Rhizobacteria of Sugar Beets: Effects of Seed Application and Root Colonization on Yield

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ABSTRACT

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Selected strains of fluorescent *Pseudomonas* spp. isolated from the rhizosphere-rhizoplane of field-grown sugar beets caused statistically significant yield increases of sugar beets in replicated greenhouse studies and field trials when applied as a seed-coating formulation. Significant increases in dry and fresh weight of seedling roots and/or shoots of plants grown in the greenhouse ranged from 20 to 85%. The effect did not occur in sterile University of California mix or in peat soils. Significant growth increases in seedling weight, mature root weight, and total sucrose yield were attained in field trials in California and Idaho over a 3-yr period. Increases in root weight and total sucrose, averaging 13% above untreated controls, were as great as 4.6 t/ha and 26.8 cwt/ha, respectively. Protocols were developed for the application and preservation of populations of up to 10^{12} colony-forming units (cfu) of plant growth-promoting rhizobacteria (PGPR) on seed, using cellulose methyl ether or gum xanthan in combination with neutralized peat or talc coatings. The seed application techniques developed were compatible with commercial planting procedures used for field trials. Colonization of roots by PGPR resistant to rifampicin and nalidixic persisted throughout the growing season, reaching populations as great as 5.2×10^4 cfu/cm of root. Populations of PGPR reached 10^3–10^4 cfu/cm of treated roots compared with natural populations of total fluorescent pseudomonads, which ranged from 90 to 500 cfu/cm of root. In vitro antibiosis by PGPR strains was observed against *Erwinia carotovora* subspp. *carotovora* and *atroseptica*; *Pseudomonas marginalis* pv. *marginalis*; *P. syringae* pv. *syringae*, pv. *phaseolicola*, and pv. *tomato*; and fungal pathogens *Rhizoctonia solani*, *Pythium ultimum*, *P. aphanidermatum*, and *P. debaryanum*. The antagonism was biostatic rather than biocidal. The establishment of high populations of antagonistic PGPR on roots appears related to plant growth promotion effects.

Additional key words: biological control, bacterization.

Various studies (3,7,9,16,17,23–25,27,28,30–33,38) implicated or suggested that bacteria such as *Pseudomonas* sp. or *Bacillus* sp. may play a role in the reduction of disease. However, considerable scepticism existed (5,8,10,29) concerning the effectiveness of bacteria in controlling diseases or influencing growth and yield because most reports were not based on several years of replicated field trials with statistical evaluations of results. Recent studies (13,14,26,43) more fully demonstrated the potential for specific bacterial strains to inhibit certain plant pathogens. In addition, in studies using commercial practices (6,20,22,40–42), specific strains of *Pseudomonas fluorescens* and *P. putida* significantly increased plant growth yield in field trials.

Rhizosphere-colonizing fluorescent pseudomonads significantly increased growth and yield of certain crops, although no specific control of major root pathogens was readily apparent (40). Application of these plant growth-promoting rhizobacteria (PGPR) to seed or seed pieces resulted in yield increases with radish (22) and potatoes (6,20) in replicated field trials. Burr et al (6) and Kloepper (20) obtained significant ($P = 0.05$ or 0.01) yield increases up to 33% with potato, using the same and different strains of PGPR, in field tests spanning six consecutive years.

This report details the ability of rhizobacteria strains, specifically selected from sugar beet rhizospheres, to increase sugar beet growth and yield in greenhouse and field trials. The development of procedures for applying PGPR to seed and preserving PGPR inoculum and the capacity of PGPR to colonize roots from treated seed are described. Preliminary reports were published (40–42).

MATERIALS AND METHODS

Initial selection of antagonistic rhizobacteria. Rhizosphere-rhizoplane colonizing bacteria were isolated from freshly harvested, field-grown sugar beet roots at both seedling and mature growth stages. Samples were taken from several geographic areas and soil types. After vigorous shaking of excised roots to remove all but tightly adhering soil, root segments (1 g) were agitated in 100 ml of sterile distilled water (SDW) for 15 min. Bacteria were isolated by dilution plating on King's medium B (KB) (18), nutrient agar, Luria's medium, Sand's fluorescent pseudomonad agar (35), potato-dextrose peptone, and yeast dextrose calcium carbonate peptone. Plates were incubated for 24 hr at 28 C and then sprayed with a suspension (10^8 colony-forming units [cfu] per milliliter) of *Erwinia carotovora* subsp. *carotovora* (Ecc) or *P. syringae* pv. *syringae* (UCBPP970) as described by Burr et al (6). Plates were then incubated an additional 24 hr and examined for colonies producing zones of inhibition. Colonies with antibiotic activity towards the two plant pathogens were purified and retested.

Eighty-four additional bacterial strains for greenhouse trials were obtained in subsequent tests by random selection of the predominant morphologically distinct colonies isolated from sugar beet roots on culture media. In vitro antibiosis was not a selection factor for these strains.

Strain *Bacillus subtilis* A-13 (obtained from K. F. Baker, USDA Ornamental Plant Res. Lab., Corvalis, OR) was tested in a field trial at Davis, CA, because it has been extensively studied for its growth-enhancing potential on many crops (2,4,27,28).

Development of seed-pelleting techniques for application of bacteria in greenhouse and field trials. Preliminary experiments indicated that inoculation of sugar beet seed with aqueous-bacterial suspensions was not satisfactory because of handling difficulties with wet seed and low survival of rhizobacteria after drying. Materials tested for use as bacterial preservatives and adhesives in seed pelleting included gum arabic, carboxymethyl cellulose, hydroxyethyl cellulose, lime-silica preparations, polyvinyl alcohol (PVA), gum xanthan, and cellulose methyl ether (MC). Water containing 0.1 M $MgSO_4$ was used as the standard to prevent decline of cell viability in distilled water (39). Materials tested for use as coating materials, necessary for rapid seed separation, included bentonite, dolomite, diatomaceous earth, azomite, talc, vermiculite, and peat.

The following protocol described for one concentration of MC

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was used in testing the preservatives-adhesives and coating materials. Bacterial strains B4, RV3, and SH5 (41) were grown on KB media for 48 hr, washed from media in 0.1 M $MgSO_4$, and diluted to a final concentration of approximately 10^9 cfu/ml. Dilutions were made with a 0.5% (w/v distilled H_2O) autoclaved solution of MC (cellulose methyl ether, 4,000 centipoises, Mallinckrodt Chemical Works, St. Louis, MO 63134). Sugar beet seeds of cultivars USH10.8, USH10.9, Utah-Idaho 3, or HH23 were sprayed with the bacterial suspension until evenly coated, then hand-shaken or mechanically rolled in a mixture of autoclaved screened peat moss (No. 16 mesh, 1 mm^2), 1.0% (w/w) dry MC, and 0.1% (w/w) $CaCO_3$. The pelleted seeds were then air-dried (~25 C) and stored in plastic petri dishes or waxed cardboard containers with lids.

Seeds thus pelleted with various coating materials were tested in greenhouse and field trials to assess their effect on germination. Fifty seeds of each treatment were planted in flats containing University of California (UC) mix (1) and evaluated for percent germination after 5, 7, and 10 days. Uncoated seed sprayed with 0.1 M $MgSO_4$ was used as the control. Pelleted seeds were also tested in a trial at Woodland, CA, to evaluate the effect of coating materials on germination under field conditions. Plot design was a randomized complete block with four replications of double row plots 14 m long. Seedling counts of four sections (4.6 m long) per plot were made at day 10, 12, and 20 from the first irrigation, which was applied 6 days after planting. Other field trials compared the effects that pelleting without bacterial treatment and pelleting with peat coatings had on seedling weight and final yield.

A different pelleting technique from that described above was used at Imperial and Tracy field trials and for greenhouse tests during 1979–80. Bacterial suspensions in 0.1 M $MgSO_4$ were prepared as before and mixed with an equal volume of autoclaved 20% gum xanthan (20) and sufficient sterilized talc (approximately 1:4) to form a slightly moistened powder. After drying, the bacterial-talc formulation was powdered in a Waring Blendor and screened to remove any lumps. Sugar beet seed was then sprayed with a 1.5% (w/v) solution of polyvinyl alcohol and coated as described above. This technique has the advantage over other bacterial inoculations of wetting seed only minimally. This is especially important when seeds are also treated with Dexon®, the effectiveness of which is reduced by exposure to water and light.

Sugar beet seed pelleted with each bacterial strain was tested at varying time intervals up to 1 yr to assess bacterial viability. Thirty seeds per treatment were evaluated by taking three 10-seed samples, mechanically agitating them on a rotary shaker for 30 min in 100 ml of SDW, and plating by serial dilutions on KB. Media were incubated for 24 hr at 28 C, and colonies of bacteria were counted.

Colonization of roots from bacterial pelleted seed. The ability of rhizobacterial strains to colonize sugar beet roots from both greenhouse plants and seedlings germinated in seed packs (diSPo®, Northrup King & Co., Scientific Products, Sunnyvale, CA 94086) were made by aseptically removing 1-cm-long sections of roots from 20 15-day-old plants and agitating them in 10 ml of SDW. Root segments removed from the tip, zone of elongation, and zone of lateral emergence were included in all isolations. Population determinations were then made separately from each region by dilution plating on KB or fluorescent pseudomonad agar.

Each bacterial strain used in field trials was tested for compatibility with the standard fungicide and insecticide seed treatment. Commercially planted sugar beet seed, provided by cooperating sugar refining companies, was treated with Lindane (gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane), pentachloronitro-benzene (PCNB), and Dexon (*p*-[dimethylamino]-benzene diazosodium sulfonate). Chemically treated sugar beet seed was pelleted with bacterial strains B4, SH5, RV3, and B2 and then germinated in seed packs. Three 10-seed samples per seed pack were examined for colonization of emerging roots, as described.

Greenhouse screening for PGPR. Strains of the rhizobacteria selected for in vitro antibiosis and the 84 randomly selected strains were pelleted onto sugar beet seeds for evaluation in greenhouse trials. Ten seeds were planted per pot and then thinned to three seedlings after development of the first true leaves. At least 15 clay pots per treatment were used. In some trials, flats with 20–36 seedlings per flat, standardized for each trial, were used instead of pots. At least three flats were used for each treatment. Loamy sand, sandy loam, clay loam, or peat field soils from various sugar beet growing areas of California were used. Plantings also were made in UC mix and UC mix-field soil combinations of 2:3 and 3:2 (v/v). Isolates were evaluated for their ability to increase plant growth under greenhouse conditions by comparing fresh and/or dry weights of shoots, roots, or whole plants to those of untreated control plants. Strains exhibiting statistically significant growth-enhancing ability were again greenhouse tested to determine the reproducibility of the phenomenon.

Evaluation of rhizobacteria to increase sugar beet yield in field experiments. Ten field experiments were made to evaluate selected rhizobacterial strains for their ability to increase seedling growth and final yield of sugar beets. Experimental design was either a randomized complete block or a Latin square, with at least four and six replications, respectively. Row length ranged from 8.5 to 16.2 m per replication in double or four-row plots. Pelleted seed was prepared as described. Rhizobacteria that gave the most consistent and greatest growth increases in greenhouse trials were used in field evaluations. Field trials in California were conducted at the University of California at Davis Agronomy Research Field D3 (Davis), the Westside Field Experiment Station in the lower San Joaquin Valley (Westside), Manteca, Tracy, El Centro, and Woodland, CA, and at Miller Farms in Minadoka, ID. For Idaho, Tracy, and El Centro field trials, seed was prepared in Berkeley and sent in number-coded packages to the cooperating grower.

Evaluations were made of seedling emergence, seedling weight, NO_3-N and PO_4-P tissue concentrations, final root weight, total roots per plot, percent processable root (percent clean), NO_3 concentration of mature root, and root sucrose concentration. Determinations of NO_3 and PO_4-P concentrations were made by petiole analysis of the first true leaves. Sucrose concentration, NO_3 concentration, and percent clean were evaluated at Westside, Davis, Idaho, and Tracy with the assistance of cooperating beet refineries. Not all of the 10 plots were evaluated completely through harvest because of the nature of the experiment or problems in growth and care of the crop not related to the experiment.

Colonization of roots in field trials. Evaluation of PGPR colonization of sugar beet roots under field conditions was done by selecting strains with resistance to both rifampicin (rif) and nalidixic acid (nal) in the following manner. Plates of KB were inoculated with a bacterial suspension of sufficient concentration to create a uniform lawn. After 2–3 hr of incubation at 28 C to allow bacterial growth to begin, plates were injected at the center with 0.2 ml of sterilized antibiotic solution. Rif concentrations were 200, 500, or 1,000 μg of active ingredient per milliliter. Resistant strains were isolated from the original plates and retested on KB plates containing 100 ppm rif. Identical procedures were repeated using nal to select for strains with double antibiotic resistance. Antibiotic-resistant strains were tested in greenhouse trials for plant growth-promotion ability. Only those strains displaying significant growth-promoting abilities were used in field trials.

Field isolations were done by removing 20-cm sections of juvenile roots with alcohol-flamed forceps and placing them in capped test tubes containing 10 ml of autoclaved 0.1 M $MgSO_4$ to prevent population decline in transit (39). Tubes containing roots were transported from the field on ice and kept refrigerated until isolations were made. Root isolations in the laboratory were done the same day or the following morning. At least 10 samples from each treatment were done, with three plates per sample. The medium used for field isolations was a modified KB (KBM) amended after autoclaving with sterilized solutions of dichlorane (Botran®; active ingredients, 100 μg/ml), nystatin (100 μg/ml), cycloheximide (150 μg/ml), benomyl (Benlate® 100 μg/ml), rif (100 μg/ml), and nal (100 μg/ml).

At field trials in Tracy, rif-nal resistant PGPR strains were identified on untreated control plants in each plot planted using KBM medium. Untreated control seed was inadvertently dusted during planting with residue pelleting material from treated seed,

which contaminated the planter box. Root samples from both PGPR and control plants were collected and isolations were made as described above, using KBM. Population determinations were made at various times throughout the growing season up to harvest.

The efficiency of recovery of KBM medium as compared to that of unamended KB was tested, using known concentrations of rif-nal resistant bacteria, before use in field trials. Recovery of resistant strain $SH5_{RN}$ on KBM was 71% of that on unamended KB from a 10^6-cfu/ml suspension. PGPR with resistance to rif-nal grew more slowly on KBM than on KB, requiring an additional 24 hr at 28 C to develop recognizable colonies.

Compatibility with pesticidal seed treatments. Three PGPR strains, B4, RV3, and SH5, were tested for compatibility with 10 common fungicidal seed treatments in addition to those used commercially on sugar beet seed. One milliliter of a bacterial suspension (10^8 cfu/ml) was placed in 100 ml of an aqueous solution (active ingredient, 1,000 μg/ml) of each fungicide and agitated for 1 hr in flasks. Three 0.1-ml aliquots were removed from each flask and spotted on KB. Relative growth of each PGPR strain was measured after a 24-hr incubation. The test fungicides were benomyl, captan, basic copper sulfate, mancozeb, carboxin, oxycarboxin, thiabendazole, thiram, diazoben, ethazol, and PCNB as a control. PGPR compatibility was rated as confluent growth greater than 10^4 cfu/ml, growth less than 10^2 cfu/ml, and no viable cells detectable.

Screening of rhizobacteria for in vitro antibiosis. Rhizobacteria were tested for in vitro antibiosis against bacterial and fungal plant pathogens, other than Ecc and *P. syringae* pv. *syringae,* on culture media. Tests were conducted against bacterial pathogens on KB, nutrient agar, Luria, yeast dextrose calcium carbonate peptone, and potato-dextrose peptone as described previously. The bacterial strains used were *E. carotovora* subsp. *atroseptica, P. marginalis* pv. *marginalis, P. syringae* pv. *tomato* (UCBPP165), *P. syringae* pv. *phaseolicola* (UCBPPHB36), and an oxidase-negative, fluorescent pseudomonad, SB24, isolated from sugar beet roots. A significant degree of antibiosis was assumed if zones of inhibition were greater than 2 mm.

Fungal pathogens were tested for susceptibility to antibiosis in vitro by PGPR strains RV3, B4, and SH5. Rhizobacteria were inoculated on KB or potato-dextrose agar (PDA) by imprinting a ring of bacteria near the outer edge of surface-dried media using a circular glass inoculator. Plates were incubated overnight at 28 C to allow growth of a ring of bacteria. Small plugs of mycelium or hyphal tips were taken from fungal cultures growing on PDA or water agar (WA) plates, then placed in the center of the bacterial ring and incubated at 25 C for 3–7 days. Test fungi used were *Rhizoctonia solani* Kuhn, *Pythium ultimum* Trow, *P. aphanidermatum* (Edson) Fitz. and *P. debaryanum* Hess. Antibiotic activity was assumed if zones of inhibition were greater than 1 cm and no hyphae extended across the bacterial growth. Hyphal tips at the margin of the zone of inhibition were removed and placed on fresh PDA or WA plates to determine if the inhibitory materials were fungicidal or fungistatic.

Rhizobacteria selected as the predominant morphologically distinct colony types obtained by dilution plates were screened for in vitro antibiosis towards Ecc, *P. marginalis* pv. *marginalis, P. syringae* pv. *syringae, R. solani,* and *P. ultimum.* Strains that were both plant growth promoters and antagonistic were retested for growth promotion in greenhouse trials in sandy loam soil for comparison to PGPR strains used in field trials.

RESULTS

Characterization of bacterial strains. Of the 450 bacterial strains isolated from sugar beet rhizospheres with in vitro antibiosis towards Ecc or *P. syringae* pv. *syringae,* 427 were strains of either *P. fluorescens* or *P. putida* based on the groupings of Stanier et al (37) and Hildebrand and Schroth (12). Other strains were *Bacillus* spp., *Actinomyces* spp., *Streptomyces* spp., and several strains of Gram-negative, facultatively anaerobic rods that were not identified. One hundred fifty of these strains caused in vitro antibiosis towards both pathogens on KB. Twenty of the *Pseudomonas* spp. and five *Bacillus* spp. induced soft-rotting of potato slices or excised lettuce leaves and were not used in further studies. Two strains of *Pseudomonas* were oxidase-negative, caused a hypersensitive reaction on tobacco (*Nicotiana glutinosa* cv. Glurk) (19) and were therefore considered potential pathogens (12). None of the remaining 123 bacterial strains caused soft rot or a hypersensitive reaction on tobacco.

Seed pelleting techniques for application of rhizobacteria. Application of rhizobacteria to seeds as an aqueous suspension was unsatisfactory because average populations per seed decreased from 10^7 to 10^4 cfu per seed within 24 hr during air-drying at ambient temperatures (25 C). Seeds were initially dipped into an aqueous suspension of 10^9 cfu/ml. By 1 wk, the population averaged 10^3 cfu per seed. None were detected after 6 mo of storage at ambient temperatures. Although relatively high populations of bacteria were obtained per seed directly after treatment, the moist seeds stuck together, which would prevent their being planted by standard commercial practices. Gum arabic, carboxymethyl-cellulose, gum xanthan, and lime-silica preparations were not suitable as bacterial preservatives because they consistently resulted in germination below 60% when applied to seed. Hydroxyethyl cellulose and PVA were toxic to rhizobacteria when incorporated directly into the adhesive solution. Subsequent tests showed that a 1.5% (w/v) solution of PVA did not significantly affect germination and could be used if bacterial strains were first incorporated into a carrier material.

Of the materials tested, only MC proved effective as a bacterial preservative and also did not significantly influence germination. Populations of strain B4 ranging from 10^{10} to 10^{12} cfu per seed were recovered after drying for 24 hr at 25 C. Seed size affected the population of rhizobacteria attainable on seed. After 2 wk of storage at ambient temperatures, populations averaged 10^8 cfu per seed. Samples taken at 6 mo and at 1 yr after storage at ambient temperatures had B4 populations of 10^5 cfu per seed. Similar results were obtained for rhizobacteria strains RV3 and SH5.

Field tests demonstrated that coating sugar beet seed with screened peat, talc, or diatomaceous earth gave the best germination compared with that of untreated controls or seeds coated with other materials (Table 1). Nonpelleted seed had an emergence rate of 15.3 seedlings per meter as compared to 11.8, 12.4, and 13.7 seedlings per meter for diatomaceous earth, talc, and peat, respectively. Greenhouse trials were not reflective of field results, in which dolomite, diatomaceous earth, and peat increased the percent germination compared to that of nonpelleted controls (Table 1). Pelleting seed without bacterial treatment did not significantly affect seedling weight at Woodland (Table 2) nor final yield at Idaho (Table 3) as compared to nonpelleted controls.

TABLE 1. Germination of sugar beet seeds coated with various pelleting materials

Seed-coating materials	Petri dish (%)[a]	Greenhouse trial (%)[b]	Field trial (seedlings/m)[c]
Uncoated control	72	73	15.3 a
Bentonite			
Unhydrated	2	22	n.d.
Hydrated	71	55	9.9 b
Azomite	18	53	n.d.
Talc	35	57	12.4 a
Dolomite	78	86	8.0 b
Diatomaceous earth	79	84	11.8 b
Peat	88	90	13.7 a

[a] Average of 100 seed placed on moistened filter paper in closed petri dishes.
[b] Average of three replications of 50 seeds planted in flats with University of California mix.
[c] Counts from a field trial in Woodland, CA. Plot design was randomized complete block with four replications; 14-m, double-row plots. Counts of four 4.6-m-long sections per plot were made at day 10 from first irrigation. Counts at day 12 and day 20 were not significantly different from counts at day 10. Numbers followed by a different letter are significantly different at $P = 0.05$. n.d. = not done.

Colonization of roots from pelleted seed. Colonization of roots of 15-day-old plants (15 days from initial irrigation) in greenhouse trials by strains B4, RV3, and SH5 from pelleted seed ranged from 10^5 to 10^7 cfu/cm of root. Colonization by rhizobacterial strains was approximately equal in samples taken from root tip, root hair, and lateral root regions. Populations of 10^5, 10^6, and 10^7 cfu/cm were detected for all strains in UC mix, sandy loam field soil, and seed packs, respectively. Colonization of roots by B2, B4, RV3, and SH5 from pelleted seed treated with the standard pesticidal seed treatment (Dexon, PCNB, Lindane) was not significantly different from that by raw seed in sandy loam soil, averaging approximately 10^6 cfu/cm of root.

Greenhouse screening of potential PGPR. Eight of 123 strains of fluorescent *Pseudomonas* spp. caused significant growth increases of sugar beets in preliminary greenhouse trials and were used for further studies (Figs. 1 and 2). Rhizobacteria strains B4, RV3, and SH5 were most consistent in causing significant growth responses in over 40 greenhouse trials in a variety of field soils. Figure 2 presents a representative sample of greenhouse trials spanning a 3-yr period; trial order does not reflect a chronological series. Significant increases, as measured by dry and fresh weight of seedling roots and/or shoots, ranged from 20 to 69% as compared to that of untreated controls. Significant growth increases averaged 40% for SH5, 41% for B4, and 43% for RV3 and were generally greatest in sandy loam soils. Growth promotion was not observed in UC mix or organic-peat soils. On the basis of these tests, three strains of beneficial rhizobacteria, B4, SH5, and RV3 were identified as PGPR and were selected for field tests.

Effect of PGPR on sugar beet growth and yield in field trials. PGPR seed treatments caused statistically significant increases of seedling weight, final root weight, and sucrose yield in field trials in three consecutive years (Tables 2 and 3). Increases in fresh weight of sugar beet shoots with PGPR treatments ranged from 21 to 77% ($P = 0.05$ or 0.01) of the weight of untreated controls and averaged 44%. Significant differences in seedling weight were not observed in comparisons of fungicide-treated seed and nonfungicide raw seed or pelleted and nonpelleted seed. Evaluations of seedling emergence, NO_3-N and PO_4-P concentration in petiole tissue, total roots per plot, percent clean, and NO_3 concentration in mature roots showed no significant differences between PGPR-treated and untreated plants. The yield increases in root weight and total sucrose were statistically significant in five of eight field trials. Root weight increases caused by PGPR strains B4, RV3, SH5, and A1 ranged from 6.1 to 8.6 t/ha in field trials with significance at $P = 0.05$ or 0.01. Rhizobacteria strain SH5 was the most consistent in increasing root weight except at the two Idaho trials. *B. subtilis* strain A-13, included as a previously reported growth promoter, caused neither growth nor yield increases in one trial at Davis. Increases in total sucrose yield ranged from 20.7 to 26.8 cwt/ha ($P = 0.05$), representing an average yield benefit of 13%. Substantial root weight (24%) and sucrose yield (19%) increases with PGPR SH5 were obtained at Imperial in 1979, although significance at $P = 0.05$ was not attained (Table 3).

Colonization of roots by PGPR in field tests. PGPR strains $SH5_{RN}$, $B4_{RN}$, $A1_{RN}$, and $E6_{RN}$ selected for resistance to rif and nal were detected on the roots of field-grown sugar beets on KBF at populations ranging from 1.4×10^3 to 3.7×10^4 cfu/cm of root (Table 4). Resistant PGPR were detected on roots as soon as emergence occurred 10 days after the first irrigation. Colonization of root segments was variable depending on sampling date and strain. PGPR $SH5_{RN}$ was detected at 5.2×10^4 cfu/cm at day 10, 1.9×10^3 cfu/cm at day 35, and 1.3×10^4 cfu/cm at day 56. Rif-nal resistant strains were not detectable on untreated controls at Woodland in 1979. The population of total fluorescent *Pseudomonas* spp. on untreated controls and sugar beet plants outside of the experimental plots (measured on unamended KB) was highly variable, ranging from 90 to 500 cfu/cm of root.

At Tracy trials, rif-nal resistant strains of PGPR were detected on the roots of untreated control plants at rates as high as 2.6×10^4 cfu/cm, resulting from contamination from residue talc-xanthan

TABLE 2. Effect of plant growth-promoting rhizobacteria (PGPR) on seedling growth in California field trials

Location and year	Plot design	Plot row (m)	Sample	Seed treatment[a]	Mean seedling weight (g)	Percent increase vs control[b]
Westside, 1977	Four randomized complete blocks	11.5	30 whole plants	Untreated[c] control (pelleted)	373.7	...
				Fungicide-treated[d]	364.7	−2
				B2	452.7	21
				SH5	463.7	24**
				B4	486.0	30**
Davis, 1978	Latin square 6 × 6	13.1	20 shoots	Fungicide-treated[d] (pelleted) control	30.4	...
				B4	41.1	35***
				E6	41.2	35***
				A1	44.0	45***
				RV3	45.4	49***
				SH5	47.5	56***
Woodland, 1979	Latin square 6 × 6	14.0	Week 5 25 shoots	Fungicide-treated[d] control		
				Pelleted	31.4	...
				Nonpelleted	32.8	4
				RV3	31.4	0
				$SH5_{RN}$	34.0	8
				$B4_{RN}$	38.8	21*
				$A1_{RN}$-$E6_{RN}$	46.1	47*
			Week 8 5 roots	Fungicide-treated[d] control		
				Pelleted	121	...
				Nonpelleted	129	6
				RV3	134	7
				$B4_{RN}$	149	19**
				$A1_{RN}$-$E6_{RN}$	190	52***
				$SH5_{RN}$	222	77***

[a] Seed treatments A1, B2, B4, E6, RV3, and SH5 were strains of PGPR. Strains A1 and E6 were originally identified as PGPR on potato.
[b] * $P = 0.05$, ** $P = 0.02$, *** $P = 0.01$.
[c] Untreated controls had no fungicide or bacterial treatment.
[d] Fungicide-treated designates seed treated commercially with Dexon®, Lindane, and PCNB.

gum pelleting material in the planter not removed between each replication. Populations of rif-nal PGPR were 0 cfu/cm in replication 1 plants, 100 cfu/cm in replication 2, and 5.2×10^3–2.6×10^4 cfu/cm in replications 3 to 6 on untreated control plants at day 10. Plants specifically pelleted with strains $A1_{RN}$ and $SH5_{RN}$ had PGPR populations of 1.9×10^4 cfu/cm and 2.7×10^4 cfu/cm at day 10. At harvest, PGPR populations on all plants had declined to less than 100 cfu/cm of root, presumably because of soil dryness.

Compatibility with pesticidal seed treatments. Most standard fungicidal seed treatments used for specific or general disease control on a variety of crops had no effect on the survival and growth of PGPR strains B4, RV3, and SH5. Benomyl, captan, carboxin, oxycarboxin, thiabendazole, thiram, diazoben, and PCNB did not affect PGPR under the test conditions. After incubation in aqueous suspensions of basic copper sulfate and mancozeb, all three strains exhibited a greater than 50% reduction in population as determined on KB media. PGPR strains RV3 and SH5 were not viable after exposure to ethazol.

In vitro antibiosis of PGPR towards bacterial and fungal pathogens. Antibiosis in vitro towards a wide spectrum of bacterial and fungal pathogens was a common characteristic among PGPR strains RV3, B4, SH5, and three strains of PGPR selected

TABLE 3. Effect of plant growth-promoting rhizobacteria (PGPR) on sugar beet yield in field trials in California and Idaho

Location and date	Seed treatment[a]	Mean root yield (t/ha)	Percent increase vs control	Mean sucrose yield (cwt/ha)	Percent increase vs control[b]
Westside, 1977	Untreated control[c]	64.0	...	147.6	...
	B4	64.0	0	140.3	−5
	B2	70.7	10	167.7	14
	SH5	71.9	12*	170.2	15*
	Fungicide-treated[d] (pelleted)	73.8	15*	172.0	17*
Davis, 1977	Untreated control	61.6	...	169.6	...
	A13	62.2	0	178.7	5
	B4	64.6	5	184.8	5
	RV3	67.7	10	196.4	16*
	Fungicide-treated (pelleted)	68.9	12	195.2	15*
Idaho, 1977	Fungicide-treated counted				
	Pelleted	65.2	...	211.7	...
	Nonpelleted	65.8	1	214.7	1
	SH5	66.5	2	212.9	0
	B4	72.6	11	236.7	12**
	RV3	73.8	13	236.7	12**
Manteca, 1978	Untreated control	54.9	...	...	...
	RV3	66.5	21	...	...
	A1	67.7	23	...	...
	B4	70.1	28	...	...
	SH5	72.6	32*	...	...
Davis, 1978	Fungicide-treated (pelleted) control	73.8	...	211.7	...
	B4	78.1	6	232.4	10**
	E6	78.1	6	232.4	10**
	RV3	79.9	8**	232.4	10**
	A1	81.7	11**	236.1	12**
	SH5	82.3	12**	238.5	13**
Idaho, 1978	Fungicide-treated (pelleted) control	45.1	...	...	...
	SH5	43.9	−3	...	...
	E6	44.5	−1	...	...
	RV3	45.1	0	...	...
	A1	45.7	1	...	...
	B4	46.9	4	...	...
Imperial, 1979	Fungicide control[e] (pelleted)	69.5	...	227.5	...
	B4	81.1	16	251.3	10
	RV3	81.1	16	244.6	8
	SH5	86.0	24	270.2	19
Tracy, 1979	Fungicide control[e] (pelleted)	64.6	...	155.5	...
	$RV3_{RN}$	57.3	−11	134.8	−13
	$A1_{RN}$	65.8	2	164.1	5
	$SH5_{RN}$	66.4	3	168.4	8
	Mix	69.5	7	173.3	11

[a] Seed treatments A1, B2, B4, E6, RV3, and SH5 were strains of PGPR. Treatment A13 was an isolate of *Bacillus subtilis* reported to cause growth promotion on a variety of crops.

[b] * $P = 0.05$, ** $P = 0.01$.

[c] Untreated control seed received no fungicide or bacterial treatments and were pelleted with peat coatings.

[d] Fungicide-treated seed were commercially treated with Dexon®, PCNB, and Lindane and were either nonpelleted or pelleted with peat coatings.

[e] Seed at Imperial and Tracy trials were pelleted with the talc-xanthan gum-bacterial coatings.

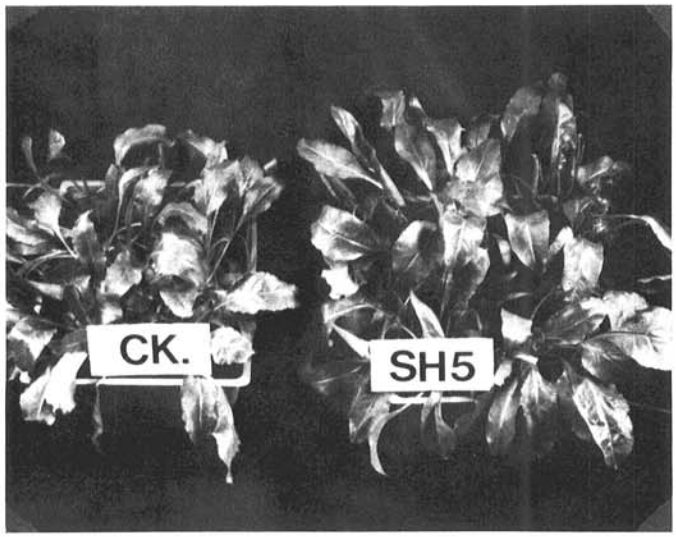

Fig. 1. Increased growth of sugar beet seedlings following seed treatment with rhizobacteria strain SH5 as compared to growth of untreated controls (CK.).

randomly from the rhizosphere of sugar beets. Bacterial pathogens Ecc, *E. carotovora* subsp. *atroseptica, E. carotovora* subsp. *betavasculorum*, *P. marginalis* pv. *marginalis, P. syringae* pv. *phaseolicola* and *tomato*, SB24, and fungal pathogens *R. solani, P. ultimum, P. aphanidermatum*, and *P. debaryanum* (*P. ultimum*) were inhibited by a diffusable product on KB, produced by all strains, which was biostatic rather than biocidal. When hyphal tips were removed from the zone of inhibition and transferred to fresh PDA or WA, rate of growth was not significantly different from that of hyphal tips removed from control fungal growth not exposed to PGPR antagonism.

Correlation of growth promotion ability to in vitro antagonism. Of 84 rhizobacteria selected as the predominant morphologically distinct colony types from commercially grown beets, three strains of fluorescent *Pseudomonas* spp., inducing significant growth increases in greenhouse trials ranging from 42 to 85%, were subsequently shown to cause antibiosis, similar to known PGPR, against all bacterial pathogens tested, and *R. solani,* and *P. ultimum*. Thirty of the original 84 colony types caused antibiosis towards at least one fungal pathogen, although none caused growth promotion in greenhouse tests. Four strains of fluorescent *Pseudomonas* spp., one *Bacillus* spp., and five unidentified strains were antagonistic to *R. solani* and *P. ultimum* and caused

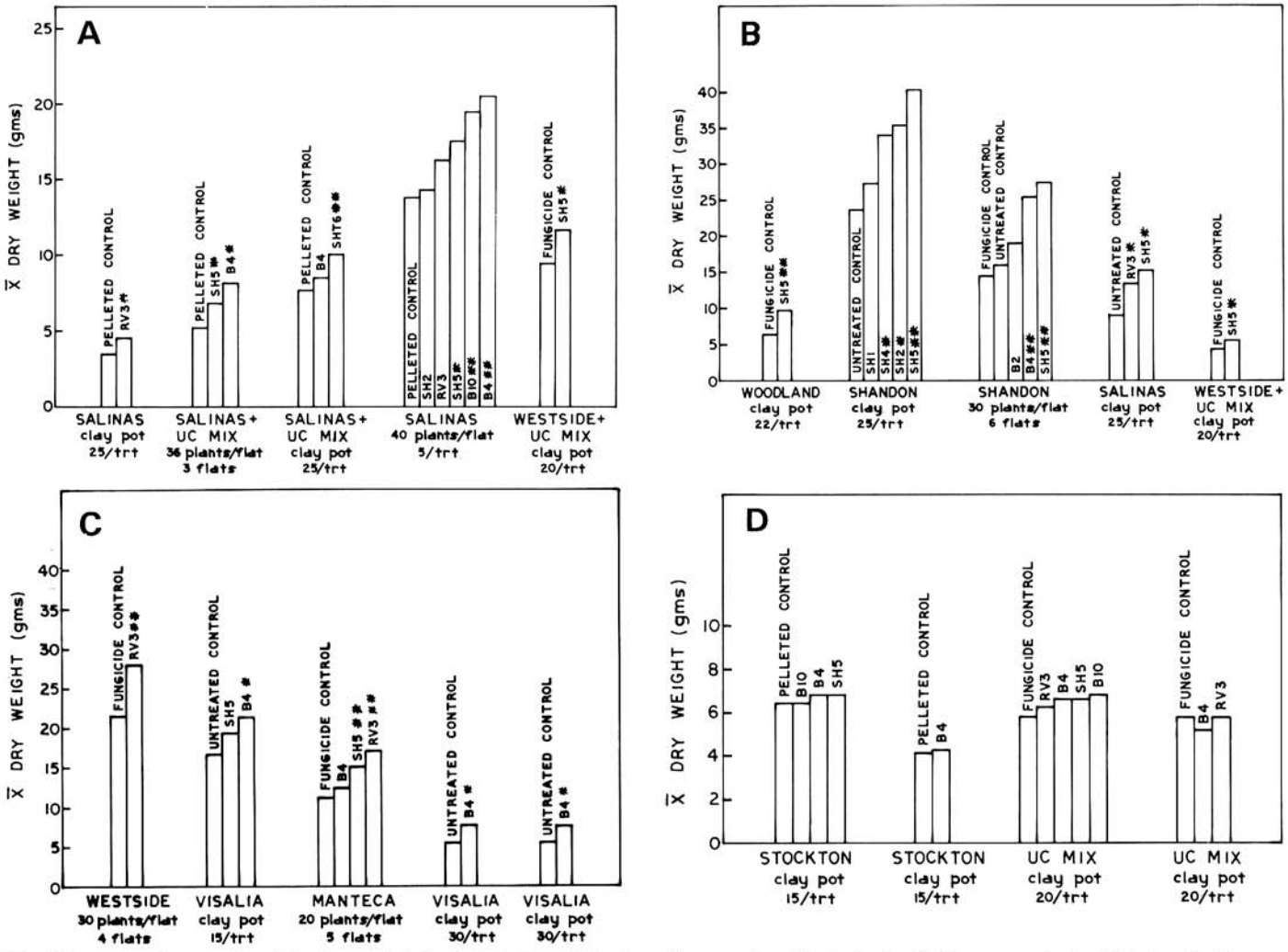

Fig. 2. Representative samples of glasshouse trials for the detection of plant growth-promoting rhizobacteria effective on sugar beets. Not all replications or treatments (trt) are presented. Fungicide controls were nonpelleted seed commercially treated with Dexon ®, PCNB, and Lindane. All natural soils used were collected from sugar beet fields located in California. UC = an artificial soil mix. **A,** Clay loam soils from central and southern Salinas Valley and the Westside Experiment Station, southwest San Joaquin Valley. **B,** Sandy loam soils from Woodland, central Sacramento Valley; Shandon, southern Calif.; Salinas; and Westside. **C,** Loamy sand soils from Manteca, central San Joaquin Valley; Visalia, southeast San Joaquin Valley; and Westside. **D,** Peat soils were from Stockton, central San Joaquin Valley. Treatments B2, B4, B10, RV3, SH1, SH2, SH4, SH5, and SHT6 are strains of plant growth-promoting rhizobacteria selected previously for growth promoting ability in glasshouse trials. The asterisks following strain designations signify that the value of the mean indicated by the bar differs statistically (* $P = 0.05$ or ** $P = 0.01$) from the control.

significant growth decreases ranging from 23 to 50% ($P = 0.05$ or 0.01). The remaining rhizobacteria had no significant effect on sugar beets in greenhouse trials. Ability to produce antibiotics in vitro was a property common to many rhizosphere bacteria as well as to PGPR and could not be used as a sole criterion for selecting plant growth promoters.

DISCUSSION

Selected strains of fluorescent *Pseudomonas* spp. isolated from the rhizospheres of sugar beets and applied as a seed-coating formulation induced statistically significant yield increases of sugar beets in replicated field trials. Three strains of these PGPR consistently increased seedling growth in both greenhouse and field trials over a 3-yr period, causing increases in root weight as great as 8.6 t/ha and in total sucrose as great as 26.8 cwt/ha, averaging a 13% increase over nonpelleted seed. Growth differences greater than 13% were attained in the seedling stage, which may represent the period of maximum benefit from PGPR treatment. This supports the findings of Mishustin and Naumova (29), who reported that the greatest benefits of bacterization were attained with plants of short cropping periods. Kloepper (22) reported yield increases up to 144% in PGPR field trials with radish, a plant with a 35-day cropping period. With sugar beets, the early season increases of plant growth, ranging from 21 to 77%, were not attained at harvest because the weight differences were not maintained over the 5–7-mo cropping period.

The pelleting materials and techniques for coating high populations of rhizobacteria on seeds were adaptable to commercial planting procedures. MC and gum xanthan were effective materials for bacterial survival on coated seed. The ephemeral nature of PGPR in nonrhizosphere soil (35) requires that seed treatments be applied at each planting to ensure that maximum rhizosphere colonization will occur. High populations of PGPR on roots of untreated controls occurring at the Tracy trials because of seed contamination from residue coating material suggests that seed inoculation other than pelleting may be possible. The mimimum population of PGPR that can be applied to seed as a dust that still allows yield increases has not been ascertained.

The specificity of PGPR for certain soils was indicated by the fact that strain SH5 increased yields in several California trials but failed in two consecutive years in Idaho. Conversely, strain B4 caused greatest yield benefits in Idaho but did not significantly affect yield in several California trials. Specificity to crop is also variable. PGPR A1 and E6, in addition to increasing growth and yield of sugar beets, cause significant yield increases on potato and radish (20,21). The use of compatible mixtures of strains effective in different soil types or on a variety of crops may increase the applicability of PGPR seed treatment.

The ability of the fluorescent *Pseudomonas* spp. to rapidly colonize the rhizosphere of most plants makes this an attractive taxonomic group of soil bacteria for bacterization studies. Other soil bacteria used as a seed or root treatment to enhance plant growth or control plant pathogens have most often not been selected from plant rhizospheres and were poor root colonizers under natural conditions. Attempts were made to adapt these strains of *Bacillus* and *Azotobacter* to a specific rhizosphere by serial passage on the roots of the target crop (5,16). Rovira (34) determined that *Bacillus* spp. only attain 1–20% of the population level attained by *P. fluorescens* on the roots of the same plant. *Azotobacter chroococcum,* a widely investigated plant growth promoter, was prevented from colonization of crop roots by antagonistic fluorescent *Pseudomonas* spp. (16). *P. fluorescens* had a 5.2-hr generation time on roots at 25 C and *Bacillus* spp. had a 39-hr generation time. The rapid growth rate of *Pseudomonas* spp. and the selectivity of roots, due to the high affinity of Gram-negative bacteria for amino acid exudates (34), give fluorescent *Pseudomonas* spp. a greater "rhizosphere competence" as defined by Schmidt (36) than other rhizosphere microflora.

Maximum population densities of PGPR are established on roots by the time shoot emergence occurs. In field tests, populations of PGPR on roots were lower than populations attained in greenhouse trials, most likely because of suboptimal water availability. PGPR populations dropped to low levels following termination of irrigation. Burr et al (6) reported that PGPR similar to strains B4, SH5, and RV3 ceased growth at water potentials below −1.7 bars. This value corresponds to moisture contents of 12% for clay soils, 7% for loam soils, and 1% for sandy soils (11). Thus, maintaining adequate soil moisture, especially at the seedling stage, is likely to be a critical factor for effective PGPR treatments. Inadequate moisture for PGPR growth and colonization of roots could explain failure to attain significant growth increases in some field trials. Under dry conditions, other rhizosphere microflora may compete with PGPR and colonize the root surface. As with *Rhizobium* inoculations to improve legume nodulations (35), the *Pseudomonas* rhizobacteria may not be able to replace resident strains after they are established on roots, even when higher rates of PGPR inoculations are used in seed treatments. Competitive growth depends on initial numbers when substrate and space are limited. Applying microorganisms to the soil for biocontrol or growth promotion, as has been pointed out by Gindrat (10), continues to be an unlikely prospect due to dilution effects. Placing PGPR directly on plant parts or seed under conditions favorable for maximal colonization gives them a competitive advantage over other rhizosphere microflora.

Studies of the interactions between PGPR and other rhizosphere microflora may provide an understanding of the growth response phenomena. PGPR treatments have been shown to alter and reduce the rhizosphere microbial composition (20,41,42), which may be the result of niche exclusion, substrate competition, antibiotic production, or other biologically active substances (15). Several antibiotics, known to be produced in media by *Pseudomonas* spp. (13,14), are inhibitory to both bacteria and fungi. Kloepper et al (21) demonstrated that some plant growth increases were related to the inhibition of root-colonizing fungi by an iron-chelating siderophore produced by specific PGPR strains. The in vitro antibiosis by PGPR strains obtained in this study was consistent with that of strains used in the subsequent studies. However, antibiosis could not be used as the sole criterion for selecting plant growth promoters from a population of rhizosphere bacteria. Beneficial strains constituted a low percentage of the rhizosphere microflora on sugar beet roots. Of the 534 isolates screened, only 12 strains, approximately 2%, could be considered plant growth promoters. Antagonistic interactions, including those caused by antibiotics, between PGPR and other root microflora result in the exclusion of deleterious bacteria and fungi from the rhizosphere and could account, in part, for growth and yield increases caused by PGPR. The lack of positive growth

TABLE 4. Colonization (cfu × 10^3/cm) of sugar beet roots[a] by plant growth-promoting rhizobacteria (PGPR) resistant to rif-nal[b] at Woodland, CA, in 1979

Treatment	Day			
	10	25	35	56
Fungicide control[c] (nonpelleted)	0[e]	...	...	...
Bacterial-treated[d]				
RV3	0	...	...	...
$SH5_{RN}$	37.0	29.0	1.4	9.0
$B4_{RN}$	22.0	27.0	13.0	23.0
$A\ 1_{RN}$-$E6_{RN}$	19.0	25.0	19.0	11.0

[a] Averages of populations from 10 root samples per treatments, 20 cm of root per sample. Root samples were taken from an area approximately equidistant from the root tip and crown.

[b] PGPR strains SH5, B4, A1, and E6 were selected for resistance to rifampicin (rif) and nalidixic acid (nal). Isolations from roots were done on King's medium B amended with rif, nal, dichlorane, nystatin, and benomyl (100 μg/ml) and cycloheximide (150 μg/ml).

[c] Fungicide control seed were commercially treated with Dexon®, PCNB, and Lindane.

[d] Bacterial-treated seed were pelleted with peat coatings.

[e] Populations of total fluoresent pseudomonads on untreated controls; roots were highly variable, ranging from 90–500 cfu/cm of root.

response in UC mix, in which the presence of potentially deleterious bacteria and fungi would be unlikely, suggests that one mode of action of PGPR is a biological control of root pathogens that depress plant growth.

The isolation and development of PGPR applicable to a variety of crops, soils, and locations will depend on the development of improved detection and screening procedures that more rapidly identify beneficial strains. The numerous greenhouse and field trials necessary to detect PGPR are, by current methods, time-consuming and involve extensive space requirements to establish statistical significance. The development of methods increasing the efficiency of detection of beneficial strains will greatly benefit future studies.

LITERATURE CITED

1. Baker, K. F., ed. 1957. The UC system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23. 332 pp.
2. Baker, K. F., and Cook, R. J. 1974. Biological control of plant pathogens. W. H. Freeman and Co., San Francisco. 433 pp.
3. Bowen, G. D. 1979. Integrated and experimental approaches to the study of growth of organisms around roots. Pages 209–227 in: B. Schippers and W. Gams, eds. Soil-Borne Plant Pathogens. Academic Press, New York. 686 pp.
4. Brown, M. E. 1973. Soil bacteriostasis limitation in growth of soil and rhizosphere bacteria. Can. J. Microbiol. 19:195-199.
5. Brown, M. E., Burlingham, S. K., and Jackson, R. M. 1964. Studies on *Azotobacter* species in soil. III. Effects of artificial inoculation on crop yields. Plant Soil 20:194-214.
6. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
7. Cook, R. J., and Rovira, A. D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil. Biol. Biochem. 8:269-273.
8. Cooper, R. 1959. Bacterial fertilizers in the Soviet Union. Soils Fert. 22:327-333.
9. Eklund, E. 1970. Secondary effects of some pseudomonads in the rhizosphere of peat-grown cucumber plants. Acta Agric. Scand. Suppl. 17:1–57.
10. Gindrat, D. 1979. Biocontrol of plant diseases by inoculation of fresh wounds, seeds, and soil with antagonists. Pages 537–551 in: B. Schippers and W. Gams, eds. Soil-Borne Plant Pathogens. Academic Press, New York. 686 pp.
11. Gray, T. R. 1976. Survival of vegetative microbes in soil. In: T. R. Gray and J. R. Postgate, eds. The Survival of Vegetative Microbes. Cambridge University Press, Cambridge. 432 pp.
12. Hildebrand, D. C., and Schroth, M. N. 1971. Identification of the fluorescent pseudomonads. Pages 281–287 in: Proc. Third Int. Conf. on Plant Pathogenic Bacteria, Pudoc, Wageningen.
13. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
14. Howell, C. R., and Stipanovic, R. D. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
15. Hussain, A., and Vancura, V. 1970. Formation of biologically active substances by rhizosphere bacteria and their effect on plant growth. Folia Microbiol. 45:468-478.
16. Jackson, R. M., and Brown, M. E. 1966. Behavior of *Azotobacter chroococcum* introduced into the plant rhizosphere. Ann. Inst. Pasteur, Suppl. III:103-112.
17. Jensen, H. L. 1942. Bacterial treatment of non-leguminous seeds as an agricultural practice. Aust. J. Sci. 1942:117-120.
18. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
19. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1944. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
20. Kloepper, J. W. 1979. The role of rhizobacteria in increasing plant growth and yield. Ph.D. dissertation, University of California, Berkeley.
21. Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:885-886.
22. Kloepper, J. W., and Schroth, M. N. 1978. Plant growth promoting rhizobacteria on radishes. Pages 879–882 in: Proc. Fourth Int. Conf. Plant Path. Bacteria. Vol. Z. Angers, France.
23. Kommedahl, T., and Chang Mew, I. P. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65:296-300.
24. Kommedahl, T., and Windels, C. E. 1976. Organism-coated seeds in disease control of peas and other vegetable crops. Proc. Am. Phytopathol. Soc. 3:272.
25. Liu, S., and Vaughan, E. 1965. Control of *Pythium* infection in table beet seedling by antagonistic microorganisms. Phytopathology 55:986-989.
26. Lynch, J. 1978. Microbial interactions around imbibed seeds. Ann. Appl. Biol. 39:165-167.
27. Merriman, P. R., Price, R. D., and Baker, K. F. 1974. The effect of inoculation of seed with antagonists of *Rhizoctonia solani* on the growth of wheat. Aust. J. Agric. Res. 25:213-218.
28. Merriman, P. R., Price, R. D., Kollmorgen, J. F., Piggott, T., and Ridge, F. H. 1974. Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. Aust. J. Agric. 25:219-226.
29. Mishustin, E. N., and Naumova, A. N. 1962. Bacterial fertilizers, their effectiveness and mode of action. Microbiologiya 31:545-555.
30. Mitchell, R., and Hurwitz, E. 1965. Suppression of *Pythium debaryanum* by lytic rhizosphere bacteria. Phytopathology 55:156-158.
31. Nair, N. G., and Fahy, P. C. 1976. Commercial application of biological control of mushroom bacterial blotch. Aust. J. Agric. Res. 27:415-422.
32. Ocampo, J. A., Barea, J. M., and Montoya, E. 1975. Interactions between *Azotobacter* and "phosphobacteria" and their establishment in the rhizosphere as affected by soil fertility. Can. J. Microbiol. 21:1160-1165.
33. Rovira, A. D. 1963. Microbial inoculation of plants. I. Establishment of free-living nitrogen-fixing bacteria in the rhizosphere and their effects on maize, tomato and wheat. Plant Soil. 3:304-314.
34. Rovira, A., and Davey, C. 1974. Biology of the rhizosphere. Pages 155–204 in: R. W. Carson, ed. The Plant Root and its Environment. University Press of Virginia, Charlottesville.
35. Sands, D. C., and Hankin, L. 1975. Ecology and physiology of fluorescent pectolytic pseudomonads. Phytopathology 65:921-924.
36. Schmidt, E. L. 1979. Initiation of plant root microbe interactions. Annu. Rev. Microbiol. 33:355-379.
37. Stanek, M. 1979. *Gaeumannomyces graminis* and bacteria in the rhizosphere of wheat. Pages 247–252 in: B. Schippers and W. Gams, eds. Soil-Borne Plant Pathogens. Academic Press, New York. 686 pp.
38. Stanier, R. Y., Palleroni, N. J., and Doudoroff, M. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-271.
39. Suslow, T. V. 1980. Increased growth and yield of sugar beets by seed treatment with specific *Pseudomonas* spp. and bacterial culture preservation in frozen and dry film cellulose methyl ether. Ph.D. dissertation, University of California, Berkeley. 144 pp.
40. Suslow, T. V., Kloepper, J., Schroth, M. N., and Burr, T. 1979. Beneficial bacteria enhance plant growth. Calif. Agric. 33:15-17.
41. Suslow, T. V., and Schroth, M. N. 1979. Role of antibiosis by growth-promoting rhizobacteria in increasing crop yield. (Abstr.) Proc. Am. Soc. Microbiol., Pacific Div.
42. Suslow, T. V., and Schroth, M. N. 1981. Interactions of growth-promoting rhizobacteria with deleterious rhizosphere bacteria and fungi. (Abstr.) Phytopathology 71:259.
43. Thirumalachar, M. J., and O'Brien, M. J. 1977. Suppression of charcoal rot in potato with a bacterial antagonist. Plant Dis. Rep. 61:543-546.