Selection of Fluorescent Pseudomonads Antagonistic to *Erwinia carotovora* and Suppressive of Potato Seed Piece Decay

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ABSTRACT

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A procedure was developed for screening fluorescent pseudomonads from potatoes for suppression of the potato soft rot pathogen, *Erwinia carotovora*, based on antagonism in vitro (i.e., siderophore and/or antibiotic production) and suppression of preemergence seed piece decay. Strains were identified as either *Pseudomonas putida* or *P. fluorescens*, the majority being *P. fluorescens* biovar III. The pseudomonads (293 strains) were screened first on King's medium B agar for ability to inhibit growth of *E. carotovora* subsp. *atroseptica*. On this medium, inhibition is due primarily to the production of siderophores. Siderophores having a diversity of iron affinities were produced; some were able to reverse iron deprivation of the producer strain at ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) concentrations up to 5,000 μg/ml. Antibiotics were produced by 56% of the pseudomonads on potato-dextrose agar. Candidate strains of *Pseudomonas* then were tested in vivo for suppression of preemergence seed piece decay. In greenhouse trials, plant emergence was significantly increased up to 64% and plant growth was increased nearly seven-fold compared to checks inoculated only with *E. c.* subsp. *atroseptica*. Strains that produced siderophores with high iron affinities were more suppressive as a group than strains that produced siderophores with low iron affinities. Furthermore, strains that produced both inhibitory siderophores and antibiotics appeared to give the strongest effect in greenhouse trials against *E. c.* subsp. *atroseptica*. In general, pseudomonads were generally less effective in suppressing seed piece decay caused by strain W3C105 of *E. c.* subsp. *carotovora* than strain W3C37 of *E. c.* subsp. *atroseptica*. The screening method, nevertheless, should prove useful in identifying potato rhizosphere pseudomonads with the greatest potential for controlling diseases caused by *E. carotovora*.

Additional key words: bacterial soft rot, blackleg, pyoverdine.

Soft rot diseases caused in potato by *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye and *Erwina carotovora* subsp. *carotovora* (Jones) Bergey et al threaten production of potatoes during all phases of plant growth and tuber storage. In the Columbia Basin in the state of Washington, *E. carotovora* causes preemergence seed piece decay, blackleg, soft stem rot, and soft rot of daughter tubers. Contaminated seed tubers, soil, and irrigation water serve as sources of inocula of *E. carotovora* (1,9,25). The pathogen colonizes potato root and tuber surfaces and under favorable environmental conditions, populations commonly exceed 10^6 colony-forming units (cfu) per gram of root by midseason (8; D. C. Gross, *unpublished*). Because soil is an important source of *E. carotovora* (primarily *E. c.* subsp. *carotovora* [25]), disease control based on planting certified pathogen-free potato seed has not been successful in the Columbia Basin. New approaches to pathogen control are needed that would integrate knowledge of the inoculum sources, the diversity of pathogenic strains, and the population dynamics and aggressiveness of the pathogen at various stages of plant growth.

Biological control is a potential method to control soft rot diseases. Potatoes could be treated with bacteria, originally selected from potato surfaces, that are inhibitory to the growth of *E. carotovora*. Fluorescent pseudomonads are attractive candidates for biological control of *E. carotovora* because they colonize the potato rhizoplane and rhizosphere and produce high populations (2,20,21,23), and they produce a variety of secondary metabolites and substances that can alter the composition of the rhizosphere microflora (3,32). Kloepper (18) reported that some fluorescent pseudomonad strains when applied to potato seed pieces reduce the populations of *E. carotovora* on root and daughter tubers by 95–100% and 28–95%, respectively, compared to untreated plants. These bacteria are known as plant growth-promoting rhizobacteria (PGPR) because of their ability to improve plant growth through suppression of "deleterious" root-colonizing microorganisms (33). Suppression was attributed to production of fluorescent siderophores that were essential for uptake of iron by the pseudomonads (19). Because the fluorescent pseudomonads produced siderophores with high affinities for iron, deleterious microorganisms which could not obtain iron from these siderophores or produced siderophores with lower affinities for iron were deprived of the iron necessary for growth. Strain B10 of *Pseudomonas fluorescens* (Trevisan) Migula-*P. putida* (Trevisan) Migula, for example, was originally isolated from potato; it produces the fluorescent siderophore, pseudobactin, which inhibited *E. carotovora* both in vitro and in vivo (19). Strain B10 suppressed growth of *E. carotovora* and its colonization of potato root and tuber surfaces (18).

Colyer and Mount (4) reported the use of fluorescent pseudomonads to control postharvest soft rot of potatoes. Preplant treatments of potato seed pieces with antibiotic-producing strain M17 of *P. putida* caused a 6.8 to 18.2% reduction in rotted tissues of the harvested daughter tubers. A mutant of strain M17 unable to produce an antibiotic was about 50% less effective than the parental strain. In a related study, Burr and Caesar (3) also noted a decreased incidence of soft rot caused by *Erwinia* spp. in potato tuber disks inoculated with a plant growth-promoting fluorescent pseudomonad.

Numerous other examples of the successful use of fluorescent pseudomonads (usually *P. fluorescens* or *P. putida*) further document their ability to thrive in root environments and provide biological control (10,11,15,16,28,30,38,40). For example, strain Pf-5 of *P. fluorescens*, which was isolated from a cotton rhizosphere, suppressed seedling diseases caused in cotton by *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow (14,15). Strain Pf-5 produced in vitro the antibiotics pyrrolnitrin and pyoluteorin, which were inhibitory to the seedling pathogens.

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Fluorescent pseudomonad siderophores also have been reported to have leading roles in the biocontrol of several important fungal plant pathogens (30,36,43).

An effective screening procedure is essential if beneficial bacteria are to be selected from the multitude of bacteria that inhabit root zones. To be functional in a given environment, an introduced bacterium would need to become established rapidly and attain a high population. Therefore, prospective biological control agents would be expected to comprise a large segment of the natural microflora and, accordingly, these predominating bacteria should be used in systematic screening procedures. Nevertheless,because only a small percentage of the total bacteria may be beneficial, a large number of bacteria may need to be screened. Schroth and Hancock (29) estimated, for example, that PGPR comprise only 2 to 5% of the root bacteria.

Following isolation, candidate bacteria are usually screened for the ability to inhibit a target pathogen in vitro. In many studies (10,16,19,42) fluorescent pseudomonads have been screened on King's medium B (KB) agar (17), which is low in available iron. Siderophore production is inversely related to iron concentration and readily occurs on KB agar. In contrast, on potato-dextrose agar (PDA), a medium high in iron and low in phosphate, fluorescent siderophores are repressed and antibiotics are produced (12). Selection may be further improved by screening for siderophores with high iron-binding affinities (36) or antibiotics with potent bactericidal properties.

Most screening procedures test candidate biocontrol agents under controlled greenhouse conditions prior to field trials (41). Measurements of disease, pathogen levels, and/or plant growth promotion have been used to identify effective strains. However, no greenhouse system has been developed for screening antagonists of *E. carotovora*.

The purpose of this study was to develop a method to screen selected fluorescent pseudomonads from potato for ability to inhibit *E. carotovora* and suppress soft rot. In particular, the role of siderophore and antibiotic production is explored relative to selection of biocontrol agents.

MATERIALS AND METHODS

Bacterial strains. Plant growth-promoting rhizobacterial strain B10 and strain Pf-5 of *P. fluorescens* (originally isolated from potato [21] and cotton [14,15], respectively) were obtained from G. D. Easton (Irrigated Agriculture Research and Extension Center, Prosser, WA). Strain W3C37 of *E. c.* subsp. *atroseptica* and strain W3C105 of *E. c.* subsp. *carotovora* were isolated from potatoes in Washington.

Isolation of antagonists. Fluorescent pseudomonads were isolated from potato stems, roots, daughter tubers, and soil collected in 1981, 1982, and 1983 from 30 commercial potato fields in the Columbia Basin of Washington. Each field sample consisted of three whole plants which were collected in August or September and chilled in transit to the laboratory. Stems, tubers, and roots were gently rinsed with tap water for 1 min to remove loosely adhering soil prior to bacterial isolation. To isolate bacteria, basal stem tissue (3–4 g per sample) was macerated in 1 ml of sterile potassium phosphate buffer (12.5 mM, pH 7.1), whereas, roots (2 g per sample), daughter tubers (nine tubers per sample) and soil (20 g per sample) were washed in a 10-fold (w/v) quantity of sterile potassium phosphate buffer by agitation for 30 min on a rotary shaker (250 rpm). Aliquots (0.1 ml) from appropriate serial dilutions were plated in duplicate on King's medium B (KB) agar (17). Plates were incubated for 48 hr at ambient temperature (approximately 24 C) and then oversprayed with a suspension of strain W3C37 of *E. c.* subsp. *atroseptica* (10^8 cfu/ml). Plates were incubated an additional 24 hr, after which zones of growth inhibition produced by UV-fluorescent colonies of *Pseudomonas* spp. against *E. carotovora* were recorded. Total fluorescent pseudomonads and bacterial populations were determined. Antagonistic and some nonantagonistic fluorescent pseudomonad colonies were selected and purified.

Cultivation and preservation of bacteria. Fluorescent pseudomonad strains were purified and routinely cultured on nutrient broth-yeast extract (NBY) agar medium (37) and then stored at 4 C for temporary maintenance. Bacteria were preserved by placing cells in a glycerol-mineral salts buffer (13) at −20 C. Reference bacterial stocks were preserved by lyophilization.

Strains of *E. carotovora* were purified on crystal violet polypectate (CVP) agar (6) and stored on NBY slants at 4 C. Long-term preservation of strains was in sterile distilled water at 4 C and as lyophilized stocks. *E. carotovora* was routinely cultured at ambient temperature (approximately 24 C) for 24 hr on NBY agar for inoculum preparation.

Screening procedure for antibiotic and siderophore production. Antibiotic production by the fluorescent pseudomonads was assayed by spotting aliquots (10 μl) of bacterial suspensions (10^9/ml) in duplicate onto two plates of PDA. After plates had been incubated at 25 C for 96 hr, they were lightly oversprayed with a suspension (10^8 cfu/ml) of *E. c.* subsp. *atroseptica* (W3C37). Zones of inhibition were measured 48 hr later. Siderophore production by fluorescent pseudomonads was assayed on KB agar in the same manner as described for antibiotic production on PDA, except that plates were incubated for 48 hr prior to overspraying them with strain W3C37 of *E. c.* subsp. *atroseptica* and inhibition zones were measured 24 hr later.

Screening procedure for pseudomonads that produce siderophores with high affinities for iron. Siderophore production by fluorescent pseudomonads was assayed by the modified methods of Cox and Graham (5) and Vandenbergh et al (36). Bacteria were grown for 24 hr on N minimal (NM) agar (37), suspended (10^6 cfu/ml) in sterile potassium phosphate buffer and spotted (10 μl) in duplicate onto two NM agar plates supplemented with purified ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) (EDDA). The EDDA was purified as described by Rogers (26) and incorporated into the minimal agar at final concentrations of 0, 250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, and 5,000 μg/ml. Plates were incubated at 25 C for 96 hr and growth was assessed as well as UV fluorescent siderophore production.

Siderophore production for strains W4F35, W4P63, W4F156, Pf-5, and B10 was assayed based on fluorescence at 400 nm in iron-deficient and nonfluorescence in iron-supplemented sucrose media (28). Strains were incubated for 24 hr in 25 ml of sucrose medium. Ferric chloride (0.5 g/L) was then added to replicate cultures of each strain and they were incubated at 25 C on a rotary shaker (250 rpm) for 24 hr. The suspensions were centrifuged at 2,500 × *g* for 15 min, supernatants were filtered (0.22 μm), adjusted to pH 5.5, and the absorbance was measured from 350 to 550 nm.

Effect of iron concentration on growth inhibition of *E. c.* subsp. *atroseptica*. Suspensions of individual fluorescent pseudomonads (10^9 cfu/ml) in sterile potassium phosphate buffer were spotted (10 μl) in duplicate onto KB agar amended with different concentrations of filter-sterilized $FeCl_3$ (0, 1, 10, 100 and 1,000 μM). Plates (four bacterial spots per plate) were incubated at 28 C for 24 hr and then oversprayed with strain W3C37 of *E. c.* subsp. *atroseptica* (10^8 cfu/ml). After 48 hr, the diameters of the zones of inhibition were measured. The experiment was repeated twice.

Inoculation of potato seed pieces with *E. carotovora*. Strain W3C37 of *E. c.* subsp. *atroseptica* from a 24-hr NBY agar culture was suspended in sterile potassium phosphate buffer and adjusted to 10^3, 10^5, 10^7, and 10^9 cfu/ml. Inocula were incorporated into the soil, coated onto potato seed pieces, or injected into seed pieces by syringe. A sandy-loam soil, pH 7.1, from Othello, WA, was fumigated (98% methyl bromide, 2% chloropicrin) and mixed with fumigated vermiculite (9:1, v/v) to maintain adequate porosity. The soil-vermiculite mix (600 g) was infested with *E. carotovora* by incorporating 10 ml of the bacterial suspension into the mix. Alternatively, seed pieces were inoculated by placing 40 ml of a 1.5% (w/v) methylcellulose-water suspension of the pathogen along with 17 seed pieces in a polyethylene bag and then shaking vigorously for 5 min. Seed pieces were injected once (1-cm deep) with a 10-μl cell suspension near a seed piece eye with a sterile 10-μl micropipet from which the tip had been cut-off. The upper part of the pipet was inserted into a disposable 18-gauge hypodermic needle. With each inoculation procedure, checks were treated with

sterile potassium phosphate buffer only. Seed piece populations of *Erwinia* were determined immediately after inoculation by shaking three potato seed pieces for 30 min in a quantity of potassium phosphate buffer ten times their weight. Soil populations were determined by shaking 20 g of soil in 200 ml of buffer on a rotary shaker (250 rpm) for 30 min. Samples (0.1 ml) from the serial dilutions were plated onto CVP medium and the numbers of colony-forming units of *Erwinia* were determined after 2 days.

Certified Montana seed potatoes (*Solanum tuberosum* L. 'Russet Burbank') were used in the greenhouse studies. Single-eye potato seed pieces were cut with a sterile, 3-cm-diameter melon-ball scoop just prior to application of the bacteria. Three seed pieces were planted per 15-cm-diameter pot containing 600 g of fumigated soil with or without *E. carotovora*, and the pots were randomized (five replications) on greenhouse benches. Plants were grown at an air temperature of 21 C during the day and 18 C at night. Daylight was supplemented 16 hr each day with cool-white fluorescent lamps 150 cm above the benches. The soil was kept moist (about −0.5 bar). Two weeks after planting, treatments were evaluated for the percentage of preemergence seed piece decay as determined by plant emergence. The experiment was repeated on two occasions.

Application of antagonistic pseudomonads to seed pieces. Strain W4P63 of *P. putida* was used to determine the optimum concentration of *Pseudomonas* per potato seed piece needed to suppress preemergence bacterial soft rot in the greenhouse. Strain W4P63 was grown for 24 hr on KB agar and suspended in sterile potassium phosphate buffer. Cell numbers were adjusted to 10^4, 10^6, 10^8, and 10^{10} cfu/ml then diluted 1:10 in a sterile 1.5% (w/v) methylcellulose-water suspension. Seedpieces were coated as described above for *E. carotovora*. Coated seed pieces were incubated in the greenhouse for 24 hr at a high humidity and then inoculated with strain W3C37 of *E. c.* subsp. *atroseptica* (10^5 cfu/ml). Seventeen seed pieces previously treated with a given concentration of the pseudomonad were mixed with inoculum of *Erwinia* (40 ml) for 5 min in a polyethylene bag and then air-dried (21 C) for 30 min. Seed pieces (three per pot) were planted in soil-vermiculite mix, and each treatment was replicated five times. Treatments were completely randomized. Two weeks after planting, emergence of plants and fresh weight of whole plants and roots were determined. Plants were gently removed from the soil, rinsed under running tap water, air-dried for 1 hr on the greenhouse benches, and fresh weights were recorded. Data were analyzed with coefficients of orthogonal polynomials to test for a linear response.

Greenhouse screening of fluorescent pseudomonads for control of preemergence seed piece decay. Thirty-one fluorescent pseudomonad strains (including strains B10 and Pf-5) that represented the different antibiotic and siderophore groups described below were screened for ability to control preemergence seed piece decay caused by strain W3C37 of *E. c.* subsp. *atroseptica*. Seed pieces were treated by coating as described above to give a final cell concentration of 10^9 cfu per seed piece. Prior to planting, the seed pieces were inoculated with a suspension of 10^5 cfu of *E. c.* subsp. *atroseptica* (W3C37) per milliliter in 1.5% (w/v) methylcellulose-water. Seedpieces treated solely with the methylcellulose-water suspension were included as controls. All treatments were completely randomized. Emergence and fresh weight of whole plants and roots were recorded 2 wk after planting. Data were analyzed by using protected least significant difference (LSD) or coefficients of orthogonal polynomials.

Identification of fluorescent pseudomonads. The fluorescent pseudomonads used in the greenhouse studies were identified to species according to the phenotypic characters and biochemical tests described by Stanier et al (31) and Sands and Rovira (27). The tests included oxidase, arginine dihydrolase, fluorescence on KB agar, gelatin hydrolysis, denitrification, levan production from sucrose, ability to cause potato soft rot, growth at 4 and 41 C, and utilization of ethanol, trehalose, sorbitol, meso-inositol, β-alanine, L-valine, and benzylamine as carbon sources. The standard tests were performed as described by Stanier et al (31). The modified tests for carbon source utilization were performed as described by Sands and Rovira (27).

Most of the strains were identified to biotype based on the GLDETS tests (i.e., gelatin hydrolysis, levan production, denitrification, ethanol utilization, trehalose utilization, and sorbitol utilization) of Sands and Rovira (27).

RESULTS

Isolation of fluorescent pseudomonads. Fluorescent pseudomonads were common inhabitants of the roots and tubers of potatoes in the Columbia Basin in the state of Washington. Populations of fluorescent pseudomonads averaged 10^7 cfu/g of root (mean of 13 locations) and 10^5 cfu/g of tuber at mid- to late season. The zone of inhibition produced by many of the strongly antagonistic strains of *Pseudomonas* spp. was typically a clear and, apparently, bactericidal zone that extended up to 13 mm from the producer colony.

A total of 293 fluorescent pseudomonad strains were isolated between 1981 and 1983 (Table 1). Most of those strains were isolated from potato roots and tuber surfaces and were, at least initially, inhibitory to strain W3C37 of *E. c.* subsp. *atroseptica* on KB agar.

In vitro antagonism of *E. c.* subsp. *atroseptica* by fluorescent pseudomonads. Of 293 fluorescent pseudomonad strains screened for inhibition of *E. c.* subsp. *atroseptica*, 88% were inhibitory on KB, PDA, or both of these media (Table 1). Of the inhibitory

TABLE 1. Influence of fluorescent pseudomonads on the growth of *Erwinia carotovora* subsp. *atroseptica* (W3C37) on King's medium B agar and potato-dextrose agar[a]

Source of strain	Strains tested (no.)	Strains (no.) of *Pseudomonas* inhibitory to *E. c.* subsp. *atroseptica* on:			Noninhibitory strains (no.)
		KB[b]	PDA[b]	KB and PDA	
Stem	33	30	18	18	3
Root	111	75	55	39	20
Tuber	124	105	79	71	11
Soil	25	24	13	13	1
Total	293	234	165	141	35

[a] Pseudomonads were inoculated onto duplicate plates, incubated at 24 C for 2 days, and then oversprayed with a suspension (10^8 colony-forming units per milliliter) of strain W3C37 of *E. c.* subsp. *atroseptica*. Inhibition of growth of *Erwinia* was noted after 48 hr of incubation.

[b] KB = King's medium B, and PDA = potato-dextrose agar.

TABLE 2. Influence of ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) (EDDA) on the growth of fluorescent strains of *Pseudomonas*

Antibiosis group[b]	Strains (no.) of *Pseudomonas* growing at an EDDA concentration (μg/ml) of:[a]									
	0	250	500	750	1,000	1,500	2,000	2,500	3,000	5,000
I	93	69	52	28	14	11	5	3	1	1
II	24	21	11	5	3	2	1	0	0	0
III	141	124	88	54	31	16	6	5	3	1
IV	35	27	22	12	2	2	1	1	1	0
Total	293	241	173	99	50	31	13	9	5	2

[a] Incorporated into N minimal (37) agar.

[b] Inhibitory to strain W3C37 of *Erwinia carotovora* subsp. *atroseptica* on: only King's medium B (KB) agar (group I), only potato-dextrose agar (PDA) (group II), both KB agar and PDA (group III), and neither KB agar nor PDA (group IV).

strains, 91% produced a zone of inhibition to *Erwinia* on KB agar that extended up to 13 mm from the margin of the producer colony; the inhibition was usually bacteriostatic since confluent growth of *Erwinia* occurred within the zone by 4 days after inoculation. This inhibitory zone generally corresponded with the area of fluorescent pigment diffusion and probably resulted from siderophore production. Only a few strains produced what appeared to be bactericidal zones of inhibition that were not associated with the inhibitory activity of the fluorescent siderophore. Fluorescent siderophores were not produced by any of the 293 strains on PDA. However, 56% of the pseudomonads produced zones of inhibition to strain W3C37 of *E. c.* subsp. *atroseptica* on PDA that apparently resulted from antibiotic production. Moreover, 48% of all 293 strains produced zones of growth inhibition on both KB agar and PDA; these were identified as antibiosis group III strains. Strains producing inhibitory zones only on KB agar (93 strains) or PDA (24 strains) were identified as antibiosis group I and II strains, respectively. Antibiosis group IV strains included 35 noninhibitory pseudomonads.

Characteristics of the siderophores produced by fluorescent pseudomonad strains. All 293 strains grew and produced a fluorescent siderophore on KB agar without EDDA (Table 2). However, at an EDDA concentration of 250 μg/ml, 52 (18%) of the strains did not grow and the growth of most of the remaining strains was greatly suppressed. With 750 μg EDDA/ml, 99 (34%) of the fluorescent pseudomonad strains grew, but at 2,500 μg EDDA/ml and above, only 9 (3%) grew. The strains were categorized into three siderophore groups based upon the apparent affinities of their fluorescent siderophores for iron. The medium iron affinity group (growth between 250 and 1,000 μg EDDA/ml) was largest consisting of 191 (65%) of the fluorescent pseudomonads. The low iron affinity group (growth at 250 μg EDDA/ml and below) consisted of 52 (18%) strains while the high iron affinity group (growth at 1,000 μg EDDA/ml and above) contained 50 (17%) strains.

Strain W4P63 of *P. putida* produced a yellow green fluorescent pigment in a low-iron medium. The siderophore had an excitation maximum of near 400 nm and an emission maximum near 500 nm. No fluorescent siderophore was produced and no absorption maximum was observed for the culture supernatant at 400 nm when $FeCl_3$ (0.1 g/L) was added. The absorption spectra of siderophores produced by four other fluorescent pseudomonad strains (W4F35, W4F156, Pf-5, and B10) were similar to that of strain W4P63.

Effect of iron concentration on the production of siderophore and antibiotics inhibitory to *E. c.* subsp. *atroseptica*. Five pseudomonad strains, including strain B10 which produces pseudobactin (19), produced fluorescent pigments on KB agar that were inhibitory to the growth of *E. c.* subsp. *atroseptica* (Table 3). Production of fluorescent pigment was completely repressed on the KB agar supplemented with 100 μM or more ferric chloride, and suppression of the growth of strain W3C37 of *E. c.* subsp. *atroseptica* was reduced when iron concentrations as low as 10 μM were added. There was no significant difference in size between the fluorescent pigment zone and the zone of inhibition to *E. c.* subsp. *atroseptica* (Table 3). The exception was strain Pf-5, which produced no fluorescent siderophore at 100 μM iron or above, but still produced a bactericidal zone of inhibition to *E. c.* subsp. *atroseptica*.

TABLE 3. Influence of iron on inhibition of *Erwinia carotovora* subsp. *atroseptica* (W3C37) by fluorescent pseudomonads

Strains of *Pseudomonas*	Zone (mm) of inhibition[a] in the presence of $FeCl_3$ at μM:				
	0	1	10	100	1,000
W4P63	8.5	8.7	4.2	0	0
W4F35	7.7	8.4	6.3	0	0
W4F156	9.1	8.8	6.6	0	0
Pf-5	8.7	9.8	6.5	7.1	8.1
B10	7.1	5.2	5.7	0	0

[a] Inhibition zones on King's medium B agar were measured from the margin of the producer colonies to the point of indicator growth. Data represent the mean of two replications repeated on two occasions.

Conditions for development of preemergence seed piece decay in the greenhouse. Application of *E. c.* subsp. *atroseptica* strain W3C37 to the surfaces of seed pieces resulted in a slightly more uniform and consistent development of preemergence seed piece decay in the greenhouse than either seed piece injection or soil infestation with the pathogen.

The population size of *Erwinia* was critical to achieving a balance between emergence and disease development (Fig. 1). Application of 10^8 cfu per seed piece or more of *E. carotovora* resulted in only one-tenth as many plants emerging as compared to uninoculated seed pieces, whereas at populations of 10^4 cfu per seed piece or less, over 60% of the seed pieces consistently emerged. An inoculum dose of 10^6 cfu per seed piece of *E. c.* subsp. *atroseptica* was chosen for greenhouse screening for strains of antagonistic *Pseudomonas* spp. because it resulted in plant emergence of approximately 30%. It was also noted that additional plants had not emerged 2 wk after planting.

Effect of strain W4P63 of *P. putida* on preemergence potato seed piece decay in the greenhouse. Potato emergence and fresh weight of whole plants and roots were increased significantly by applying strain W4P63 to seed pieces 24 hr prior to inoculating them with strain W3C37 of *E. c.* subsp. *atroseptica* and planting (Fig. 2; Table 4). Strain W4P63 applied at a minimum of 10^6 cfu per seed piece increased emergence (32%) and more than doubled the fresh weights of whole plants compared to those untreated with the pseudomonads. At a pseudomonad concentration of 10^{10} cfu per seed piece, emergence exceeded 96%. All seed pieces not treated with either strain W4P63 of *P. putida* or strain W3C37 of *E. c.* subsp. *atroseptica* (check) emerged with an average (2-wk whole plant) fresh weight of approximately 24 g. Treatment with strain W4P63 at 10^4 cfu per seed piece resulted in emergence and plant fresh weight not significantly different ($P = 0.05$) from the uninoculated check.

Screening of fluorescent pseudomonads for control of preemergence seed piece decay of potato caused by *E. c.* subsp. *atroseptica*. Of the 31 strains tested, all improved plant emergence by 6.7 to 63.5% compared to the untreated check, but no strain gave complete control (Table 5). Strain B10, which produces pseudobactin (19), increased plant emergence by 50% and fresh

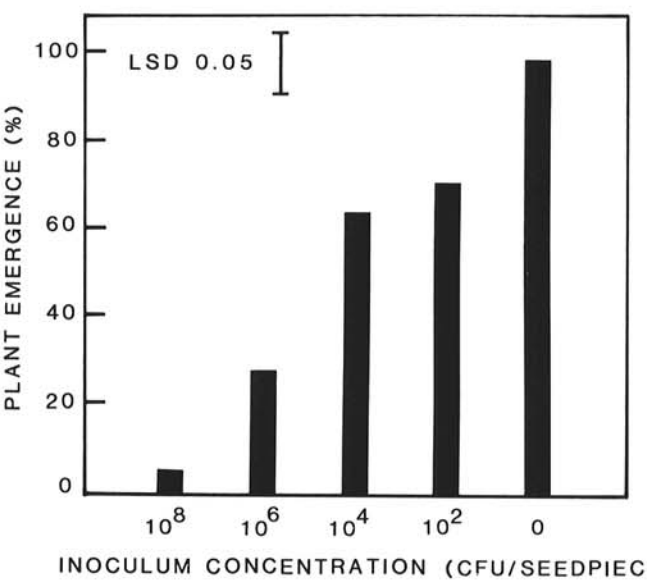

Fig. 1. Effect of different inoculum concentrations of *Erwinia carotovora* subsp. *atroseptica* (W3C37) as a seed piece coating on preemergence seed piece decay of Russet Burbank potatoes as measured by percent emergence. Each treatment was replicated five times, three seed pieces per pot, in fumigated soil. Results shown are averages from two trials completed on different dates. Inoculum concentrations of *E. c.* subsp. *atroseptica* per seed piece were 10^8 cfu, 10^6 cfu, 10^4 cfu, 10^2 cfu, and 0 cfu.

TABLE 4. Influence of strain W4P63 of *Pseudomonas putida* on preemergence seed piece decay of potato (as measured by plant emergence) caused by strain W3C37 of *Erwinia carotovora* subsp. *atroseptica*

Population[a]	Emergence[b] (%)	Fresh weight[b] Whole plant (g/pot)	Fresh weight[b] Roots (g/pot)
10^{10}	96.6	17.7	2.2
10^{8}	91.1	15.2	1.8
10^{6}	77.7	15.0	2.3
10^{4}	50.0	6.1	1.5
Check[c]	45.5	6.1	0.9
Uninoculated	100.0	23.8	3.9
Linear MS[d]	9,683.0**	718.7**	21.1**

[a] Colony-forming units (cfu) of W4P63 per Russet Burbank seed piece.
[b] Mean of 15 replicated pots, three seed pieces per pot. Emergence and weights were recorded 2 wk after planting.
[c] Treatments with *E. c.* subsp. *atroseptica* only. Inoculated with 10^6 cfu per seed piece of the pathogen prior to planting in fumigated soil.
[d] Asterisks (**) indicate significance of the experiment at $P = 0.01$ for a linear model based on coefficients of orthogonal polynomials.

weight over five-fold as compared to the check. In contrast, strain Pf-5, which produces pyrrolnitrin and pyoluteorin (14,15), increased plant emergence by 66.7% but did not greatly increase fresh weight.

Strains that produced siderophores with high affinities for iron improved plant emergence by about 9% more than did strains that produced siderophores with low iron affinities (Table 6). A significant ($P = 0.01$) linear relationship between increasing iron affinity and either emergence or fresh weight was observed. As a group, pseudomonad strains applied to seed pieces improved emergence by at least 45%. Moreover, whole plant and root fresh weights were about eight times greater than those of the untreated check plants (Table 6). Strains that produced a zone of inhibition on both PDA and KB agar had the strongest effect, increasing emergence by 51% compared to the check (Table 7). However, there was less than a 5% difference in the average emergence for strains that produced inhibitory siderophores and/or antibiotics (groups I, II, and III). In contrast, noninhibitory strains (antibiosis group IV) significantly ($P = 0.05$) improved emergence and fresh weights relative to the check, but tended to be 15–20% less effective

TABLE 5. List of representative fluorescent strains of *Pseudomonas* spp. isolated from potatoes and their antagonistic properties toward strain W3C37 of *Erwinia carotovora* subsp. *atroseptica*

Species	Strain	Biovar[a]	Siderophore group[b]	Antibiosis group[c]	Plant emergence[d] (%)	Fresh weight of whole plant[d] (g/pot)
P. fluorescens	W4F111	III	high	I	60.0	17.2
P. fluorescens	W4F166	III	high	I	86.7	20.3
P. putida	W4P396		high	I	70.0	16.9
P. fluorescens	W4F164	ND[e]	high	II	70.0	19.9
P. fluorescens	W4F188	V	high	II	73.3	18.3
P. putida	W4P5		high	III	86.7	22.4
P. fluorescens	W4F35	III	high	III	86.7	20.6
P. putida	W4P144		high	III	63.4	15.4
P. fluorescens	W4F151	III	high	III	80.0	22.7
P. fluorescens	W4F156	III	high	III	50.0	9.9
P. fluorescens	W4F131	ND	high	IV	80.0	20.8
P. putida	W4P31		medium	I	63.4	12.7
P. fluorescens	W4F68	III	medium	I	66.7	14.5
P. putida	W4P312		medium	I	73.3	24.0
P. fluorescens	W4F586	III	medium	I	30.0	1.5
P. putida	W4P143		medium	II	53.3	16.7
P. putida	W4P174		medium	II	73.4	23.2
P. putida	W4P63		medium	III	60.0	13.9
P. putida	W4P64		medium	III	80.0	21.4
P. fluorescens	W4F382	III	medium	III	70.0	11.4
P. fluorescens	W4F509	III	medium	III	83.3	25.1
P. fluorescens	W4F560	ND	medium	III	70.0	22.8
P. fluorescens	W4F8	III	low	I	80.0	18.3
P. fluorescens	W4F16	III	low	I	33.3	4.9
P. fluorescens	W4F58	III	low	I	86.7	15.6
P. fluorescens	W4F159	III	low	II	63.4	15.3
P. putida	W4P11		low	III	63.3	18.4
P. fluorescens	W4F15	III	low	III	50.0	12.8
P. fluorescens	W4F548	III	low	III	83.3	21.0
P. fluorescens -putida	B10		medium	I	73.3	19.2
P. fluorescens	Pf-5	ND	high	III	80.0	8.0
Check[f]					23.3	3.6
Uninoculated[f]					100.0	32.6
LSD[g] 0.10					38.5	5.9
LSD[g] 0.05					46.3	7.1

[a] Strains of *P. fluorescens* were identified to biovar based on the GLDETS method of Sands and Rovira (27) (i.e., gelatin hydrolysis, levan production, denitrification, ethanol utilization, trehalose utilization, and sorbitol utilization).
[b] Strains of *Pseudomonas* that produce siderophores with high ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) (EDDA) $\geq$ 1,000 μg/ml), medium (EDDA $>$250–$<$1,000 μg/ml), and low (EDDA $\leq$250 μg/ml) affinities for iron.
[c] Inhibition of strain W3C37 of *E. c.* subsp. *atroseptica* on: only King's medium B (KB) agar (group I strains), only potato-dextrose agar (PDA) (group II strains), both KB agar and PDA (group III strains), and neither KB agar nor PDA (group IV strains).
[d] Standard preemergence seed piece decay assays of Russet Burbank potatoes. Fluorescent pseudomonads and *E. carotovora* were added at 10^9 and 10^6 cfu per seed piece, respectively. All treatments were planted into fumigated soil. Each strain was replicated ten times, three seed pieces per pot. Emergence and weights were recorded 2 wk after planting.
[e] Strains could not be differentiated as to biovar.
[f] Check seed pieces were inoculated only with *E. c.* subsp. *atroseptica*. Uninoculated seed pieces were treated with methylcellulose-water only.
[g] Protected least significant difference, LSD.

in improving emergence than strains that produced inhibitory siderophores and/or antibiotics. However, some strains in antibiosis group IV, such as W4F131 (Table 5), produced siderophores that did not inhibit growth of strain W3C37 of *E. c.* subsp. *atroseptica* on KB agar despite high affinities for iron.

Suppression of preemergence potato seed piece decay caused by *E. c.* subsp. *atroseptica* or *E. c.* subsp. *carotovora*. Seven strains that suppressed preemergence seed piece decay caused by strain W3C37 of *E. c.* subsp. *atroseptica* were tested against strain W3C105 of *E. c.* subsp. *carotovora* (Fig. 3). Plant emergence was from 16.7% to 53.4% greater for seed pieces treated with the pseudomonads and strain W3C37 of *E. c.* subsp. *atroseptica* compared with those inoculated with *E. c.* subsp. *atroseptica* only. The level of protection produced by all strains except strain W4F156 was significant ($P = 0.05$). However, the same pseudomonad strains were not as effective in suppressing preemergence seed piece decay caused by strain W3C105 of *E. c.* subsp. *carotovora*; six pseudomonad strains increased emergence between only 3.3 and 33.3% compared to the untreated check. While seed pieces treated with strain W4F156 had lower emergence (23.4%) than check seed pieces, only strains W4F68, W4P5, W4F35, and W4P144 increased emergence significantly ($P = 0.05$). Depending on the strain of *Pseudomonas*, 0 to 43.3% less control of preemergence seed piece decay occurred when tubers were

Fig. 2. Effect of the cell concentration of strain W4P63 of *Pseudomonas putida* on preemergence seed piece decay (as measured by plant emergence) caused in Russet Burbank potato by strain W3C37 of *Erwinia carotovora* subsp. *atroseptica*. Treated seed pieces were planted, three seed pieces per pot replicated five times, in fumigated soil. Results shown are based on data collected 2 wk after planting. Treatments A to E were treated with 10^6 cfu of strain W3C37 of *E. c.* subsp. *atroseptica* per seed piece. Cell concentrations of strain W4P63 of *P. putida* per seed piece were **A,** 0 (*E. c.* subsp. *atroseptica* only); **B,** 10^4 cfu; **C,** 10^6 cfu; **D,** 10^8 cfu; **E,** 10^{10} cfu; and **F,** 0.

TABLE 6. Effect of fluorescent pseudomonads that produce siderophores with high, medium, or low affinities for iron on preemergence seed piece decay (as measured by plant emergence) of Russet Burbank potato caused by strain W3C37 of *Erwinia carotovora* subsp. *atroseptica*

Iron affinities[a]	Emergence[b] (%)	Fresh weight (g/pot)[b]	
		Whole plant	Roots
High[c]	75.2	11.5	2.1
Medium[c]	64.9	9.4	1.8
Low[c]	66.1	9.9	1.8
Check[d]	20.0	1.4	0.2
Uninoculated[d]	93.4	17.3	4.1
Linear MS[e]	9,469.6**	452.4**	33.3**

[a] Strains of *Pseudomonas* spp. that produce siderophores with high ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) (EDDA $\geq$1,000 μg/ml), medium (EDDA $>$250–$<$1,000 μg/ml), and low (EDDA $\leq$250 μg/ml) affinities for iron. Cell concentrations per seed piece were 10^9 cfu for the pseudomonads and 10^6 cfu for *E. c.* subsp. *carotovora*. All treatments were planted into fumigated soil. The check was treated only with *E. c.* subsp. *atroseptica*.

[b] Each strain was replicated five times, three seed pieces per pot. Emergence and weights were recorded 2 wk after planting.

[c] Means of 11 strains; all four antibiosis groups (Table 7) are represented.

[d] Check seed pieces were inoculated only with *E. c.* subsp. *atroseptica*. Uninoculated seed pieces were treated with methylcellulose-water only.

[e] Asterisks (**) indicate significance of the experiment at $P = 0.01$ for a linear model based on coefficients of orthogonal polynomials.

TABLE 7. Effect of fluorescent pseudomonads of different antibiosis groups on preemergence seed piece decay (as measured by plant emergence) of Russet Burbank potato caused by strain W3C37 of *Erwinia carotovora* subsp. *atroseptica*

Antibiosis group[a]	Emergence[b] (%)	Fresh weight (g/pot)	
		Whole plant	Roots
I[c]	69.3	11.0	2.1
II[c]	66.7	9.9	1.9
III[c]	70.7	12.1	2.3
IV[c]	52.0	9.5	1.8
Check[d]	20.0	1.4	0.2
Uninoculated[d]	93.4	17.3	4.1
LSD[e] 0.10	24.2	5.0	1.2
LSD[e] 0.05	28.8	5.9	1.4

[a] Antagonistic to strain W3C37 of *E. c.* subsp. *atroseptica* on: only King's medium B (KB) agar (group I strains), only potato-dextrose agar (PDA) (group II strains), both KB agar and PDA (group III strains), and neither KB agar nor PDA (group IV strains). Cell concentrations per seed piece were 10^9 cfu for the pseudomonads and 10^6 cfu for *E. carotovora*. All seed pieces were planted into fumigated soil. Check seed pieces were inoculated only with *E. c.* subsp. *atroseptica*.

[b] Each strain was replicated five times, three seed pieces per pot. Emergence and weights were recorded 2 wk after planting.

[c] Means of five strains.

[d] Check seed pieces were inoculated only with *E. c.* subsp. *atroseptica*. Uninoculated seed pieces were treated with methylcellulose-water only.

[e] Protected least significant difference, LSD.

inoculated with strain W3C105 of *E. c.* subsp. *carotovora* as when inoculated with strain W3C37 of *E. c.* subsp. *atroseptica*. Treatments with fluorescent pseudomonads increased the average plant weight 1.3 times more for seed pieces inoculated with strain W3C37 of *E. c.* subsp. *atroseptica* compared to seed pieces inoculated with strain W3C105 of *E. c.* subsp. *carotovora*. Furthermore, the average plant weight produced by seed pieces treated with the pseudomonads and strain W3C37 of *E. c.* subsp. *atroseptica* was 5.2 times greater than that produced by seed pieces treated with strain W3C37 alone.

Species and biovars of fluorescent pseudomonads. A collection (Table 5) of representative fluorescent pseudomonads isolated from Columbia Basin potatoes were identified as either *P. putida* (10 strains) or *P. fluorescens* (19 strains). The majority of the strains of *P. fluorescens* were subdivided into biovar III (15 strains); the remainder were either biovar V (one strain) or could not be clearly differentiated as to biovar (three strains). Within each species and biovar, diversity in siderophore and antibiotic group was evident; although nearly half of the strains of *P. putida* produced siderophores with high affinities for iron.

DISCUSSION

The procedure developed for screening fluorescent pseudomonads for biological control of potato soft rot is based on antagonism of *E. carotovora* by siderophore and/or antibiotic production and suppression of seed piece decay (expressed as improved plant emergence) in the greenhouse. This screening method is rapid and should prove reliable for identifying the set of fluorescent pseudomonad strains that naturally thrive in the potato root zone and have the greatest potential for controlling potato soft rot diseases in the field. Several strains significantly reduced seed piece decay caused by strain W3C37 of *E. c.* subsp. *atroseptica*, as indicated by improved emergence (up to 63%) and increased plant growth (up to six- or seven-fold) of treated potatoes compared to the checks inoculated with *Erwinia*. However, not all fluorescent pseudomonad strains were effective, which reinforces the value of the greenhouse assay as a means to eliminate ineffective strains. Strain W4F586, in fact, caused a reduction in plant weights relative to the check.

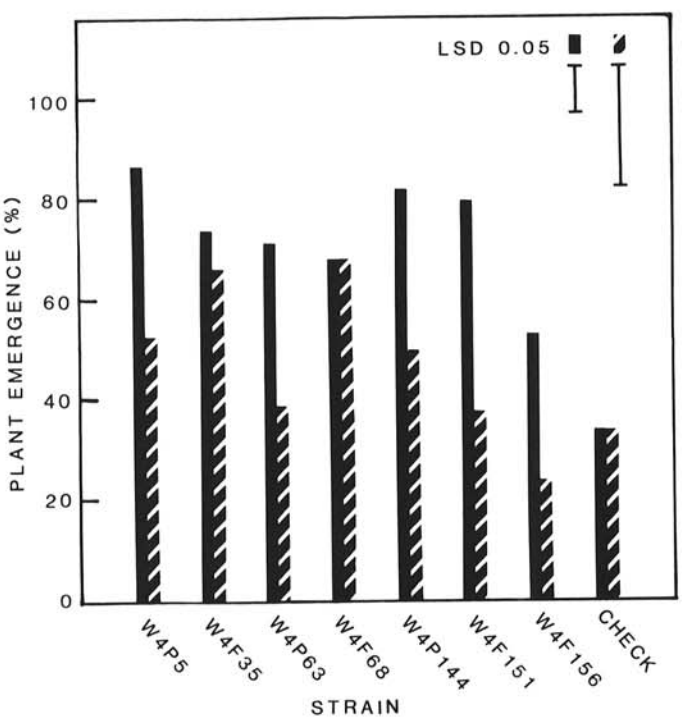

Fig. 3. Effects of antagonistic fluorescent pseudomonads on preemergence seed piece decay (measured as percent emergence) caused by strain W3C37 of *Erwinia carotovora* subsp. *atroseptica* (solid bars) or strain W3C105 of *E. c.* subsp. *carotovora* (diagonally shaded bars). Data are means of five replications (three seed pieces per pot) 2 wk after planting. Strains of *Pseudomonas* were added at 10^9 cfu per seed piece and those of *Erwinia* at 10^6 cfu per seed piece. Check seed pieces were treated with *E. carotovora* only.

The greenhouse screening procedure was based on a balance between the *E. carotovora* and pseudomonad populations that could clearly identify the pseudomonad strains that were effective in biocontrol. *E. carotovora* at a concentration of 10^6 cfu per seed piece generally reduced emergence by about 80%; highly effective pseudomonads improved emergence to over 80% with the emerged plants exhibiting vigorous growth. Dosage with *Pseudomonas* at least 10^6 cfu per seed piece was necessary to achieve significant reductions in seed piece decay. These results are encouraging because populations of indigenous fluorescent pseudomonads on potato roots can exceed 10^6 cfu/g (2,8). The environmental conditions of the greenhouse assay are favorable for both soft rot disease development and growth of the soil pseudomonads. *E. c.* subsp. *atroseptica* is favored by relatively cool and moist soil conditions (24). Even when *E. c.* subsp. *carotovora*, which prefers a warmer temperature range for growth and pathogenesis (24), is used as the pathogen, plant emergence is still suppressed by approximately 80% under the standard greenhouse screening conditions.

The level of disease control by the pseudomonads was less when strain W3C105 of *E. c.* subsp. *carotovora* was the pathogen than when strain W3C37 of *E. c.* subsp. *atroseptica* was used. Indeed, many pseudomonads which gave significant control of seed piece decay caused by strain W3C37 (e.g., W4P63, W4F151, W4F156) were ineffective against strain W3C105. Of the strains tested (Fig. 3), only strain W4F68 of *P. fluorescens* provided equivalent suppression of seed piece decay when either strain W3C37 or W3C105 was used. Thus, not all strains of *E. carotovora* were suppressed to the same extent.

The pseudomonads used in this study were targeted only for control of *E. carotovora* on potato. However, the growth increases are consistent with those reported for potatoes and sugar beets treated with PGPR (32,34), where growth promotion results from suppression of a group of lesser-known pathogens and not a single pathogen. For example, Kloepper et al (19) reported that in greenhouse assays strain B10 improved potato growth 2.3 times over that of water checks. Greater or lesser increases in plant growth were obtained for strain B10 depending on the type of soil used in the assay (21). In the present study, strain B10 improved plant emergence by 50% and increased plant weights approximately five-fold. Interestingly, strain Pf-5 of *P. fluorescens* improved emergence of potato by 57% but did not increase plant weights; although relatively free of soft rot, plants grown from seed pieces treated with strain Pf-5 were stunted and exhibited interveinal leaf chlorosis. The reason for these effects are unknown, but they may have resulted from the production of a phytotoxic antibiotic (15).

Screening fluorescent pseudomonads for antagonism of *Erwinia* on both KB agar and PDA resulted in the selection of strains that produce a diverse array of inhibitory compounds. Antibiotic production occurred in 56% of the fluorescent pseudomonads tested on PDA, an iron-rich medium low in phosphate (12). Secondary metabolism is necessary for antibiotic production in *Pseudomonas* and this process requires iron concentrations of at least 2 μM for maximum yield (12). PDA has the additional advantage of being low in inorganic phosphate, a nutrient that strongly suppresses secondary metabolism in *Pseudomonas* at concentrations greater than 1 mM. Inorganic phosphate apparently suppresses the synthesis of alkaline and acid phosphatases required for formation of secondary metabolites (39). Thus, PDA provides a favorable nutritional environment for secondary metabolism and antibiotic production. In contrast, iron levels of this magnitude are inhibitory to fluorescent pigment production (35). This is consistent with our observations that none of the potato pseudomonads produced fluorescent siderophores on PDA. Siderophore production, being repressed at high concentrations of iron, readily occurred for all strains on KB agar.

Although all of the pseudomonads produced a fluorescent siderophore on this medium, about 20% of these strains did not produce a siderophore inhibitory to the growth of *E. c.* subsp. *atroseptica*. Similarly, Geels and Schippers (10) reported that 70% of the fluorescent pseudomonads tested were antagonistic to *E. c.* subsp. *atroseptica* on KB agar. Only a few of our strains produced inhibition zones on KB agar that were bactericidal and that were not reduced by additions of iron. Strains such as Pf-5 apparently produce antibiotics that are not highly regulated by iron. Because relatively small quantities of iron are available in the root environment, strains that produce both inhibitory siderophores and bactericidal antibiotics may have a greater potential for suppressing pathogens.

The fluorescent pseudomonads from potato produced fluorescent siderophores with wide variations in iron-binding affinities. Of 293 strains tested, 17% of these strains produced siderophores with high affinities for iron (i.e., $\geqslant$1,000 μg EDDA/ml). Few fluorescent pseudomonad strains have been reported to grow at such high levels of EDDA. Kloepper et al (19) suggested that growth-promoting strains of *Pseudomonas* spp., (e.g., strain B10 which produces pseudobactin) inhibited growth of *E. carotovora* perhaps because they produced fluorescent siderophores with higher iron affinities than those of *E. carotovora*, thereby resulting in iron deprivation in the pathogen. Our results indicate that pseudobactin is intermediate in its ability to reverse iron deprivation as compared to siderophores produced by some pseudomonads isolated from potatoes in the Columbia Basin. Vandenbergh et al (36) furthermore reported that PGPR strain B10 grew in the presence of 620 μg EDDA/ml, an EDDA concentration considerably lower than that tolerated by a strain of *P. putida* suppressive to Fusarium wilt of tomato. However, the iron-binding properties of pseudobactin appear sufficiently high to be inhibitory to *E. carotovora* because strain W3C37 of *E. c.* subsp. *atroseptica* was unable to grow under mild iron deficient conditions (media supplemented with 100 μg EDDA/ml).

Our results also show that the inhibition of *E. carotovora* on KB agar by strains such as W4P63 resulted from production of a siderophore. Siderophore production was repressed by addition of 10 μM or more iron and the siderophore had an absorption maximum of approximately 400 nm, which is typical for the pyoverdine class of siderophores produced by *Pseudomonas* (22). Although most, but not all, fluorescent siderophores produced on KB agar were bacteriostatic to growth of *E. carotovora*, this inhibition could not be directly related to its effectiveness as a biocontrol agent. Nevertheless, strains that produced siderophores with high affinities for iron (Table 6) tended, as a group, to be somewhat more effective in reducing seed piece decay. Many strains in the low and medium iron affinity groups, however, gave good control of *E. c.* subsp. *atroseptica* (Table 6). Consequently, most of the fluorescent siderophores may have a sufficiently high iron-binding affinity to successfully suppress growth of *E. carotovora* on potato.

The pseudomonads isolated from potatoes grown in the Columbia Basin were identified as either *P. fluorescens* or *P. putida* and exhibited a large diversity of characteristics. Of the 29 strains identified, 19 were *P. fluorescens* and 15 of these strains were biovar III; only one strain was identified as biovar V (Table 5). Cuppels and Kelman (7), in a survey of pectolytic pseudomonads isolated from Wisconsin-grown potatoes, identified 87% of the pseudomonads as *P. fluorescens* biovar II [*P. marginalis* (Brown) Stevens]. The remaining 13% were classified as *P. fluorescens* but could not be further identified to biovar, although they most closely resembled biovar II strains. Pectolytic pseudomonads commonly occurred in high populations on potatoes grown in the Columbia Basin. They were not considered as candidates for use in biocontrol because of a general lack of antagonism of *E. carotovora*. Furthermore, none of the beneficial strains were classified as biovar II. The dominance of biovar III of *P. fluorescens* on potato roots is in sharp contrast with wheat rhizospheres where biovar V is reported to be the dominant fluorescent pseudomonad (27). Despite the diverse nature of fluorescent pseudomonads in the potato rhizosphere, the specific composition of the pseudomonad population may differ distinctly from other crops such as wheat, a common rotation crop in the Columbia Basin.

Diversity in the isolated pseudomonads was further apparent in antibiotic production and in the production of siderophores with wide ranges in affinities for iron. Because the pseudomonads are the major group of nondifferentiating microorganisms that produce antibiotics (22), the mechanistic basis of antagonism of *E. carotovora* and control of seed piece decay may be highly varied. The challenge in establishing effective biocontrol is to select strains that utilize different mechanisms to control diseases caused by *E. carotovora*, to understand specific interactions between pseudomonads and *E. carotovora*, and to combine complementary factors that will have an additive effect in suppressing growth and/or viability of *E. carotovora*. This may be accomplished by treating potatoes with two or more strains that are nonantagonistic to one another, or genetically modifying a particular strain that would then be able to antagonize *E. carotovora* on the basis of more than one mechanism.

The success of strain Pf-5 of *P. fluorescens* as a biocontrol agent on cotton appears to result from its ability to produce pyrrolnitrin (14), pyoluteorin (15) and a fluorescent siderophore with a very high affinity for iron (Table 5). Although strain Pf-5 is highly antagonistic to *E. carotovora* in vitro and controls potato seed piece decay in the greenhouse, it causes stunting and intervienal chlorosis. Thus, careful methods must be employed to select pseudomonads that can colonize roots to high populations and readily produce those metabolites that suppress the target pathogen without deleteriously affecting the plant.

Few systematic procedures have been developed for selecting fluorescent pseudomonads for biological control. This is the first detailed description of a method for selecting agents specifically for control of potato soft rot diseases caused by *E. carotovora*. Candidate pseudomonads then can be further screened for antagonism and control of other soilborne potato pathogens, such as *Verticillium dahliae* Kleb. Such strategies may result in selection of fluorescent pseudomonads that are antagonistic to several pathogens. Beyond initial selection, however, the pseudomonads need to be carefully evaluated in the field for survival, suppression of the pathogen, and adverse effects on potato yield and quality. Field evaluations of representative strains are summarized (43). Greenhouse evaluations are indicative of a high degree of biological control of *E. carotovora* by fluorescent pseudomonads; consequently, an important achievement would be the successful adaptation of these bacteria to commercial potato production.

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