Plant Growth-Promoting Rhizobacteria and Plant Growth Under Gnotobiotic Conditions

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ABSTRACT

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Increases in radish and potato plant growth caused by inoculating seeds or seed pieces with plant growth-promoting rhizobacteria (PGPR) were apparently not related to the production of bacterial products that directly stimulated growth. Radish seeds inoculated with PGPR and grown under gnotobiotic conditions did not produce larger plants than water-treated controls, even though the PGPR colonized the plant roots. When radishes were grown under the same, but unsterile conditions, plants grown from seeds treated with PGPR exhibited significantly greater growth ($\leq$150%) than did untreated controls. Radish seeds treated with rhizobacteria in sterile cellophane growth packets produced hormonal-type increases in branching or total length of roots; however, there was no relationship between increased root development in growth packets and subsequent growth responses by radish inoculated with the same PGPR. These results suggest that PGPR increase plant growth indirectly by interacting with the native root microflora rather than directly by producing growth-promoting substances.

The capacity of specific plant growth-promoting rhizobacteria (PGPR) to cause yield increases of potatoes up to 33% (5) and of radishes up to 100% (13) prompted an investigation to determine the mechanism. One hypothesis is that the PGPR elaborate substances that directly stimulate plant growth such as nitrogen, hormones, or compounds, which promote the mineralization of phosphates. Another proposed mechanism concerns the interactions of PGPR with rhizosphere microflora and the possible displacement of detrimental microorganisms.

The hypothesis that elaboration of bacterial products is related to plant growth promotion and yield increase is consistent with the idea that bacterial products play an important role in stimulating plant growth. This hypothesis, however, is controversial because there are no definitive supporting data. For example, *Azotobacter*, *Bacillus*, and *Clostridium* spp. were thought to increase plant growth by nitrogen fixation or solubilization of soil phosphates (1,3,9,10) but Mishustin and Naumova (16) calculated that the amount of nitrogen or phosphates available to plants as a result of bacterial metabolism could not account for the observed growth increases. Several workers suggested that plant hormones, sometimes produced in vitro by *Bacillus* spp. and fluorescent pseudomonads, may increase plant growth (2,4,6–8,11,17). However, data on the activity of hormones in the rhizosphere are lacking.

The hypothesis that PGPR increase plant growth by interacting with root microflora seems likely since PGPR aggressively colonize roots at populations up to 9×10^5 colony forming units per

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centimeter of root (cfu/cm) (14). Such populations should alter the composition of rhizosphere microflora.

This paper reports the results of experiments in which plants were grown under gnotobiotic and nongnotobiotic conditions to determine whether plant growth promotion is caused by the elaboration of products from PGPR. If plant growth enhancement by PGPR occurs only when plants are grown in field-collected soils, this would strongly suggest that the mechanism is related to interactions with soil microflora.

MATERIALS AND METHODS

Effect of PGPR on plants under gnotobiotic conditions. Three experiments were done in which rifampicin and nalidixic acid-resistant PGPR (rif, nal PGPR) (13) were applied to radish seeds under gnotobiotic conditions. In all experiments, seeds were agitated for 2.5 min in 1.5% sodium hypochlorite and rinsed three times with sterile water. Ten seeds were individually transferred to tubes containing 5 ml of sterile nutrient broth and incubated for 72 hr at 28 C to check the effectiveness of the sterilization. Treated seeds were agitated in 10^9 cfu/ml PGPR suspensions and planted in 600 g of twice-autoclaved field soil (sandy loam from Shafter, CA) contained in 2-L flasks sealed with cotton and aluminum foil. The soil was allowed to stabilize for 2 wk after autoclaving before it was used in the experiment. The persistence of sterile conditions during the experimentation was checked by adding three 1-g soil samples from flasks at planting and harvesttime to 100 ml of sterile nutrient broth. In addition, three 1-g soil samples were placed directly onto agar plates of King's medium B (KB), nutrient agar (NA), and potato-dextrose agar (PDA) and incubated at 27 C for 14 days. Flasks were planted and watered with half-strength Hoagland's solution inside a laminar flow hood. Five to seven replicate flasks, each with two to three plants, were used per treatment. Plants were harvested and weighed 5 wk after planting. Roots from nontreated plants and PGPR-treated plants were suspended in 10 ml of sterile water, agitated, and 1-ml aliquots of each were plated on NA, KB, and rif, nal KB.

PGPR strains E10 and E8 were used in the first experiment, strains E6, E8, and E10 in the second experiment, and strains E2, E6, and E8 in the third experiment. Water-treated controls were used in each experiment. The third experiment was duplicated with unsterilized field soil in sealed flasks as a positive control to detect growth promotion. Data from each experiment were analyzed by using a two-way analysis of variance. If a significant F-test resulted, means were separated by using the LSD test.

Bacterial effects on root development in growth packets. Radish seeds were surface-sterilized by agitating for 2.5 min in 1.5% sodium hypochlorite, rinsed in sterile water and agitated in 10^9 cfu/ml suspensions of 30 bacterial isolates from rhizospheres of healthy radish plants grown in field soil. Seeds were transferred to sterilized cellophane growth packets (diSPO Seed Packs, Northrup King & Co., Minneapolis, MN 55413), watered with sterile Hoagland's solution, and placed under nonsterile conditions. After incubation for seven days at 22 C, roots of bacteria-treated seedlings were examined under ×10 magnification and compared to roots on water-treated controls for morphological alterations such as total length or amount of branching.

The same 30 isolates were screened in the greenhouse for the plant growth-promoting activity. Radish seeds were agitated in 10^9 cfu/ml bacterial suspensions and were planted in field soil in the greenhouse. Plants were harvested and roots were weighed 8 wk after planting. Bacteria significantly promoting radish growth were compared with those stimulating root development in the growth packets.

TABLE 1. Comparison of radish growth promotion by rhizobacteria under gnotobiotic and nonsterile conditions

Seed treatment[a]	Treatment conditions	Average plant weight (g)
E8	Nonsterile[b]	1.5*[c]
E2		0.9*
E6		1.3*
Control		0.6
E10	Gnotobiotic[d]	1.4
E8		1.3
Control		1.3
E6	Gnotobiotic[e]	1.9
E8		1.6
E10		1.4
Control		1.7
E8	Gnotobiotic[f]	0.6
E2		0.8
E6		0.8
Control		0.8

[a] PGPR strains: E10 is an unidentified gram-negative rhizobacterium from celery roots; E2, E6, and E8 are fluorescent pseudomonads from celery roots.
[b] Average of six replications; five plants per replication.
[c] *Indicates significant difference ($LSD_{0.01}$= 0.3).
[d] Average of five replications; two plants per replication.
[e] Average of seven replications; two plants per replication.
[f] Average of six replications; five plants per replication.

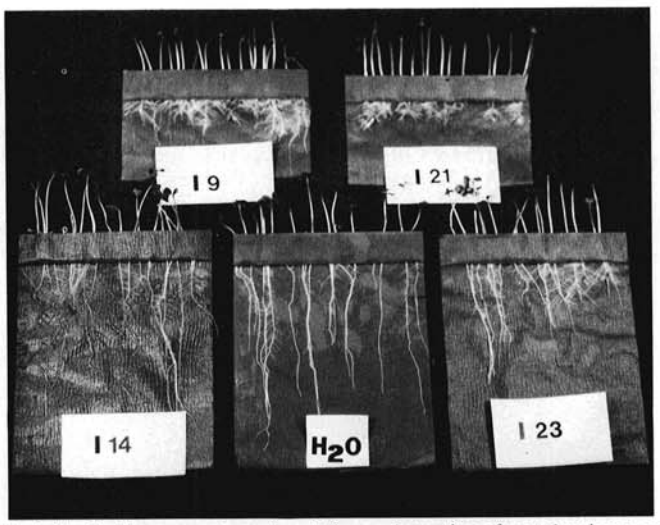

Fig. 1. Typical hormone-type stimulation and stunting of root development following inoculation of radish seed with rhizobacteria and planting in unsterile soil. Isolates I9 and I23 increased fibrous root development, I14 increased lateral branching, and I21 stunted root development.

TABLE 2. Independence of root development effects caused by plant-growth-promoting rhizobacteria (PGPR) in gnotobiotic growth packets and growth promotion in unsterilized soil

Seed treatment[a]	Appearance of roots in growth packets[b,c]	Increase in plant growth in soil[d] (%)
I2	Short and fibrous	...
I9	Short and fibrous	...
I16	Short and fibrous	...
I18	Short and fibrous	...
I23	Short and fibrous	...
I14	Thin with increased lateral branching	...
I24	Thin with increased lateral branching	364
I21	Stunted with brown root tips	342
I4	Similar branching with less total growth[c]	364
I5	Similar branching with less total growth[c]	329
I7	Same as control	221
I11	Same as control	257

[a] Unidentified rhizobacteria isolated from roots of healthy radish plants grown in field soils.
[b] Plants were grown 7 days in sterile cellophane packets.
[c] The descriptions are relative to the control roots.
[d] Average of six replications, three plants per replication. Growth was measured by weighing radish roots.

RESULTS

Effect of PGPR on plants under gnotobiotic conditions. There was no difference in growth of PGPR-treated and nontreated radish plants grown in autoclaved field soil under gnotobiotic conditions (Table 1). The same PGPR, however, caused a significant increase in plant growth (50–150%) when grown in unsterilized field soil in sealed flasks (Table 1). Seed germination was 95% in flasks under gnotobiotic conditions compared to 76% in raw soil. Plants in the gnotobiotic system developed normally, with no noticeable adverse effects from autoclaving of the soil.

There was no evidence of contamination on surface-sterilized radish seed, autoclaved soil, or roots from nontreated control plants grown under gnotobiotic conditions. All four PGPR colonized roots of plants under both gnotobiotic and nonsterile conditions with populations that ranged from 1×10^4 to 7×10^4 cfu/cm.

Bacterial effects on root development in growth packets. Although many of the bacteria inoculated onto radish seed affected root growth in growth packets, this was not related to their plant growth-promoting activity in soil.Twenty-three of the 30 bacteria that were screened for effects on root development either did not affect or inhibit root growth (Table 2). Seven bacteria caused an increase in total root length or increases in the fibrous root hair branching (Fig. 1) similar to that observed in bioassays for plant growth-promoting substances. However, only strain 124, which promoted root growth in growth packets, also promoted radish plant growth in the soil. Strain 121 increased growth of radish roots in soil but caused root-tip necrosis and overall stunting in growth packets. Strains 14, 15, 17, and 111 promoted plant growth in nonsterile soil, but had either no effect on or decreased root development in the packets.

DISCUSSION

The gnotobiotic experiments with radish and PGPR suggest that enhancement of plant growth is caused by the interaction of PGPR with the native rhizosphere microflora and not the production of metabolic products that could directly increase growth. Although PGPR grown under gnotobiotic conditions readily colonized radish roots, growth responses occurred only when radish seeds inoculated with PGPR were grown in unsterile field soil. Some rhizobacteria caused hormonal-type effects on plant roots as revealed in growth packets; however, there was no relationship between these results and subsequent plant growth increases when plants were grown in nontreated field soil.

The possibility that the use of autoclaved field soils in the gnotobiotic tests was not conducive for the elaboration of growth-promoting substances seems unlikely. The soil was allowed to stabilize 2 wk after autoclaving, and the uninoculated plants grew as well or better in this soil as in the nonautoclaved soil. Furthermore, PGPR in various greenhouse experiments with radish only increased plant growth when they were grown in nontreated field soil and never when grown in autoclaved UC Mix under unsterile conditions.

The contention that plant growth promotion by PGPR is related to reductions in populations of pathogens in the rhizosphere (3,5,15) rather than to production of growth-promoting substances is also supported by the findings that they substantially affect the bacterial and fungal composition of the root zone microflora (12,18,19). However, our continuing studies of PGPR indicate that they are a diverse assemblage of bacteria representing several bacterial groups. Thus, it is likely that other mechanisms affecting plant growth will be discovered in future experiments.

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