Relationship Between In Vitro Inhibition of *Gaeumannomyces graminis* var. *tritici* and Suppression of Take-All of Wheat by Fluorescent Pseudomonads

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ABSTRACT

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Fluorescent *Pseudomonas* spp. isolated from roots of wheat that had been grown in a take-all suppressive soil were more inhibitory to *Gaeumannomyces graminis* var. *tritici* in vitro and more suppressive to take-all as seed treatments than fluorescent pseudomonads isolated from roots that had been grown in a take-all conducive (nonsuppressive) soil. The relationship between the ability of fluorescent pseudomonads to inhibit *G. g. tritici* in vitro and suppress take-all of wheat in soil was then examined. *Pseudomonas fluorescens* strains 2-79RN₁₀, R1a-80R, and R7z-80R were treated with N-methyl-N′-nitro-N-nitrosoguanidine, and mutants were selected that lost or were reduced in the ability to produce antibiotics and/or siderophores in vitro. These mutants also lost some or all of their ability to suppress take-all but still colonized wheat roots to the same degree as their respective parents. The addition to the soil of excess Fe(III) in the form of ferric-ethylenediamine-tetraacetic (FeEDTA) acid to repress siderophore production eliminated suppression of take-all by *Pseudomonas putida* L30b-80, reduced suppression by strain 2-79, and had no effect on strain R1a-80. The results confirm previous conclusions that fluorescent pseudomonads have a role in take-all decline and that suppression of take-all by some fluorescent pseudomonads is mediated in part by production of antibiotics and/or siderophores.

Additional keywords: biological control, soilborne pathogen, *Triticum aestivum*.

Take-all, caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker, is probably the most important root disease of wheat worldwide (1). Take-all decline (37) is a natural form of biological control whereby the severity of take-all declines and wheat yields increase following one or two outbreaks of the disease and continuous monoculture of wheat. Soil from a field following take-all decline is highly suppressive to *G. g. tritici* (suppressive soil). Several mechanisms have been proposed to explain this phenomenon (16,32), including that pseudomonads are partly responsible for the disease suppression (9,12,38,39,49).

Fluorescent pseudomonads have been studied as agents for biological control of well-known pathogens (10,17,18,34,52), including *G. g. tritici* (12,45,47,51) and lesser-known pathogens (deleterious rhizosphere microorganisms) (24–26,35,36,43,53) of

roots. The characteristics of strains that function as biocontrol agents are not totally known (41) but probably include ability to colonize roots, or survive in soil, inhibit the pathogen directly, or elicit host defense responses.

The production of siderophores apparently is necessary for some fluorescent pseudomonads to suppress root and seedling diseases. Siderophores are low-molecular-weight Fe(III) chelators that have a high affinity for iron and are involved in iron transport across the bacterial outer membrane (28,31). Kloepper et al (23) reported that either *Pseudomonas* sp. B10, applied as a seed treatment, or its siderophore (pseudobactin), applied as a drench to take-all conducive (nonsuppressive) soil infested with *G. g. tritici*, suppressed take-all on barley. Further, the addition of Fe(III) to a take-all suppressive soil converted it to a conducive soil. Wong and Baker (51) obtained control of take-all by adding either fluorescent pseudomonads or the synthetic chelator Fe ethylenediamine-di-(*o*-hydroxyphenylacetic acid) (Fe EDDA) to the soil and concluded that suppression by the bacteria was due to iron competition at the rhizoplane or in the rhizosphere. Fluorescent pseudomonad

strains of plant growth-promoting rhizobacteria (PGPR), when applied to seeds or seed pieces, are thought to improve plant growth by displacing or excluding deleterious rhizosphere microorganisms (35,36,42). PGPR apparently produce siderophores that sequester Fe(III) and starve other organisms for iron (24,35,36). *Pseudomonas putida* strain WCS358 improved the growth of potato by suppressing deleterious rhizosphere microorganisms, but a siderophore-negative Tn5 mutant had no effect (5,6). Similarly, siderophores appear to be responsible for the suppression of *Pythium* spp. (7,29) and formae speciales of *Fusarium oxysporum* (3,4,13,34,40) by *Pseudomonas* spp.

The production of antibiotics apparently is necessary for other fluorescent pseudomonads to suppress disease. Howell and Stipanovic (17,18) demonstrated that *Pseudomonas fluorescens* Pf-5 suppressed damping-off of cotton seedlings by producing the antibiotics pyoluteorin and pyrrolnitrin, which inhibited *Pythium ultimum* and *Rhizoctonia solani*, respectively. Howie and Suslow (20) reported that *P. fluorescens* strain Hv37aR2, which produces an antifungal compound (15,21), was more suppressive of infection by *P. ultimum* in cotton than an antifungal-minus isogenic mutant. Colyer and Mount (10) reported that *P. putida* M17, which produces an antibiotic inhibitory to *Erwinia* spp., controlled potato tuber soft rot but that the antibiotic-negative strain M74 was less effective.

The purpose of this research was to determine the role of both antibiotics and siderophores in the suppression of take-all by fluorescent pseudomonads. A preliminary account of this work was published (48).

MATERIALS AND METHODS

Culture of *G. g. tritici* and preparation of inoculum. The strain of *G. g. tritici*, R3-111a-1, used throughout this study was started from a single ascospore. The fungus was grown in vitro on dilute potato-dextrose agar (PDA) (fresh potatoes, 40 g; glucose, 4 g; Difco agar [Difco Laboratories, Detroit, MI], 15 g; deionized water, 1,000 ml), and oat-kernel inoculum of the pathogen was prepared as previously described (47).

Soils and pot bioassay. A soil suppressive to take-all was collected from a field located near Moses Lake, WA (Shano silt loam [50]; pH 6.0, determined in 0.01 M CaCl₂) that had been cropped to wheat for 22 consecutive years. Conducive (nonsuppressive) soils were collected from fields near Mt. Vernon (Puget silt loam [49], pH 5.8) and Lind, WA (Ritzville silt loam, pH 7.5) that had been cropped to a pea-wheat rotation and to alfalfa, respectively. Each soil was tested for its suppressiveness to take-all by a modification of the pot bioassay (11,47). Soil was mixed 1:10 with fumigated (methyl bromide) virgin Ritzville silt loam (50). The mixture was then amended with fragmented oat-kernel inoculum (1.0%, w/w) and added (400 g) to 500-ml pots. For the controls, the oat-kernel inoculum was autoclaved before it was added to the soil. Five seeds of the spring wheat cultivar Fielder were sown per pot (first planting). After 5 wk, the plants were harvested and the soil was reinfested with additional oat-kernel inoculum of *G. g. tritici* (1.0%, w/w) and again planted to wheat (second planting). The mixing of all of the test soils with fumigated Ritzville silt loam was done to minimize differences in soil physical and chemical factors that can affect the severity of take-all in the pot bioassay. Each treatment was replicated four times and treatments were arranged in a randomized complete block design.

Isolation, culture, and storage of bacteria. Bacteria were isolated from roots of 5-wk-old seedlings of the second planting of the pot bioassay. Roots with tightly adhering soil were macerated in 30 ml of 0.01 M phosphate buffer (pH 7.2) with a mortar and pestle. Serial dilutions were made and aliquots (0.1 ml) were plated in duplicate on various media. Fluorescent pseudomonads were isolated on King's medium B (KMB) agar (22) supplemented with novobiocin (45 μg/ml), penicillin G (75 units/ml), and cycloheximide (75 μg/ml) (NPC) (33); plates were incubated at 25 C; and fluorescent colonies were counted after 48 hr. Total aerobic bacteria were isolated on dilute tryptic soy agar (TSA) (Difco tryptic soy broth, 3.0 g; Difco agar, 15 g; and deionized water,

1,000 ml) (30). Bacteria were subcultured on nutrient broth yeast extract agar (NBY) (44) and then preserved in 40% glycerol at −20 C or lyophilized.

Fluorescent *Pseudomonas* strains. *P. fluorescens* strains 2-79 (NRRL B-15132), R1a-80 (NRRL B-15135), and R7z-80 (biovars III, II, and II, respectively) (27) are suppressive to take-all and were described previously (19,47). Other strains used in this study were isolated from roots of wheat grown in the pot bioassay described above.

Bacterial inhibition of *G. g. tritici* in vitro. Tests for ability of fluorescent *Pseudomonas* strains to inhibit *G. g. tritici* were conducted in standard petri dishes (100 × 15 mm) containing KMB agar or PDA (fresh potatoes, 200 g; glucose, 20 g; deionized water, 1,000 ml; and Difco agar, 15 g). For some studies these media were amended with 10, 50, or 100 μM filter-sterilized FeCl₃. A single *Pseudomonas* strain was spotted twice, 5 mm from opposite edges of the dish, and incubated at 24 C; 2 days later a 6-mm-diameter plug from a fresh culture of *G. g. tritici* from dilute PDA was placed in the center of the plate. Inhibition of fungal growth was rated after 4–5 days. Bacterial inhibition of *G. g. tritici* on PDA (high-iron medium) was assumed to be due mainly to the production of antibiotics, whereas inhibition on KMB agar (low-iron medium) was assumed to be due to the production of a siderophore if inhibition was reduced by addition of FeCl₃ to KMB agar. If inhibition of the fungus was not reduced by FeCl₃, an antibiotic was assumed to be responsible.

Selection of mutants. Rifampin-resistant strains of *P. fluorescens* R1a-80 and R7z-80, designated R1a-80R and R7z-80R, were selected as previously described (47). Each strain was similar to its respective parent with respect to fluorescent pigment production, colony morphology, and ability to inhibit *G. g. tritici* on both KMB and PDA. The rifampin and nalidixic acid-resistant strain of 2-79 (2-79RN₁₀) was described previously (47).

Rifampin-resistant strains were treated with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) as previously described (19). Mutants were selected that were nonfluorescent or reduced in ability to inhibit *G. g. tritici* on KMB or PDA but that maintained growth rates similar to their respective parental strains.

Bacterial treatment of wheat seeds. Seeds were treated with bacteria by methods similar to those previously described (47). A 1.0% suspension of methylcellulose (Methocell A-15, Dow Chemical, Midland, MI) (4 ml) was added to a 48-hr-old bacterial culture on KMB agar. The bacteria were scraped into a test tube, vortexed for 30 sec, and then mixed with 5.0 g of surface-sterilized wheat seeds. Treated seeds were dried under a stream of sterile air.

Tube assay for take-all suppression. The assay for ability of bacteria to suppress take-all was essentially as described (49). Plastic tubes (2.5 cm diameter × 16.5 cm long) (Ray Leach Cone-tainer, Canby, OR) that hung in plastic racks, 200 per rack, were plugged in the bottom with cotton and then filled with a 6.5-cm-deep column of sterile vermiculite (pH 6.1) followed by 5.0 g of fumigated Shano silt loam (pH 6.3 after fumigation) amended with *G. g. tritici*. *G. g. tritici* was added into the soil as colonized oat kernels that were fragmented and then sieved into fractions of known particle sizes. Particles 0.25–0.5 mm in diameter were added to the soil at 0.45%, w/w (50). Two seeds treated with bacteria (1–2 × 10⁸ colony-forming units [cfu] per seed) were sown and then covered with vermiculite. Each tube received 10 ml of deionized water (pH 6.4), and then the rack was covered with plastic for 4 days and incubated at 15–18 C in a dark-light cycle of 12 hr. After the plastic was removed, and unless otherwise stated, each cone received 5 ml of diluted (1:3 v/v) Hoagland's solution (macroelements only, pH 5.5) twice a week. After 3–4 wk, the seedlings were washed and evaluated for disease severity on a previously described (49) scale of 0 to 5, where 0 = no disease evident and 5 = plants dead or nearly so.

Studies on the suppressiveness of fluorescent pseudomonads from suppressive and conducive soils. Fluorescent pseudomonads, isolated from roots of wheat grown in the pot bioassay, were tested in the tube assay. In one experiment, nine strains from the Shano silt loam mix (suppressive) and nine strains from the Ritzville silt loam mix (conducive) were tested; in a second experiment, the

same strains from the Shano silt loam and nine strains from the Puget silt loam mix (conducive) were tested. In both experiments, the treatments were replicated 10 times, with each replicate consisting of a single tube with two plants. Treatments were arranged in a completely randomized design.

Studies on the effect of loss of in vitro inhibition of *G. g. tritici* on root colonization and take-all suppression by *P. fluorescens*. NTG-generated mutants of strains 2-79RN$_{10}$, R1a-80R, and R7z-80R, deficient in ability to inhibit *G. g. tritici* in vitro, and their respective parental strains were tested for ability to suppress take-all in the tube assay. Seeds (cultivar Fielder) were treated with approximately $1-2 \times 10^8$ cfu/seed. In these experiments, each treatment was replicated five times for strain 2-79RN$_{10}$ and its mutants, six times for R1a-80 and its mutants, and three times for R7z-80 and its mutants. Each replicate consisted of 10 separate cones with two plants per cone. Treatments were arranged in a randomized complete block design.

Some of the mutants and the respective parental strains were tested for ability to colonize wheat roots. Thatuna silt loam (19) was wetted to −0.3 bars matric potential and added to the plastic tubes; one bacteria-treated seed (cultivar Daws) (approximately 5 × 10^7 cfu/seed) was then placed in each tube and covered with a 1.5-cm layer of soil. Soil matric potential was monitored with a tensiometer (Irriometer Co., Inc., Riverside, CA) and maintained between −0.3 and −0.5 bar by adding water. After incubation at 15–18 C for 14 days, plants were removed from the tubes and roots were gently shaken to remove excess soil. A section of root with tightly adhering rhizosphere soil 3–5 cm from the seed was cut from two roots of a single plant and vortexed with glass beads for 1 min as previously described (19). Serial dilutions were made and aliquots (0.1 ml) were plated in duplicate on KMB agar supplemented with 100 μg/ml of rifampin and cycloheximide. Each replicate consisted of two root sections from a single plant; each treatment was replicated six times. Treatments were arranged in a randomized complete block design.

Studies on the effect of iron and iron chelators on suppression of take-all by fluorescent pseudomonads. Ferric ethylenediamine-tetraacetic acid (Fe EDTA) and EDDA were added to the soil to give a final concentration of 1,000 μg/g of soil using the methods of Scher and Baker (34). EDDA was dissolved in 0.1 N NaOH and the pH was adjusted to approximately 9.0 with 1 N HCl. Fe EDTA was dissolved in water. Soil infested or noninfested with *G. g. tritici* (0.45%, w/w) was misted with the appropriate chelator while mixing the soil, and the soil was dried overnight. Controls consisted of soil misted with water that was adjusted to the same pH as its respective treatment. The tube assay was modified for these studies such that there were two 5-g layers of soil in each cone with only the bottom layer containing *G. g. tritici*. Each tube received 15 ml of deionized water at the time the seed was sown; thereafter it received three 10-ml waterings at weekly intervals with dilute Hoagland's solution containing 5 mM of the appropriate iron or iron chelator solution. Controls received diluted Hoaglunds plus water adjusted to the same pH as their respective EDDA or Fe EDTA solutions. Treatments were replicated 20 times, with each replicate consisting of a single tube with two plants.

Statistics. Data in all experiments were analyzed by analysis of variance. Following a significant *F* test, a least significant difference analysis (LSD) was performed.

RESULTS

Pot bioassay. In the first planting cycle in the pot bioassay, wheat grown in the soil mix with the Shano silt loam (suppressive soil) had the same amount of disease as the wheat in the soil mixes with Ritzville silt loam or Puget silt loam (conducive soils) (Table 1). However, in the second cycle, the mix with the Shano silt loam was highly suppressive to take-all; the plants had significantly less root disease and were taller than plants in the two conducive (nonsuppressive) soil mixes (Table 1). A portion of the roots with adhering rhizosphere soil from the second cycle with live inoculum of *G. g. tritici* was assayed to determine the populations of

rhizosphere bacteria. Roots from all three soil mixes had similar populations of total aerobic bacteria, estimated at 8.03, 8.08, and 8.10 log cfu/0.1 g root in the Shano silt loam, Ritzville silt loam, and Puget silt loam mixes, respectively. However, roots from the Shano silt loam mix had significantly ($P = 0.05$) more fluorescent pseudomonads (7.38 log cfu/0.1 g root) than the roots from the mixes containing either the Ritzville silt loam (6.11 log cfu/0.1 g root) or Puget silt loam (6.08 log cfu/0.1 g root). Further, 85% of the fluorescent pseudomonads from the Shano silt loam mix caused a distinct zone of inhibition (at least a + rating) against *G. g. tritici* on KMB agar, whereas only 21 and 26% of the fluorescent pseudomonads from the Puget silt loam and Ritzville silt loam mixes were as inhibitory to *G. g. tritici*.

Suppressiveness of fluorescent pseudomonads from take-all suppressive and conducive soils. Fluorescent pseudomonads from the Shano silt loam mix (suppressive soil) were more inhibitory of *G. g. tritici* on both KMB agar and PDA than strains from the Ritzville and Puget silt loam mixes (conducive soils) (Table 2). In the tube assay, most of the strains from the Shano silt loam mix were significantly ($P = 0.05$) more suppressive of take-all than the strains from the Ritzville (experiment 1) or Puget silt loam (experiment 2) mixes (Table 2). Further, when compared as groups, strains from the Shano silt loam were significantly ($P = 0.05$) more suppressive of take-all than strains from either the Ritzville or Puget silt loam mixes. Experiment 1 was repeated and the results were similar to those reported.

Effect of loss of in vitro inhibition of *G. g. tritici* on take-all suppression and root colonization by *P. fluorescens*. *P. fluorescens* 2-79RN$_{10}$, R7z-80R, and R1a-80R originally were from roots of wheat grown in suppressive soil from either this study or a previous study and they were highly inhibitory to *G. g. tritici* on both PDA and KMB agar (Table 3). The ability of these strains to inhibit *G. g. tritici* was not altered on PDA by the addition of FeCl$_3$. On KMB agar all three strains produced a fluorescent pigment, presumed to be a siderophore because pigment production was suppressed by the addition of 10–50 μM FeCl$_3$ to the medium. The addition of 10–100 μM of FeCl$_3$ to KMB completely eliminated inhibition of *G. g. tritici* by R1a-80R and almost completely eliminated inhibition by R7z-80R; however, even with 100 μM FeCl$_3$ in KMB, R7z-80R was still slightly inhibitory to *G. g. tritici*. On KMB agar, antibiotic production by R1a-80R was not stimulated by FeCl$_3$. The correlation between loss of the fluorescent siderophore and at

TABLE 1. Severity of take-all caused by *Gaeumannomyces graminis* var. *tritici* in a pot bioassay using take-all conducive and suppressive soils

| Soil and history[a] | Live *G. g. tritici*[a] | | Dead *G. g. tritici*[a] |
	Plant height (cm)	Root disease rating[b]	Plant height (cm)
First cycle[c]			
SSL (suppressive)	10.6	3.81	16.9
RSL (conducive)	12.4	3.54	17.3
PuSL (conducive)	10.5	3.93	17.0
LSD ($P = 0.05$)	3.8	0.52	2.6
Second cycle[c]			
SSL	24.6	3.0	26.2
RSL	15.0	4.1	26.2
PuSL	13.7	4.8	24.2
LSD ($P = 0.05$)	5.4	0.8	3.1

[a] Test soils were diluted 1:10 with fumigated virgin Ritzville silt loam and amended with 1.0%, w/w, ground oat-kernel inoculum (live or dead) of variable sizes. SSL = Shano silt loam from a field cropped many years to continuous wheat; RSL = Ritzville silt loam from a field cropped to alfalfa; PuSL = Puget silt loam from a field rotated between peas and wheat; LSD = least significant difference.

[b] Root disease was rated on a 0–5 scale, where 0 = no disease and 5 = plant dead or nearly so.

[c] In the first cycle, the pot bioassay was established as described above and plants were grown for 4 wk. In the second cycle, plants of the first cycle were harvested and soil from each treatment was air dried, sieved, amended again with 1.0%, w/w, oat inoculum, and sown to wheat. Plants were grown for 5 wk.

least partial loss of in vitro inhibition on KMB amended with iron suggested that one probable mechanism of inhibition of *G. g. tritici* by these strains was iron deprivation caused by the fluorescent siderophore. With strain 2-79RN$_{10}$, the zone of inhibition against *G. g. tritici* on KMB agar was reduced by 10 μM FeCl$_3$, but with higher concentrations of iron the zone size actually increased even though no fluorescent pigment was evident. These results suggest that, when siderophore production by 2-79RN$_{10}$ was inhibited by

iron, production of an antibiotic was stimulated on KMB.

All five mutants of 2-79RN$_{10}$, partially or completely impaired in ability to inhibit *G. g. tritici* on KMB or PDA, were less suppressive of take-all (indicated by greater root disease ratings) than strain 2-79RN$_{10}$ (Table 3). For example, wheat treated with strains 892B (no antibiotic-mediated inhibition) and 117R (almost no siderophore-mediated inhibition) had similar root disease ratings (3.10 and 3.27, respectively), and these ratings were intermediate between those of 2-79RN$_{10}$ (2.71) and the methylcellulose control (3.77). Likewise, the five mutants of strain R7z-80R provided less suppression of take-all than the parental strain (Table 3). Wheat treated with mutant 28, which showed little or no antibiotic and siderophore-mediated inhibition in vitro, had as much disease as wheat treated with methylcellulose alone. Five of the six mutants of R1a-80R were less suppressive of take-all than the parental strain. Loss of antibiotic-mediated inhibition had a greater effect on ability of R1a-80R to suppress take-all than loss of siderophore-mediated inhibition (Table 3). For example, the amount of root disease on plants treated with mutant 302 (2.40) (lacked a fluorescent pigment and inhibition of *G. g. tritici* on KMB was not regulated by iron) was not significantly different than the amount of disease on plants treated with the parental strain (2.15).

In general, the NTG-derived mutants of 2-79RN$_{10}$, R7z-80R, and R1a-80R reached populations on the 3–5-cm section of wheat roots comparable to their respective parents. The populations (log cfu/cm root) of 2-79RN$_{10}$ (parent), 892B, 117R, 79R, and 541G (mutants) were 2.68, 2.52, 3.86, 3.69, and 3.32, respectively, on the 3–5-cm-section of root (LSD 0.05 = 0.61); populations of R7z-80R (parent), 105, 88, and 56 (mutants) were 3.45, 3.36, 3.71, and 2.94, respectively (LSD 0.05 = 0.48); populations of R1a-80R (parent), 303, 302, A643, and A653 (mutants) were 3.73, 3.33, 3.50, 3.80, and 3.11, respectively (LSD 0.05 = 0.69). One exception was strain 56, which had a population on the 3–5-cm root section significantly less than that of R7z-80R; however, the populations of mutant strains 117, 79R, and 541G were significantly greater than the population of parental strain 2-79RN$_{10}$.

Effect of iron and iron chelators on the take-all suppressiveness of fluorescent pseudomonads to take-all. Strains 2-79, R1a-80, and L30b-80 each were tested in the tube assay in the presence or absence of Fe EDTA and EDDA to further assess the role of siderophores in take-all suppression. *P. putida* L30b-80 was included because it suppressed take-all in previous tests (Table 2), but in vitro inhibition of *G. g. tritici* occurred only on KMB agar and was eliminated when KMB agar was supplemented with FeCl$_3$. Wheat treated with 2-79, R1a-80, or L30b-80 had significantly less disease than wheat treated only with methylcellulose (Table 4). When EDDA was added to the soil, take-all was less severe, suggesting that *G. g. tritici* was sensitive to iron deprivation in the soil used in the tube assay. Further, EDDA enhanced the suppressiveness of all three strains tested. When Fe EDTA was added to the soil, take-all was increased but not significantly. The take-all suppressiveness of strain L30b-80 was nullified and that of strain 2-79 was only reduced by Fe EDTA. In contrast, Fe EDTA enhanced, but not significantly, the suppressiveness of R1a-80 (Table 4).

DISCUSSION

This study suggests that both antibiotics and siderophores have a role in biological control of take-all by certain strains of fluorescent *Pseudomonas* spp. These conclusions are based on several lines of evidence. First, strains showing strong in vitro inhibition of *G. g. tritici* on PDA and KMB agar were more suppressive than strains that showed weak or no inhibition on these media. Second, mutants that were partially or totally deficient in antibiotic and/or siderophore production colonized roots as extensively as the parental strains but were less suppressive of take-all. Finally, adding iron to the soil as a means to suppress siderophore production by the bacteria reduced the suppressiveness of some strains.

The relative importance of antibiotics and siderophores in

TABLE 2. Effect of source (soil and cropping history) of fluorescent *Pseudomonas* strains on ability to suppress take-all as a seed treatment in the tube assay

Seed treatments[a]	Soil source[b]	In vitro inhibition of Ggt on:[c]		Disease rating[d]	
		PDA	KMB	Exp. 2	Exp. 2
R30a-80	SSL	+++	+++	2.94	3.22
R10a-80	SSL	+++	+++	2.95	3.36
R1a-80	SSL	+++	+++	2.98	3.22
R17a-80	SSL	+++	+++	3.00	3.29
R11b-80	SSL	+++	+++	3.25	3.28
R4a-80	SSL	+++	+++	3.33	3.40
R15b-80	SSL	+++	+++	3.35	3.20
R7a-80	SSL	+++	+++	3.60	3.61
R1b-80	SSL	++	++	3.78	3.60
L30b-80	RSL	+−	++*	3.58	
L23a-80	RSL	−	−	3.70	
L3a-80	RSL	−	+*	3.83	
L1b-80	RSL	−	−	3.90	
L25a-80	RSL	−	+*	3.90	
L18b-80	RSL	+−	−	3.95	
L34b-80	RSL	−	−	3.98	
L18a-80	RSL	−	+−	3.98	
L36a-80	RSL	−	−	4.10	
M4-80	PuSL	+	++		3.85
M7-80	PuSL	+−	+*		3.85
M5-80	PuSL	−	+*		3.88
M6-80	PuSL	+−	+*		3.90
M3-80	PuSL	−	+*		3.98
M8-80	PuSL	−	+*		4.00
M9-80	PuSL	+−	+*		4.00
M1-80	PuSL	−	+−	4.15	
M2-80	PuSL	+−	+*	4.15	
Methylcellulose				4.07	4.25
None				4.00	4.43
LSD (P = 0.05)				0.31	0.34

[a] Bacteria were grown on King's medium B agar for 48 hr, mixed with a suspension of 1.0% methylcellulose, and then added to wheat seeds. Each seed received approximately 1–2 × 10^8 colony-forming units per seed. The controls received only methylcellulose or no treatment at all. LSD = least significant difference.

[b] SSL = Shano silt loam (take-all suppressive) from a field cropped continuously to wheat for 22 yr, where take-all had declined; RSL = Ritzville silt loam (take-all conducive) from a field previously cropped to alfalfa; and PuSL = Puget silt loam (take-all conducive) from a field where wheat was grown in rotation with peas. Bacteria were from roots of wheat grown in the pot bioassay described in Table 1.

[c] Bacteria were spotted 5 mm from the edge of the agar plate, and 2 days later a 6-mm-diameter agar disk of *G. graminis* var. *tritici* (Ggt) was placed in the center of the plate: − = no zone of inhibition (i.e., abrupt cessation of fungal growth) and the fungus overgrew the bacterial colony; +− = no sharp zone of inhibition but the fungus did not overgrow the bacterial colony; +* and ++* = no sharp zone of inhibition and the fungus grew up to the bacterial colony but the mycelium was less dense out to 6 mm (+*) and 10 mm (++*), respectively, from the point where the bacteria were spotted; + = a sharp zone of inhibition with fungal growth initially inhibited less than 6 mm from the point where the bacteria were spotted; ++ = a sharp zone of inhibition with fungal growth initially inhibited 6–10 mm from the point where the bacteria were spotted; +++ = a sharp zone of inhibition with fungal growth initially inhibited greater than 10 mm from the point where the bacteria were spotted. PDA = potato-dextrose agar; KMB = King's medium B agar.

[d] Strains from the SSL and RSL were compared in one experiment (Exp. 1); the same SSL strains were compared to PuSL strains in a separate experiment (Exp. 2). Disease was rated on a 0–5 scale, where 0 = no disease and 5 = plant dead or nearly so.

TABLE 3. Suppression of take-all by *Pseudomonas fluorescens* strains 2-79RN$_{10}$, R7z-80R, and R1a-80R and their respective mutants deficient in in vitro antagonism of *Gaeumannomyces graminis* var. *tritici* (Ggt)

Experiment[a]	Seed treatment[b]	Fluorescent pigment[c]	Inhibition of Ggt on:[d]		Disease rating[e]
			KMB	PDA	
1	2-79RN$_{10}$	++	+++	+++	2.71
	892B	++	++	−	3.10
	117R	−	+−	++	3.27
	302G	++	+	++	3.29
	79R	−	+	++	3.31
	541B	++	++	−	3.36
	Methylcellulose				3.77
	LSD (P = 0.05)				0.20
2	R7z-80R	++	+++	+++	1.28
	88	−	+++[f]	++	1.77
	105	++	++	++	2.10
	56	−	+++	+−	2.16
	99a	−	+−	+−	2.22
	28	−	+−	−	2.58
	Methylcellulose				2.52
	LSD (P = 0.05)				0.31
3	R1a-80R	++	+++	+++	2.15
	302	−	++[f]	++	2.40
	347	+	++[f]	+	2.45
	A643	++	++	+	2.82
	303	++	+	−	2.90
	B139	−	+−	+−	2.95
	A653	+−	++[f]	+−	2.99
	Methylcellulose				3.11
	LSD (P = 0.05)				0.27

[a] A parental strain, its mutants, and the methylcellulose control represent a single experiment.

[b] Bacteria were grown on King's medium B agar for 48 hr, mixed with a suspension of 1.0% methylcellulose, and then applied to wheat seed. Each seed received approximately $1-2 \times 10^8$ colony-forming units. The control was treated with 1.0% methylcellulose. The N-methyl-N'-nitro-N-nitrosoguanidine mutants are listed under their respective parental strains: 2-79RN$_{10}$, R7z-80R, or R1a-80R. LSD = least significant difference.

[c] Fluorescent pigment production was detected on King's medium B agar.

[d] Bacteria were spotted 5 mm from the edge of the agar plate, and 2 days later a 6-mm-diameter agar disk of *G. g. tritici* was placed in the center of the plate: − = no zone of inhibition (i.e., abrupt cessation of fungal growth) and the fungus overgrew the bacterial colony; +− = no sharp zone of inhibition but the fungus did not overgrow the bacterial colony; + = a sharp zone of inhibition with fungal growth initially inhibited less than 6 mm from the point where the bacteria were spotted; ++ = a sharp zone of inhibition with fungal growth initially inhibited 6–10 mm from the point where the bacteria were spotted; +++ = a sharp zone of inhibition with fungal growth initially inhibited greater than 10 mm from the point where the bacteria were originally spotted. KMB = King's medium B agar, PDA = potato-dextrose agar.

[e] Disease was rated on a 0–5 scale, where 0 = no disease and 5 = plant dead or nearly so.

[f] Fungal inhibition on King's medium B agar was not reversible by the addition of FeCl$_3$ to the medium.

TABLE 4. Effect of EDDA or Fe EDTA[a], added to the soil, on suppression of take-all caused by *Gaeumannomyces graminis* var. *tritici* by fluorescent pseudomonads

Seed treatment[b]	Disease rating[c]	EDDA[d]	Disease rating[c]	Fe EDTA[d]	Disease rating[c]
Methylcellulose	4.18	−	4.16	−	4.14
		+	3.46	+	4.31
L30b-80	3.84	−	4.08	−	3.90
		+	3.41	+	4.31
2-79	3.74	−	3.95	−	3.74
		+	3.43	+	4.03
R1a-80	2.93	−	3.08	−	3.25
		+	2.21	+	3.07
LSD (0.05)			0.28[e]		

[a] EDDA = ethylenediamine-di-(o-hydroxyphenylacetic acid); Fe EDTA = ferric ethylenediamine-tetraacetic acid.

[b] Bacteria were grown on King's medium B agar for 48 hr, mixed with a suspension of 1.0% methylcellulose, and then added to wheat seed. Each seed received approximately $1-2 \times 10^8$ colony-forming units. The control was treated with 1.0% methylcellulose.

[c] Root disease was rated on a 0–5 scale, where 0 = no disease and 5 = plant dead or nearly so.

[d] EDDA and Fe EDTA initially were added (+) to soil at 1,000 μg/g of soil. Subsequently, the soil was watered three times (10 ml/tube) with dilute Hoagland's solution containing 0.005 M of EDDA or Fe EDTA; controls received dilute Hoagland's solution plus water adjusted to the same pH as their respective treatments. Treatments with means shown in the second column received only dilute Hoagland's solution.

[e] For comparison of all treatments. LSD = least significant difference.

suppression of take-all depends on the strain tested. For example, with strain R1a-80R, antibiotic production appeared to be of major importance because a siderophore-minus, antibiotic-plus mutant (302) of R1a-80R was not significantly different than the parent strain in suppressiveness to take-all and Fe(III) added into the soil did not reduce the suppressiveness of R1a-80. In contrast, strain L30b-80 appeared to rely to a large extent on siderophore production because Fe(III) added to the soil eliminated its suppressiveness. Both inhibitors appeared to have a role in the suppressiveness of strain 2-79; both siderophore- and antibiotic-deficient mutants were less suppressive than 2-79, and Fe(III) only partially reduced the suppressiveness of 2-79. Studies with genetically defined mutants deficient in antibiotic and/or siderophore production are now needed to better define the relative roles of these mechanisms in biological control of take-all. It is important to remember that other mechanisms besides antibiotics and siderophores, such as competition for carbon and nitrogen sources and favored sites on the root (41), also probably contribute to biocontrol of take-all by these strains.

The relative importance of antibiotics and siderophores in disease suppression by a strain also may depend on environmental conditions. Factors such as soil matric potential (19) and rhizosphere pH influence the colonization of wheat roots by introduced bacteria, and it is likely that the regulation of antibiotic and siderophore production would be even more sensitive to soil physical and chemical factors. Baker (3) pointed out that soil pH has an indirect role in siderophore-mediated disease suppression because pH affects the amount of iron available to plants and

microorganisms. Thus, it is possible that, at a higher soil pH, strain R1a-80 might also show siderophore-mediated suppression, whereas at a low pH the siderophore-mediated suppression of strain 2-79 or L30b-80 may not function because iron would be more available. Further studies on the effects of soil physical and chemical factors on disease suppression by introduced bacteria are greatly needed.

The relative importance of antibiotics and siderophores produced by these strains also may vary during different phases of pathogenesis by *G. g. tritici*. Based on previous studies in the field (46), it is known that the pattern of root colonization by introduced fluorescent pseudomonads closely follows the growth of the take-all fungus on the roots. The ability of introduced bacteria to become established along roots and in the lesions is important for pathogen suppression to occur. We speculate that, under natural conditions, *Pseudomonas* siderophores may be primarily responsible for inhibiting *G. g. tritici* early in the parasitic phase when runner hyphae initially colonize the surface of the wheat root. The fungus might be especially vulnerable to iron deprivation at this time because iron, as well as other nutrients, could become limiting at the root surface due to the intense activity of the indigenous rhizosphere microflora and the introduced pseudomonads. This process by which iron would become limiting for *G. g. tritici* might be similar to that speculated to occur when introduced *Pseudomonas* spp. suppress formae specialis of *Fusarium oxysporum* (3,4,34). On the other hand, *Pseudomonas* antibiotics might function primarily in take-all lesions and thereby limit secondary infections by the fungus. Bacteria proliferate within lesions (32,46) presumably in response to the leakage of nutrients from the tissue, which would probably be sufficient to support antibiotic production.

For strain 2-79, production of both a siderophore and an antibiotic provides a continuum of substances important to the suppression of *G. g. tritici*. For example, on media low in iron, such as KMB agar, a fluorescent siderophore was produced, but as the concentration of iron increased, siderophore production was repressed and antibiotic production was initiated. The major antibiotic produced by strain 2-79 originally was reported to be a dimer of phenazine-1-carboxylic acid (14); however, in another study (8), it was reported to be the monomer phenazine-1-carboxylic acid. Production of this phenazine as well as other phenazines appears to respond to iron. Perhaps for strain 2-79 the siderophore supplies essential quantities of iron needed for production of this antibiotic. The phenazine is probably responsible for at least a portion of the antibiotic-mediated suppression of take-all by strain 2-79.

This study provides further support for a previous conclusion (12,49) that fluorescent pseudomonads have a role in take-all decline. First, plants that were grown in the pot assay with suppressive soil (from a field where take-all decline had occurred) had less take-all than plants grown in conducive soils. Second, the roots in the suppressive soil as compared to conducive soils harbored both higher total numbers of fluorescent pseudomonads and a greater proportion of fluorescent pseudomonads that were inhibitory to *G. g. tritici* in vitro. Finally, the *Pseudomonas* strains from the suppressive soil, in general, were more suppressive of take-all in the tube assay than the strains from the conducive soils.

It is well accepted that biocontrol agents cannot be selected only on the basis of an agar-plate bioassay (2). Antibiosis is only one of many characteristics that may be important to a strain enabling it to provide biological control. Strains effective in biocontrol of a root pathogen also must be able to colonize roots and avoid displacement by the indigenous microorganisms. Antibiotics or siderophores must be produced under conditions far different than those in vitro, and for some strains the conditions in the rhizosphere may not be suitable. In spite of what is usually observed, we found an association between antibiotic and siderophore production in vitro and biological control when the strains from roots grown in take-all suppressive soils were compared to strains from roots grown in conducive soils. We speculate that during take-all decline a natural selection occurs in favor of bacteria that are rhizosphere competent, are highly

antagonistic to *G. g. tritici*, and possess other unknown traits essential for biocontrol activity. The importance of these unknown traits to take-all suppression is suggested in the data of a previous study (49); in a test of 66 strains from roots grown in take-all suppressive and conducive soils, 90% of which inhibited *G. g. tritici* on both PDA and KMB agar, a significantly greater proportion of the strains from suppressive soils as compared to strains from conducive soils were effective against take-all when tested as seed treatments.

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