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Pseudomonas Siderophores: A Mechanism Explaining Disease-Suppressive Soils

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Abstract. The addition of either fluorescent *Pseudomonas* strain B10, isolated from a take-all suppressive soil, or its siderophore, pseudobactin, to both *Fusarium*-wilt and take-all conducive soils inoculated with *Fusarium oxysporum* f. sp. *lini* or *Gaeumannomyces graminis* var. *tritici*, respectively, rendered them disease suppressive. Our findings suggest that disease suppressiveness is caused in part by microbial siderophores which efficiently complex iron(III) in soils, making it unavailable to pathogens, thus inhibiting their growth. Amendment of exogenous iron(III) to disease-suppressive soils converted them to conducive soils presumably by repressing siderophore production.

Vascular wilt diseases caused by *Fusarium oxysporum* Schlecht. are considered among the most commonly encountered plant diseases in agriculture. Control of these diseases is difficult because of persistence of the fungi in soils. Take-all disease of wheat and other cereals, caused by *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* (*Ggt*), another soil-borne pathogen, also occurs throughout the world. However, some soils are not conducive to disease even though *Fusarium* or *Ggt* are present; these soils are termed disease-suppressive soils. The search for the mechanism(s) of disease-suppressive soils has been intensive, beginning about 1935 [4], because of the widespread nature of the phenomenon and the hope that an understanding of the mechanism(s) would lead to the development of biological controls for *Fusarium* wilt and take-all diseases. Views on why some soils are suppressive include both biotic and abiotic factors [4]. However, a biological agent appears to be involved since suppressiveness is transferable from one soil to another and is destroyed by heat [1,3,8,10]. The elucidation of this biological agent has attracted the attention of many plant scientists since it could, if organismic in nature, enable the practical conversion of disease-conducive soils to suppressive ones.

We previously reported that specific plant growth-promoting rhizobacteria (PGPR) in the *Pseudomonas fluorescens-putida* group vigorously colonized plant roots and caused significant increases in yields [7,13]. PGPR exert their plant growth-promoting activity in part by depriving native microflora of iron [6]. PGPR produce extracellular siderophores (microbial iron transport agents [9]) which efficiently complex environmental iron, making it less available to certain native microflora and thus inhibiting their growth. We now report that a specific plant growth-promoting *Pseudomonas* strain (B10, isolated from a plant disease-suppressive soil) or its siderophore (pseudobactin, whose chemical structure is currently under investigation) converts disease-conducive soils to disease-suppressive soils.

Materials and Methods

Microorganisms and chemicals. *Pseudomonas* B10, which was isolated from potato [7] in take-all suppressive soils, was propagated at room temperature either in King's medium B (KB) liquid cultures or on KB agar plates [5]. *Gaeumannomyces graminis* var. *tritici (Ggt)* and *Fusarium oxysporum* Schlecht. f. sp. *lini* (Bolley) Snyd. & Hans. (*Fol*) were grown at room temperature either in one-fifth strength potato dextrose broth (PD) (Difco Laboratories, Detroit, Michigan) or on PD agar plates. Pseudobactin and ferric pseudobactin were isolated from *Pseudomonas* B10 and were purified to homogeneity as described previously [6].

Assays for disease-suppressiveness of soils. Take-all suppressive soils (TASS) (clay loam, pH 7.0) were collected from wheat fields near Medford, Oregon, and disease-conducive soils (sandy loam, pH 7.0) were from Moss Landing, California. The suppressive nature of TASS was confirmed using the following greenhouse assay. *Ggt* was grown for 2 weeks on sterile barley seeds, which were then dried and used to inoculate soils. Ten of these barley seeds were

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planted in each of six replicate 15-cm pots containing either TASS or conducive soils. Pots were maintained at 16°C, and the number of surviving 2-week-old barley seedlings was recorded.

Fusarium-wilt suppressive soils (FWSS) (Chualar sandy loam, pH 7.1) were collected from agricultural fields near Soledad, California, and conducive soils (fine sandy loam, pH 7.2) were from Kern County, California. The suppressiveness of FWSS was confirmed as follows. Ten flax seeds were planted in each of six replicate 15-cm pots containing either inoculated FWSS or inoculated conducive soils. Soils were inoculated with 20 ml of an aqueous suspension of *Fol*, obtained from 7-day-old agar plates. Pots were maintained at 27°C, and the number of surviving 2-week-old flax seedlings was recorded.

Effect of iron(III) on take-all and *Fusarium*-wilt disease-suppressiveness of soils. One hundred milliliters of 50 μM ethylenediaminetetraacetatoferrate(III) ($Fe^{III}edta^-$) were added on alternate days to pots containing disease-suppressive or conducive soils inoculated with *Ggt* or *Fol*. The number of surviving 2-week-old seedlings was recorded and was compared to treatments without iron(III). The *Fol*-assay was repeated in a field experiment in FWSS near Soledad, California. Ten replicate plots were planted with 25 flax seeds each for each of the following four treatments: *Fol*-inoculated with 50 μM $Fe^{III}edta^-$, *Fol*-inoculated without $Fe^{III}edta^-$, noninoculated with 50 μM $Fe^{III}edta^-$, and noninoculated without $Fe^{III}edta^-$. The number of surviving 4-week-old flax seedlings was recorded.

Effect of *Pseudomonas* B10 and its siderophore on conducive soils. Flax and barley seeds were dipped in a suspension (10^9 colony-forming units [CFU]/ml) of *Pseudomonas* B10 prior to planting in *Fol*-inoculated conducive soils or *Ggt*-inoculated conducive soils, respectively. In treatments receiving pseudobactin at 10 or 50 μM, pots were watered with 100 ml of the appropriate solution on alternate days. The number of surviving 2-week-old seedlings was recorded.

The effect of strain B10 on *Fol*-inoculated conducive soils was also determined using 4-week-old flax transplants. Plant roots were dipped in a bacterial suspension (10^9 CFU/ml) immediately prior to planting. The number of surviving 2-month-old flax transplants was recorded.

In vitro antibiosis against *Fol* and *Ggt* by pseudobactin. KB agar plates were modified by reducing proteose peptone no. 3 content to 1% in *Ggt* experiments and by substituting 1% Casamino Acids (Difco) for proteose peptone no. 3 in *Fol* experiments. After paper disks containing 20 μl of 10 mM pseudobactin were applied to the periphery of 4-day-old modified KB agar plates previously inoculated with *Ggt* or *Fol*, growth inhibition of the fungi about the disks was examined.

Results and Discussion

The suppressiveness of take-all suppressive soils (TASS) was confirmed in the greenhouse assay which compared the number of surviving 2-week-old barley seedlings in suppressive and conducive soils, both inoculated with *Ggt*. The suppressive nature of *Fusarium*-wilt suppressive soils (FWSS) was determined in the greenhouse assay which compared the number of surviving 2-week-old flax seedlings at 27°C in suppressive and conducive soils, both inoculated with *Fusarium oxysporum* Schlecht. f. sp. *lini* (Bolley) Snyd. & Hans. (*Fol*). An average of 83% and 82% of the plants survived in repeated assays in both inoculated TASS and FWSS, respectively, whereas only 27% and 48% survived in the corresponding conducive soils (Tables 1 and 2). However,

Table 1. Take-all disease conductive soils made suppressive by the addition of *Pseudomonas* strain B10 or its siderophore, pseudobactin.

	Soil[a]	*Ggt*-inoculated[b]	Treatment[c]	Average of surviving barley seedlings (%)[d]
Experiment 1	S	+	H_2O	83[e]
	S	+	50 μM $Fe^{III}edta^-$	38
	S	–	50 μM $Fe^{III}edta^-$	85[e]
	C	+	H_2O control	27
	C	+	50 μM $Fe^{III}edta^-$ control	25
	C	–	50 μM $Fe^{III}edta^-$	87[e]
	C	+	B10	88[e]
	C	+	B10 + 50 μM $Fe^{III}edta^-$	25
Experiment 2	C	+	H_2O control	15
	C	+	10 μM pseudobactin	13
	C	+	50 μM pseudobactin	73[e]
	C	+	50 μM ferric pseudobactin	20

[a] S, take-all disease-suppressive soils; C, disease-conductive soils.

[b] *Gaeumannomyces graminis* var. *tritici* (*Ggt*) was grown for 2 weeks on sterile barley seeds which were then added to pots of *Ggt*-inoculated treatments.

[c] In treatments with *Pseudomonas* strain B10, *Ggt*-treated seeds were dipped in a bacterial suspension prior to planting. Pots were watered with 100 ml of the appropriate solution on alternate days.

[d] Data were recorded after 2 weeks at 16°C. Average of 6 replications with 10 plants each.

[e] Indicates statistically significant increase ($P = 0.05$) compared to inoculated controls.

Table 2. *Fusarium*-suppressive soil made conducive by the addition of iron(III) and conducive soil made suppressive by the addition of *Pseudomonas* strain B10 or pseudobactin.

Soil[a]	*Fol*-inoculated[b]	Treatment[c]	Average of surviving flax seedlings (%)[d]
S	+	H_2O	82[e]
S	+	50 μM $Fe^{III}edta^-$	47
S	−	50 μM $Fe^{III}edta^-$	90[e]
C	+	H_2O control	48
C	+	50 μM $Fe^{III}edta^-$ control	52
C	−	50 μM $Fe^{III}edta^-$	92[e]
C	+	B10	87[e]
C	+	B10 + 50 μM $Fe^{III}edta^-$	48
C	+	50 μM pseudobactin	90[e]
C	+	50 μM ferric pseudobactin	50

[a] S, *Fusarium*-wilt disease-suppressive soils; C, disease-conducive soils.
[b] *Fol*-inoculated treatments received 20 ml of a suspension of *Fusarium oxysporum* f. sp. *lini* (*Fol*) per pot.
[c] In treatments with *Pseudomonas* strain B10, flax seeds were dipped in a suspension prior to planting. Pots were watered with 100 ml of the appropriate solution on alternate days.
[d] Data were collected after 2 weeks at 27°C. Average of 6 replications with 10 plants each.
[e] Indicates statistically significant increase ($P = 0.05$) compared to inoculated controls.

Table 3. *Fusarium*-wilt conducive soil made suppressive by the addition of *Pseudomonas* strain B10.

Fol-inoculated[a]	Treatment[b]	Average of surviving flax transplants (%)[c]
+	H_2O control	45
+	50 μM $Fe^{III}edta^-$ control	25
+	B10	83[d]
+	B10 + 50 μM $Fe^{III}edta^-$	25
−	50 μM $Fe^{III}edta^-$	83[d]

[a] *Fol*-inoculated treatments were performed as described in Table 2. Four-week-old flax transplants were dipped into a suspension of *Fusarium oxysporum* f. sp. *lini* (*Fol*) prior to planting.
[b] In treatments with *Pseudomonas* strain B10, *Fol*-treated transplants were dipped in a suspension prior to planting. Pots were watered as described in Table 2.
[c] Data were recorded after 2 months at 27°C. Average of 6 replications with 4 plants each.
[d] Indicates statistically significant increase ($P = 0.05$) compared to inoculated controls.

when $Fe^{III}edta^-$ at 50 μM was added to these soils, there was no significant statistical difference in the number of plants surviving in both inoculated suppressive and conducive soils (38% for TASS amended with $Fe^{III}edta^-$ and 47% for amended FWSS (Tables 1 and 2). Hence the suppressiveness was eliminated. These results were further substantiated in the field experiment. When $Fe^{III}edta^-$ at 50 μM was added to FWSS at Soledad, California, 47% fewer flax seedlings survived after 4 weeks in *Fol*-inoculated soils amended with $Fe^{III}edta^-$ than in inoculated soils without added $Fe^{III}edta^-$. The numbers of flax seedlings which survived in noninoculated soils with and without added $Fe^{III}edta^-$ were the same as that of inoculated soils without $Fe^{III}edta^-$. The elimination of suppressiveness in both FWSS and TASS by the addition of $Fe^{III}edta^-$ suggested that siderophores, which are only produced by microorganisms in response to iron-limiting conditions, were sequestering iron in suppressive soils, making it unavailable to the pathogen.

We next tested the possibility of converting a conducive soil to a suppressive soil by the addition of either *Pseudomonas* strain B10, isolated from a TASS, or its siderophore, pseudobactin [6], to the conducive soil. Inoculation of flax seeds with a suspension (10^9 CFU/ml) of strain B10, followed by planting in *Fol*-inoculated conducive soils, resulted in increased survival of seedlings from 48% to 87% when compared to noninoculated seeds (Table 2). Strain B10 extensively colonized root systems of the inoculated flax at levels averaging 2.1×10^3 CFU/cm root using a previously described method [7]. In a second *Fusarium*-wilt assay, dipping of flax transplants in a suspension of strain B10 caused increased survival of flax from 45% to 83% in *Fol*-inoculated conducive soils (Table 3). Inoculation of barley seeds with strain B10 prior to planting in *Ggt*-inoculated conducive soils increased survival of seedlings from 27% to 88% when compared to noninoculated seeds (Table 1). Similar results were obtained when *Ggt*-inoculated conducive soils were amended with 10^5 CFU of strain B10 per g of soil immediately prior to planting of noninoculated barley seeds. When $Fe^{III}edta^-$ and B10 were used together as a treatment in *Fol*- and *Ggt*-inoculated conducive soils, the soils remained conducive in all three assays (Tables 1–3). The addition of pseudobactin purified from strain B10 to fungi-inoculated conducive soils caused them to become suppressive in both the take-all (Table 1) and the *Fusarium*-wilt (Table 2) seedling assays. In the take-all assay, treatment with pseudobactin at 50 μM effectively caused suppressiveness (73% survival of seedlings) in conducive soils, whereas pseudobactin at 10 μM had no significant effect (13% survival) (Table 1). The addition of ferric pseudobactin

at 50 μM did not cause suppressiveness in *Ggt*-inoculated conducive soils (20% survival of seedlings) (Table 1) nor in *Fol*-inoculated conducive soils (50% survival) (Table 2).

We have therefore shown that either *Pseudomonas* strain B10 or its siderophore, pseudobactin, can cause conducive soils to become suppressive. These results suggest that field suppressiveness to take-all and *Fusarium*-wilt diseases is caused in part by microbial siderophores which effectively complex the already limited supply of iron(III) in soils, making it unavailable to the respective pathogen. Our data on in vitro antibiosis against *Fol* and *Ggt* by pseudobactin support this conclusion. (*Ggt* and *Fol* apparently are unable to obtain essential quantities of iron for growth in the presence of pseudobactin either becuase they do not produce siderophores or produce siderophores which have less affinity for iron than that of pseudobactin. The production of siderophores by *Ggt* and *Fol* is currently being investigated.) However, the plant must be able to utilize the iron from ferric pseudobactin, otherwise, it would become chlorotic. This likelihood is being investigated. Various microorganisms including a pseudomonad [10] have also been implicated in both TASS and FWSS [3,11,12]. However, other bacteria and fungi, which also produce siderophores in suppressive soils, may be important suppressive agents in field soils.

The ability to convert conducive soils into suppressive soils implies that certain fluorescent pseudomonads or other siderophore-producing microorganisms may be successfully used for biological control of take-all and *Fusarium*-wilts. The capacity of these bacteria to protect plants from disease by merely treating seeds is indicative of their root colonizing ability, and these seed treatments could easily become a standard commercial practice. The techniques for applying high numbers of viable pseudomonads to field crops in commercial fields have been extensively and successfully tested with plant growth-promoting rhizobacteria [2,7,13].

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