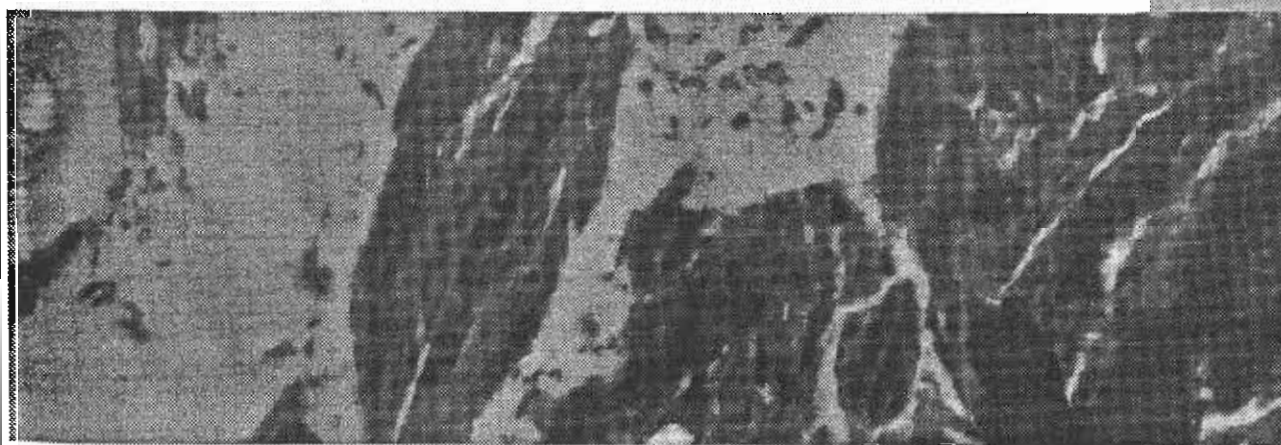


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# *A*pplications of Plant Growth-Promoting Rhizobacteria in Sustainable Agriculture

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## *Summary*

*This review focuses on the beneficial effects of plant growth-promoting rhizobacteria (PGPR) and the application of PGPR in agriculture. Particular emphasis is given to the potential and current use of PGPR in sustainable agriculture systems. PGPR are a diverse subgroup of beneficial rhizobacteria that actively colonize plant roots. Beneficial effects such as suppressing pathogens, direct growth promotion, increasing nutrient availability, enhancing legume nodulation by rhizobia, and inducing systemic disease resistance have resulted from application of specific PGPR strains and may account for increased yields associated with their application. Some PGPR are bacterial endophytes, and thus the use of bacterial endophytes in agriculture and their beneficial effects on plant growth are also addressed. Several examples of the commercial application of PGPR are summarized. Future challenges for the successful implementation of PGPR are also discussed.*

## *Introduction*

The modern concept of sustainable agriculture has many definitions (Bohlool *et al.*, 1992; Lopez-Real, 1986) and includes biological, organic, and low-input farming, but the commonality among all definitions is that sustainable agriculture is based on conserving resources and maintaining or enhancing the quality of the environment while preserving the ability to sustain the world's food needs. This is a very difficult goal that can only be met if alternatives to fossil-fuel-based agricultural inputs are developed. Technologies and methodologies must be developed that address the problems associated with sustaining agricultural production and that increase production above current levels in order to meet the needs of the ever growing population. One problem that limits agricultural production, even with the use of pesticides, is plant diseases, especially soilborne diseases.

A healthy root system is, perhaps, the most important parameter in determining the production capability of a crop (Cook, 1986). The plant root system is responsible for uptake of nutrients and water

that are essential for growth, and any improvement in the efficiency at which this uptake occurs, be it from disease control or increased root growth, would result in increased crop productivity. Cook (1986) stated that root health, improved by controlling both major and minor pathogens, contributed as much to growth and yield of a crop as did high rates of fertilizers, indicating that root health is a primary growth-limiting parameter in most crops. Thus, not only is the soil important in determining root health, but so are the microorganisms that exist in the soil. The area of soil under the influence of the root was described as the rhizosphere (Hiltner, 1904). Hiltner recognized that plant health and growth were affected by the association of rhizosphere-residing bacteria with plant roots and that this association could be beneficial to the plant.

The capacity of bacteria to increase plant growth or health was first realized when symbiotic, nitrogen-fixing rhizobia were recognized for their growth-promoting properties on legumes. Subsequently,

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inoculation of legumes with rhizobia has become a significant agricultural practice.

By the 1930s other bacterial inoculants were being investigated for their plant growth-increasing properties (Brown, 1974); most of these inoculants were *Bacillus* and *Arthrobacter* species. In 1958, over 35 million hectares of crop land had been treated with bacterial inoculants in Russia. With yield increases of 10 to 70% in bacterized vegetable and field crops, this early work indicated that inoculation of plants with bacteria could be used to increase agricultural production. Subsequent investigations outside of Russia employed modern statistical analysis of experimental field trials (Broadbent *et al.*, 1971, 1977; Rovira, 1965; Rovira and Sands, 1971) and confirmed that some bacterial inoculants increased plant growth, thereby establishing the foundations on which the concept of plant growth-promoting rhizobacteria were built.

#### Plant Growth-Promoting Rhizobacteria

During the late 1970s and early 1980s with the advent of bacterial marking systems, it was demonstrated that specific bacterial strains used as seed inoculants colonized plant roots and increased plant growth (Kloepper and Schroth, 1978; Kloepper *et al.*, 1980; Kloepper and Schroth, 1981a and 1981b). The term plant growth-promoting rhizobacteria (PGPR) was coined to describe bacteria that actively colonize roots and increase plant growth (Kloepper and Schroth, 1978).

The mechanisms by which PGPR affect plant health or growth are direct growth promotion (Frommel *et al.*, 1991), induced systemic resistance (Wei, *et al.*, 1991), mineralization (Lifshitz *et al.*, 1986), substrate competition (Elad and Chet, 1987), niche exclusion (Cooksey and Moore, 1982), detoxification of surrounding soil (Walton and Anderson, 1990), increased nodulation of legumes by rhizobia (Halverson and Handelsman, 1991), and production of antibiotics (Thomashow and Weller, 1990), chitinases (Ordentlich *et al.*, 1988), cyanide (Ahl, *et al.*, 1986), and siderophores (Thomashow and Weller, 1990). It is unlikely that a single mechanism could be responsible for the beneficial effects of a single PGPR strain. It is more likely that several mechanisms are involved with one or two being more prominent, and several reports demonstrate that multiple mechanisms are involved with several PGPR strains (Thomashow and Weller, 1990; Ahl *et al.*, 1986; Chanway *et al.*, 1989). The sustained use of PGPR in agriculture would also be facilitated if multiple mechanisms were operable, because resistance would be less likely to develop.

PGPR strains have been reported within numerous bacterial genera and species, including *Actinobacter* sp. (Tanii *et al.*, 1990), *Aeromonas caviae* (Inbar and Chet, 1991), *Agrobacterium radiobacter* (Ryder and Jones, 1990), *Alcaligenes* sp. (Yeun *et al.*, 1985), *Bacillus brevis* (Chen *et al.*, 1993), *B. cereus* (Handelsman *et al.*, 1990), *B. circulans* (Berge *et al.*, 1990), *B. firmus*, *B.*

*licheniformis* (Chen *et al.*, 1993), *B. subtilis* (Turner and Backman, 1991), *Enterobacter agglomerans* (Tanii *et al.*, 1990), *E. cloacae*, *Erwinia herbicola* (Nelson, 1988), *Flavobacterium* spp. (Tanii *et al.*, 1990), *Phyllobacterium* sp (Lambert *et al.*, 1990), *Pseudomonas aureofaciens* (Kluepfel and McInnis, 1991), *P. cepacia* (Parke, 1987), *P. fluorescens* (Voisard *et al.*, 1989), *P. gladioli* (Hasegawa *et al.*, 1991), *P. putida* (de Freitas and Germida, 1991), *Serratia fonticola* (Chanway *et al.*, 1991), *S. marcescens* (Ordentlich *et al.*, 1988), and *S. proteamaculans* (Chanway *et al.*, 1991). While the predominance of reports with PGPR involves strains of fluorescent pseudomonads, the preceding list clearly demonstrates that PGPR activity is not confirmed to a single genus or family. Although rhizobia and *Azospirillum* spp. can be considered PGPR, they are usually treated separately and will not be included within this review.

#### Beneficial Effects

##### Biological control

PGPR have been shown to suppress numerous plant pathogenic bacteria, fungi, nematodes, and viruses that infect root or foliar tissues on numerous agronomic crops (Table 1). Experimental field applications of PGPR have been encouraging, with yield increases over 20% reported in several studies (Kloepper *et al.*, 1991). Some of the beneficial field results include biological control of several pathogens (Kloepper, 1991; Weller, 1988). As with growth promotion, results from multiple-year field trials with PGPR as biological control agents indicate inconsistency of performance. These inconsistencies are due in part to the lack of knowledge about how PGPR interact with the host plant and resident microflora to increase growth or suppress disease. The subject of biological control is covered more extensively by J. Cook (this volume).

##### Direct growth promotion

PGPR-mediated direct growth promotion is the increase in plant growth by production of bacterial metabolites that directly affect the plant, irregardless of the indigenous microflora (Kloepper, 1991). While specific mechanisms of PGPR-mediated direct growth promotion remain to be elucidated, experimental evidence indicates that hormone production (de Freitas *et al.*, 1990; Frommel *et al.*, 1991; Holl *et al.*, 1988) and inhibition of ethylene (Young *et al.*, 1993) could be considered as mechanisms. Frommel *et al.* (1991) concluded that growth promotion of cultured potato plantlets *in vitro* due to inoculation with *Pseudomonas* sp. (strain Ps JN) was likely caused by production of plant growth hormones by Ps JN. Holl *et al.* (1988) demonstrated that growth promotion, afforded by inoculation of forage plants with *Bacillus polymyxa*, was mimicked by the addition of IAA and that neither nitrogen fixation nor phosphate mineralization was involved. Alternatively, inhibition of ethylene by a *Pseudomonas putida* (GR12-2) was

Table 1. Examples of various plant pathogens that Plant Growth-Promoting Rhizobacteria have been shown to suppress and the host crop.

Plant Pathogen	Crop	Reference
<b>Bacteria</b>		
<i>Agrobacterium</i> spp.	Almond, Cherry, Tomato	Ryder and Jones, 1990
<i>Curtobacterium michiganensis</i>	Potato	de la Cruz <i>et al.</i> , 1992
<i>Erwinia carotovora</i>	Potato	Kloepper <i>et al.</i> , 1980; Xu and Gross, 1986
<i>Pseudomonas solanaceorum</i>	Potato	Kempe and Sequiera, 1983
<i>R. syzygii</i> pv. <i>phaseolicola</i>	Bean	Alström, 1991
<i>Streptomyces scabies</i>	Potato	Tanii <i>et al.</i> , 1990
<i>Streptomyces campestris</i> pv. <i>oryza</i>	Rice	Chen <i>et al.</i> , 1993
<b>Fungi</b>		
<i>Aphanomyces</i>	Pea	Défago <i>et al.</i> , 1990
<i>Colletotrichum lintrista</i>	Sweet Potato	Chen <i>et al.</i> , 1993
<i>Colletotrichum</i> spp.	Cucumber	Wei <i>et al.</i> , 1991
<i>Colletotrichum</i> spp.	Chinese Chestnut	Chen <i>et al.</i> , 1993
<i>Fluoroglucium</i> spp.	Bean, Cucumber, Pea, Radish	Elad and Baker, 1985
<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Wheat	Thomashow and Weller, 1990
<i>Gymnospora cingulata</i>	Apple	Chen <i>et al.</i> , 1993
<i>Gymnospora ulmi</i>	Elm	Scheffer, 1983
<i>Phytophthora</i> spp.	Soybean	Lifshitz <i>et al.</i> , 1986
<i>Pseudoperonospora cubensis</i>	Cucumber	Chen <i>et al.</i> , 1993
<i>Pythium</i> spp.	Wheat, Lettuce	Thomashow & Weller, 1990; Lynch <i>et al.</i> , 1991
<i>Rhizoctonia solani</i>	Cotton, Peanut, Potato	Turner and Backman, 1991; Tanii <i>et al.</i> , 1990
<i>Sclerotium oryzae</i>	Rice	Sakthivel and Gnanamanickam, 1987
<i>Sclerotium rolfsii</i>	Tomato	Ordentlich <i>et al.</i> , 1987
<i>Thielaviopsis basicola</i>	Tobacco	Ahi <i>et al.</i> , 1986
<i>Ustilago maydis</i>	Corn	Chen <i>et al.</i> , 1993
<i>Verticillium dahliae</i>	Potato	Leben <i>et al.</i> , 1987
<b>Nematodes</b>		
<i>Criconemella xenoplax</i>	Peach	Kluepfel and McInnis, 1991
<i>Heterodera glycines</i>	Soybean	Kloepper <i>et al.</i> , 1992
<i>Meloidogyne incognita</i>	Cotton, Cucumber, Peanut, Tomato	Becker <i>et al.</i> , 1988; Kloepper <i>et al.</i> , 1992; Sikora, 1988
<i>Rotylenchulus reniformis</i>	Cotton	Sikora, 1988
<b>Viruses</b>		
Cucumber Mosaic Virus	Cucumber	Liu <i>et al.</i> , 1992

proposed as mechanism of direct growth promotion by Young *et al.* (1993). They observed that seed inoculation with gibberellins produced similar plant growth responses in canola as did inoculation with GR12-2; however, a similar response was not observed when a gibberellin-deficient cultivar was used. These studies indicate that PGPR are able directly to increase plant growth, although the mechanism(s) by which this growth promotion is accomplished still remains unclear.

#### Increased nutrient availability to plants

While the agricultural significance of mineralization and nitrogen fixation by PGPR is still debatable, increased nutrient availability in PGPR-treated plants has been demonstrated for several minerals, including P, Fe, and nitrogen (Bar-Ness *et al.*, 1991; Chanway and Holl, 1991; Lifshitz, *et al.*, 1987). Lifshitz *et al.* (1987) demonstrated that under gnotobiotic conditions growth of canola was stimulated by inoculation with *Pseudomonas putida* strain GR12-2, and phosphorus uptake was increased while uptake of other minerals was not. They concluded that growth promotion by GR12-2 was due to increased phosphorus uptake and that this uptake resulted either from stimulation of root growth by bacterial production of plant growth regulators which resulted in increased phosphorus uptake, or from direct

stimulation of phosphorous uptake which caused increased root growth.

Nitrogen fixation by free-living bacteria is well accepted and has been demonstrated for several PGPR strains (Chanway and Holl, 1991; Lifshitz *et al.*, 1986; Ruppel, 1989). Chanway and Holl (1991) calculated that nitrogen fixation by a *Bacillus* PGPR strain accounted for 4% of the foliar nitrogen of lodgepole pine grown in a N-limited medium. Other PGPR strains have been shown to fix nitrogen *in vitro* (Lifshitz *et al.*, 1986; Ruppel, 1989), but the significance of this fixation in the field is not known. In general, it is unlikely that the limited amount of nitrogen fixation reported for PGPR accounts for the growth promotion reported for these strains; however, it is possible that nitrogen fixation may play a role in the overall growth promotion afforded by inoculation with specific PGPR.

Mineralization of iron by PGPR can be accomplished by plant utilization of PGPR-produced siderophores (Bar-Ness *et al.*, 1991; Crowely *et al.*, 1988). Numerous microbial siderophores have been shown to be used by plants as an iron source. Bar-Ness *et al.* (1991) reported that a siderophore from the rhizobacterium *Pseudomonas putida* strain P-3 was used as an iron source by both dicots and monocots. Experiments by Crowely *et al.* (1988) established that oat plants have a microbial siderophore-mediated iron

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transport system. It is apparent that PGPR siderophores may serve to increase plant growth through mineralization (Bar-Ness *et al.*, 1991; Crowley *et al.*, 1988) as well as suppress plant pathogens by limiting available iron (Loper and Ishimaru, 1991).

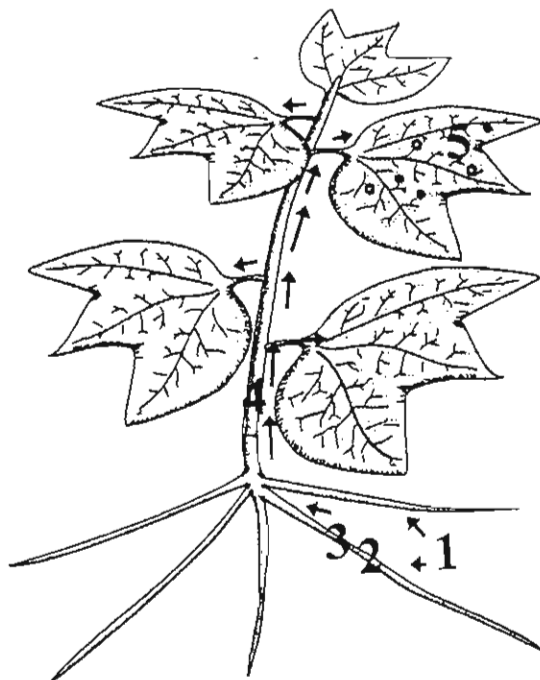
PGPR have also been shown to increase mineralization of nutrients by increasing and inducing colonization of plant roots by mycorrhizal fungi (Myer and Linderman, 1986; Raj *et al.*, 1981). Myer and Linderman (1986) demonstrated that dual inoculation of subterranean clover with mycorrhizal fungi and a PGPR strain resulted in increased accumulation of Fe, Al, Cu, Zn, Co, and Ni and suggested that this accumulation was a result of bacterial chelating action combined with fungal uptake. The interactions between PGPR and mycorrhizae are important in sustainable agriculture systems and could prove important in the implementation of PGPR-based biological control agents.

#### Increased nodulation by rhizobia

Several PGPR strains have been shown to increase nodulation of rhizobia spp. on legumes in field trials (Burn *et al.*, 1981; Grimes and Mounr, 1984; Halverson and Handelsman, 1991; Turner and Backman, 1991). Grimes and Mount (1984) demonstrated that *Pseudomonas putida* strain M17 increased nodulation of *Rhizobium phaseoli* on common bean in the field. Nodulation was increased by M17 with natural populations of *R. phaseoli* or when co-inoculated with *R. phaseoli*. Similarly, Halverson and Handelsman (1991) and Turner and Backman (1991) demonstrated that *Bacillus subtilis* strains increased nodulation of soybean and peanut, respectively, by natural rhizobia populations.

A slightly different approach to the use of PGPR and rhizobia was used by Li and Alexander (1988). Rhizobacteria which produced antimicrobial agents that inhibited rhizobia were used specifically to enhance nodulation of two rhizobia strains resistant to this antimicrobial compound. Increased nodulation by the introduced rhizobia strains was observed. They concluded that populations of indigenous rhizobia were suppressed by the introduced rhizobacteria, giving introduced rhizobial strains a competitive advantage for nodulation sites. Such approaches using PGPR with rhizobia hold potential for future use in sustainable agriculture of legumes.

Production of phytohormones with resulting changes in root morphology (Myer and Linderman, 1986), increased phosphorous availability (Grimes and Mount, 1984), production of antimicrobial agents (Li and Alexander, 1992) and cell membrane-bond proteins (Burns *et al.*, 1988) have all been proposed as mechanisms by which PGPR increase nodulation by rhizobia; however, there is only circumstantial evidence for any of these mechanisms. There are also reports of nodulation being increased as a result of enhanced root colonization by mycorrhizal fungi following PGPR treatment (Meyer and Linderman, 1986; Staley *et al.*, 1992).



**Figure 1** Schematic representation of possible steps in PGPR-mediated induced resistance. (1) An introduced PGPR strain colonizes the plant root; (2) a trigger is recognized by the host plant; (3) a plant derived signal is produced and translocated through the plant; (4) host defense genes are activated and subsequent physiological changes occur in the plant; (5) induced resistance is expressed as evidenced by reduction in disease following challenge by a pathogen.

#### Induced disease resistance

PGPR-mediated induced systemic disease resistance (PGPR-ISR) is the activation of host plant defence mechanisms by colonizing PGPR. The process of PGPR-ISR is a multistep process (Fig. 1) that involves recognition by the plant of the colonizing PGPR strain, translocation of a signal, and a response by the plant. Several recent reports indicate that PGPR can induce disease resistance to a wide range of foliar and root pathogens (reviewed by Kloepper *et al.*, 1993) in glasshouse and growth chamber experiments. Only one field investigation has been reported for PGPR-mediated ISR (Wei, 1993). Fields trials in two consecutive years demonstrated that PGPR seed treatments on cucumber significantly decreased the total lesion diameter of bacterial angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*) and increased yield and vegetative growth when compared to a non-induced and nonbacterized control (Wei, 1993).

PGPR-ISR is still in the preliminary stages of investigation. Much is not understood about this process. The specific mechanism(s) of how PGPR trigger the host is unknown but may involve PGPR-produced compounds, e.g. HCN (Voisard, *et al.*, 1989), or plant recognition of bacterial cell membrane components (van Peer *et al.*, 1992) as in the hypersensitive response. The duration and spectrum of pathogens suppressed due to PGPR-ISR is also unknown. Preliminary indications are that PGPR-mediated induced systemic resistance may be strain

specific, in that protection may only be induced against certain pathogens or pests by an individual PGPR strain, while other PGPR strains may protect against multiple pathogens (Li *et al.*, 1992).

All of the above beneficial effects could result in increased root health and ultimately in increased yields. It is important to note that many of these beneficial effects have common proposed mechanisms by which PGPR cause the effects; indicating the complexity of plant - PGPR interactions and suggesting that care must be taken in assessing the effects of a particular PGPR character on the host plant. One must not only look at a single character but must also examine the effects on the entire system, including the indigenous microflora and symbiotes.

#### Endophytes

Bacterial endophytes are bacteria that reside in the internal tissues of plants and have been isolated from inside healthy plant tissues, including flowers (Misaghi and Donnedelinger, 1990), fruits (Samish *et al.*, 1961), stems (Hollis, 1951; McInroy and Kloepper, 1993), roots (McInroy and Kloepper, 1993; Philipson and Blair, 1957), and seed (Mundt and Hinkle, 1976) in numerous crops (i.e. cotton, corn, cucumber, potato, tomato). Over 113 different bacterial species from 38 bacterial genera have been identified as endophytes (Gardner *et al.*, 1982; McInroy and Kloepper, 1993; Mundt and Hinkle, 1976). There are also reports of known PGPR colonizing the root interior after inoculation (Kloepper *et al.*, 1992; Scheffer *et al.*, 1989) and hence, these PGPR are endophytes as well.

Some bacterial endophytes suppress disease (Kempe and Sequeria, 1983; Kloepper *et al.*, 1992; Scheffer, 1983) and deleterious rhizobacteria (van Peer *et al.*, 1989) and promote plant growth (Frommel *et al.*, 1991; van Peer *et al.*, 1989; Gardner *et al.*, 1982; Lalande *et al.*, 1989). van Peer *et al.* (1989) demonstrated that one endophytic bacterium (*Pseudomonas* sp. WCS417r) reduced populations of indigenous bacteria in the root interior and increased plant growth up to 50%. Kempe and Sequeria (1983) demonstrated that two endophytic bacterial strains suppressed development of bacterial wilt on potato.

There is also evidence that endophytes may be involved in resistance of multi-adversity resistance (MAR) cultivars of cotton. Inoculation of susceptible cultivars with bacteria isolated from inside leaf blades and flower buds of MAR cultivars was shown to suppress boll weevil feeding and development of bacterial angular leaf spot disease, caused by *Xanthomonas campestris* pv *malvacearum* (Bird, 1982; Bird *et al.*, 1980).

Endophytic bacteria may serve as delivery systems for generically engineered biopesticides and plant growth regulators. The biological control product InCide (Crop Genetics International) is based on this premise. The bacterial endophyte *Clavibacter xyli* subsp. *cynodontis* was genetically modified to produce

delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki* (Bt) to control the European corn borer on corn (Dimock *et al.*, 1989). While consistent increases in yields were not observed, corn borer damage was reduced without application of chemicals (Fahey *et al.*), thus saving the farmer money in application costs and reducing the use of pesticides.

Endophytes could prove useful as biological control agents because they are able systemically to colonize the host plant (Dimock *et al.*, 1989; Mahaffee, unpublished data). In addition, endophytes form an intimate relationship with the host plant, where they may be protected from environmental stresses and microbial competition. As our understanding of plant-endophyte interactions and ecology of endophytes increases, the possibility of exploiting these organisms for the improvement of agricultural systems should increase.

#### Use of PGPR in Agricultural Systems

The most successful commercial application of PGPR is the use of *Agrobacterium radiobacter* strain K84 to control crown gall on several plant families (eg. Compositae, Juglandaceae, Rosaceae and Salicaceae). K84 was isolated by New and Kerr (1972) in Australia and has been commercially available since 1973 in Australia. This strain is now available in Europe and North America as a commercial product. Commercial application of K84 has proved to be more effective, often attaining 100% control, and economical than methods employing chemicals (Ryder and Jones, 1990). Application of K84 usually occurs at the time of transplanting when damage to the root system makes the transplants most susceptible to infection by *A. tumefaciens*. Plant roots are treated by dipping or spraying an aqueous suspension containing  $10^8$ - $10^9$  cfu/ml. Biological control of *A. tumefaciens* by K84 is mainly due to production of agrocin, which is encoded by a transferable plasmid in K84. Due to the possibility of transfer of the agrocin-84 plasmid into pathogenic strains of *A. tumefaciens* and the resultant development of an agrocin-resistant *A. tumefaciens* strain, a transfer-deficient mutant of K84 (K1026) was created (reviewed by Ryder and Jones, 1990). The *tra* region from the agrocin-84 plasmid was deleted without the addition of foreign DNA to the plasmid, thus preventing transfer of the plasmid. K1026 is now available as a commercial biological control agent (NOGALL) in Australia, making it the first genetic engineered biological control agent. The use of K1026 is recommended instead of K84 because of the possible transfer of the agrocin-84 plasmid to pathogenic *A. tumefaciens* strains from K84.

Several PGPR-based inoculants are commercially available and extensively applied in the People's Republic of China where they are known as YIB (reviewed by Chen *et al.*, 1993). Research on YIB in China began shortly after the end of the cultural revolution in 1978 and quickly led to the first commercial field application of YIB on vegetables in 1985. By 1990, over 3.35 million ha were treated with

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1132 tonnes of YIB inoculants. YIB treatments are primarily used to increase yields, with reported average yield increases of 10-20%; however, biological control has also been demonstrated on numerous crops. YIB treatments were reported to increase seedling emergence, vegetative vigour, and crop quality parameters (e.g. protein, amino acid content, length on cotton fibre, and sugar and oil content). Commercial formulations include both liquid-based and powdered inoculants; liquid formulations are mainly used as dips for seedling transplants, while powdered formulations are used for seed treatments. The majority of YIB strains commercially applied in China are *Bacillus* species.

Another PGPR-based biocontrol product, which contains *Bacillus subtilis* strain GB03, was released in 1993 under the product name KODIAK. KODIAK is marketed by Gustafson, Inc. of Dallas, Texas as a technical powder for commercial seed treatment and a planter-box formulation. KODIAK is registered with the U.S. Environmental Protection Agency for use on cotton and common bean to suppress *Rhizoctonia solani* infection. GB03 is an "improved" variant of the *B. subtilis* strain A-13 isolated by Broadbent *et al.* A-13 was shown to suppress *Rhizoctonia solani* and increase yield, seedling stand, and root growth on peanut (Turner and Backman, 1991). Turner and Backman (1991) conducted a regional field trial, which included 24 locations in Alabama, Georgia and Florida, to evaluate peanut responses to inoculation with A-13. The average multi-site yield increase with bacterization was 7.6%, with yield increases up to 37% at individual sites. When locations were analyzed by planting time and rotation history, fields that were planted early and had a poor rotation history (legumes in either of two previous years) had the greatest yield response (average 14.5%). Hence, it was concluded that fields with poor rotation history and those planted in cool soils would likely benefit most from inoculation with A-13. Thus, it may be possible to shorten rotation times by applying PGPR to counter the build-up of deleterious microorganisms normally associated with monoculture and sometimes with short rotations (Schippers *et al.*, 1987). Turner and Backman (1991) also noted that populations of A-13 were consistently greater than log 4 cfu/g root 120 days after planting, indicating that A-13 is relatively insensitive to environmental conditions. This environmental insensitivity may be due to formation of endospores which increase the probability of strain survival under stress.

Recent work by Kastelein *et al.* (1992) has combined the use of PGPR with the technique of green-crop-harvesting to control disease on seed potatoes. Green-crop-harvesting is used in seed potato production to replace the use of herbicides to kill potato vines just before harvest to prevent virus infection. This procedure involves the removal of vines by pulling or cutting at the soil level, followed by digging with a lifter and placing the tubers back on a soil bed which is then recovered with soil and allowed to mature before final harvest (Mulder *et al.*, 1992).

Since the tubers are exposed on the lifter for a few seconds, there is an opportunity to apply antagonistic microorganism to suppress development of bacterial rots and other diseases. Kastelein *et al.* (1992; *pers. comm.*) applied PGPR to immature tubers at the time of green-crop-harvest to suppress development of bacteria associated with potato blackleg disease, caused by *E. chrysanthemi*. In field trials, populations of *E. chrysanthemi* were reduced by log 4 cfu/ml peel extract immediately after harvest (Kastelein *et al.*, 1992) and log 2 cfu/ml peel extract after storage for 6 months (Kastelein, *pers. comm.*). Thus, application of PGPR to immature seed potatoes reduced the probability of blackleg symptom development in the next year's crop. The combination of these two sustainable agriculture techniques reduces the use of chemicals and improves product quality beyond that offered by traditional chemical-based methods.

The above are just a few examples of the use of PGPR to increase plant growth and improve agricultural production while decreasing reliance on synthetic pesticides. By integrating the application of PGPR with other methods for sustainable agriculture, we are beginning to assemble the tools needed to sustain agriculture production well into the future without heavy reliance on fossil-fuel-based agriculture inputs.

#### Future Challenges

Understanding PGPR ecology is perhaps the greatest challenge that we face. In order to access and develop future PGPR based products effectively, we must understand how PGPR colonize and survive on plant roots; how PGPR interact with pathogens, beneficial symbionts (introduced and indigenous), and other soil organisms; and how other environmental conditions affect the above. Without this knowledge we will be trying to develop PGPR-based biological control agents by randomly applying bacteria to plants under environmental conditions that are not conducive to survival, growth, and expression of beneficial attributes of the applied PGPR. This would be similar to spraying a pesticide in the rain, which clearly would be ineffective.

As we begin to understand the environmental parameters that govern the efficacy of PGPR inoculants, it becomes apparent that the host plant plays an important role in the success of PGPR. It may be possible and beneficial to create new varieties, through breeding or genetic engineering, that are more responsive to, and better suited for, colonization by PGPR. It is also probable that once we understand how PGPR colonize plants, suppress disease, and increase plant growth, genetic engineering could be used to enhance PGPR efficacy. While the use of genetically modified PGPR may offer future opportunities to improve performance, a thorough understanding of how gene products function in the environment is necessary to avoid unanticipated consequences. Maurhofer *et al.* (1992) have already demonstrated that over-production of an antibiotic

responsible for control of *Pythium ultimum* on cucumber, cress and sweet corn results in some deleterious effects on the plant.

### Conclusion

PGPR have such diverse beneficial effects as disease suppression, direct growth promotion, increased nutrient availability to plants, stimulation of rhizobium nodulation, and induced systemic disease resistance. These beneficial effects lead to improved root health and ultimately to increased yields. The application of PGPR could serve as an alternative to fossil-fuel-based agricultural inputs, thereby reducing negative environmental impacts of agricultural systems while improving yields and food quality. Also by incorporating the use of PGPR with other sustainable agriculture methods, it may be possible to improve the efficiency of both systems. The continued successful commercial development of PGPR requires an improved understanding of interactions between PGPR and the host plant and indigenous microflora. With this understanding, we will be better able to manage the problem of inconsistent results with PGPR inoculants. Thus, farmers will accept PGPR-based products as alternatives to synthetic pesticides, and we will have gained a valuable tool for the implementation of sustainable agriculture.

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