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Increased emergence of spring wheat after inoculation with *Pseudomonas chlororaphis* isolate 2E3 under field and laboratory conditions

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Abstract Inoculation at the time of planting with *Pseudomonas chlororaphis* strain 2E3 increased the emergence of spring wheat by 8% and 6% at two different sites in northern Utah. Isolate 2E3 strongly inhibited the growth of the wheat pathogen *Fusarium culmorum* on artificial media. A second isolate of *P. chlororaphis* (strain O6) also inhibited fungal growth on artifical media but did not increase emergence of the spring wheat at the same field sites. Inoculation of winter wheat by 2E3 did not promote emergence when planted into field soil sterilized by fumigation with methyl bromide. Under laboratory conditions, emergence of spring wheat in sterilized soils from both sites was at least 90%. In the soils that were not sterilized, emergence was below 25% in soil from one site and below 50% in soil from the other. Treating seeds with 2E3 significantly improved emergence in a sterile soil-containing matrix that had been inoculated with the wheat pathogen *Fusarium culmorum*. Consequently, we propose that increases in wheat emergence can be attributed to the suppression by 2E3 of pathogenic organisms present in the native field soils. A strain of *Rhizoctonia solani*, shown to the pathogenic on winter wheat, was isolated from one of these soils.

Key words Plant growth-promoting rhizobacteria · *Pseudomonas* spp. · Wheat · *Rhizoctonia solani*

Introduction

Both spring and winter wheat are grown under dryland conditions in northern Utah. Since very little precipitation is received during the growing season, success often depends on early emergence and establishment to utilize accumulated soil moisture. In this paper, we examine whether treatment of wheat seeds at the time of planting with potential biocontrol-active pseudomonads can enhance emergence.

The plant growth-promoting effects of certain bacteria have been well established (Kloepper et al. 1980a, b; Brisbane et al. 1989; DeFreitas and Germida 1990; DeFreitas and Germida 1992; Klumar and Dube 1992). Bacterial seed treatments increase the emergence and growth of plants in a number of agricultural systems under greenhouse and field conditions (Kloepper 1983; Lifshitz et al. 1987; Callan et al. 1991; Chanway et al. 1991; DeFreitas and Germida 1992a, b; Gagné et al. 1993). Winter wheat responds positively to treatment with *Pseudomonas cepacia* and *P. putida* in the field (DeFreitas and Germida 1992b). Biocontrol of take-all of wheat under field conditions has also been demonstrated for several fluorescent pseudomonads (Weller and Cook 1983; Sarniquet and Lucas 1992).

This promotion of plant growth by bacterial colonization is attributed to a number of factors. Some bacterial strains produce indole acetic acid, which stimulates plant growth (Dubiekovsky et al. 1993; Loper and Schroth 1986). Other rhizosphere-inhabiting bacteria increase plant growth by solubilization of phosphorus (Goldstein 1986). Plant growth is also stimulated by the inhibition of deleterious root microbes through the production of siderophores or antibiotics (Kloepper et al. 1980a, b; Kloepper and Schroth 1981; Leong 1986; Elad et al. 1987).

In the present work, two bacterial strains, which inhibited the growth on artificial medium of *Fusarium culmorum*, a wheat root pathogen, were tested in the field for the ability to improve emergence of spring wheat in northern Utah. These isolates were selected because they have be ability to produce phenazine antibiotics. Fluorescent pseudomonads that produce phenazines have been previously shown to be effective for biocontrol of the take-all fungus of wheat (Thomashow and Weller 1988).

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Materials and methods

Microbial strains

Pseudomonas chlororaphis strains 2E3 and O6 were isolated by dilution plating form soils collected in northern Utah. Numerous bacteria were initially selected at random from soil extracts plated onto potato dextrose agar (PDA) (Difco, Detroit, MI). Single colonies of these strains were obtained by streaking isolates onto PDA plates and subculturing onto fresh medium. The bacterial strains used in these studies were chosen based on a test for antagonism to *F. culmorum* done as follows: PDA plates were inoculated with 0.5 ml of a 24-h liquid culture of the bacterium and the inoculum was spread evenly across the surface of each plate. After 24 h growth at 25°C, the entire surface of each plate was covered by confluent colonies. A 6-cm-diameter piece of sterilized glass tubing was used to imprint a ring of bacterial inoculum from the confluent plates of each strain onto three replicate plates of PDA. A 5-mm-diameter plug of inoculum from the margin of a 3-day culture of *F. culmorum* growing on PDA at 25°C was simultaneously placed at the center of the ring. Control plates lacking the bacterial ring were also prepared. Diameters of the *Fusarium* colonies were measured after 3 days.

The identities of the isolates were determined by the Bacterial Strain Identification and Mutant Analysis Service at Auburn University using fatty acid analysis. The cultures were stored at –80°C in nutrient broth (Difco, Detroit, MI) containing 15% (v/v) glycerol. The isolate of *F. culomorum* used in this work was obtained from Dr. D. Mathre of the Department of Plant Pathology at Montana State University.

Pigments from *P. chlororaphis* strains 2E3 and O6 were extracted from filtrates of 2-day cultures in potato dextrose broth (PDB) (Difco, Detroit, MI) following the methods described by Radtke et al. (1994). In brief, the filtrates were acidified to pH 2 using hydrochloric acid and extracted with an equal volume of benzene. Benzene was removed by evaporation at 40°C. The concentrated extract subjected to thin-layer chromatography (TLC) on 250-µm-thick Rediplate Silica Gel-G (Fisher Scientific, Pittsburgh, PA) and developed in glacial acetic acid and benzene (5:95). Yellow and orange pigments were extracted with chloroform and absorbance spectra were measured on a spectrophotometer. Extracts prepared similarly from *P. fluorescence* 2–79 (a gift from Dr. D. Weller, Washington State University) and *P. aureofaciens* 30–84 (a gift from Dr. L.S. Pierson, University of Arizona) were used as standards.

Bacterization of seed

Bacterial wheat seed inoculum of *P. chlororaphis* strain 2E3 or O6 was prepared by inoculating 125 ml PDB with a single loopful of bacteria from a 48-h culture on a plate of PDA. The culture was grown for 48 h at 25°C in an orbital shaker operating at 200 rpm. After growth, the culture was amended with sterilized 1% carboxymethylcellulose (CMC). Wheat seed was surface sterilized by immersion in 3% sodium hypochlorite (v/v) for 5 min. The seed was rinsed in eight changes (500 ml each) of sterile distilled water to remove the sodium hypochlorite. Surface-sterilized seeds were immersed for 30 min in the bacterial suspension. The treated seed were air dried overnight at room temperature inside a laminar flow hood before use.

The bacterial populations adhering to the treated seed were determined by shaking ten treated seeds in 1 ml sterile distilled water at 200 rpm for 30 min followed by dilution plating onto PDA. The seeds carried a population of between 10^7 and 10^8 colony-forming units (CFU) per seed.

Control seeds were treated as described above using 1% CMC but with no added bacteria. No bacteria were present upon dilution plating of washes from these seeds onto PDA.

Field studies

Field studies were carried out at two experimental farms operated by the Utah Agricultural Experiment Station in northern Utah, the Greenville Experimental Farm located at Logan, Utah, and the Blue Creek Experimental Farm about 30 miles northwest of Logan, Utah. The studies were designed to determine whether the emergence of wheat treated with bacterial isolates 2E3 and O6 differed from that of untreated seed. The wheat cultivar Fremont was planted in May because it is a spring wheat cultivar commonly grown in northern Utah. In September, the winter wheat cultivar Manning was used to enable the overwintering potential of the pseudomonads to be examined. Viability tests for the untreated seed showed 97% seed germination on moist filter paper under laboratory conditions. Seed was treated with bacterial strains 2E3 and O6 as indicated above for use in field experiments.

Two field plots, one at each farm, were planted in mid-May 1994 using a randomized block design with six replicate blocks containing both the untreated and treated wheat seed. Each treatment consisted of one row, 1.8 m long, into which 100 seeds were planted at a depth of approximately 4 cm. The bacterial populations were supplemented by simultaneously adding 75 ml milled corn cobs (which had been sterilized by autoclaving at 121°C for 1 h on two consecutive days and treated with a 48-h culture of isolates 2E3 or O6) into the soil of each row. An equal amount of untreated sterile granules was added to each control row. Because of drought conditions at planting, each row was watered by hand with 3.8 l water 48 h prior to planting and again on the day of planting.

A second field plot was established using a modification of the design described above in fumigated soil during September 1994 at the Greenville farm. Soil was fumigated by applying methyl bromide under a plastic tarp at a rate of 0.083 kg m^{-2}. The experiment was a randomized block design with six replicate blocks containing the treated and untreated seed. One hundred seeds of winter wheat cultivar Manning were planted in each of two 1.2-m rows per treatment at a depth of about 4 cm. One of these rows was used to determine emergence and the other was used for removing roots to sample bacterial survival. The roots of plants inoculated with 06 and 2E3 were examined for colonization by these strains after overwintering in April 1995. Roots were carefully dug from the soil and segments immersed into 10 ml sterile H_2O. The sample was vigorously agitated and the suspension dilution plated onto PDA. Colonies that were green or orange pigmented were scored as being 2E3 or 06-like.

Percentage emergence for both experiments was determined by counting the number of plants in each row 5 weeks after planting. The data from each plot were statistically analyzed using standard ANOVA techniques.

Seed emergence in sterile and nonsterile soil

One explanation for the improvement in emergence of wheat seed treated with bacteria is that the bacteria restrict the deleterious effects of minor soil pathogens. To evaluate this possibility, soil samples from the experimental plots at each farm were collected in May and stored at 4°C. Half of the soil samples from each farm was sterilized by autoclaving for 1 h at 121°C at 15 psi. To avoid compaction, the sterile and nonsterile soil samples were mixed in a 1:4 ratio by volume with a 1:1:1 mixture by volume of sterilized sand, peat and vermiculite. Sterile oats (10% v/v) were added as an organic amendment simulating the corn cob granules in the field. Ten surface-sterilized Fremont spring wheat seeds were planted in each of six replicate 6.3-cm^2 pots containing mix with sterilized or nonsterilized soil from each experimental farm. The pots were incubated in a growth room for 11 days at 25°C under a 12-h photoperiod and the number of plants that emerged from each treatment was counted.

The seeds that did not germinate from the pots containing nonsterile soil from the Greenville farm site were plated directly onto PDA to determine whether they were infested with microorganisms. An isolate of *Rhizoctonia* was obtained from ungerminated wheat seed in pots containing nonsterile soil from the Greenville farm site. This isolate was identified as *R. solani* because of its multinucleate, buff mycelia having undifferentiated sclerotia and hyphae larger than 7 µm wide. To determine whether it was pathogenic, wheat seeds were surface sterilized twice by treatment with 10.0% sodium hypochlorite plus 0.01% Tween 20 for 30 min and washed with 10 volumes of sterile distilled water. Seeds were immersed for 30 min in a suspen-

sion of *Rhizoctonia* mycelia in 1% CMC. Mycelia were removed from the surface of a 7-day PDA culture by scraping in 10 ml sterile distilled water using a sterile spatula and the suspension diluted to 20 ml using sterile water. Infested seed along with uninoculated controls were planted separately into magenta boxes containing 350 ml sterile vermiculite and 150 ml sterile water. Three boxes were planted for each treatment with five seeds in each box. The plants were grown at 26°C for 10 days when the number of diseased plants were scored.

Characterization of soils

The soil at Greenville is classified as Millville series, coarse-silty, carbonatic, mesic Typic Haploxeroll. Maize was grown in the experimental plot at Greenville Farm for at least 2 years prior to our work. The Blue Creek soil is in the Timpanogos series as a fine-loamy, mixed, mesic Calcic Argixeroll. The soil in this plot had lain fallow for at least 2 years before these studies were carried out.

Three soil samples were taken at a depth of approximately 5 cm from the experimental plots at both sites for characterization. The samples were kept at 4°C until analyzed. The chemical and physical properties of the samples were determined by the Utah State University Soil Testing Laboratory. To characterize the microbial properties of each soil, 1 g soil from each sample was added to 10 ml sterile distilled water. After being vigorously shaken, a 1:1000 dilution was made from each and 0.25 ml of the dilution was plated onto ten replicate petri plates of PDA to determine total bacterial populations, PDA plus 60 μm ml^{-1} rifampin to determine fungal populations and King's medium B (Atlas 1993) to determine the total number of fluorescent pseudomonads. Rhizosphere populations were determined by planting noninoculated, sterilized Fremont wheat seeds into ten 6 cm pots for each soil sample from both sites. Each pot contained 100 ml of a 50/50 (v/v) mixture of nonsterile soil and sterile sand. The plants were grown for 3 weeks under a 12-h photoperiod at room temperature. Five healthy plants from each soil sample were selected and shaken to remove loose soil from the roots. The entire root system of each sampled plant was submerged in 10 ml sterile water in a 15-ml test tube and shaken for 15 min at 200 rpm using a mechanical shaker. Bacterial populations were determined from the resulting suspension by serial dilution using the media described above and the roots were dried to allow determination of bacterial populations on a per gram (root dry weight) basis. Between 100 and 200 randomly selected bacterial strains cultured from the soil and rhizosphere populations from each site were tested for growth inhibition of *F. culmorum* on PDA.

Laboratory assays using *F. culmorum*

To determine whether *P. chlororaphis* 2E3 has the potential to counter the deleterious effects of fungal pathogens, the emergence of seed treated with *P. chlororaphis* strain 2E3 was compared with the emergence of untreated seed in potting mix containing *F. culmorum*.

Inoculum of *F. culmorum* was grown in 1-Erhlenmeyer flasks containing a mixture of 250 ml oats and 150 ml water that had been autoclaved at 121°C for 1 h. Each flask was inoculated with five 5-mm-diameter agar plugs from the margin of a 3-day colony of *F. culmorum* growing on PDA. The inoculated oats were incubated at room temperature for 14 days before use.

Seed treated with 2E3 and the controls were planted in 6-cm^2 plastic pots each containing 100 ml potting mix (1:1:1:1 peat:sand:vermiculite:soil by volume) which had been sterilized previously by autoclaving for 1 h. Inoculation with *F. culmorum* was done by mixing the oat inoculum (5%) into the potting mix. Sterile oats (5%) were added to the control treatment lacking *F. culmorum*. Nine seeds were planted in each pot and lightly covered with sterile sand. The plants were grown in a growth room held at room temperature with a 12-h photoperiod and watered as needed using 20 ml sterile distilled water per pot. Each treatment was replicated 4 times and arranged in a completely randomized design. Data were gathered on precentage seedling emergence at the end of a 14-day growth period.

Results

The physical characteristics of the soils from Blue Creek and Greenville farms were quite similar (Table 1). The chemical characteristics of the soils differed primarily in pH; the Blue Creek soil was more acidic and had a more silty texture than the soil at Greenville. The two soils were also very similar in biological characteristics (Table 2). Samples from both soils yielded between 0.9 and 1.4×10^5 CFU total bacteria g^{-1} soil. The numbers of microfungi cultured from each of the soils were similar: 0.13×10^5 CFU g^{-1} soil in the Greenville samples and 0.1×10^5 CFU g^{-1} in the Blue Creek samples. Soil samples from the Greenville farm had a higher proportion of fluorescent pseudomonads to nonfluorescent organisms than those from Blue Creek.

Treatment of spring wheat seed with *P. chlororaphis* strain 2E3 significantly increased emergence in unfumigated soil at both experimental farms. Treatment with 2E3

Table 1 Comparison of physical characteristics of soils from the experimental plots (*CEC* cation exchange capacity measured as meq 100 g^{-1} soil; NO_3^-, K and P are measured as mg kg^{-1} soil extractable with $NaHCO_3$)

	Greenville Farm	Blue Creek Farm
Sand (%)	28	28
Silt (%)	45	51
Clay (%)	17	21
pH (H_2O)	7.5	6.5
CEC	11.1	19.7
Total carbon (%)	1.45	1.28
Total nitrogen (%)	0.13	0.13
NO_3^-	23.6	45.1
K	407.0	439.0
P	36.0	27.0

Table 2 Comparison of biological properties of soils from the experimental plots

	Greenville Farm		Blue Creek Farm	
	Soil	Rhizosphere	Soil	Rhizosphere
Total bacteria (CFU g^{-1} soil)	0.9×10^5	116×10^5	1.4×10^5	432×10^5
Fluorescent pseudomonads (CFU g^{-1} soil)	0.06×10^5	1.1×10^5	0.003×10^5	31.2×10^5
Microfungi (CFU g^{-1} soil)	0.13×10^5	1.9×10^5	0.1×10^5	0.51×10^5
Fungal antagonist (%)[a]	38	35	35	28

[a] Percentage of soil bacterial isolates inhibiting the growth of *F. culmorum* on PDA

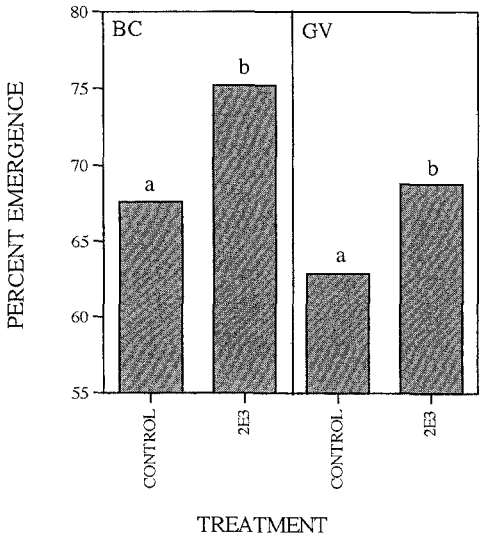

Fig. 1 Percentage emergence of Fremont wheat treated with *Pseudomonas chlororaphis* strain 2E3 and planted at two different field sites. Values with different letters are significantly different from one another ($P=0.05$). *GV* Greenville, *BC* Blue Creek

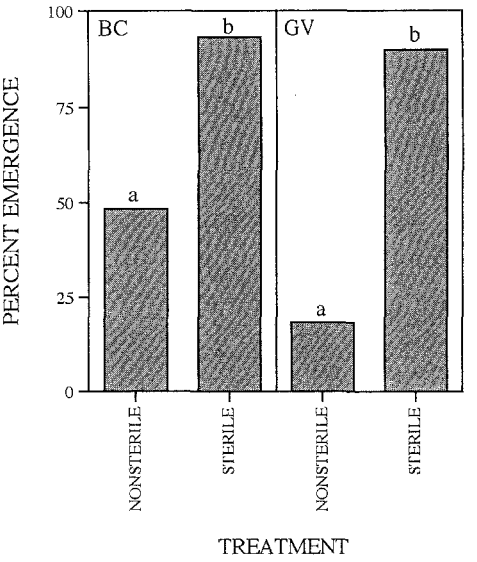

Fig. 3 Emergence of untreated Fremont wheat planted in pots containing sterile and nonsterile soil from two different field sites. Emergence values are based on ten seeds for each of six replicates. Values with different letters are significantly different from one another ($P=0.05$). *GV* Greenville, *BC* Blue Creek

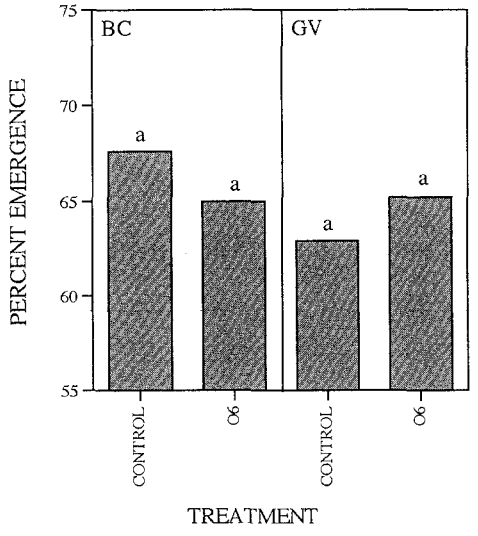

Fig. 2 Percentage emergence of Fremont wheat treated with *Pseudomonas chlororaphis* strain O6 and planted at two different sites. Values with the same letters are not significantly different from one another ($P=0.05$). *GV* Greenville, *BC* Blue Creek

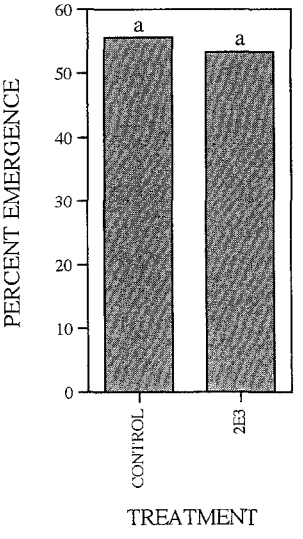

Fig. 4 Percentage emergence of Manning wheat treated with *Pseudomonas chlororaphis* strain 2E3 and planted into fumigated soil. Values with the same letters are not significantly different from one another ($P=0.05$)

increased the emergence at Blue Creek from 68% to 75% from 63% to 69% at Greenville 3 weeks after planting (Fig. 1). Emergence at the Greenville Farm was significantly lower than at the Blue Creek farm. Treatment with *P. chlororaphis* strain O6 did not significantly alter emergence at either site (Fig. 2).

Under laboratory conditions, the emergence of spring wheat planted in soil from both farms was greatly increased by sterilizing the soils. Only 18% of the seed planted into pots containing nonsterile Greenville soil emerged, while 48% emergence occurred for seed planted into nonsterile Blue Creek soil. When the soils were sterilized, emergence improved to 90% for the Greenville soil and 93% for the Blue Creek soil (Fig. 3). These differences indicate that a biological soil factor reduced emergence at both of these farms. Seed that failed to germinate in the nonsterile Greenville soil under laboratory conditions yielded cultures of *Fusarium* sp. and *Rhizoctonia solani*. When Manning wheat seed were inoculated with these isolates, the *Fusarium* strain did not cause disease. However, all wheat seedlings growing from seed inoculated with the *Rhizoctonia* strain were diseased, displaying brown lesions on the stems and roots.

Table 3 Emergence values under laboratory conditions for Manning and Fremont wheat treated with *Pseudomonas chlororaphis* strain 2E3 and inoculated with *Fusarium culmorum*. Emergence values and analysis of variance were based on nine seeds in each of four replicates; emergence is expressed as a percentage for clarity. Different letters indicate significantly different values at the 0.05 probability level

Treatment	Emergence	
	Fremont	Manning
Uninoculated	86a	78a
2E3	86a	78a
2E3+*Fusarium*	53b	48b
Fusarium	25c	23c

The studies indicate that isolate 2E3 increased wheat emergence by exerting biological control of fungal pathogens. When 2E3-treated seed was grown at Greenville farm after fumigation, emergence did not increase (Fig. 4). No fungal colonies grew on dilution plates from the methyl bromide-treated soil at the time of planting the winter wheat. Emergence of wheat also was not enhanced by treatment with 2E3 in an autoclaved growth matrix in the laboratory (Table 3), although under these conditions 2E3 suppressed disease caused by the pathogen *F. culmorum*. Survival of wheat in the presence of this pathogen was increased about twofold by the treatment with 2E3 (Table 3).

Isolate 2E3 increased emergence at the farm sites even though the field soils already possessed bacteria with antagonistic potential. Between 32% and 38% of the bacteria recovered from the soil at both field plots were antagonistic to the growth of *F. culmorum* when tested on PDA plates (Table 2). Similarly, between 28% and 35% of the bacteria recovered from the rhizospheres of wheat planted into soil from these sites in the laboratory were antagonistic towards *Fusarium* (Table 2). Only a very small percentage of the fluorescent pseudomonads from the Greenville and Blue Creek soils prior to planting had the green-pigmented phenotype of 2E3. No colonies with the pigmentation characteristic of strain O6 were detected. However, survival of both 2E3 and O6 was demonstrated on the surfaces of roots of the winter wheat planted in September 1994 and sampled in April 1995. Populations of 3.7×10^4 CFU 2E3 and 1.8×10^4 CFU O6 were recovered per gram root dry weight.

The extracted pigments from 2E3 grown in PDB produced a yellow band on TLC plates that migrated to the same position as phenazine-1-carboxylic acid extracted from *P. fluorescens* 2-79 (Table 4). Phenazine-1-carboxylic acid has been implicated in the biological control of take-all of wheat (Thomashow and Weller 1988). Strain O6 was at least as strongly antagonistic toward growth of *F. culmorum* on PDA plates as 2E3 (Table 5). This strain, when grown in PDB, produced two yellow-orange pigments that migrated on TLC plates to the same positions as the hydroxy-phenazines extracted from *P. aureofaciens* 30-84 (Table 4).

Table 4 Characterization of pigments isolated from *Pseudomonas chlororaphis* isolates 2E3 and O6 relative to pigments from known strains

Isolate	Color	Rf value	Absorbance maxima (nm)	
30–84	Light yellow	0.385	251.5	368.5
	Light yellow	0.352	251.5	369.5
O6	Yellow	0.381	252.0	367.5
	Yellow	0.350	251.5	365.0
2–79	Yellow	0.339	251.5	370.0
2E3	Yellow	0.340	251.5	370.0

Table 5 Radial growth of *Fusarium culmorum* confronted with the isolates of *Pseudomonas chlororaphis* on agar medium using a ring bioassay. Radial growth of colonies is expressed in centimeters. Different letters indicate significantly different values at the 0.05 probability level

Isolate	Diameter (cm)
Control	8.50a
2E3	4.36b
O6	3.78b

Discussion

Plant growth-promoting bacteria act through a number of potential mechanisms (Kloepper et al. 1980a, b; Goldstein 1986; Loper and Schroth 1986). The increased emergence of spring wheat that we observed under field conditions could have been due to one or a combination of several factors. However, we propose that the primary mechanism by which isolate 2E3 increased emergence in our studies was by exerting biological control activity of pathogens in the field.

Our comparison of the emergence of seed grown in potting mix containing nonsterile and sterile soils under laboratory conditions indicates that pathogenic organisms were present in the soil from both experimental farms. A strain of *R. solani* pathogenic on wheat was isolated from seed that had failed to emerge after being planted in the nonsterile field soil. The laboratory conditions under which this comparison of nonsterile and sterile soils was made apparently favored pathogenic activity because emergence of untreated seed in nonsterile mix under laboratory conditions was lower than that observed for untreated seed in the field. Enhanced pathogenesis may have been related to the well-watered status of the pots and more favorable temperatures for pathogen growth.

Our experiments demonstrate that pathogenic fungi needed to be present to increase emergence upon seed treatment with 2E3. Treatment of seed with 2E3 did not enhance emergence when seed was grown in a sterilized matrix in the laboratory or in soil at Greenville farm after fumigation with methyl bromide. No culturable fungi were isolated from these methyl bromide-treated soils, suggesting that potential soil-borne fungal pathogens had been eliminated.

We propose that the increase in emergence by 2E3 was by suppression of pathogenic organisms. Although a pathogenic strain of *R. solani* was present at the Greenville site, we cannot be certain that enhanced emergence was due to inhibition of this fungus alone. Other root pathogens may have been controlled by 2E3. Certain minor pathogens such as *Penicillium pinophilum* Hedgc., which have been shown to be suppressed by fluorescent pseudomonads (Gamliel and Katan 1993), may have affected emergence at our sites.

One of the major mechanisms by which bacteria exert biological control of plant pathogens is antibiotic production (O'Sullivan and O'Gara 1992). Strain 2E3 produces phenazine-1-carboxylic acid, an antibiotic previously implicated in the suppression of take-all by *P. fluorescens* and *P. aureofaciens*. This antibiotic is produced in the rhizosphere of wheat (Thomashow and Weller 1988; Thomashow et al. 1990). Isolate O6 produces a complement of phenazines identical to those from *P. aureofaciens* 30-84 which acts as a biocontrol agent in the field for take-all of wheat (Pierson and Thomashow 1992). In this context, it is interesting that O6 unlike 2E3 did not promote wheat emergence. The fact that O6 is less effective in the field than 2E3 indicates that the production of more than one antibiotic may not be correlated with greater antagonism of fungal pathogens in the soil. Although both *P. chlororaphis* strains are impressive fungal antagonists on agar medium, this may not be a predictor of field performance.

The fact that the emergence of spring wheat responded to treatment with 2E3 in the same way at both farms could be attributed to the similarities in the physical and biological properties of the soils. Physical differences between the soils were minor. Both soils contained a similar number of culturable bacteria which was low compared to that reported from other soils. The average number of microorganisms isolated from soil has been reported to be between 1.1 and 4.5×10^6 g^{-1} soil (Atlas and Bartha 1981). However, reports of bacterial populations in soil vary from as low as 1.1×10^3 to 1.2×10^8 (Gray and Williams 1971; Leyns et al. 1990) and determinations of bacterial populations differ with factors such as soil type, sampling depth and culture medium (Atlas and Bartha 1981: Leyns et al. 1990). Another point of interest is that roughly a third of the indigenous bacteria from both sites were found to be antagonistic to pathogenic fungi (Table 2). This level of potential antagonists is probably typical of many soils. For example, Leyns et al. (1990) reported the same proportion of all bacteria isolated from the rhizospheres of seven different crops had antifungal properties. In spite of the existence of an indigenous population with antagonistic potential, adding 2E3 promoted emergence. This might be explained by the relatively low total numbers of competing bacteria. In addition, the inoculation of 2E3 directly onto the seed where it could readily colonize the rhizosphere during the critical period of emergence could be significant. Further work is required to determine how plant growth promotion by pseudomonads is affected either by soil physical factors or by indigenous soil microbial populations.

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