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Pseudomonas Inoculants to Benefit Plant Production

Certain *Pseudomonas* strains, particularly *P. fluorescens* and *P. putida* (fluorescent pseudomonads), are adapted to colonize plant roots. Selected strains can significantly promote plant growth, following seed inoculation under field conditions. This bacterium-induced growth promotion may result from the following mechanisms: 1) production of siderophores [high-affinity iron(III) chelators] and the resulting iron deprivation from deleterious rhizosphere microorganisms; 2) production of antibiotics which are toxic to soil-borne plant pathogens; 3) production of plant growth regulators which stimulate plant growth; and 4) enhancement of phosphate uptake by the plant. Field trials with specific pseudomonad strains have demonstrated yield increases, control of soil-borne plant pathogens, promotion of seedling emergence, and promotion of legume nodulation by nitrogen-fixing *Rhizobium* spp. Several companies are now developing products which consist of *Pseudomonas* strains for use in agriculture.

BACKGROUND

Inoculation of crop seeds with beneficial bacteria has been a component of agricultural production throughout the twentieth century. The most common inoculants consist of nitrogen-fixing strains of *Rhizobium*, which form nodules on legumes and hence are termed symbiotic. The potential of free-living (nonsymbiotic or "associative") bacteria to promote plant growth has been reported over the past 30 years.

Early attempts to improve plant growth with free-living bacteria concentrated on phosphate-solubilizing bacteria

such as *Bacillus megaterium* var. *phosphiticum* or on free-living nitrogen-fixing bacteria such as *Azotobacter* (1,2). The pioneering work with free-living phosphate-solubilizing and nitrogen-fixing bacteria demonstrated the potential benefits of inoculation, as yield increases of up to 25% were sometimes obtained (3). However, these bacteria had a low rhizosphere (root zone) competence and did not compete effectively with the native soil microflora, as evidenced by their declining populations after introduction in the rhizosphere. This observation prompted the "biological balance" (4) or "microbial equilibrium" (5) theory, which suggested that the rhizosphere microflora is a diverse collection of organisms in equilibrium. The premise was that the population of an introduced organism would decline following its introduction to the rhizosphere, and the original microbial balance would be reestablished quickly.

However, in the late 1970s, researchers at the University of California in Berkeley discovered that the microbiological balance of the rhizosphere could be altered by inoculating plant parts with specific strains of rhizosphere pseudomonads. These pseudomonads changed the balance of both fungal and bacterial microfloras by colonizing roots throughout the growing season (6). Bacterial colonization of roots was strain-specific in that not all fluorescent pseudomonads efficiently competed and persisted in the presence of other soil microorganisms. Some pseudomonads induced increased plant growth and were termed PGPR (plant growth-promoting rhizobacteria). Pseudomonad PGPR were associated with yield increases of 10 to 30% with root crops such as potato, radish, and sugarbeet (7). Recent work has confirmed the potential for obtaining yield increases with PGPR on seed crops (8). Pseudomonad PGPR also inhibited a number of soil-borne plant pathogens (reviewed in 9).

The number of research groups working with PGPR is growing each year, and therefore an understanding of the beneficial effects of PGPR and of the mechanisms by which PGPR affect plant growth is also increasing. The First International Workshop on PGPR was held in 1987 in Canada and was attended by 60 people involved with PGPR research, development, and commercialization. This review is intended to summarize the range of research activities on pseudomonad PGPR and current directions toward commercializing them.

CURRENT STATUS

Mode of Action

The siderophore theory Promotion of plant growth by the pseudomonad PGPR strains reported by the Berkeley group was associated with siderophore production (10). Siderophores are high-affinity iron(III) transport agents which are produced under conditions of low iron concentrations (11). Certain pseudomonads produce siderophores which chelate the ferric iron in the rhizosphere, thus inhibiting other microorganisms, including plant pathogenic or deleterious species, with less affinity for iron.

The siderophore theory is supported by the following findings:

1. Addition of dissolved iron(III) to the environment prevents the inhibition of in vitro antagonism and also nullifies the bacterium-induced plant growth promotion (10).
2. Pure siderophores produced by certain strains inhibit plant pathogens in vitro and also promote plant growth upon addition to soil (10).
3. In field trials of continuously cropped potato, a siderophore-producing pseudomonad PGPR (WCS358) consistently promoted plant growth, while a siderophore-negative mutant, obtained by transposon mutagenesis, did not (12).

The siderophore mechanism is operable only under conditions of low iron availability. Iron concentration in the soil solution is related directly to soil pH. As soil pH decreases to below 6, iron availability increases and the siderophores become ineffective (13). The critical level of iron necessary for the suppression of a fungal pathogen by a siderophore-producing *P. putida* strain was determined by Simeoni and Baker (14). They found that the fungus *Fusarium oxysporum* was suppressed by the bacteria only at an Fe(III) level lower than 10^{-19} M. The optimal suppression took place between Fe(III) levels of 10^{-22} M and 10^{-27} M.

Production of antibiotic compounds Fluorescent pseudomonads produce a range of secondary metabolites which are toxic to soil-borne plant pathogens (antibiotics) (15). The production of antibiotics in the rhizosphere has been suggested to be the mechanism for biological control of plant diseases by pseudomonad PGPR. While current techniques make it difficult to detect antibiotics in the rhizosphere, several examples which relate antibiotic reduction to disease control have been reviewed (7). The linkage between in vitro production of antibiotics by PGPR and the biological control capacity of the PGPR has been strengthened by using mutants. *P. fluorescens* strain HV37a (16) protects cotton seedlings from premergence damping-off caused by *Pythium ultimum*, and the strain produces an antifungal metabolite in vitro. A mutant produced by site-directed marker-exchange procedures showed no in vitro antagonism to *Pythium* and exhibited reduced disease protection capacity. The researchers concluded that 60 to 75% of the disease suppression of the wild-type strain was accounted for by the antibiotic.

Production of plant growth regulators It is generally believed that plant growth promotion, induced by pseudomonad PGPR, occurs as a result of suppression of the native rhizosphere microflora. For example, plant growth promotion did not occur when sugarbeet was

grown in soil-less media or in autoclaved soil (7). Growth promotion was observed when small volumes of field soil or strains of deleterious microflora were introduced. Similarly, no plant growth promotion occurred with pseudomonad PGPR on radish under gnotobiotic conditions. However, it was recently reported (17) that some pseudomonad PGPR strains promote plant growth directly in the absence of deleterious microorganisms. Inoculation of canola seeds with a *P. putida* PGPR strain (GR12-2) induced significant increases in root length, root dry weight, shoot length, and shoot dry weight, under soil-free, gnotobiotic conditions.

Potential PGPR are commonly selected on the basis of siderophore or antibiotic production in vitro. A different approach was taken recently (8,18) in which over 10 000 bacterial strains were isolated randomly from plant rhizospheres in diverse habitats. After laboratory testing for growth at low temperatures, the strains were screened in greenhouse trials with field soils for repeatable growth promotion on soybean and canola (rapeseed), without regard to taxonomy or any single mechanism. Selected strains were then tested in field trials, and some induced significant yield increases. While the majority of the yield-promoting PGPR were subsequently identified as fluorescent pseudomonads, some non-fluorescent pseudomonads and other groups were identified.

Enhanced phosphate uptake Considerable research efforts have been directed toward the evaluation of phosphate-solubilizing bacteria. These bacteria, which include fluorescent pseudomonads, may increase the availability of phosphorus to plants by either mineralization (solubilization of organic phosphate via the action of phosphatase) or solubilization of unavailable inorganic phosphates via the action of organic acids. This topic was previously reviewed by Brown (2), who concluded that inoculation in raw soil did not result in increased phosphate levels.

In contrast to the numerous studies with bacterium-induced phosphate solubilization, relatively little attention has been given to the potential effect of rhizobacteria on phosphorus uptake by plants. Benians and Barber (19) found that plants grown in sterile soil take up more phosphorus and obtain greater dry weight than plants grown in non-sterile soil. They concluded that microorganisms and plants probably compete for available phosphorus. However, a recent study (17) showed that a *P. putida* PGPR strain increased the uptake of 32 P-labeled phosphate by canola seedlings. Inoculation of seeds with a pseudomonad PGPR resulted in a significant increase of 32 P levels in roots and in shoots. Linear regression analysis indicated significant correlations between root length and the level of 32 P in roots and between root length and the level of 32 P in shoots.

Effects of *Pseudomonas* Inoculation on Plants in Field Trials

Yield increases Significant crop yield increases with PGPR under diverse field conditions have been reported previously (7,9,20,21). These reports indicate that seed inoculation with PGPR does not always lead to significant yield increases. There are many potential reasons for this inconsistency, including soil-type, iron availability, and the nature of the soil microflora. It is also important to examine the fate of the inoculant by monitoring the population on seeds and roots, since the

application procedures may not be conducive to establishment of the introduced bacterium in the rhizosphere.

Biological control of soil-borne plant pathogens

During the past decade, specific fluorescent pseudomonads have been used successfully as seed inoculants to control soil-borne plant pathogens, as reviewed recently (22). In general, bacterial protection from soil-borne plant pathogens is thought to depend on two aspects: the root-colonization capacity of the biocontrol agent and the production of siderophores or antibiotics that suppress the growth of the plant pathogens.

Promotion of seedling emergence Certain root-colonizing bacteria, including pseudomonad PGPR, induce increases in seedling emergence. This phenomenon was first reported with strains that caused significant increases in emergence rates of soybean and canola (rape-seed) seedlings under cold field conditions in Canada (18). The new class of PGPR strains was termed emergence-promoting rhizobacteria (EPR). The mode of action of EPR is not known.

Promotion of *Rhizobium* and *Bradyrhizobium* nodulation of legumes Specific pseudomonad strains stimulate nodulation of leguminous crops by *Rhizobium* and *Bradyrhizobium*. Grimes and Mount (23) found that a *P. putido* strain (M17), which was selected as a potential biological control agent, markedly increased *Rhizobium* nodulation of bean in field soils. Similarly, Polonenko et al. (24) tested the effects of certain rhizobacteria (primarily fluorescent pseudomonads) on nodulation of soybean roots by *Bradyrhizobium japonicum*. Select PGPR strains enhanced *Bradyrhizobium* nodulation and soybean plant growth in field soil. These strains were termed nodulation-promoting rhizobacteria (NPR). The authors suggested that NPR could be incorporated into rhizobial inoculant formulations to improve the performance of these products. The mode of action of NPR is currently under investigation.

Ecology of the Introduced Bacteria in the Rhizosphere

Root colonization PGPR multiply and persist in the rhizosphere following inoculation onto crop seed. The bacteria are distributed in the rhizosphere in a log-normal pattern (25) and are sporadically located along roots (26). Irrigation causes redistribution of the bacteria. Colonization, although difficult to measure, is a prerequisite for causing an interaction with the plant and other members of the microflora. The role of root colonization by PGPR has been reviewed (7,9).

Fluorescent pseudomonads, isolated from crop rhizospheres, are highly rhizosphere competent (capable of root colonization), which most likely accounts for the predominance of pseudomonads among the PGPR. Pseudomonads have several traits which appear to aid seed colonization, including fast growth, motility, and chemotaxis to root exudates (27-29). However, these traits may not relate to subsequent root colonization. For example, Howie et al. (30) found that three nonmotile mutants of *P. fluorescens* colonized wheat roots as well as their respective motile parents. Scher et al. (31) also showed that chemotaxis and motility may not relate to root colonization.

Biological interactions in the rhizosphere Beneficial bacteria which are introduced to the rhizosphere are

involved in a complex of biological interactions with the host plant and with the surrounding rhizosphere microorganisms. The introduced bacteria are nourished by the root exudates and are thus dependent on the host plant. At the same time, the introduced bacteria may affect the host by inducing physiological changes in the plant. Interactions with indigenous rhizosphere microorganisms may be neutral, antagonistic (e.g., competition for nutrients, production of antibiotic compounds, parasitism, or predation), or synergistic (i.e., the promotion of *Rhizobium*-induced nodulation of legumes). These microbial interactions are greatly influenced by many environmental parameters, including temperature, moisture, fertilizer regimen, and soil type (Fig. 1).

The ecology of pseudomonad PGPR is a relatively new research area. Consequently, there is little understanding of how environmental factors will affect bacterial colonization effects and persistence on roots and the resulting effects on plant growth.

FUTURE DIRECTIONS

The commercial development of PGPR is an example of the need for technology transfer from basic research to product development. For PGPR to be exploited effectively, public sector research groups must increase the number of basic investigations, determining factors such as the modes of action of PGPR strains and the ecological complexities of microbial interactions in the rhizosphere. Corporate research groups must then apply the results of this research, for example, by conducting extensive regional field trials, developing formulations, and developing improved delivery systems.

Several companies are now involved in the development of products based on pseudomonad PGPR research. Their plans call for product introductions in the early 1990s. The first such products will consist of PGPR which act as biological control agents. Use of such biological control PGPR is likely to increase over the next two decades as policies are developed, by governmental agencies, to reduce the use of pesticides in agriculture. For example, recent policies by some states in the United States and some European countries are aimed at reducing the ground water contamination by agricultural chemicals via reducing the amount of pesticides used in crop production. Strategies for using biological control PGPR should not necessarily be limited to viewing PGPR as chemical replacements. PGPR can be used together with specific chemicals in an integrated strategy to reduce the total amount of pesticides.

Another type of envisioned PGPR product will contain PGPR which directly promote plant growth via produc-

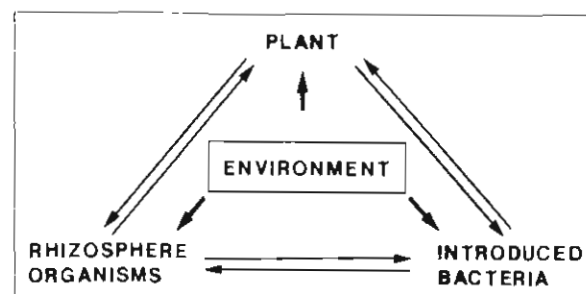


Figure 1 Schematic representation of the multiple biological interactions in the rhizosphere.

tion of growth-promoting compounds. Such products will be used initially to increase crop production in areas where crops are grown under suboptimal conditions, i.e., where early season growth is limited as a result of low soil temperatures, drought, and soil crusting. Under such conditions, an inoculant user should see a clear benefit from use of the product.

KEY CONTRIBUTORS

This list represents some of the laboratories which have made a major contribution to the field of pseudomonad PGPR. As stated in the review, this area is expanding rapidly, and hence, new contributors are publishing relevant research each year.

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GLOSSARY

rhizosphere: The zone around plant roots which is affected by the root exudates. In comparison with the non-rhizosphere soil, the rhizosphere contains more abundant nutrients and, hence, more abundant microflora.

rhizobacteria: Specific strains of bacteria which are isolated from the rhizosphere and which can colonize and persist on roots.

root colonization: The capacity to multiply and persist on roots, in the presence of the native soil microflora, after inoculation onto seeds (or vegetatively propagated plant parts).

PGPR: Acronym for plant growth-promoting rhizobacteria, i.e., specific strains of rhizobacteria which can promote growth of plants following inoculation onto seeds or subterranean plant parts.

EPR: Acronym for emergence-promoting rhizobacteria, i.e., a subclass of PGPR which can enhance the emergence rate of seedlings.

NPR: Acronym for nodulation-promoting rhizobacteria, i.e., a subclass of PGPR which can increase nodulation

of legumes by nitrogen-fixing *Rhizobium* or *Brodyrhizobium* spp.

antagonism: A general microbial interaction in which one organism is adversely affected by another. Antagonism includes the specific examples of antibiosis, competition, parasitism, and predation.

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