et al (20).

Extraction of antibiotics. Strains CHA0 and CHA0/pME3090 were cultivated on malt or King's B agar at 18 C for 3 days. Two agar plates were cut into pieces of 1 cm^2 and extracted with 50 ml of 80% aqueous acetone (10). The agar that failed to dissolve in the acetone was removed from the extract by filtration through glass wool. After the acetone was evaporated in vacuo, the remaining extract was acidified with HCl to pH 2 and extracted with 50 ml of ethyl acetate. The organic phase was separated from the aqueous phase by filtering through silicone-coated filter paper (Macherey and Nagel, 5160 Düren, Germany) and brought to dryness in vacuo. The pellet was dissolved in methanol.

Detection and quantification of antibiotics. Aliquots of the extracts were analyzed in a Hewlett Packard 1090 Liquid Chromatograph equipped with a diode-array detector, using a column (100 × 4 mm) packed with Nucleosil 120-5-C18 (Macherey and Nagel). The samples (10 μl) were eluted with a three-step linear methanol gradient from 18 to 23% (0–5 min), from 23 to 53% (5–6 min), and from 53 to 68% (6–15 min) in 0.43% o-phosphoric acid. The flow rate was 1 ml/min. Plt and Phl were detected by UV-absorption at 313 and 270 nm, respectively. The retention time of authentic Plt, synthesized according to Cue et al (3), and authentic Phl, synthesized according to Campbell and Coppinger (2), were 12.2 and 9.6 min, respectively. The purity grade

of synthetic Plt was 86–87%, that of synthetic Phl was more than 97%. Stock solutions of Plt and Phl were prepared in methanol and ethanol, respectively. The detection limit was 0.2 nmol for Plt and 0.02 nmol for Phl. Plt and Phl produced were quantified according to a standard curve prepared by injecting known amounts of synthetic antibiotics.

Suppression of *P. ultimum* under gnotobiotic conditions. Disease suppression by *P. fluorescens* strain CHA0 and its antibiotic-overproducing derivative CHA0/pME3090 was tested in a sterile flask containing a pure vermiculitic clay mineral mixed with quartz sand of different sizes (14). Known amounts of the pathogen and biocontrol agent were added, and then the plants were planted and moistened with modified Knop solution (14) as briefly described here.

For cress, flat-bottomed flasks (100 ml) with openings of 3 cm (diameter) were filled with 60 g of artificial soil. The flasks were plugged with cotton wool and autoclaved at 121 C for 30 min. Bacteria were added to the soil with a sterile syringe to a final concentration of 10^7 cfu/g of soil. Four days later 0.05 g of millet covered with *P. ultimum* was mixed into the soil. Control flasks without *P. ultimum* contained 0.05 g of autoclaved millet. On the same day, 0.2 g of cress seeds (*Lepidium sativum* L. 'Gartenkresse einfach,' Altorfer Samen, Zürich, Switzerland) was placed on the soil, and 2 ml of modified Knop nutrient solution was added. Cress seeds showed practically no evidence for contamination with microorganisms when placed on nutrient agar for 3 days. We were therefore able to avoid surface sterilization with sodium hypochlorite, because this causes an extensive swelling of the seeds. The flasks were placed in randomized blocks in a growth chamber with 70% relative humidity and incubated at 22 C with light (80 μmol·m^{-2}·sec^{-1}) for 16 h, followed by an 8-h dark period at 15 C. After seven days, plants were removed from the flasks by flushing with tap water. Roots were separated from adhering soil by washing. Roots and shoots were briefly dried with paper tissues and weighed separately.

For cucumber, seeds (*Cucumis sativus* L. 'Chinesische Schlange,' Altorfer Samen, Zürich, Switzerland) were surface-disinfected for 30 min with 1% sodium hypochlorite (v/v), rinsed with sterilized double-distilled water, and then grown on 0.6% water agar in the dark for 3 days. For sweet corn, seeds (*Zea mays* L. var. *saccharata* (Sturtev.) L. H. Bailey 'meritosa,' Samen Mauser, Dübendorf, Switzerland) were surface-disinfected in 5% sodium hypochlorite (v/v) for 10 min, rinsed with sterilized double-distilled water, and then grown on 0.6% water agar in the dark for 3 days. Erlenmeyer flasks (1,000 ml) with an opening of 5 cm (diameter) were filled with 400 g of artificial soil, plugged, and autoclaved. Bacteria were added as described above. Four days later, millet covered with *P. ultimum* (0.2 g for cucumber, 1.5 g for sweet corn) was mixed into the soil. Control flasks without the pathogen received the same amount of autoclaved millet. On the same day, three seedlings of cucumber or five seedlings of sweet corn were planted per flask, and 15 ml of

TABLE 1. Production of pyoluteorin (Plt) and 2,4-diacetylphloroglucinol (Phl) by *Pseudomonas fluorescens* strain CHA0 and CHA0/pME3090 on malt and King's B agar plates

Bacteria[x]	Malt agar[y,z]		King's B agar[y,z]	
	Plt	Phl	Plt	Phl
CHA0	<0.1 a	9.3 a	8.3 a	<0.1 a
CHA0/pME3090	2.8 b	14 b	26.4 b	<0.1 a

[x] CHA0 = wild-type strain of *P. fluorescens*, and CHA0/pME3090 = pyoluteorin and 2,4-diacetylphloroglucinol overproducing transconjugant of CHA0. The bacteria were grown on agar plates for 3 days at 18 C. The agar plates were extracted as described in Materials and Methods.
[y] Antibiotic production in micrograms per milliliter of medium.
[z] Means within the same column followed by the same letter are not significantly different at $P = 0.05$ according to the Student's t test. Each value is the mean of six experiments with one replicate and one extraction per replicate.

sterile modified Knop nutrient solution was added. After 2 wk in a growth chamber with 70% relative humidity and 16 h of light (160 μmol·m^{-2}·sec^{-1}) at 22 C and a 8-h dark period at 18 C, the plants were removed from the flasks by flushing with tap water, briefly dried with paper tissues, and weighed.

Bacterial root colonization. After being weighed, plants were assessed for bacterial root colonization as described earlier (14). Colonies of strain CHA0/pME3090 growing on King's B agar were transferred by replica plating onto nutrient agar (26) containing 125 μg of tetracycline per milliliter. After 48 h at 27 C, the percentage of tetracycline-resistant colonies indicative of pME3090 was evaluated.

Fungitoxicity of Plt and Phl. Plugs (6 mm in diameter) of *P. ultimum* were placed on malt agar plates containing different amounts of synthetic Plt or synthetic Phl. The plates were incubated at 27 C for 7 days. Toxicity was scored by measuring the radial growth of the fungal mycelium.

Phytotoxicity of Plt and Phl. Cucumber and sweet corn seeds were surface-disinfected as described above and grown on 0.6% water agar for 4 days. Four cucumber seedlings, four sweet corn seedlings, or 0.2 g of cress seeds were placed into sterile plant tissue culture containers (Flow Laboratories, Inc., McLean, VA) containing Knop nutrient solution agar, with or without different amounts of synthetic Plt or Phl. After 7 days in a growth chamber with 70% relative humidity and 16 h of light (80 μmol·m^{-2}·sec^{-1}) at 22 C and a dark period of 8 h at 15 C, the plants were carefully taken out from the agar, briefly dried with paper tissues, and weighed.

Statistics. Each experiment was repeated at different times; means of at least three experiments are presented. Each mean was compared with all other means by the Student's *t* test, considering one independent experiment as a repetition. An analysis of variance could not be performed, because the variation among the treatments was not homogenous (Bartlett test) due to the erratic loss of the plasmid by the transconjugant CHA0/pME3090.

RESULTS

Screening for increased inhibition of *P. ultimum* in vitro. The sensitivity of *P. ultimum* to Plt prompted us to construct a derivative of *P. fluorescens* strain CHA0 overproducing this antibiotic. To screen for such a strain, we mobilized a genomic library of strain CHA0 (established in the cosmid pVK100) from *Escherichia coli* to strain CHA0 and tested the 2,112 transconjugants for increased inhibition of *P. ultimum* on King's B agar. One recombinant cosmid (pME3090) that in strain CHA0 caused an inhibition zone of 11 mm on King's B agar was identified, whereas the wild type CHA0 showed an inhibition zone of 4 mm. In similar tests on malt agar, strain CHA0/pME3090 gave an inhibition zone of 3 mm, the inhibition zone produced by the wild-type strain being 2 mm. Six other transconjugants also showed an increased inhibition on King's B agar but not to the same extent as strain CHA0/pME3090. None of the transconjugant strains showed less fungal inhibition compared to strain CHA0.

Southern hybridization of the CHA0 genome. Plasmid pME3090 contained an insert of about 22 kb of *P. fluorescens* DNA composed of two *Hind*III fragments (data not shown). Southern hybridization of the CHA0 genome with pME3090 as the probe showed that both *Hind*III fragments in the insert consisted of *P. fluorescens* DNA (data not shown).

Antibiotic production. Strains CHA0 and CHA0/pME3090 and the other six transconjugant strains that showed increased inhibition of *P. ultimum* were grown on malt and King's B agar. Cosmid pME3090 enhanced the production of both antibiotic compounds, in particular that of Plt (Table 1). On malt agar, cosmid pME3090 rendered strain CHA0 capable of producing Plt and increased the production of Phl by 50%. On King's B agar, strain CHA0/pME3090 produced about three times more Plt compared to the wild-type strain (Table 1). Both strains did not produce Phl on King's B agar (Table 1). Strain CHA0/pME3090 produced pyoverdine and hydrogen cyanide to the same

TABLE 2. Influence of increased pyoluteorin (Plt) and 2,4-diacetylphloroglucinol (Phl) production in *Pseudomonas fluorescens* strain CHA0 on the suppression of damping-off of cucumber, cress, and sweet corn caused by *Pythium ultimum*

| Plant tested | Microorganisms added | | Root fresh weight^{x,y} (mg) | Plant fresh weight^{x,y} (mg) | Fluorescent pseudomonads^y (10^8 cfu/g)^z |
	Bacteria^v	*P. ultimum*^{w}			
Cucumber	None	−	547 a	1,147 ab	0.0 a
	CHA0	−	544 a	1,175 a	1.0 b
	CHA0/pME3090	−	498 a	1,138 ab	0.4 b
	None	+	71 d	91 d	0.0 a
	CHA0	+	158 c	471 c	18.0 c
	CHA0/pME3090	+	394 b	948 b	1.8 b
Cress	None	−	1,032 a	2,696 a	0.0 a
	CHA0	−	1,045 a	2,627 a	14.0 b
	CHA0/pME3090	−	462 b	1,317 b	30.0 b
	None	+	47 d	176 d	0.0 a
	CHA0	+	618 b	1,578 b	23.0 b
	CHA0/pME3090	+	247 c	751 c	49.0 b
Sweet corn	None	−	1,489 a	2,111 a	0.0 a
	CHA0	−	1,317 a	1,917 a	1.1 b
	CHA0/pME3090	−	982 b	1,430 b	1.2 b
	None	+	576 d	929 c	0.0 a
	CHA0	+	952 b	1,471 b	1.2 b
	CHA0/pME3090	+	665 c	1,010 c	1.7 b

^{v} CHA0 = wild-type strain of *P. fluorescens*, and CHA0/pME3090 = Plt and Phl overproducing transconjugant of strain CHA0. Bacteria were added as described in Materials and Methods.

^{w} *P. ultimum* was added as described in Materials and Methods.

^{x} Fresh weight per plant for cucumber and sweet corn, fresh weight per flask for cress.

^{y} 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's *t* test considering one independent experiment as a repetition. Cucumber: each value is the mean of three experiments with three replicates per experiment and one flask with three plates per replicate. Cress: each value is the mean of six experiments with five replicates per experiment and one flask with plants grown from 0.2 g of seeds per replicate. Sweet corn: each value is the mean of six experiments with three replicates per experiment and one flask with five plants per replicate.

^{z} Colony-forming units per gram of root.

extent as did the wild-type strain (data not shown). The six other transconjugants did not differ from the wild-type strain in the amounts of Plt and Phl they produced.

Disease suppression. In the presence of *P. ultimum* the final fresh weight of cucumber plants was drastically reduced (Table 2). Fifty percent of the plants were dead. Strain CHA0 provided partial protection against the pathogen. Fresh weights were five times higher compared to plants grown in the presence of *P. ultimum* alone; however, they reached only 40% of the fresh weights of plants grown in absence of microorganisms (Table 2). Strain CHA0/pME3090 provided better protection with plant weights twice as high as those found after treatment with wild-type CHA0 (Table 2). These results show that in this host-pathogen system, cosmid pME3090 improves the disease suppressive capacity of strain CHA0. In the absence of *P. ultimum*, cucumber

plants that had been treated with either CHA0 or CHA0/pME3090 had the same root and total weights as had plants grown without bacteria (Table 2). In treatments with strain CHA0 and *P. ultimum* the bacterial root colonization was 2×10^9 colony forming units (cfu) per gram of roots (Table 2). In all other treatments, the bacterial root colonization was significantly lower (4×10^7–2×10^8 cfu/g roots).

In the absence of the pathogen, the antibiotic overproducing recombinant strain was toxic to cress and sweet corn in that it reduced plant fresh weight by 51% on cress and 32% on sweet corn, whereas strain CHA0 had no influence on plant growth in either case (Table 2). Both strains CHA0/pME3090 and CHA0 protected cress and sweet corn against *P. ultimum* disease, but root and total fresh weights of plants protected with strain CHA0/pME3090 were 50% lower for cress or 30% lower for sweet corn, compared to plants protected by the wild-type strain (Table 2). Within the system cress-*P. ultimum* and within the system sweet corn-*P. ultimum*, strain CHA0 and strain CHA0/pME3090 colonized the roots to the same extent (Table 2).

In the three plant-pathogen systems tested, 70–80% of the bacteria reisolated from the roots that still contained cosmid pME3090.

Fungi and phytotoxicity of Plt and Phl. Because enhanced antibiotic production in strain CHA0 resulted in a toxic effect on cress and sweet corn but not on cucumber, the toxicity of synthetic Plt and Phl on these plants and on *P. ultimum* was tested on Knop and malt agar plates, respectively, containing different amounts of either of the two antibiotics. The first visible inhibitory effect of Plt on *P. ultimum* and the plants tested could be observed at concentrations between 16 and 32 μM (Fig. 1A; Fig. 2). At a concentration of 128 μM of Plt, the growth of *P. ultimum* was completely inhibited; the growth of cucumber, cress, and sweet corn in terms of plant fresh weight was lowered by 72, 90, and 95%, respectively (Fig. 1A; Fig. 2). Phl was much less toxic to growth of *P. ultimum* than was Plt and slightly less for the plants (Fig. 1A,B; Fig. 2). Substantial growth reduction of the three plant species tested could be observed at 80 μM of Phl, a concentration that had no effect on the growth of the pathogen (Fig. 1B; Fig. 2). A concentration of 320 μM of Phl was necessary to reduce the growth of *P. ultimum* (Fig. 2). Cucumber was less sensitive to concentrations between 80 and 640 μM of Phl compared to cress and sweet corn. At a concentration of 640 μM, the growth of *P. ultimum* and cress was completely inhibited by Phl, whereas the growth of sweet corn and cucumber was lowered by 80 and 63%, respectively (Fig. 1B; Fig. 2).

DISCUSSION

Cosmid pME3090 in *P. fluorescens* strain CHA0 enhanced the in vitro production of Plt strongly and that of Phl weakly (Table 1). Enhanced Plt production could explain increased inhibition of *P. ultimum* on King's B agar, because neither strain CHA0 nor strain CHA0/pME3090 produced Phl on this medium. On malt agar, Phl production by strain CHA0/pME3090 was probably not high enough to inhibit the growth of *P. ultimum* (Table 1; Fig. 2). Therefore, the ability of the mutant to produce Plt on malt agar may account for the slightly improved antibiosis on this medium. The reason for the small inhibition zone strain CHA0 was able to cause on malt agar was probably the production of hydrogen cyanide by strain CHA0.

The mechanism by which pME3090 enhances antibiotic production in strain CHA0 is not clear, because the genes carried by the insert of this plasmid have not been identified. The vector used for the construction of pME3090 has several copies. Hence, a gene dosage effect of some sort can be envisaged. There are different possibilities, for example, that pME3090 carries either structural genes for Plt and Phl synthesis or a gene or genes for positive regulation of Plt and Phl synthesis, or that it permits the production of increased amounts of precursor molecules that are converted to Plt and Phl.
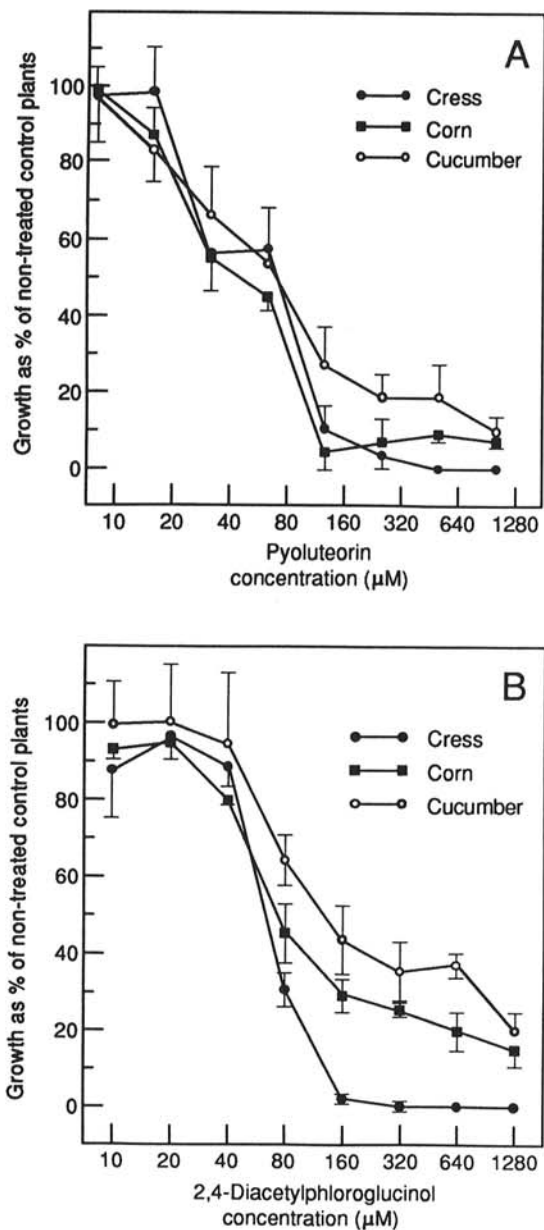
Fig. 1. Influence of increasing concentrations of **A,** synthetic pyoluteorin (Plt) and **B,** synthetic 2,4-diacetylphloroglucinol (Phl) on the growth of cress, sweet corn, and cucumber (plant fresh weights). Plants were grown for 7 days on Knop agar plates supplemented with different amounts of Plt or Phl. Each value is the mean of three experiments with three replicates per experiment and four plants (cucumber, sweet corn) or plants grown from 0.2 g of seeds (cress) per replicate. Error bars give the standard deviations of the means.

In the gnotobiotic system, strain CHA0/pME3090 was superior to its parent in the protection of cucumber against *P. ultimum* (Table 2). We suggest that the improved production of Plt rather than that of Phl may be responsible for this beneficial effect for the following reasons: *P. ultimum* was found to be much more sensitive to Plt than to Phl (Fig. 2). Moreover, a Phl negative Tn5 insertion mutant of strain CHA0 showed no reduction in the capacity to protect cucumber against *P. ultimum* (M. Maurhofer, *unpublished results*), indicating that Phl has no role in the suppression of damping-off of cucumber by strain CHA0. Kraus and Loper (18,19) investigated the role of Plt in the suppression of Pythium damping-off of cucumber and cotton. Two Plt negative Tn5 mutants of *P. fluorescens* strain Pf-5 also suppressed Pythium damping-off of cucumber, but one of these was less suppressive than the parental strain. Kraus and Loper (19) suggest that Plt may have contributed to the biocontrol activity of Pf-5, but it did not wholly determine this activity. However, in a Willamette sandy loam containing indigenous *Pythium* spp. Pf-5 and Plt negative Tn5 mutants of Pf-5 were statistically indistinguishable with respect to biocontrol of cotton damping-off (18). The role of Plt in disease suppression may depend on the plant-pathogen system.

In the system described here, the improvement of the antibiotic production resulting in improvement of disease suppression was due to a gene dosage effect. Gutterson and collaborators also have constructed an antibiotic overproducing strain of *P. fluorescens*. These authors fused the *E. coli tac* promoter to a chromosomal gene cluster essential for the biosynthesis of the antibiotic oomycin A in *P. fluorescens* Hv37a, and observed enhanced production of oomycin A and improved suppression of damping-off of cotton (6,7). Control of *P. ultimum* by this strain was improved by 20–25% in field soil (6), whereas the disease suppression of strain CHA0/pME3090 under gnotobiotic conditions was twofold more effective compared to that of strain CHA0 (Table 2).

Cosmid pME3090 could not improve the protection of cress and sweet corn against *P. ultimum*. Moreover, in the absence of the pathogen, strain CHA0/pME3090 reduced the growth of cress and corn. In the presence of the pathogen, fresh weights of cress and sweet corn plants protected by strain CHA0/pME3090 were lower than those of plants protected by strain CHA0 (Table 2); the reason for this may have been that strain CHA0/pME3090 was deleterious to the plants rather than that it wa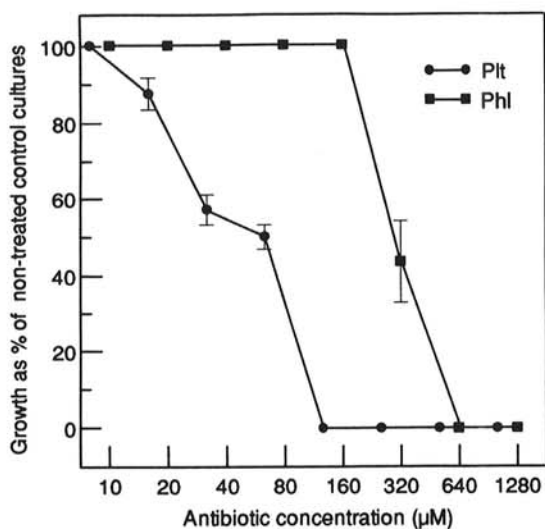s less efficient in disease suppression. Although we have not determined the amounts of Plt and Phl produced in the rhizosphere of cress and sweet corn, and we have not tested the phytotoxic effects of combinations of Plt and Phl, we suggest that in the rhizosphere the recombinant strain CHA0/pME3090 synthesized Plt and Phl in sufficient quantities to inhibit the growth of cress and sweet corn. In contrast, cucumber was not affected, either because too low an amount of these compounds was produced in the rhizosphere or because this plant was more resistant to the combination of Plt and Phl. Cucumber was less sensitive to Phl than were cress and sweet corn at concentrations between 80 and 640 μM (Fig. 1). It is known that the phytotoxic effect of Plt and Phl depends on the plant species tested (13,15, 21,23). In seed germination tests Reddi et al (23) also found cucumber to be less sensitive to Phl compared to other plants tested.

Increased inhibition of a pathogen in vitro or increased antibiotic production does not necessarily lead to improved disease suppression; this has been shown previously. Two Tn5 mutants of *P. fluorescens* strain NRRL B-15135 that were selected for increased antibiosis against *Gaeumannomyces graminis* var. *tritici* in vitro had a reduced capacity to suppress take-all disease (22). Shim et al (25) showed that overproduction of agrocin 84 in *Agrobacterium radiobacter* strain K84 does not lead to improved control of crown gall; in one case it even reduces drastically the biocontrol capacity of strain K84 (25).

In conclusion, genetic alteration of *P. fluorescens* strains to increase their antibiosis capacity in vitro can, depending on the host-pathogen system, either improve their disease suppressive capacity or render them deleterious to the plant.

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Fig. 2. Inhibition of *Pythium ultimum* by synthetic pyoluteorin (Plt) and synthetic 2,4-diacetylphloroglucinol (Phl). *P. ultimum* was grown on malt agar plates supplemented with different amounts of Plt or Phl at 27 C for 7 days. Each value is the mean of three experiments with three replicates per experiment. Error bars give the standard deviations of the means.

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