# Suppression of Black Root Rot of Tobacco and Other Root Diseases by Strains of *Pseudomonas fluorescens*: Potential Applications and Mechanisms

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# Chapter 7

# Suppression of Black Root Rot of Tobacco and Other Root Diseases by Strains of *Pseudomonas fluorescens*: Potential Applications and Mechanisms

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## INTRODUCTION

*Pseudomonas fluorescens* strain CHA0 was isolated from a soil at Morens (near Payerne, Switzerland). This soil is naturally suppressive to black root rot of tobacco, a disease caused by *Thielaviopsis basicola* (Gasser and Défago, 1981; Stutz *et al.*, 1986), and populations of fluorescent pseudomonads tightly bound to roots were shown to be involved in disease suppression (Stutz *et al.*, 1986; Défago and Haas, 1989). This suppressive soil is derived from a weathered ground moraine and contains more copper, iron, and zinc than the neighbouring soil which is derived from a weathered molasse and which is naturally conducive to black root rot of tobacco (Stutz *et al.*, 1985). The weathered ground moraine contains vermiculitic clay minerals, whereas the weathered molasse contains illitic-smectitic clay minerals (Stutz *et al.*, 1989). *P. fluorescens* strain CHA0 was able to suppress disease in natural soil containing vermiculitic clay minerals and in artificial soil consisting of quartz grains and native vermiculite or clay minerals derived from the suppressive soil (Stutz *et al.*, 1986 and 1989). No, or little, suppression occurred in natural soil containing illitic clay minerals or in artificial soil composed of quartz grains and illite or clays derived from the conducive soil. In soil containing smectitic clays, disease suppression was slightly less than in vermiculite (Stutz *et al.*, 1989). Many regions of Switzerland contain vermiculitic or smectitic clay minerals. In the light of these results, the presence of these types of clays in soil appears to be important for the potential application of strain CHA0 as a biocontrol agent.

# FIELD TRIALS WITH CEREALS

*P. fluorescens* strain CHA0 or its spontaneous mutant C03 (resistant to nalidixic acid) were applied to field plots naturally infested with *Gaeumannomyces graminis* var. *tritici* (*Ggt*). In one set of experiments, plots of 1 m × 2 m were sown in October 1987 with winter wheat cv. Partizanka at 6 rows per 1 m. Strain CHA0 was applied as a suspension in the furrow at $2.5 \times 10^{12}$ colony-forming units (cfu)/$m^2$; Baytan F was applied to seeds at 60–100 g/100 kg of seeds. The number of healthy heads in the four middle rows was counted in May. The plots were separated from each other by one metre planted with non-treated wheat and the plots were randomized. The field was near Geneva and the soil type was similar to that of the soil VC1 (Stutz, 1985; Stutz *et al.*, 1986). At harvest in May, about two-thirds of the heads of wheat in the non-treated plots were white (symptoms often associated with *Ggt*) and runner hyphae were observed on the roots. In the plots inoculated with strain CHA0 the

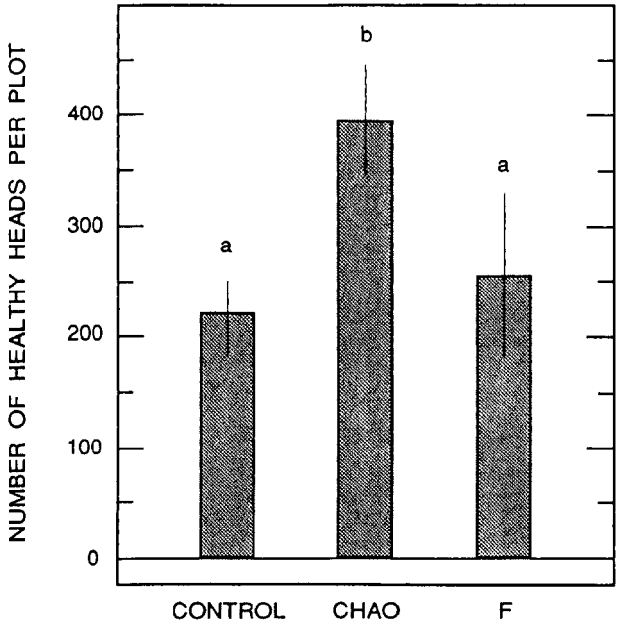


*Figure 7.1* Suppression by *Pseudomonas fluorescens* strain CHA0 of take-all and whiteheads caused by a natural infestation of *Gaeumannomyces graminis* var. *tritici* in field plots. CONTROL = mean of five non-treated plots; CHA0 = mean of five plots treated with strain CHA0; F = mean of five plots treated with Baytan F (triadimenol and fuberidazole). Columns with the same letter are not significantly different at $P=0.05$ according to Student's t-test; each mean was compared with each of the other means. Bars indicate the standard error of the mean.

number of healthy heads was increased by about 80% (Figure 7.1). In the plots treated with Baytan F the increase was about 17%. This indicates that strain CHA0 was able to suppress disease caused by *Ggt* efficiently under these field conditions.

When spring wheat was sown in 1986 in a field in which the previous three crops had also been wheat, the emergence was poor, and *Pythium* could be isolated. Plots of 1 m × 1 m were sown with spring wheat cv. Kärtner précoce. Bacteria were incorporated into granules of native vermiculite at the rate of $10^5$ cfu/g of vermiculite and the granules applied to the furrow between the seeds. Plots were separated from each other by 2.5 m planted with wheat. The field was located at Eschikon, near Zürich, where the soil has clay minerals and a geological origin similar to the soil MS1 (Défago *et al.*, 1987; Stutz *et al.*, 1989). In plots inoculated with strain CO3, the number of shoots was increased by 31% (Figure 7.2). Strain CO3 colonized the roots and the rhizosphere soil down to a depth of one metre in two and a half months. During the first two months after inoculation the number of bacteria of strain CO3 which could be reisolated from

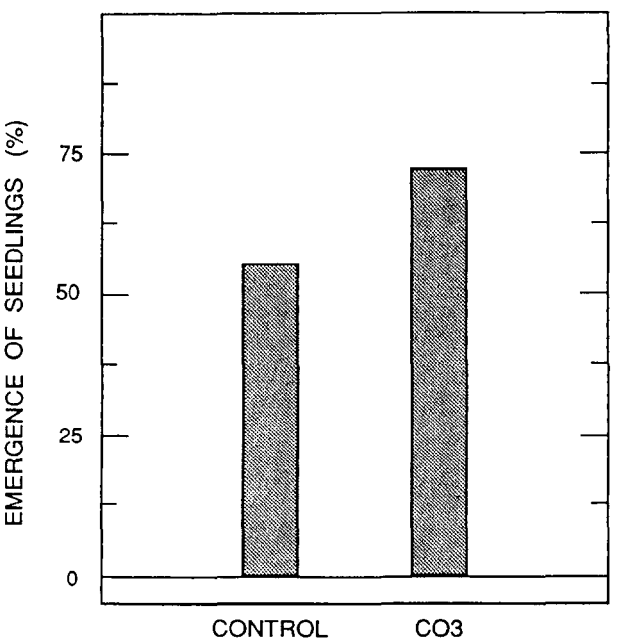


*Figure 7.2* Influence of *Pseudomonas fluorescens* strain CO3, a spontaneous mutant of strain CHA0 resistant to nalidixic acid, on emergence of spring wheat grown as a fourth consecutive wheat crop. CONTROL = mean of three non-treated plots; CO3 = mean of three plots treated with 2 x $10^7$ cfu of strain CO3 per $m^2$. The treatment differed significantly from the control at $P$=0.05, according to Student's t-test.

fresh roots decreased from $10^6$ to $10^4$ cfu/g and remained constant after this period. Nine months later, when a soil sample was taken to a greenhouse and planted with wheat, we were able to isolate $10^4$ cfu/g of fresh roots (Défago *et al.*, 1987).

We have tried different methods for the application of bacteria to field plots: freshly prepared suspensions added in the furrow, incorporation in sawdust or in granules of native vermiculite before applying in the furrow between the seeds, or seed coating. The last three allowed good survival of the bacteria on the shelf at room temperature for at least 2 months. The emergence of wheat and the number of bacteria reisolated from the rhizosphere soil were higher when the bacteria were applied in vermiculite granules than when applied as a seed coating. The granules of vermiculite gave better results than sawdust (Défago *et al.*, 1987).

These results were obtained in fields with similar soil types in different regions of Switzerland between 1985 and 1988. However, further field trials are necessary to assess the beneficial effect of strain CHA0. From other work reviewed by Schippers (1988) and Weller (1988), it is known that disease suppression showed considerable variation when analysed over several consecutive years. However, in some of those experiments, the fields used in different years may have differed in soil type. It will be interesting to see the long-term effect of strain CHA0 when it is used in fields with known vermiculitic clay minerals.

## GREENHOUSE AND GROWTH CHAMBER EXPERIMENTS

Strain CHA0 suppressed efficiently diseases caused by *T. basicola* on tobacco, cotton and cherry trees, and take-all of wheat due to *Ggt*, in natural, non-sterilized soil. These diseases and those caused by *Aphanomyces euteiches* on peas, *Fusarium oxysporum* f.sp. *lycopersici* on tomatoes, *F. oxysporum* f.sp. *lini* on flax and *Rhizoctonia solani* on cotton were also suppressed in partially sterilized soil or in artificial soil under gnotobiotic conditions (Stutz *et al.*, 1986; Défago *et al.*, 1987; Keel *et al.*, 1989; J. Fuchs, and E. Stutz, personal communications).

## MECHANISMS OF DISEASE SUPPRESSION

Different mechanisms have been proposed to explain how pseudomonads suppress diseases: (i) inhibition of the pathogen by competition for Fe(III) (siderophore hypothesis); (ii) inhibition of the pathogen by diffusible or volatile products (antibiosis); (iii) induction of resistance in the plant; and (iv) aggressive root colonization and stimulation of plant growth (Howell and Stipanovic, 1980; Kloepper *et al.*, 1980; Leong, 1986; Loper, 1988;

Thomashow and Weller, 1988; Schippers, 1988; Weller, 1988; Défago and Haas, 1990). These hypotheses are not mutually exclusive. Their diversity arises from the array of extracellular metabolites produced by pseudomonads which may be of importance in disease suppression (Leisinger and Margraff, 1979; Kiprianova and Smirnow, 1981; Défago and Haas, 1990). The synthesis and the fungitoxicity of these metabolites interact in an extremely complex way with environmental conditions, so we decided to use genetic tools to investigate the mechanisms of disease suppression and to develop a gnotobiotic system for suppression tests under standardized conditions.

## THE GNOTOBIOTIC SYSTEM

The gnotobiotic system allows us to measure the suppressive effect quantitatively (plant weight), qualitatively (disease index) and reproducibly (Keel *et al.*, 1989). This system consists of a sterilized artificial soil composed of quartz grains and pure clay minerals, a tobacco plant grown from surface sterilized seed, and known numbers of *T. basicola* endoconidia and *P. fluorescens* cells in a flask plugged with cotton wool. Water is added to the soil only at the beginning of the experiment. This arrangement enables us to control the essential environmental parameters and has proved to be useful in our studies on the molecular mechanism of disease suppression by *P. fluorescens*. The gnotobiotic system, however, excludes competition with other organisms and hence does not reflect the situation in the field. The suppressive capacity is therefore tested in both pasteurized and untreated natural soils as well. Although strain CHA0 seems to protect the plants better in natural soil than in the gnotobiotic system (compare Figures 7.3 and 7.7), this discrepancy arises from the high inoculum density of the pathogen in the gnotobiotic system, which is deliberate to reduce variation between the replicates.

## GENETIC CHARACTERIZATION OF STRAIN CHA0

The successful genetic manipulation of a soil bacterium requires several tools, i.e. methods for transposon insertion mutagenesis and for transfer of recombinant cosmids to the strain of interest (allowing complementation tests), as well as gene replacement techniques (Défago and Haas, 1989). The success of these methods depends on efficient conjugation systems that enable transfer of plasmids from *Escherichia coli* to the bacterium of interest. The most commonly used transfer functions are those of the broad-host-range plasmid RP1 (= RP4 = RK2) (Simon, 1989). However, RP1 could not be transferred from *E. coli* to *P. fluorescens* strain CHA0. Fortuitously it was found that this conjugative transfer was possible with

RP1 derivatives which had large deletions extending from the primase gene towards the *Tra-2* transfer gene region, thus lacking the kanamycin-resistance gene and insertion sequence IS*21*. Such RP1 deletion derivatives permitted IncP cosmid mobilization to *P. fluorescens* CHA0 and could be used as vectors for transposon mutagenesis in strain CHA0 (Voisard *et al.*, 1988). The IncI1 transposon delivery vector pLG221 (Boulnois *et al.*, 1985) was also found suitable for mutagenizing strain CHA0. A genomic bank of this strain was constructed in the IncP vector pVK100. Using these recombinant plasmids we have been able to do complementation tests of HCN-non-producing mutants of strain CHA0 (see below). We then devised a system which allows gene replacement in *P. fluorescens* CHA0; this system is also based on the transfer functions of IncI1-vectors (Voisard *et al.*, 1989).

## IDENTIFICATION OF SECONDARY METABOLITES PRODUCED BY STRAIN CHA0

Strain CHA0 produces several extracellular metabolites (Table 7.1) and a number of additional, unidentified compounds. Some of these metabolites might be important for disease suppression. The efforts reported here were focused on the role of two compounds, HCN and pyoverdine. About 800

*Table 7.1* Extracellular metabolites produced by *Pseudomonas fluorescens* strain CHA0.

| Metabolites |
|---|
| HCN[a] |
| Siderophore(s) (pyoverdine)[a] |
| 2,4-Diacetylphloroglucinol[b] |
| Pyoluteorin[b] |
| Monoacetylphloroglucinol[b] |
| Salicylic acid[c] |

[a]Ahl *et al.*, 1986

[b]These metabolites were isolated by standard acetone extraction techniques (Howell and Stipanovic, 1980), and purified by thin layer chromatography on silica gel (chloroform/methanol, 20:1).

2,4-Diacetylphloroglucinol, the main antibiotic constituent ($R_f = 0.55$) was found by $^1$H-NMR, UV, IR, and mass spectrometry to be identical to a sample synthesized according to Campbell and Coppinger (1951).

Pyoluteorin, the second most abundant antibiotic ($R_f = 0.35$), was identical by standard spectroscopic methods to an authentic sample synthesized according to Birch *et al.* (1964) and Cue *et al.* (1981).

The structure of monoacetylphloroglucinol (2,4,6-trihydroxyacetophenone) ($R_f = 0.32$) was assigned to a very minor third antibiotic metabolite based on spectroscopic comparison with a commercial sample (Aldrich Chemicals).

[c]M. A. Abdallah (personal communication)

plant species are known to produce HCN after wounding or fungal attack. Successful plant pathogens of these species are less HCN-sensitive than unsuccessful ones. There is speculation that liberation of HCN by the plant contributes to disease resistance (Mansfield, 1983). In contrast, many deleterious pseudomonads (rhizobacteria which inhibit plant growth without visibly damaging the roots) also produce HCN. HCN inhibits rooting of potatoes in liquid culture. There is a hypothesis that HCN is responsible for the deleterious effect of these bacteria (Bakker and Schippers, 1987). The first tests in our system were to see whether HCN has a beneficial or deleterious effect on the plant.

## ROLE OF HCN IN DISEASE SUPPRESSION

The importance of HCN was tested in the gnotobiotic system containing an artificial, iron-rich vermiculite soil. An HCN-negative (*hcn*) mutant, CHA5, constructed by gene replacement, gave about 25%, whereas the wild-type strain CHA0 afforded about 70% protection (100% protection being equivalent to the yield of tobacco plants in the absence of *T. basicola*). Complementation of strain CHA5 by the *hcn*$^+$ genes cloned in pVK100 restored the strain's ability to suppress black root rot (Figure 7.3A). A derivative of transposon Tn*5* carrying the *hcn*$^+$ genes of strain CHA0 (Tn*hcn*) was constructed and inserted into the genome of *P. fluorescens* P3, a strain which does not naturally produce HCN and gives poor protection of tobacco (Figure 7.3B). The P3::Tn*hcn* derivative synthesized HCN and exhibited an improved ability to suppress disease but did not reach the suppressive capacity of strain CHA0 (Voisard *et al.*, 1989). All bacterial strains colonized the roots similarly and did not reduce significantly the number of *T. basicola* propagules in soil, indicating that the bacteria killed little or no fungus in the artificial soil, despite the fact that *T. basicola* is sensitive to HCN on malt agar (Ahl *et al.*, 1986). However, it is possible that *P. fluorescens* inhibits the pathogenic activity of *T. basicola* locally, i.e. on the rhizoplane. Glycine, a component of root exudates (Rovira and Davey, 1971), may locally stimulate the formation of HCN by *P. fluorescens*. *In vitro*, glycine is known to induce cyanogenesis in *Pseudomonas* (Askeland and Morrison, 1983; Voisard *et al.*, 1989). The production of HCN did not decrease the weight and growth of tobacco plants in the absence of the pathogen. These results lead us to conclude that HCN produced by strain CHA0 contributes significantly to suppression of black root rot, but clearly other metabolites (presumably antifungal compounds) are also important for suppression (Voisard *et al.*, 1989).

HCN seemed to be a major factor involved in the suppression of take-all disease of wheat in natural, partially sterilized soil under greenhouse conditions. Autoclaved soil from Eschikon (see Figure 7.2) was placed in pots, inoculated with $10^7$ cfu of bacteria per ml of soil and, one week

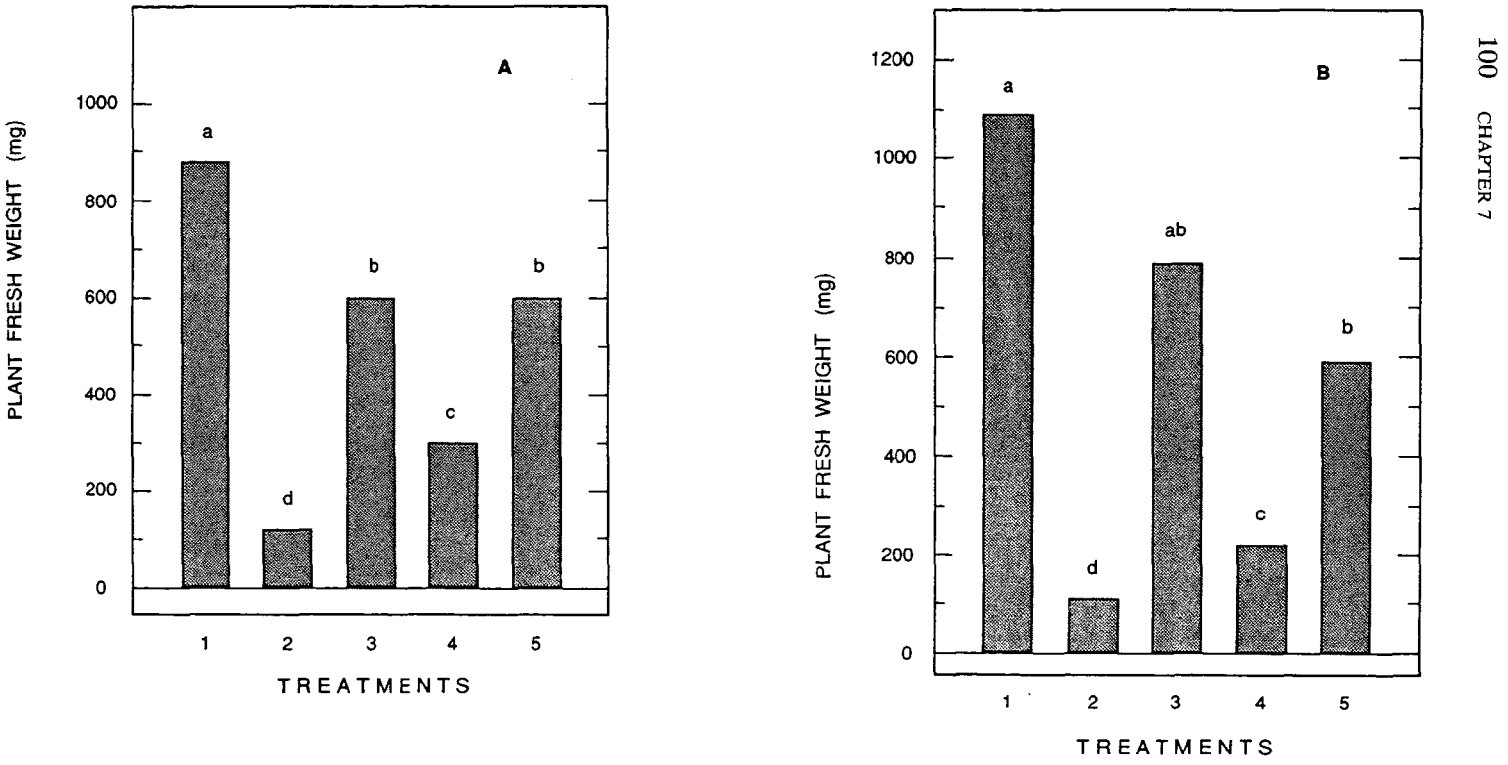


*Figure 7.3* Role of HCN in suppression by *Pseudomonas fluorescens* of tobacco black root rot caused by *Thielaviopsis basicola* (Tb) revealed in gnotobiotic conditions using an artificial soil containing vermiculite. A: treatment 1, plant growing in the absence of Micro-organisms; treatment 2, plant infected with Tb; treatment 3, plant infected with Tb and protected with strain CHA0 ($Hcn^+$); treatment 4, plant infected with Tb and protected with strain CHA5 ($Hcn^-$); treatment 5, plant infected with Tb and protected with CHA5/pME3013 ($Hcn^+$). B: treatments 1, 2 and 3 as in A; treatment 4, plant infected with Tb and protected with P3 ($Hcn^-$); treatment 5, plant infected with Tb and protected with P3::Tn*hcn* ($Hcn^+$). Treatments with the same letter are not significantly different at $P=0.05$ according to Student's t-test; each mean was compared with each of the other means.

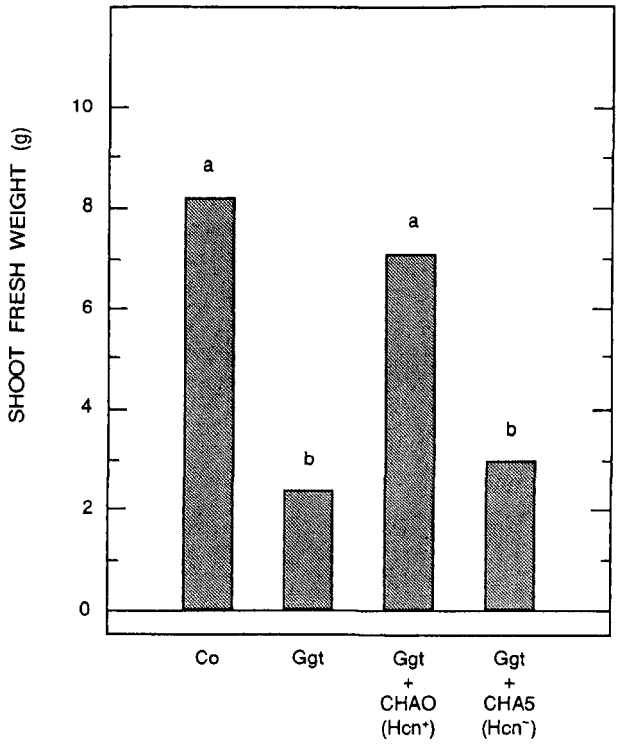


*Figure 7.4* Role of HCN in suppression by *Pseudomonas fluorescens* of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in natural soil. The columns represent means of 3 experiments with 6 pots per replicate. Co = soil without added micro-organisms. Ggt = soil infested with *Ggt*. Ggt + CHA0 ($Hcn^+$) = soil infested with *Ggt* and protected with CHA0. Ggt + CHA5 ($Hcn^-$) = soil infested with *Ggt* and protected by CHA5. Columns with the same letter are not significantly different at $P=0.05$ according to Student's t-test; each mean was compared with each of the other means.

later, with *Ggt* grown on dead millet seeds. Two days after inoculation with *Ggt*, 5 seedlings of winter wheat cv. Arina were planted per pot and grown for 6 weeks in a greenhouse. Strain CHA0 increased by 360% the weight of seven-week-old wheat plants infected with *Ggt* grown on dead millet seeds and reduced the number of runner hyphae on roots. The HCN-negative mutant CHA5 had no suppressive effect (Figure 7.4).

## STIMULATION OF ROOT HAIR FORMATION BY STRAIN CHA0

The root tips of tobacco growing under gnotobiotic conditions had short root hairs (Figure 7.5A). In the presence of *P. fluorescens* CHA0, the roots

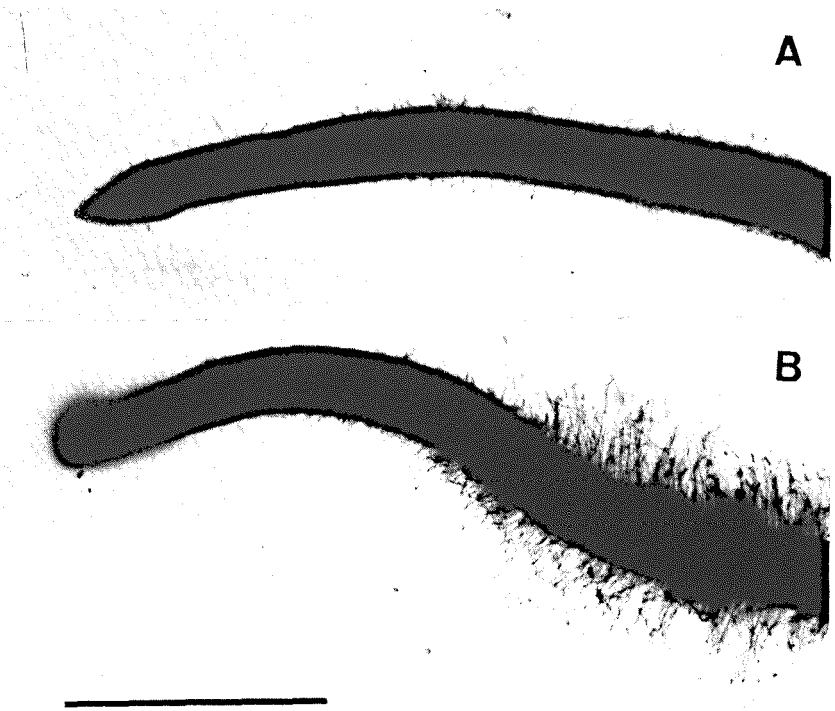


*Figure 7.5* Root tips from a tobacco plant after 3 weeks' growth under gnotobiotic conditions in an artificial soil containing vermiculite. A = without bacteria; B = with strain CHA0 of *Pseudomonas fluorescens*. (Root hairs cleaned of sand and clay; bar = 1 mm.)

were stunted and had numerous long hairs (Figure 7.5B). The root hairs trapped quartz and clay particles. The increased hair formation in the presence of strain CHA0 ($Hcn^+$) was observed on about 50% of the root surface of plants growing in vermiculite, whether or not this soil was infested with *T. basicola*. In the presence of strain CHA5 ($Hcn^-$) only 6% of the root surface had increased root hair formation (Table 7.2). Complementation of strain CHA5 by the *hcn*$^+$ genes cloned in pME3013 restored the ability to induce luxuriant root hair formation. Strain P3 ($Hcn^-$) did not increase root hair formation. In contrast, the P3::Tn*hcn* derivative synthesized HCN and improved root hair formation. Thus, HCN production, root hair formation and the ability of the bacteria to suppress black root rot are positively correlated.

After indirect immunofluorescence staining, the bacteria were found not only on the root surface of tobacco but also in the root cortex and in small wounds (C. H. Berling, personal communication). This points to the possibility that strain CHA0 might produce a stress effect inside the roots.

*Table 7.2* Influence of *Pseudomonas fluorescens* strains CHA0 and P3 and their derivatives on root hair formation by *Nicotiana glutinosa* and on black root rot, caused by *Thielaviopsis basicola* (Tb), under gnotobiotic conditions in artificial soil containing vermiculite.

| Strains | Increased root hair formation[x] % | Disease suppression[y] % |
|---|---|---|
| CHA0 ($Hcn^+$) | 51 a[z] | 66 ab[z] |
| CHA5 ($Hcn^-$) | 5 c | 24 c |
| CHA5/pME3013 ($Hcn^+$) | 50 a | 65 ab |
| P3 ($Hcn^-$) | 3 c | 11 c |
| P3::Tn*hcn* ($Hcn^+$) | 35 b | 49 b |

[x]The percentage of the root surface showing increased root hair formation, estimated by the scale used by Stutz *et al.* (1989) for evaluation of black root rot severity.
[y]100% indicates the same fresh weight as plants growing in the absence of Tb and of bacteria; 0% indicates the same weight as plants growing in the presence of Tb alone (actual values in Figure 7.3).
[z]Means within columns with the same letter are not significantly different at $P = 0.05$ according to Student's t-test; each mean was compared with each of the other means. For details see Voisard *et al.* (1989) and Keel *et al.* (1989).

It is known that cyanide stress induces cyanide-resistant respiration and production of phytoalexins in potato slices (Alves *et al.*, 1979; Laties, 1982). Thus, it seems possible that bacterial HCN might modify the metabolism of tobacco in a way that induces some plant defence mechanisms against the pathogen. The effects of strain CHA0 on the root physiology deserve further study.

## ROLE OF IRON

Iron is necessary for HCN production by pseudomonads *in vitro* (Askeland and Morrison, 1983; Knowles and Bunch, 1986; Bakker and Schippers, 1987). Addition of iron to batch cultures of strain CHA0 in minimal medium induced HCN production. When iron was replaced by vermiculite, cyanogenesis was also induced, but when illite was added instead of vermiculite or iron, cyanogenesis was not induced (Keel *et al.*, 1989). This indicates that in the presence of strain CHA0 vermiculite releases more iron than illite. Strain CHA0 was an effective biocontrol agent in the gnotobiotic system containing vermiculite as a clay mineral. When illite replaced vermiculite, it protected tobacco poorly. When $FeCl_3$ or FeEDDHA (Fe(III)-ethylenediamine-di-*o*-hydroxyphenylacetic acid) was added to illite, strain CHA0 afforded good protection (Figure 7.6). Strain CHA400 (a siderophore-negative, non-fluorescent mutant obtained after transposon insertion) was unable to use $Fe^{3+}$ chelated by EDDHA. This

mutant had the same disease suppressive capacity as strain CHA0 in vermiculite, illite and illite amended with $FeCl_3$. However, strain CHA400, in contrast to strain CHA0, protected plants poorly in illite amended with FeEDDHA. From these experiments we conclude that iron sufficiency is necessary for the metabolism of bacterial strains to suppress black root rot (Keel *et al.*, 1989). We think that the available iron in soil regulates the amount of HCN formed by *P. fluorescens* and, in parallel, the degree of disease suppression. This may explain in part why strain CHA0 is able to suppress disease in vermiculite soil but not in illite soil.

Strains CHA0 and CHA400 increased root hair formation on about 50% of the root surface of tobacco in vermiculite soil infested with *T. basicola* (Table 7.3). With illite instead of vermiculite only 2-7% of the root surface showed increased root hair formation. However, when FeEDDHA was added to this soil, strain CHA0 was able to induce a luxuriant hair formation, whereas strain CHA400 was not (Table 7.3). Again, the induction of luxuriant root hair formation seems to correlate with the capacity of the bacteria to suppress disease.

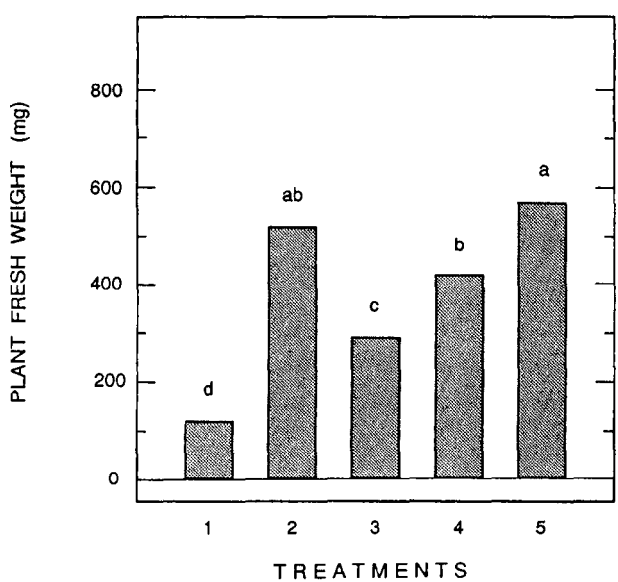


*Figure 7.6* Influence of clay minerals and iron on suppression of tobacco black root rot, caused by *Thielaviopsis basicola* (Tb), by strain CHA0 of *Pseudomonas fluorescens* under gnotobiotic conditions. Treatment 1, vermiculite or illite soil plus Tb. Remainder with Tb and strain CHA0 in soil containing: treatment 2, vermiculite; treatment 3, illite; treatment 4, illite amended with $FeC1_3$; treatment 5, illite amended with FeEDDHA (Fe(III) 0.177 μMol/g dry soil). Columns with the same letter are not different at $P=0.05$ according to Student's t-test; each mean was compared with each of the other means. (Details in Keel *et al.*, 1989).

*Table 7.3* Influence of *Pseudomonas fluorescens* strain CHA0 and its siderophore-negative mutant CHA400 on root hair formation by *Nicotiana glutinosa* and on black root rot caused by *Thielaviopsis basicola* in artificial soil with different clay minerals and iron supplements.

| Strains | Clay minerals and iron supplements | Increased root hair formation[x] % | Disease suppression[y] % |
|---|---|---|---|
| CHA0 | vermiculite | 53 a[z] | 54 ab[z] |
| | illite | 7 bc | 29 c |
| | illite + FeEDDHA* | 56 a | 81 a |
| CHA400 | vermiculite | 53 a | 60 ab |
| | illite | 2 c | 23 c |
| | illite + FeEDDHA* | 9 b | 36 bc |

x, y, z see Table 7.2.
* 0.177 μMol Fe(III) per g dry soil.

## ROLE OF SIDEROPHORES IN DISEASE SUPPRESSION

In a natural, non-sterile soil in pots, strains CHA0 and CHA400 revealed no significant difference in the suppression of tobacco black root rot (Figure 7.7). Therefore, we have no evidence for the involvement of siderophores of the pyoverdine type in suppression of black root rot and it is unlikely that competition for iron (Kloepper *et al.*, 1980; Leong, 1986; Bakker *et al.*, 1986 and 1987; Loper, 1988) is a mechanism of suppression in this soil.

## CONCLUSION

*P. fluorescens* strain CHA0 is able to suppress different diseases in field plots and greenhouse experiments. However, the effectiveness of disease suppression is limited to certain types of soil. In these soils, the determinant factor is the nature and the origin of the clay minerals. Disease suppression is effective in clay minerals weathered directly from rocks (e.g. native vermiculite), but is poor in clay minerals formed by neogenesis (e.g. illite taken from sea sediment). In the first type of clays, some iron is still available to the bacteria but in the second type all the available iron had already disappeared before neogenesis.

Production of HCN by *P. fluorescens*, induced by the presence of iron, appears to be a major factor involved in suppression of black root rot and take-all. This explains in part why strain CHA0 is able to suppress disease in vermiculitic soil and not in illitic soil. The mechanism by which HCN suppresses disease is not fully understood. On one hand HCN is toxic for fungi and on the other, HCN-producing bacteria influence the metabolism

of the plant and induce a luxuriant root hair formation. It is possible that apart from these effects HCN may also increase the resistance of the host plant. Furthermore, *P. fluorescens* strain CHA0 produces siderophore(s) of the pyoverdine type, pyoluteorin, 2,4-diacetylphloroglucinol and monoacetylphloroglucinol, but there is no evidence of a role for pyoverdine in disease suppression. The significance of the other metabolites and their interaction with different clay minerals will be investigated in future work.

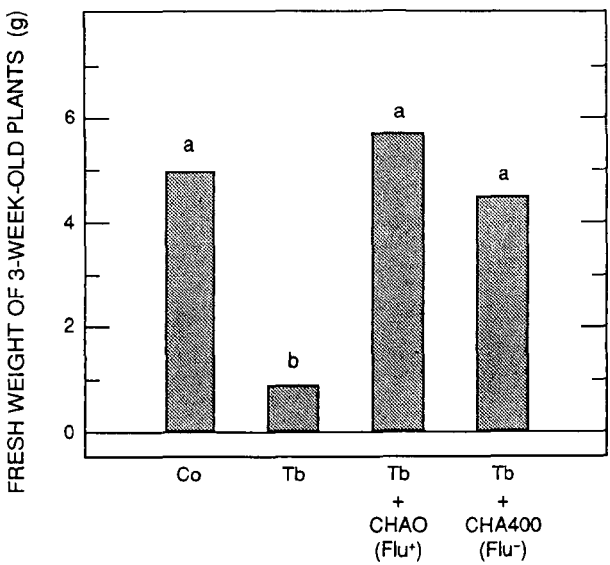


*Figure 7.7* Suppression of black root rot of tobacco by *Pseudomonas fluorescens* strains CHA0 and CHA400, a siderophore-negative, non-fluorescent [Flu−] mutant, in natural soil. The columns represent means of 3 experiments with 10 pots per replicate. Co = natural VC1 soil without added micro-organisms; Tb = soil inoculated with $10^4$ endoconidia of *Thielaviopsis basicola* (Tb) per g of soil; Tb + CHA0 = soil inoculated with Tb and $10^7$ cfu of strain CHA0 per g of soil; Tb + CHA400 = soil inoculated with Tb and $10^4$ cfu of strain CHA400 per g of soil. Columns with the same letter are not significantly different at $P$=0.05 according to Student's t-test; each mean was compared with each of the other means. (More details in Stutz *et al.*, 1986).

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