Beneficial bacteria enhance plant growth

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Potato field trial in Kern County testing several strains of plant-growth-promoting rhizobacteria in replicated trials.

Direct use of microorganisms to promote plant growth and to control plant pests continues to be an area of rapidly expanding research. The capacity of specific root-colonizing bacteria, or rhizobacteria, to increase growth and yield of crop plants currently is attracting considerable attention. The process by which bacteria are applied to soil and plant parts has been termed bacterization and was pioneered in the Soviet Union.

By 1958, more than 35 million hectares of crop lands were treated with strains of *Azotobacter* and *Bacillus* spp., which were considered to be bacterial fertilizers. Benefits were reported on 50 to 70 percent of field crops tested, with yield increases ranging from 10 to 20 percent. Greatest benefits were reported on vegetable crops grown on well-watered, fertile soil, although the most extensive application of beneficial bacteria was on grain crops. Soviet scientists E.N. Mishutin and A.N. Naumova, in a critical evaluation of the many claims of success with bacterial fertilizers, concluded that about two-thirds of the applications had no significant effect on crop yield. When positive results were reported, they usually were not obtained from replicated trials and had not been statistically evaluated. Nevertheless, these reviewers concluded that under cetain conditions yield increases from 10 to 13 percent were obtained and confirmed by statistical analysis, establishing increased crop yield from bacterization as a real phenomenon.

Our interest in bacterization increased markedly when we obtained substantial increases in root and foliar growth with several crop plants. In field trials, yields of sugar beets increased up to 15 percent, potatoes up to 33 percent, and radishes as much as 144 percent when seed or seed pieces were treated with selected bacterial strains before planting. This report summarizes results from our studies on the use of specific plant-growth-promoting rhizobacteria (PGPR) and their mode of action.

Selection and characterization of strains

Specific strains of PGPR were initially selected from several hundred root-colonizing bacteria isolated from excised roots of field-grown plants. Potential PGPR were selected for their ability to inhibit growth of various bacterial and fungal pathogens or miscellaneous rhizosphere (root-zone) bacteria and fungi in the laboratory. Pure cultures of antagonistic bacterial strains were then screened in greenhouse trials using field soils. Seed or seed pieces were treated with a 10^8 colony-forming-unit (cfu)/ml bacterial suspension and planted in replicated pot tests. Strains that consistently caused statistically significant increases in root or shoot development, or both, were selected for further testing in field trials.

The vast majority of bacteria that exhibit antibiosis towards some test organisms do not stimulate plant growth in greenhouse or field trials. However, *in vitro* (laboratory) antibiosis against a wide spectrum of microorganisms is a consistent property of PGPR. Recently, selections for PGPR have been made by mass screening all rhizosphere bacteria, obtained in dilution platings, for plant-growth-promoting potential. Strains that caused increased plant growth were subsequently shown to have antagonistic properties, *in vitro*, similar to those of the original PGPR strains. In general, 1 to 4 percent of the rhizosphere bacteria isolated from potato and sugar beet proved to have plant-growth-promoting ability.

Biochemical-physiological tests show that most PGPR belong in the *Pseudomonas putida* and *Pseudomonas fluorescens* groups but do not coincide with any of the described strains in Bergey's Manual. Six PGPR from radish, currently unidentified, are facultatively anaerobic, gram-negative rods.

Inoculum preparation for field tests

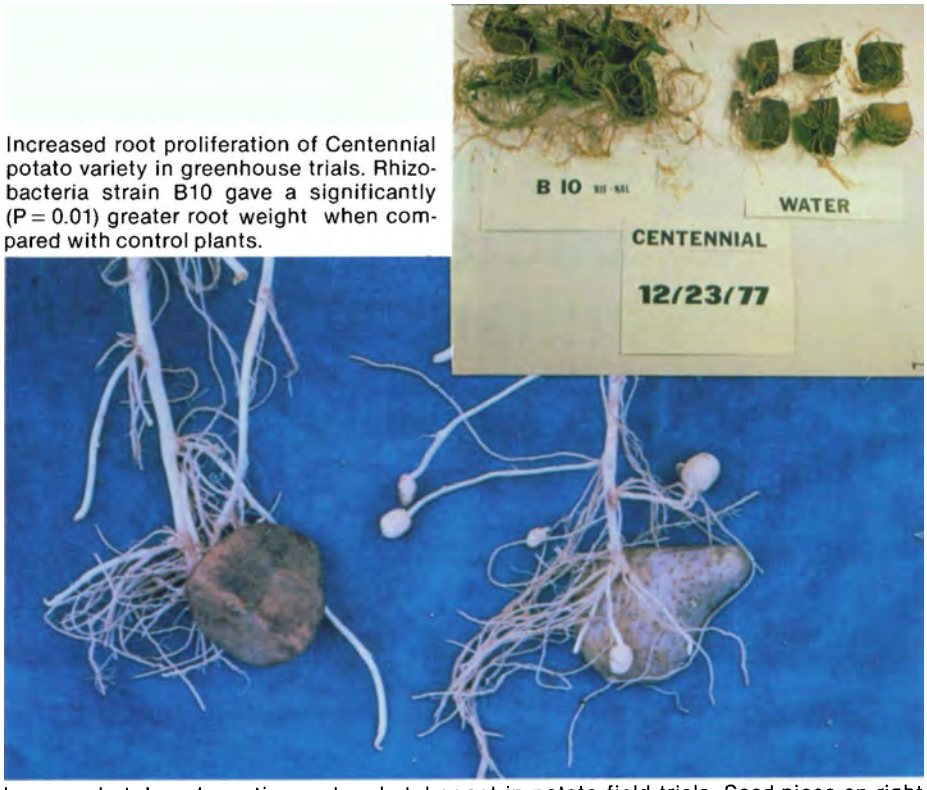
Field testing of potential PGPR requires an inoculum delivery system that maintains high populations of viable bacteria in storage and on plant parts. Treating seed with liquid suspensions of bacteria in the initial field experiments was unsatisfactory, because it was difficult to apply suspension in the field, liquids were not compatible with planting systems, and the bacteria rapidly lost viability in storage.

Incorporating these non-spore forming bacteria directly into dry powders such as talc or clays is not possible, because the bacteria do not survive for extended periods in these materials. Recently, however, the coating of bacteria with certain gums and polysaccharides allowed their formulation as a dry powder without appreciable loss of viability. Populations of 10^8 cfu or greater can be obtained per sugar beet seed when pelleted with these formulations. PGPR powder applications to potato seed pieces, sugar beet, and radish seed have been very effective in field tests.

Summary of field results

The initial emphasis was to determine whether specific PGPR could significantly increase plant growth and yield under field conditions and to evaluate the consistency of the phenomenon in the same and different locations. A total of 33 replicated

Seedling growth of sugar beet increased by pelleting seed with plant-growth-promoting rhizobacteria.



Increased root proliferation of Centennial potato variety in greenhouse trials. Rhizobacteria strain B10 gave a significantly (P = 0.01) greater root weight when compared with control plants.

Increased stolon elongation and early tuber set in potato field trials. Seed-piece on right was treated with strain A-1.

field plots, each testing three to six different PGPR for ability to increase growth and yield of potato, radish, and sugar beet, have been evaluated since 1975. The experimental design was either a randomized complete block or a Latin square with five to eight replications. In some cases plots were not followed through to harvest due to the nature of the test being conducted or because of problems in growth and care of the crop that were not related to the experiment.

The first observable effects of PGPR were increased seedling vigor, root growth, and top growth with radish and sugar beets. Differences in top growth of treated and nontreated potato plants are difficult to discern, especially after complete emergence. The most striking difference observed on potato was a significant ($P = 0.05$) increase in stolon length 2 weeks after plant emergence in 7 of 7 field tests. Associated with the increased elongation is a consistent early tuber set and increases in final yield.

Statistically significant increases in potato yields have ranged from 5 to 33 percent in 7 of 11 field plots in California and Idaho. Yield increases due to pelleting of sugar beet seed with PGPR ranged from 4.4 to 8.4 tons per hectare with increases in total sucrose ranging from 20.7 to 26.9 cwt per hectare ($P = 0.05$ or 0.01) in 4 out of 6 plots.

Field results with radish were especially striking; significant increases of 60 to 144 percent in root weight occurred in 7 of 7 trials ($P = 0.01$). The span of only 35 days from planting to harvest of the radish favors maximum benefit from bacterial treatment. These results agree with observations of Soviet scientists, who noted that bacterization effects were greatest with crops of short cropping period.

There is little definitive information to support any of the theories of how bacterization increases yield. Two of the principal hypotheses are that the bacteria produce growth hormones, or that they may act as biological control agents by displacing harmful microorganisms that commonly colonize roots. We favor the latter hypothesis on the basis of several experiments.

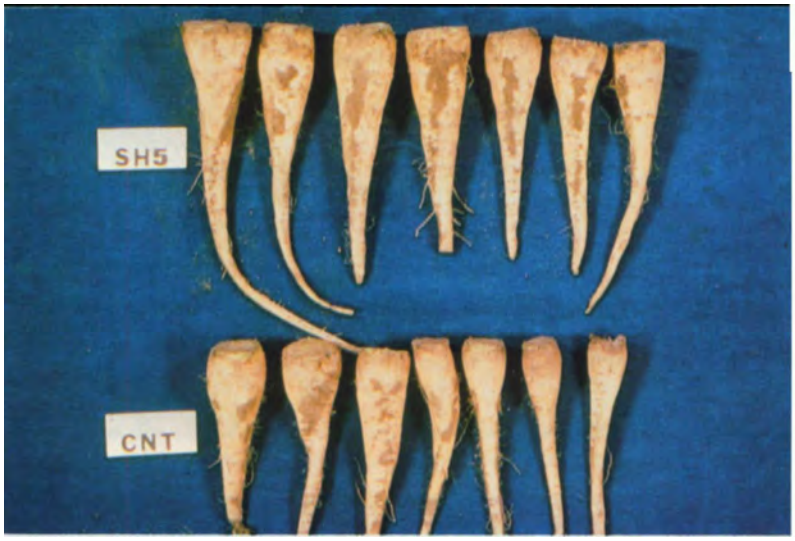
In greenhouse trials, radish seeds sterilized with sodium hypochlorite were planted in autoclaved or nonautoclaved field soil inside sealed Erlenmeyer flasks. The PGPR strain E6 increased radish root weight (>100 percent compared with the untreated control) only when nonautoclaved field soils were used. In autoclaved soils, there was no significant weight difference between radishes in PGPR-treated flasks and those in nontreated flasks, indicating that the effects of PGPR bacteria are due to interactions with microflora on roots rather than to elaboration of hormones or growth factors.

This conclusion also is supported by the finding that PGPR seldom affect growth of sugar beets or potatoes when sterilized U.C. mix is used in greenhouse tests. Growth increases generally are greater in field soils that have been previously used to grow the test crop. In a few tests with potato, growth enhancement occurred in U.C. mix, probably because potato seed pieces are colonized by high populations of various microorganisms that have contaminated the sterilized planting mix. It is not possible to sterilize potato seed pieces, since sterilization agents do not eliminate many microorganisms that colonize the lenticels.

High populations of viable PGPR on seed ensure the rapid colonization of roots once germination and root growth begins. The capability, extent, and duration of PGPR colonization of roots were tested with strains genetically marked for resistance to the antibiotics rifampicin (RIF) and nalidixic acid (NAL). Roots were removed from soil throughout the season and plated on a medium containing the antibiotics. Since the antibiotics inhibited native, nonresistant rhizosphere bacteria, populations of PGPR resistant to RIF-NAL were easily quantified. Populations of RIF-NAL-resistant PGPR were as great as 9×10^5 cfu/cm on potato. In a separate test, populations of these bacteria remained on roots throughout the season, but decreased sharply to low levels when irrigation was terminated. Similar results were obtained with antibiotic-marked PGPR specific for sugar beets.

Effect of PGPR populations on other root microflora

High populations of PGPR may affect colonization of roots by *Rhizoctonia, Verticillium, Pythium,* and other classical plant pathogens. However, the greatest benefit of seed treatment may be inhibition of slightly parasitic or nonparasitic but toxigenic microorganisms. We believe that toxigenic bacteria in plant rhizospheres may have a considerable effect on plant growth. Although little is known about this group of root-colonizing bacteria, they are abundant and were encountered frequently when screening bacteria for growth-promoting ability. Some strains caused signifi-



Early season root weight increase in field trial. Rhizobacteria strain SH5 caused a 72 percent increase in root weight at week 8.

Dry powder formulation of plant-growth-promoting rhizobacteria for seed or seed piece treatment.

cant decreases in plant growth, ranging up to a 69 percent reduction in seedling weight of sugar beets. The PGPR were antagonistic *in vitro* to most of these deleterious bacteria. The role of deleterious bacteria in depressing root growth is under study.

PGPR treatment caused significant quantitative changes in both bacterial and fungal populations of root systems. A 60 percent decrease in the total gram-positive bacterial population and up to an 80 percent reduction of root-colonizing fungi were detected with PGPR-treated roots when compared with untreated controls. Qualitative measurements of microbial population changes are currently being investigated.

Discussion

The application of beneficial bacteria to seed to increase yields and reduce pesticide use is an attractive and likely prospect; the benefits could be considerable. The commercial success of bacterization as a common agricultural practice will depend on such aspects as cost-benefit ratios, widespread applicability of specific strains, development of improved inoculum delivery systems, and consistent positive results.

Selection of efficacious strains of PGPR is highly complicated; variation in watering, soil compaction and aeration, or light intensity may prevent the detection of PGPR in greenhouse trials. Watering is particularly critical, because PGPR do not become established on root systems when the soil is allowed to dry below -1.7 bars. To increase reliability of greenhouse trials, we recently have employed a drip irrigation system, operated with a timer for more uniform watering and prevention of soil crusting. Greenhouse tests with crops such as potatoes and sugar beets often are difficult to evaluate, because the commercial plant part cannot be grown to maturity in pot trials. Potential PGPR selected in greenhouse trials have been ineffective in field tests, where environmental conditions are more variable. Greenhouse tests only serve as a preliminary indication of field potential. As with any beneficial microbe, PGPR cannot be effectively used in the field unless the ecological conditions that favor their growth and survival are met.

Positive results in field trials have become more consistent as greater efforts have been made to prevent contamination of control plots with PGPR during the planting process. Another principal reason for inconsistencies in yield increases obtained when working in different locations was that some bacterial strains appeared to work only in certain soil types. One strain of sugar beet PGPR that increased yield in California field tests failed consistently in Idaho, while a different PGPR caused greatest yield benefits in Idaho but had no significant effect in some California trials. A similar situation occurred with potato: some PGPR increased yield in peat soil but not in loamy sand, whereas others functioned inversely.

The commercial use of PGPR must await the development of coating technology to improve methods of storing and applying bacteria without loss of viability, and in a form that allows rapid growth and colonization of roots. An exciting possibility that may increase the future potential of beneficial bacteria is their incorporation into seed tapes, matrixes containing transplant seedlings, and gels used in gel extrusion planting. These methodologies would permit the introduction of very high populations of PGPR to plant parts, providing ideal conditions for rapid colonization of roots.

(Registration of new soil amendments and microbial agents by the California Department of Food and Agriculture requires submission of valid scientific evidence with statistical analyses of replicated tests. Currently, only microorganisms of the *Rhizobium* species, *Agrobacterium radiobacter, Bacillus thuringiensis*, and some ecto- and endomycorrhizal fungus species are registered with CDFA as soil amendments and microbial agents.)

TABLE 1. Effect of PGPR on Seedling Growth of Sugar Beets

Location	Bacterial Strain	Avg wt Seedling (gm)*	Incr. (%)
Westside Field Station (1977)	Nontreated control	12.1a	—
	B2	15.0b	23
	SH5	15.5b	28
	B4	16.2b	33
U.C. Davis (1978)	Nontreated control	30.4a	—
	B4	41.1b	35
	E6	41.2b	35
	A-1	44.0b	45
	RV3	45.4b	49
	SH5	47.5c	56

*Numbers followed by different letters indicate significant differences at P = .02 (seeds in all trials treated with Dexon, Lindane, and PCNB)

TABLE 2. Length of Potato Stolons 2 Weeks after Emergence

Treatment	Avg Stolon Length per Plant (cm)*
Control	4a
A1	17b
E6	18b
B10	27c

*Average of 4 replications, 3 plants per replication numbers followed by different letters indicate significantly different means LSD 0.05 = 8.

TABLE 3. Potato Root Populations of Plant-Growth-Promoting Rhizobacteria

Treatment	Avg cfu/cm root* 2 wks after emergence	1 wk before harvest
Control	0	0
B10	3.3×10^4	4.7×10^2
E6	3.6×10^4	6.3×10^2
A1	2.8×10^3	2.4×10^2

*Average of five replications, three plants per replication, 50 cm root/plant; all bacteria strains were marked with resistance to the antibiotics rifampicin and nalidixic acid.

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