Role of Siderophores in Suppression of *Pythium* Species and Production of Increased-Growth Response of Wheat by Fluorescent Pseudomonads

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ABSTRACT

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About 7% of nearly 5,000 strains of bacteria isolated from roots of wheat produced a zone of inhibition (≥ 10 mm) against *Pythium ultimum* var. *sporangiiferum* (pathogenic to wheat) on either King's medium B (KMB), potato-dextrose agar (PDA), or both media. One-third of these strains, in turn, resulted in 10–30% taller seedlings (equal to the response to metalaxyl) when applied at 10^7–10^8 colony-forming units per wheat seed sown in a Thatuna silt loam (pH 5.3 in 0.01 M $CaCl_2$) naturally infested with *Pythium*. None of the isolates were positive for growth promotion on wheat in the same soil made *Pythium*-free with methyl bromide. Of 10 growth-promoting fluorescent pseudomonads, six were inhibitory to one or more of seven *Pythium* isolates (representing five species or varieties, all pathogenic on wheat) on KMB and four on both KMB and PDA. All seven *Pythium* isolates were inhibited on KMB amended with ethylene-diamine-di-o-hydroxphenylacetic acid (EDDA), but none were inhibited on KMB amended with EDDA + $FeCl_3$ at 50 μg/ml, nor by any of the six fluorescent pseudomonads on KMB amended with $FeCl_3$ at 10 μg/ml. Strain B324, with characteristics intermediate between *Pseudomonas fluorescens* and *P. putida*, was inhibitory to all seven *Pythium* isolates on KMB but not on PDA and produced a strong growth-promoting effect on wheat in the Thatuna silt loam that was nullified by $FeCl_3$. None of five siderophore-negative mutants, produced by N-methyl-N′-nitro-N-nitrosoguanidine mutagenesis of B324, were inhibitory to *Pythium* on KMB, and none produced a significant growth response on wheat in natural soil. The results indicate that the plant growth-promoting activity of some strains of fluorescent pseudomonads on wheat results from ability of the strains to suppress *Pythium* by production of siderophores, including in relatively low-pH soil.

Additional keywords: bacterization, biological control.

Pythium root rot, caused by up to 10 species of *Pythium* (7), is a chronic problem of wheat (*Triticum aestivum* L.) in eastern Washington, adjacent northern Idaho, and northeastern Oregon (Inland Northwest) in areas averaging 40–45 cm available water or more per crop-year (8–10). The disease begins with a generally nonlethal infection of the embryos of germinating wheat seeds during the first 24–48 hr after sowing into moist soil (13). Embryo infections are followed by root infections that result in the loss of root hairs and fine rootlets of wheat (9). Of 39 wheat fields sampled between 1983 and 1986, all had more than 100 propagules of *Pythium* per gram of soil in the top 15 cm (9). Highly significant negative correlations occurred between the population of *Pythium* spp. in soil at sowing and heights of adult plants 8–9 mo later. Treatment of soil in field plots with metalaxyl has resulted in up to 28% greater yield of wheat, equal to the yield response of wheat to soil fumigated with methyl bromide (10).

Weller and Cook (30) reported that the increased growth and yield of wheat in response to seed treatment with fluorescent *Pseudomonas* strains in soils from eastern Washington were probably the result of protection against *Pythium* damage to the plants. Plants were taller in response to the bacteria in *Pythium*-infested soil but not in *Pythium*-free (fumigated or pasteurized) soil, and yields in a field plot averaged 25% more in response to seed treatment with a strain of *Pseudomonas fluorescens*. The protection equaled or surpassed that provided by seed treatment with metalaxyl.

Siderophores have been shown to play a role in the increased-growth response of certain plants to treatment of the planting material with fluorescent *Pseudomonas* spp. (1,18,25). This response is thought to involve suppression of deleterious rhizosphere microorganisms in some cases (18,25,26) and to suppression of known soilborne plant pathogens (1,25,27,33) in other cases, including suppression of *Pythium* spp. pathogenic to cotton (20). *Pythium* spp. are ubiquitious inhabitants of agricultural soils, and Wilhelm (32) presented good evidence that the increased-growth response of several plants to soil fumigation was a response to reduction in the *Pythium* population in the soil. In the case of wheat, simply protecting the germinating seeds against embryo infections could account for part of the increased-growth response of young plants to seed treatment with fluorescent pseudomonads.

This study was conducted to determine the role, if any, of siderophore production by fluorescent *Pseudomonas* spp. in the increased-growth response of wheat, which is attributed to control of *Pythium* by *Pseudomonas* bacteria. A preliminary report of this work has been published (4).

MATERIALS AND METHODS

Isolation, culture, and storage of bacterial strains and *Pythium* spp. All candidate bacteria were isolated originally from roots of the tallest seedlings (in the 2- to 3-leaf stage) among those growing in soil naturally infested with *Pythium* spp. (30) at 800–1,000 propagules/g. The soils were collected from several locations in eastern Washington and selected because of their high populations of *Pythium* spp. and hence the high probability of severe stunting of seedlings caused by *Pythium* spp. (8,9,13). Pots (13 cm in

diameter) were filled with fresh soil, seeded with the winter wheat cultivar Daws at 10 seeds per pot, and incubated in a growth chamber at 13–15 C with light provided on a 12-hr day-night cycle. During plant growth, the soil was allowed to dry to a water potential no lower than −1.0 bar before watering (13). To isolate candidate bacteria, roots with tightly adhering soil were macerated in 0.01 M phosphate buffer (pH 7.2) with a mortar and pestle, and then serial dilutions were plated on King's medium B (KMB) agar (17), the medium of Sands and Rovira (24), 10% tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) (21), and dilute (1/5-strength) Difco potato-dextrose agar (PDA) (8 g of Difco product plus 15 g of agar). Isolates were subcultured in nutrient broth (Difco) on a rotary shaker for 24 hr at 25 C and then stored in 40% glycerol at −15 C.

Seven *Pythium* isolates were tested in vitro for sensitivity to the bacterial strains: *P. ultimum* var. *ultimum* Trow, *P. u. sporangiiferum* Drechs., *P. torulosum* Coker and Patterson, *Pythium* species D (7), and *P. irregulare* Buis. (three isolates). All were from the collection maintained at the U.S. Department of Agriculture-Agricultural Research Service Root Disease and Biological Control Research Unit and all were originally isolated from and shown to be pathogenic to wheat. All isolates of *Pythium* were maintained on 1/5-strength PDA at 10 C and routinely transferred to fresh slants about every 4 wk.

Selection of strains inhibitory to *Pythium* in vitro. Candidate strains were tested for ability to inhibit *Pythium* in vitro on both KMB and full-strength (39 g of Difco product) PDA. Four strains from 24-hr-old nutrient-broth cultures were spotted equidistantly around the perimeter, and a 5-mm-diameter plug of agar-culture of *Pythium* was placed at the center of each plate. The bacterial strains were spotted on the plates 48 hr before the two isolates of *P. ultimum* and simultaneously for the other five slower growing isolates of *Pythium*. Plates were incubated at 22 C until the test fungus reached the edge of a control (no bacteria) plate. Bacteria that inhibited one or more isolates of *Pythium* at a distance of 10 mm or more on at least one medium were chosen for further testing.

Treatment of seeds with bacteria. To prepare the bacterial inoculum for treatment of seed, candidate strains from storage were streaked onto nutrient-broth agar supplemented with 2 g of yeast extract (Difco) per liter and incubated at 22 C for 1 day. Bacteria from this medium were suspended in sterile deionized water, and then 0.1 ml of this suspension was spread on the surface of KMB agar and incubated at 22 C for 2 days. Four milliliters of a suspension of methylcellulose (4,000 centipoises) (Sigma Chemical Co., St. Louis, MO) was added to each plate, and the bacterial-methylcellulose contents of one plate were scraped into a sterile beaker containing 10 g of wheat seed of either cv. Daws or cv. Fielder. Before treatment, and to eliminate possible seedborne pathogens, the seed was emersed in a 30% solution of commercial bleach (5.25% sodium hypochlorite) for 3 min, rinsed for 30 min with tap water, and air dried. The seeds were then coated with bacteria, air dried overnight under a flow of sterile air (laminar flow), and stored at 4 C until sowing 1–2 days later. The number of colony-forming units (cfu) of the candidate strain per seed was determined by macerating five seeds (held back at the time of planting) in 0.1 M phosphate buffer (pH 7.2) with a mortar and pestle and dilution-plating onto KMB.

Assessment of plant growth-promoting activity. Bacteria-treated seeds were sown in tapered plastic tubes 2.5 cm in diameter × 15 cm long (Ray Leach Conetainer Co., Canby, OR), two seeds per tube and 10 tubes per candidate strain. Each tube contained vermiculite in the bottom, a 3-cm-thick layer of Thatuna silt loam (20 g, naturally infested with *Pythium* spp. at about 500 propagules/g) in the middle, and a 1-cm-thick layer of a 1:1 mixture of the natural Thatuna silt loam and vermiculite on top. No fertilizer was added to the soil. Two seeds were placed on the layer of soil and covered with the soil-vermiculite mixture. Ten milliliters of water was added at the top of each tube, and the tubes were incubated in a growth chamber at 13–15 C with a 12-hr light-dark cycle. Each experiment included at least three checks: seed treated with the methylcellulose only and sown in the natural Thatuna silt loam; seed treated with methylcellulose and sown in the Thatuna silt loam made free of *Pythium* by fumigation with methyl bromide; and seed treated with metalaxyl (1 g active ingredient / kg seed) and sown in the natural soil. The height of the shoot (from the soil surface to the tip of the second leaf) and the length of the first true leaf (8,9,22) were determined after 3 wk. All candidate strains were tested at least twice.

The Thatuna silt loam (15), a fine-silty, mixed, mesic xeric Argia boll, was pH 5.3 (measured in 0.01 M $CaCl_2$), contained 59.8 ppm DTPA-extractable Fe^{3+}, and was used unmodified in all but one experiment in which enough lime $Ca(OH)_2$ was added to elevate the pH to 7.0 at the time of planting. The soil was collected from the Plant Pathology Farm near Pullman, WA, at the beginning of the trials, sieved, and stored outdoors in the shade until used.

Evaluation of the relationship of siderophore production by bacteria to the growth promotion of wheat. *Tests with soil amendments.* The relationship of siderophore production to plant growth promotion was evaluated using *Pseudomonas* strain B324. This strain was selected from the initial screening because of its ability to inhibit all seven *Pythium* isolates on KMB (but not on PDA) and its ability to improve the growth of wheat in the Thatuna silt loam. The experimental approach was similar to that of Scher and Baker (21) in which the soil was treated with chelators to simulate the activity of siderophores or with iron to shut down the production of siderophores. The experiments were conducted with the soil tube system as described above. Eight treatments (and checks) were used: 1) B324-treated seeds sown in the Thatuna silt loam and the soil watered as previously described; 2) same as the first treatment except that the 10 ml of water added initially to the tubes contained $FeCl_3$ at 100 μg/ml; 3) seed treated with B324 and sown into natural soil amended with ethylenediaminetetracetic acid (EDTA), sprayed as an aqueous solution onto a thin layer of the soil (1 mg/g of soil) followed by mixing and incubation moist for 2 days to allow metal chelation before use; 4) same as third treatment except that the seed was not treated with B324 before being sown in the EDTA-amended natural soil; 5) nontreated seed sown in natural soil amended with ethylene-diamine-di-o-hydroxyphenylacetic acid (EDDA), applied at 1 mg/g of soil in the same way described for EDTA; 6) seed treated with metalaxyl at 1 g active ingredient/kg seed and sown in natural soil; 7) nontreated seed sown into fumigated (methyl bromide) soil; and 8) nontreated seed sown into natural soil.

Tests with siderophore-negative mutants. As a second experimental approach, the growth-promoting activity of siderophore-negative mutants of strain B324R (resistant to rifampin) was compared with the parent strain B324R. The rifampin-resistant strain of B324 was selected 4 days after spreading a turbid cell suspension onto the surface of KMB agar containing rifampin (100 μg/ml). Several rifampin-resistant strains were grown in nutrient broth for 1 day on a rotary shaker at 25 C, and the resultant cells were spun down at 4,000 *g* for 30 min, resuspended in 0.1 M $MgSO_4$, and used to coat wheat seeds. These seeds were then sown in pots of the natural, *Pythium*-infested Thatuna silt loam. After 4 wk, plants were removed, and 2-cm lengths from the root tips were macerated in 0.1 M phosphate buffer and dilution-plated onto KMB + rifampin (100 μg/ml). This procedure was used to help insure the rhizosphere competence of the rifampin-resistant strains. Rifampin-resistant strain B324R was then treated with N-methyl-N′-nitro-N-nitrosoguanidine (NTG) (22). Strain B324R was incubated in 10 ml of nutrient broth to produce a density of about 5×10^8 cells/ml. The liquid culture was centrifuged for 5 min at 5,000 *g*, and the pellet was resuspended in an equal volume of Tris-maleate buffer at pH 6. A freshly prepared solution of NTG in sterile water (1 mg/ml) was added to the cell suspension to produce a final NTG concentration of 100 μg/ml. The NTG-treated culture was then incubated at 28 C for 30 min without shaking and centrifuged, and the pellet was resuspended in an equal volume of mineral salts (MS6) minimal medium. After incubation for another 4 hr, the cells were plated onto KMB with rifampin at 100 μg/ml. Nonfluorescent colonies on KMB were selected under ultraviolet light. Nonfluorescent strains were verified as siderophore-negative by their inability to

grow on KMB amended with EDDA at 1.25 mg/ml. Mutants were passaged on wheat once as described for rifampin-resistant strains to eliminate unfit isolates and insure their rhizosphere competence.

Evaluation of the role of siderophores and iron deprivation in the inhibition of *Pythium* in vitro. The sensitivity of *Pythium* isolates to siderophores was studied in vitro using six strains of bacteria inhibitory only on KMB and four strains of bacteria inhibitory to one or more *Pythium* isolates on both KMB and PDA. All 10 strains of bacteria produced an increased-growth response of wheat in tubes containing the natural, *Pythium*-infested Thatuna silt loam. Each strain was spotted on one side of a plate of KMB amended with $FeCl_3$ at 1, 5, 10, or 50 μg/ml. A disk (5 mm in diameter) of an agar culture of *P. torulosum* was placed on the opposite side of each plate. To verify the sensitivity of each of the seven *Pythium* isolates to iron deprivation, 40 μl of a solution (0.1 or 0.2 mg/ml) of EDDA was added in a well (4 mm in diameter) on one side of a plate of KMB (where otherwise spotted with a bacterial culture), and the *Pythium* isolate was placed as a disk of mycelium in agar on the opposite side 2 days later. The EDDA was dissolved in 1 N NaOH, and this solution was adjusted to pH 9.0 with 1 N HCl to produce the final product placed in each well in combination with each of the four concentrations of $FeCl_3$ added to the medium. The control was adjusted with deionized water to pH 9 with NaOH. The plates with EDDA and the water controls were incubated for 2 days at 22 C (to allow diffusion from the well and metal chelation) before the test *Pythium* isolate was introduced.

All experiments were repeated at least twice.

RESULTS

Inhibitory activity of bacterial strains to *Pythium* spp. in vitro. About 5,000 colonies of bacteria from wheat roots were selected at random (mainly from KMB but some from TSA) and tested against the *Pythium* spp. on KMB and PDA. About 90% of the bacteria selected were pseudomonads. *P. torulosum* was inhibited on KMB by nearly every fluorescent *Pseudomonas* strain tested and was therefore not useful in this initial screen. *P. ultimum* var. *sporangiiferum* was not as sensitive to inhibition by fluorescent pseudomonads and therefore was used to screen all 5,000 colonies on both KMB and PDA. Only 350 isolates produced a zone of inhibition of this *Pythium* isolate $\geq$ 10 mm on one medium or the other.

Growth-promoting activity of bacterial strains in vivo. About one-third of the approximately 350 strains inhibitory to *P. u. sporangiiferum* in vitro, when tested as seed treatments, resulted in 10–30% taller seedlings and 20–50% longer first true leaves compared with the untreated controls; results with representative bacterial strains are shown in Table 1. Plants grown in *Pythium*-free (fumigated) soil were consistently the tallest and appeared to be the healthiest. Of 10 strains (B324, B509, A708, A48, A606, A52, C791, C4, E591, and C611) that gave growth promotion in natural Thatuna silt loam, none produced any improvement in seedling size when tested in the same soil fumigated.

Strains B324 and A708 were tested further, each applied at $3.5–5.5 \times 10^8$ cfu per seed of both Daws and Fielder and sown in tubes with the Thatuna silt loam limed to pH 7.0 with $Ca(OH)_2$. These two strains were inhibitory to the test isolates of *Pythium* on KMB but not on PDA; both strains produced zones of inhibition about 10 mm across for the two isolates of *P. ultimum* and 10–20 mm across for the other five isolates of *Pythium*. Strain B324 had characteristics intermediate between *P. fluorescens* and *P. putida*. Both strains resulted in percentages of emergence of the two cultivars (Table 2) that were midway between the percentages for no control of *Pythium* spp. (natural soil with no seed treatment) and the best control of *Pythium* spp. (metalaxyl-treated seeds or fumigated soil). Of seedlings that emerged, those grown from bacteria-treated seed were significantly taller and/or their first true leaves were longer than those grown from untreated seed in natural soil with no control of *Pythium* spp.; the plant growth responses were as good or better than those that occurred with metalaxyl on the seed (Table 2).

Inhibition of *Pythium* isolates by siderophores and iron deprivation. None of the six growth-promoting strains that inhibited *Pythium* on KMB were inhibitory on KMB + $FeCl_3$ at 10 μg/ml. In contrast, all four plant growth-promoting strains inhibitory on both KMB and PDA were still inhibitory to *Pythium* spp. on KMB amended with $FeCl_3$ at 50 μg/ml. Moreover, on KMB, *P. u. ultimum* and *P. u. sporangiiferum* were least sensitive, and the other five isolates were almost totally inhibited by either EDDA or the siderophore-producing strain B324 (Table 3). The addition of $FeCl_3$ to the KMB at 10 μg/ml eliminated the inhibitory effect of both EDDA and the wild-type, siderophore-producing strain of B324. None of the seven isolates of *Pythium* were inhibited on KMB by any of five siderophore-negative mutants.

Evidence for a role of siderophores in the plant growth response. Strain B324 was positive for growth promotion on wheat in the

TABLE 1. Increased-growth response of wheat seedlings after 3 wk to treatment of seeds with strains of fluorescent pseudomonads and grown in a Thatuna silt loam naturally infested with *Pythium* species

Treatment[y]	First leaf (cm)	Shoot (cm)[z]
Fumigated soil (control)	10.8 a	17.3 a
Natural soil (control)	6.5 e	14.1 b
Natural soil/treated seed		
Metalaxyl	10.5 ab	16.7 a
B324	9.8 abc	17.1 a
A708	9.6 abc	16.7 a
C4	9.0 bcd	16.3 a
A606	9.2 bcd	16.3 a
B509	8.8 cd	16.2 a
Q72a	9.0 bcd	16.1 ab
E591	9.1 bcd	16.1 ab

[y] All treatments involved the same Thatuna silt loam (pH 5.3 in 0.1 M $CaCl_2$, 59.8 ppm DPTA-extractable Fe^{3+}). Methyl bromide was used to fumigate the soil. The natural soil contained a population of *Pythium* spp. estimated at 500 propagules/g. All seed treatments were tested in the natural soil. Metalaxyl was applied at 1.0 g active ingredient/kg of seed. Bacterial strains were applied at an estimated 1×10^8 cfu/seed.

[z] 20 plants per treatment, grown in tubes for 3 wk at 13–15 C. Numbers followed by a different letter are significantly different at $P = 0.05$ according to Duncan's multiple range test.

TABLE 2. Growth response of wheat cultivars Daws and Fielder to seed treatments with metalaxyl and strains B324 and A708 of fluorescent pseudomonads in a limed Thatuna silt loam

Treatment[x]	Emergence[y] No.	First leaf[y] (cm)	Shoot (cm)
Cultivar Daws			
Natural soil (control)	15/20	4.8 c	9.8 c
Metalaxyl seed	20/20	7.8 b	14.8 b
B324 seed	17/20	8.2 ab	19.8 a
A708 seed	18/20	7.9 ab	16.6 b
Fumigated soil (control)	20/20	9.3 a	20.4 a
Cultivar Fielder			
Natural soil (control)	14/20	5.5 c	14.8 a
Metalaxyl seed	20/20	8.0 ab	14.6 a
B324 seed	16/20	7.1 bc	14.6 a
A708 seed	16/20	6.2 c	15.5 a
Fumigated soil (control)	20/20	9.2 a	16.4 a

[x] All treatments involved the same Thatuna silt loam (pH 5.3 in 0.1 M $CaCl_2$, 4.98 ppm DPTA-extractable Fe^{3+}). Methyl bromide was used to fumigate the soil. The natural soil contained a population of *Pythium* spp. estimated at 500 propagules/g. All seed treatments were tested in the natural soil. Metalaxyl was applied at 1.0 g active ingredient/kg seed. Inoculation with bacterial strains B324 and A708 resulted in $3–5 \times 10^8$ cfu per seed of each strain on each cultivar at the time of sowing (seeds dried).

[y] Each value is based on emergence and growth of wheat in 10 tubes, two seeds per tube, and seedlings measured after 2 wk at 13–15 C. The soil was Thatuna silt loam (original pH 5.3 in $CaCl_2$) naturally infested with *Pythium* spp. and limed with $Ca(OH)_2$ to pH 7.0. Numbers followed by a different letter are significantly different at $P = 0.05$ according to Duncan's multiple range test.

natural Thatuna silt loam but not in this soil amended with either $FeCl_3$ or EDTA (Table 4). EDTA by itself had no effect on wheat, but EDDA by itself improved seedling growth to the same extent as either B324 or metalaxyl. As usual, plant growth promotion was greatest in response to treatment of the soil with methyl bromide.

Four of five siderophore-negative mutants of strain B324R resulted in plants intermediate in size between those produced with strain B324R on the seed and those produced with no seed treatment (Table 5). The fifth siderophore-negative mutant (strain S-6) tested was significantly ($P = 0.05$) less active than the parent and resulted in plants virtually identical to those produced with nontreated seed.

DISCUSSION

Growth promotion of plants by fluorescent pseudomonads has been reported to result from suppression of known root pathogens in some cases (1,20,25,27,29,31,33) and of ill-defined deleterious microorganisms (subclinical pathogens) in the rhizosphere in other cases (3,6,18,26,28,34). Our study supports a report by Weller and Cook (30) that, for wheat in soil naturally infested with *Pythium* spp., the growth promotion in response to seed treatment with fluorescent pseudomonads results largely or entirely from protection of the plants against the *Pythium* spp. Of 10 strains of bacteria inhibitory to *Pythium* spp. in vitro, all produced an increased-growth response of wheat in a natural Thatuna silt loam that was near or equal to the growth response of wheat in the same soil to seed treatment with metalaxyl. This chemical inhibits the oomyceteous fungi, of which *Pythium* spp. are the only examples known or likely to affect wheat in the soil used. None of the 10 strains produced a growth response in the soil fumigated with methyl bromide to eliminate the *Pythium* inoculum. A growth response to seed treatment with fluorescent pseudomonads would not be expected of seedlings in *Pythium*-free soil where presumably the plants are already growing near or at their full growth potential. It is doubtful that wheat seedlings equal to or approaching the size of those in fumigated soil, or those produced with metalaxyl-treated seed, could have been produced with any of the bacteria-treated seeds unless the bacteria somehow protected against or counteracted the damaging effects of *Pythium*. Even protection against embryo infection (11) could account for part of the increased growth response for wheat obtained with the bacteria.

The results with *Pseudomonas* strain B324 strongly support previous reports (3,19,20,25) that siderophore production is an important mechanism by which some strains of bacteria protect plants against root pathogens. This conclusion is based on several lines of evidence, including: 1) strain B324 was inhibitory to all seven *Pythium* isolates on KMB agar (low iron content) but was not inhibitory to any of the isolates on PDA (high iron content), suggesting that the inhibitory effect of this strain results from production of siderophores and not antibiotics; 2) both the inhibitory effect of this strain on KMB agar and its growth-promoting activity on wheat in the natural Thatuna silt loam were reduced or nullified by the addition of $FeCl_3$ to the KMB or the soil; 3) the synthetic iron chelator EDDA added to the soil resulted in a growth-promoting effect on wheat equal to that obtained with strain B324; and 4) siderophore-negative mutants of B324R were less effective than the parent strain or ineffective in growth promotion of wheat.

Disease suppression by production of siderophores might seem unexpected in a soil at pH 5.3 and with 59.8 ppm DTPA-extractable iron, considering reports that this mechanism of inhibition of pathogens by their antagonists is likely to be important mainly or only at higher pH values (e.g., neutral to alkaline soil) or with low available iron (2). However, our results are not surprising in view of the report of Bossier and Verstraete (5) that the concentration of siderophores in several soils related more strongly to the amount of organic substrates available for microbial growth in the soils than to either soil pH or extractable iron. In particular, a highly significant correlation was found between siderophore concentrations and the amount of grass production in seven soils that had the same texture but received different combinations of mineral fertilizers and ranged in pH from as low as 3.97 to as high as 7.50 (5). The higher concentration of siderophores in the rhizosphere than in bulk soil (23) and the dense rooting of grasses (5) could account for the evidence of a siderophore effect in our wheat system in spite of the soil having a relatively low pH and high content of available iron.

TABLE 3. Relative sensitivities of *Pythium* isolates to strains B324 and A708 of *Pseudomonas putida* on King's medium B (KMB)

Pythium isolate	Inhibition per strain[a]	
	B324	A708
P ultimum var. *sporangiiferum*	+	+
P. ultimum var. *ultimum*	+	+
P. irregulare-1	+++	+
P. irregulare-2	+++	+
P. irregulare-3	+++	++
P. torulosum	+++	+++

[a] Indicates approximate size of the zone of inhibition on KMB (13): + ≤ 1.0 cm, ++ = 1.0–2.0 cm, and +++ ≥ 2.0 cm. No zones of inhibition developed with any combinations of *Pythium* isolate and bacterial strain on KMB amended with $FeCl_3$ at 10 μg/ml.

TABLE 4. Growth response of wheat seedlings to treatment of seeds with strain B324 of *Pseudomonas putida* and grown in a Thatuna silt loam treated to modify the availability of iron

Treatment[y]	First leaf (cm)[z]
Natural soil (control)	6.3 e
Metalaxyl seed	10.3 ab
B324 seed	9.9 ab
EDDA soil	8.8 bc
Ethylenediaminetetraacetic acid (EDTA) soil	6.5 de
B324 seed + EDTA soil	7.5 cde
B324 seed + $FeCl_3$ soil	6.3 e
Fumigated soil (control)	11.0 a

[y] All treatments involved the same Thatuna silt loam (pH 5.3 in 0.1 M $CaCl_2$, 59.8 ppm DPTA-extractable Fe^{3+}). Methyl bromide was used to fumigate the soil. The natural soil contained a population of *Pythium* spp. estimated at 500 propagules/g. All seed treatments were tested in the natural soil. Metalaxyl was applied at 0.75 g active ingredient/kg of seed. Bacterial strains were applied at an estimated 1×10^8 cfu/seed. EDDA and EDTA were applied at 1 mg/g of soi; $FeCl_3$ was applied at 100 μg/ml with the irrigation water.

[z] 20 plants per treatment, grown in tubes for 3 wk at 13–15 C. Numbers followed by a different letter are significantly different at $P = 0.05$ according to Duncan's multiple range test.

TABLE 5. Growth response of wheat seedlings to treatment of seeds with siderophore-negative mutants of *Pseudomonas* strain B324R and grown in a Thatuna silt loam naturally infested with *Pythium* species

Treatment[y]	First leaf (cm)	Shoot (cm)[z]
Fumigated soil (control)	9.8 a	22.2 a
Natural soil (control)	4.7 ef	14.9 c
Metalaxyl seed	8.3 ab	18.1 bc
B324R (parent) seed	6.5 bcde	18.2 b
S-2	5.2 cdef	15.1 bc
S-5	5.7 cdef	15.4 bc
S-6	4.1 f	14.8 c
S-10	5.3 def	15.4 bc
S-12	5.6 cdef	15.9 bc

[y] All treatments involved the same Thatuna silt loam (pH 5.3 in 0.1 M $CaCl_2$, 59.8 ppm DPTA-extractable Fe^{3+}). Methyl bromide was used to fumigate the soil. The natural soil contained a population of *Pythium* spp. estimated at 500 propagules/g. All seed treatments were tested in the natural soil. Metalaxyl was applied at 0.75 g active ingredient/kg of seed. Bacterial strains were applied at an estimated 1×10^8 cfu/seed.

[z] Each value is the average of 20 seedlings. Numbers followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Four strains of bacteria were inhibitory to *Pythium* on both PDA and on KMB amended with iron. Possibly these strains inhibit *Pythium* by production of antibiotic substances as reported by Howell and Stipanovic (14) and Gutterson et al (12) for *Pythium* on cotton. Inhibition by production of siderophores, antibiotics, or both kinds of inhibitors could be operative depending on the strain. The selection process in finding effective antagonists should attempt to obtain strains with more than one mechanism of inhibition of the target pathogen to help insure protection over a greater range of conditions and to minimize the chances of the pathogen population becoming insensitive or resistant to the strain.

Neither strain B324 nor strain A708 were as effective as metalaxyl based on percentage of seedlings emerged, but both strains were equivalent to metalaxyl based on size and vigor of emerged seedlings. Preemergence seedling blight of wheat in soils of the Inland Northwest is caused mainly by *P. ultimum* (16). Isolates of this species were highly sensitive to metalaxyl (11) but were least sensitive among the test isolates compared in vitro for inhibition by strains B324 and A708. *P. irregulare*, on the other hand, stunts wheat mainly by damage to roots (5,16), is relatively insensitive to metalaxyl (11), but was more sensitive than *P. ultimum* to strains B324 and A708 in vitro. The generally poor performance of the bacteria based on seedling emergence and their good performance based on seedling vigor in our experiments may reflect relatively poor control of *P. ultimum* on seeds but good control of *P. irregulare* and other species on roots.

Control of *Pythium* spp. gives the appearance that seedling growth has been stimulated by the treatment when actually the seedlings are only revealing more nearly their full growth potential (32). Because *Pythium* species are ubiquitious in agricultural soils and are known to have subtle but significant effects on seedling growth (8,9,13,32), more attention should be given to the possible effect of siderophore-producing and antibiotic-producing bacteria on this pathogen in tests with seed bacterization in natural soil.

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