Effects of Rhizosphere Colonization by Plant Growth-Promoting Rhizobacteria on Potato Plant Development and Yield

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ABSTRACT

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Two strains of fluorescent *Pseudomonas* spp. isolated from potato periderms and from celery roots significantly increased growth of potato plants up to 500% greater than controls in greenhouse assays. Mutants of these plant growth-promoting rhizobacteria (PGPR) resistant to rifampicin and nalidixic acid rapidly colonized rhizospheres of roots emerging from treated seedpieces in field tests. The bacteria colonized the entire rhizosphere of treated plants including developing daughter tubers and the apical roots of adjacent nontreated plants. PGPR rhizosphere populations were as great as 9.6×10^5 colony-forming units per centimeter (cfu/cm) of root 2 wk after plant emergence and averaged 10^3 cfu/cm throughout the season, but declined to approximately 10^2 cfu/cm at harvest time. PGPR populations were relatively constant throughout the root system. Field tests were done in various soil types with pH ranges from 6.8 to 7.8. PGPR rhizosphere colonization resulted in significant increases in stolon length 2 wk after plant emergence in all six sampled fields. The early season plant growth promotion caused by PGPR was followed by significant yield increases up to 17% compared to untreated controls in four of five harvested fields.

Understanding the dynamics of root colonization by specific microbial components of the rhizoplane/rhizosphere is basic to the development of biological controls of soilborne pathogens and the effective use of beneficial microorganisms to enhance plant growth. Most studies of bacteria in the rhizosphere were involved with the populations of general bacterial groups such as amino acid-requiring, Gram-positive vs Gram-negative, or aerobic vs anaerobic rather than the quantitative monitoring of particular species and strains during different stages of plant growth (1,3,6,7,14). Thus, little information is available on the initial colonization of seeds by specific bacteria, their subsequent colonization of growing roots, and their persistence during the growing period.

Although highly significant increases in yield have resulted from the inoculation of seed with specific bacteria (bacterization) (2,4,10), the results have been erratic, which raises questions about the validity and importance of the phenomenon (5,11,15). These investigations did not determine whether the growth response was related to successful root colonization by the inoculated bacteria. Our experimentation led us to speculate that much of the past variability in results reflected differences in root colonization of the applied bacteria under specific growing conditions. Various factors such as the nature of the inoculum, soil type, and soil moisture greatly affect bacterial survival, root colonization, and persistence.

The genetic marking of bacteria with antibiotic resistance for identification purposes allows the study of population dynamics of soil-inhabiting bacteria. Specific plant growth promoting rhizobacteria (PGPR) that caused marked increases in potato plant growth and yield were marked to follow their populations during the various stages of potato development. This study presents quantitative data concerning the relationship between PGPR

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colonization of potato roots, their persistence during the life of the plant, and their effects on early-season plant growth and potato yield.

MATERIALS AND METHODS

Isolation of plant growth-promoting rhizobacteria and greenhouse assay. Bacteria tested for plant growth-promoting activity were isolated from potato tuber periderms of cultivars White Rose (Shafter, CA), Netted Gem (Tulelake, CA), and Centennial (Monte Vista, CO); from potato roots of cultivars White Rose and Netted Gem; and from celery roots of cultivar 52-70 R in Salinas, CA. Serial dilutions were made from periderm and root washings and plated onto King's medium B (KB) (8). Bacteria present in the greatest numbers were selected to test for plant growth-promoting activity. Fluorescent *Pseudomonas* strain E6 was isolated from celery roots and was previously shown to promote radish growth in greenhouse and field trials (9).

Fifty-eight strains were screened in the greenhouse (temperature 25–29 C during the day and 18–20 C at night) for ability to increase plant growth in an assay modified from Burr et al (4). PGPR strains TL3A, TL3B1, TL3B2, and BK1 were included in some tests because of proven capacity to increase plant growth (4). Single-eye potato seedpieces were dipped into bacterial suspensions containing 10^9 colony forming units per milliliter (cfu/ml) prepared from 24-hr-old cultures scraped from KB plates and resuspended in sterile water, and were immediately planted in moist, sandy loam field soil from Shafter, CA (pH 7.2), in 12.7-cm (5-in) diameter clay pots. Control seedpieces were dipped either in sterile water or in autoclaved suspensions ($\sim 10^9$ cfu/ml) of a test isolate. Typical experiments included six treatments, with six replicate pots per treatment, and three seedpieces per pot. In initial experiments, pots were not watered until plants emerged (10–20 days). In subsequent tests, pots were watered every other day until emergence and then daily until harvest.

Plants were harvested 2 wk after emergence and the weight of either the root system or the entire plant was recorded. The data were analyzed by using a two-way analysis of variance. If a significant F-test result was obtained, differences in treatment and control means were separated by using the LSD test.

Genetic marking of plant growth-promoting rhizobacteria for antibiotic resistance. PGPR isolates A1, B10, TL3, BK1, and E6 were used to obtain antibiotic-resistant mutants. A loopful of each isolate was suspended in 10 ml of sterile distilled water, and 0.1 ml of the suspension was spread on each of six KB plates. After 2 hr of incubation at 28 C, 0.1 to 0.3 ml of 100 μg/ml rifampicin was placed on the center of three plates and 1,000 μg/ml rifampicin on three different plates for each PGPR isolate. The plates were not disturbed until all of rifampicin solution was absorbed into the agar. Following an additional 48–72 hr of incubation, colonies in the center or the edge of inhibition zones were transferred to KB plates containing 100 μg/ml rifampicin. Colonies resistant to rifampicin and with colony size similar to wild PGPR colonies were selected. The marking procedure was repeated with the rifampicin-resistant mutants by exposing them to 100 and 1,000 μg/ml nalidixic acid; purification was done on KB containing 100 μg/ml each of rifampicin (rif) and nalidixic acid (nal). The rif, nal-resistant PGPR were screened for plant growth-promoting capacity in the greenhouse before they were used in field tests.

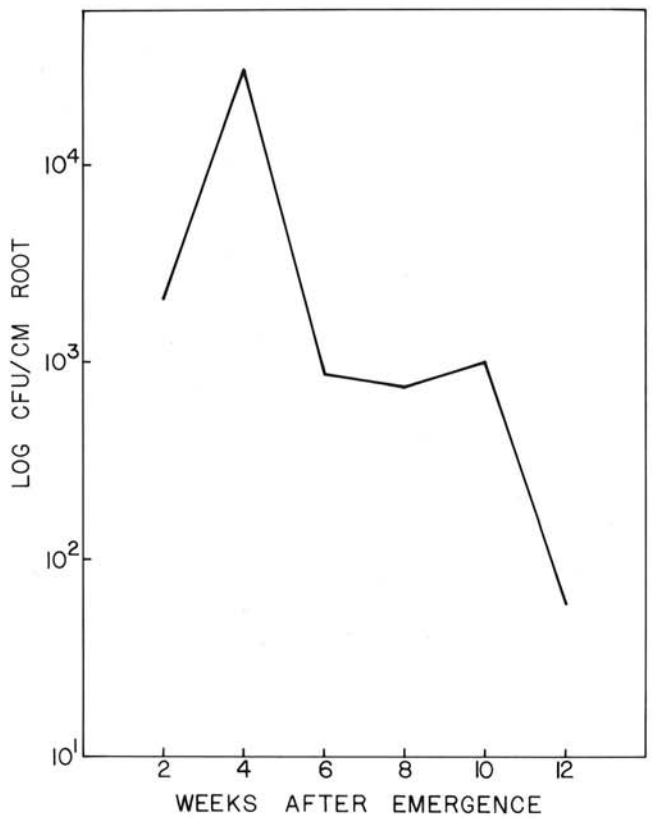

Fig. 1. Potato root populations of plant growth-promoting rhizobacteria in a fine sandy loam (pH 7.1) 1978. Irrigation was terminated after week 10.

TABLE 1. Plant growth increases resulting from inoculation of potato seedpieces with plant growth-promoting rhizobacteria in the greenhouse

Seedpiece treatment[a]	Field soil source[b]	Potato cultivar	Average plant weight[c] (g)	Increase compared to control (%)
B10	Shafter	White Rose	3.6	47
Water control			1.9	···
E6	Shafter	White Rose	1.3	86
Water control			0.7	···
A1	Shafter	Netted Gem	2.8	47
B10			3.1	64
BK1			3.3	74
T13B2			3.0	58
Autoclaved A1 control			1.9	···
A1	Tulelake	Centennial	0.7	250
B10			1.2	500
BK1			0.9	350
Water control			0.2	···

[a] *Pseudomonas* strains A1 and B10 are from potato periderm, E6 from celery root, and TL3B2 and BK1 from potato roots.
[b] Shafter, CA soil is a sandy loam, pH 7.2; Tulelake, CA soil is a peat, pH 6.8.
[c] In all cases inoculation of seedpieces with live bacteria resulted in a significant increase in plant weight ($P = 0.05$). Each value is based on six replications with three plants per replication. Plants were harvested 2 wk after emergence.

TABLE 2. Plant growth increases caused by treatment of potato seedpieces with plant growth-promoting rhizobacteria resistant to rifampicin and nalidixic acid[a] (rif, nal).

Seedpiece treatment[b]	Plant weight (g)[c]	Increase compared to control (%)
rif, nal A1	1.7*[d]	325
rif, nal B10	1.2*	300
rif, nal BK1	1.5*	275
rif, nal TL3B1	1.5*	275
rif, nal TL3B2	2.0*	500
water control	0.4*	···

[a] Greenhouse test using field soil from Shafter, CA.
[b] *Pseudomonas* strains A1 and B10 are from potato periderm; BK1, TL3B1, and TL3B2 are from potato roots.
[c] Average of six replications, three plants per replication.
[d] * indicates significant difference (LSD 0.01 = 0.5) from mean.

Root colonization by plant growth-promoting rhizobacteria. The greenhouse-tested PGPR resistant to rif and nal (rif, nal PGPR) were used in five and three fields in 1978 and 1979, respectively. Inoculum was prepared immediately before planting for the first two fields by suspending 48-hr-old bacteria from 12, 150 × 15-mm diameter KB plates in 4 L of water. In subsequent field tests, inoculum was prepared by suspending PGPR in water containing 0.1 M $MgSO_4$ to maintain the viability of the pseudomonads. Typical field tests were randomized blocks with six treatments and eight to 12 replications of 15.2 m (50 ft) or 7.6 m (25 ft) two-row plots.

Seedpieces were dipped in PGPR-suspensions, or in water for a control treatment, and placed in opaque plastic bags until planting. The planter was not sterilized between PGPR treatments in the first two fields of 1978. In later plantings, the machine was sprayed between treatments with either 95% EtOH or 20% formaldehyde and allowed to air-dry.

The PGPR populations on roots of treated plants were determined 2 wk after plant emergence in all fields during 1978 and 1979, at 2-wk intervals throughout the 1978 season in one field (Fig. 1), and at harvest in one field in 1978. Rhizosphere populations were determined by sampling 50 cm of root per plant, three plants per

TABLE 3. Rhizosphere colonization of potato in field trials by plant growth-promoting rhizobacteria (PGPR) and effects on early season plant growth and yield

Field number, location, date, soil type, pH	Seedpiece treatment[a]	PGPR population (colony-forming units/cm root)	Stolon length (cm)[c]	Average yield (kg/plot)[d]	Increase compared to control[g]
1. Shafter, CA	A1	4.2×10^3	47*[h]	...	...
1978	B10	...	40*	...	...
Sandy loam	TL3B2	...	50*	...	...
pH 7.2	E6	...	54*	...	...
	Control[e]	4.9×10^2	27	...	...
			LSD $_{P=0.05} = 8$		
2. Idaho	A1	5.2×10^3	7.6*	105	5
1978	B10	1.2×10^4	7.1*	102	3
Silt loam	Control	0	2.2	98	...
pH 7.8					
			LSD $_{P=0.01} = 3.6$		
3. Tenneco	A1	9.6×10^5	...	...	...
Ranch	B10	3.8×10^4	...	...	...
1978	E6	8.5×10^5	...	...	...
sandy loam	Control	0	...	...	...
pH 7.0					
4. Tulelake, CA	A1	6.8×10^2	27*	38*	9*
1978	BK1	3.4×10^2	22*	36	0
peat	B10	7.4×10^1	28*	39*	11*
pH 7.0	Control	0	17	35	...
			LSD $_{P=0.05} = 3$	LSD $_{P=0.05} = 2$	
5. Tulelake, CA	A1	7	17*	40*	8*
1979	E6	0	18*	40*	8*
Peat	B10	18	27*	38	3
pH 7.0	Control	0	4	37	...
			LSD $_{P=0.01} = 8$	LSD $_{P=0.01} = 3$	
6. Tulelake, CA	A1	...	...	39*	14*
1979	E6	3×10^2	39*	38*	12*
peat	B10	7	26*	39*	14*
pH 7.8	T13B1	...	...	38*	12*
	Control	0	6	34	...
			LSD $_{P=0.01} = 9$	LSD $_{P=0.01} = 3$	
7. Tenneco Ranch	A1	2.2×10^3	...	...	...
1978	B10	2.3×10^2	...	...	...
fine sandy loam	TL3B2	1.9×10^2	...	...	...
pH 7.1	E6	5.4×10^2	...	...	...
	Control[e]	6.9×10^2	...	...	...
8. Tenneco Ranch	BK1	4.1×10^5	20	54*	17*
1979	TL3B1	2.7×10^4	22*	53*	15*
fine sandy loam	A1	2.8×10^5	25*	49*	7*
pH 7.1	E6	3.6×10^4	30*	48	4
	B10	3.3×10^4	22*	47	1
	Control	0	16	46	...
			LSD $_{P=0.10} = 6$	LSD $_{P=0.01} = 3$	

[a] Fluorescent *Pseudomonas* strains TL3B1, TL3B2, and BK1 were isolated from potato roots, A1 and B10 from potato periderm, and E6 from celery roots.
[b] Average populations were determined from three-to-six replications with three plants per replication and 50 cm root per plant.
[c] Average of five replications with three plants per replication.
[d] Fields 1, 2, 5, and 8 had 15.2 m (50 ft) plots. Fields 3, 4, 6, 7, and 9 had 7.6 m (25 ft) plots.
[e] Control was contaminated with rhizobacteria during planting by splashed inoculum on the mechanical planter.
[f] Field was dry at planting time and maintained at drier-than-normal conditions throughout the season.
[g] Fields not harvested were designed only to measure rhizoplane colonization or increased stolon growth.
[h] * indicates significant increase compared to control.

replication, and usually five to six replications per treatment. Individual plants were dug, roots were shaken to remove excess soil, and 50 cm of root was cut into approximately 5 cm segments by using surface-disinfested scissors. The segments were placed in tubes containing 10 ml of sterile 0.1 M $MgSO_4$. Root samples were processed immediately at a field station or within 24 hr in Berkeley at the University of California, by agitating them in suspension for 1 min, allowing them to settle for 5–10 min, and reagitating for 1 min. Serial 10-fold dilutions were prepared up to 10^{-3}, and a 0.1 ml aliquot of each was plated onto KB containing 100 μg/ml rifampicin, 100 μg/ml nalidixic acid, 100 μg/ml cycloheximide, 30 μg/ml benomyl, 20 μg/ml nystatin, and 20 μg/ml dicloran (botran) to control soil fungi. Colony counts were made after 48 hr of incubation at 28 C, and populations of PGPR per centimeter of root were determined.

Effect of plant growth-promoting rhizobacteria on early-season plant development and yield. Plants in one field in 1978 were weighed 2 wk after emergence to detect early-season increases in plant growth. Five replications per treatment with three plants per replication were selected at random, dug, washed, air-dried, and weighed. In the remaining fields, PGPR effects on early-season plant growth were evaluated by determining the average stolon length 2 wk after plant emergence. Five fields were mechanically harvested and yields were recorded as total weight of tubers.

RESULTS

Isolation of plant growth-promoting rhizobacteria and greenhouse assay. Two strains of fluorescent *Pseudomonas* spp. (A1 and B10) isolated from potato periderm caused significant increases in plant growth when inoculated onto potato seedpieces in the greenhouse (Table 1). Isolates from potato roots did not enhance plant growth. One of four celery root isolates which previously increased radish growth (E6) (9) significantly increased potato growth. The plant growth-promoting activity of *Pseudomonas* strains TL3 and BK1 reported by Burr et al (4) were confirmed.

Genetic marking of plant growth-promoting rhizobacteria for antibiotic resistance. Five PGPR mutants resistant to rif and nal (rif, nal PGPR) retained plant growth-promoting activity in the greenhouse assay (Table 2). The mutants caused an increase in plant weights up to 500% greater than uninoculated controls. This was similar to increases obtained with wild type PGPR (Table 1). Compared to the controls, the PGPR-treated seedpieces developed larger root systems with increased branching.

Root colonization by plant growth-promoting rhizobacteria. The rif, nal PGPR which induced significant weight increases in greenhouse-grown plants colonized roots of treated seedpieces in seven fields with populations ranging from 4.0×10^2 to 9.6×10^5 cfu/cm root in sandy loam fields (pH 7.0–7.2) 2 wk after emergence (Table 3). The PGPR colonization of roots was lower in peat soils (pH 6.8–7.0) (fields 5–7) with populations ranging from 0 to 6.8×10^2 cfu/cm root.

As the season progressed, the PGPR colonized the developing plant roots with populations similar to those on older roots. Root populations in field 3 at 6 wk after emergence averaged 1.3×10^4 cfu/cm at root tips (1–2 mm) and 2.4×10^4/cm on root segments 1–5 cm distal to the tip. There was no difference in the average PGPR population on roots originating from the seedpiece and roots originating from the stem near the soil surface. PGPR were detected on root tips of neighboring untreated border plants outside the test plot with populations averaging 300 cfu/cm, which indicated that the PGPR are efficient root colonizers.

Colonization persisted throughout the growing season with levels fluctuating between 10^2 to 10^3 cfu/cm root (Fig. 1). Root populations of PGPR dropped approximately 1.5 log units when the irrigation was terminated in preparation for harvest. A decrease in PGPR rhizoplane populations at the end of the season also was observed in field 9 (Table 4). The magnitude of population decreases over the season varied markedly with different PGPR isolates, with decreases up to four log units occurring with BK1 and as little as 1.5 with TL3B1 (Table 4).

Effect of plant growth-promoting rhizobacteria on early-season plant development and yield. An increase in total plant weight similar to that obtained in the greenhouse assay was the first detectable plant response to PGPR root colonization in the field (Table 5). Increased plant weights up to 100% greater than controls occurred with PGPR-treated plants dug at random in field plots 1 wk after plant emergence. At 2 wk after emergence, statistically significant increases in stolon length were evident (Fig. 2) in all six sampled fields (Table 3). Enhancement of stolonization was associated with PGPR colonization regardless of soil type or potato cultivar; it ranged from 25 to 550%.

Yield increases occurred in all five harvested fields and were significant at $P = 0.01$ in four fields (Table 3). Significant increases

TABLE 4. Early and late-season potato root colonization by plant growth-promoting rhizobacteria[a]

Seedpiece treatment[b]	Bacterial root populations (colony forming units/cm root)	
	2 wk after plant emergence	1 wk before harvest
A1	2.8×10^5	2.4×10^3
B10	3.3×10^4	4.7×10^2
TL3B1	2.7×10^4	6.9×10^2
BK1	4.1×10^5	5.4×10^1
E6	3.6×10^4	6.3×10^2
Control	0	0

[a] Field was a fine sandy loam soil near Shafter, CA (pH 7.1) 1979.
[b] *Pseudomonas* strains A1 and B10 are from potato periderm, E6 from celery root, and TL3B2 and TL3B1 from potato roots.

TABLE 5. Increased plant weight following potato seedpiece inoculation with plant growth-promoting rhizobacteria[a]

Seedpiece treatment[b]	Average plant weight (g)[c]
A1	50*[d]
B10	39*
TL3B2	33*
E6	32*
Control	25*

[a] Field was a fine sandy loam soil near Shafter, CA (pH 7.1). Samples were taken 1 wk after emergence, 1978.
[b] *Pseudomonas* strains A1 and B10 are from potato periderm, E6 from celery root, and T13B2 and BK1 from potato roots.
[c] Average of five replications, three plants per replication.
[d] * indicates significant difference from check (LSD $P = 0.05 = 4.0$).

Fig. 2. Typical increase in potato stolonization and early tuberization resulting from treatment of seedpieces with plant growth-promoting rhizobacteria (on right) compared with untreated control (on left). The increases were statistically significant in all field tests.

of 17 and 15% with BK1 and TL3B1, respectively, occurred in sandy loam soils, and 12% to 14% with A1, B10, T13B1, and E6 in peat soils.

DISCUSSION

Recovery from field-grown potato plants of genetically marked strains showed that PGPR aggressively colonized the rhizospheres of roots of developing plants up to 9.6×10^5 cfu/cm (Table 3), and that they persisted on roots for the duration of the growing season. PGPR colonization of roots was related to early-season plant growth increases as evidenced by increased total plant weights and length of stolons. These early-season plant responses to PGPR subsequently were followed by increased yields.

PGPR colonized various portions of the rapidly-growing root system at relatively uniform levels 6 wk after plant emergence. There was little difference in populations of PGPR detected in the rhizosphere of root tips. The aggressive nature of the PGPR in colonizing all parts of the potato rhizosphere indicates that they are natural root-colonizing bacteria and are vigorous rhizosphere competitors. An indication of the aggressive colonization capacity of the PGPR was their spread from treated to nontreated plants in border rows.

Some of the variability observed in past bacterization studies in obtaining yield increases is probably caused by the failure of the introduced bacteria to colonize the rhizosphere. Absence of colonization could result from any number of factors such as ineffective strains, viability of the introduced bacteria, soil moisture, soil type, and soil temperature. Broadbent et al (2) reported yield variability from season to season in field tests using *Bacillus* sp. as treatment and attributed this to uncontrolled physical or biological factors without presenting any data on rhizoplane colonization of the introduced bacteria. The great variation in results with *Azotobacter* spp. were related to failure of the bacteria to colonize roots in various experiments (3,14). These and other studies on the capacity of specific bacteria to colonize the rhizosphere were limited by the lack of methods to identify the strains. Identification was generally made by colony morphology (4,14), in vitro requirements for amino acids (6), antigenic reactions (4), or biochemical reactions in vitro (1). The use of genetically marked PGPR strains resistant to antibiotics allows a precise determination of rhizoplane colonization.

Rhizosphere populations of PGPR were greater than populations reported for most bacteria used in bacterization studies. The maximum populations reported for inoculated *Azotobacter* on plant roots was 1.1×10^4 bacteria on the entire root system (7) which is markedly less than populations of our PGPR which frequently reached 10^4 cfu/cm. Our populations also were greater than those reported for total bacterial rhizosphere colonization by several workers. Rouatt and Katznelson (13), for example, reported total rhizosphere bacterial population averages of 300×10^6 cells per gram of root (wet weight) and Peshakov (12) reported 1×10^6 bacteria per gram (dry weight). While a direct comparison of these values based on population per gram with ours is difficult, they are log units less than the PGPR root populations detected in our study.

The PGPR-induced increases in plant growth and yield reported here confirm the work of Burr et al (4) with strains BK1 and TL3 and extend the work with three additional PGPR strains A1, B10, and E6. In this study statistically significant yield increases were obtained for the first time in fields with peat soils using strains A1 and E6. Only one of the four PGPR which increased radish growth (E6) also increased potato growth, suggesting a possible host specificity of PGPR strains.

The mechanism by which PGPR promoted increased plant growth is unclear. However, these data indicate that plant response is associated with rhizosphere colonization by the bacteria. Whether the bacteria elaborate products which directly influence plant growth and/or affect the composition of the root microflora by antagonism remains to be discerned.

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