17. Plant Growth Promoting Rhizobacteria and Mycorrhizal Fungi in Sustainable Agriculture and Forestry

Muhammad A.B. Mallik[1] and Robert D. Williams[2]

[1] Research and Extension, Langston University, Langston, Oklahoma. mmallik@luresext.edu
[2] Research and Extension, Langston University, Langston, Oklahoma, USDA, ARS, USA. robert.williams@ars.usda.gov

Abstract. Plant-growth promoting rhizobacteria (PGPR) encourage plant growth by producing growth regulators, facilitating nutrient uptake, accelerating mineralization, reducing plant stress, stimulating nodulation, providing nitrogen fixation, promoting mycorrhizal fungi, suppressing plant diseases, and functioning as nematicides and insecticides. Many of the PGPR are fluorescent pseudomonads (*Pseudomonas fluorescens*), but other bacteria (*Bacillus* sp., *Azotobacter* sp., *Acetobacter* sp., *Azospirillum* sp.) are known as well. Many of these organisms have been formulated into biofertilizers and are commercially available. However, there is a disconnect between the demonstration of the growth-promoting activity of these organisms in laboratory and field studies versus their use in commercial production. The reason for this is two-fold. First, there have been inconsistent results between experimental studies and practical field applications where the growth-promoting activities of the rhizobacteria are masked by other environmental and management factors. Second, there is a lack of technology transfer and education, thus limiting the farmers' use of biofertilizers. Here we review the role of rhizobacteria stimulating plant growth and their use as biofertilizers; indicate that the use of biofertilizers may be of more benefit in unproductive and stressful environments; and recommend that commercially available biofertilizers be evaluated in standardized field tests.

17.1 Introduction

As stated recently by An (2005) "Allelopathy arises from the release of chemicals by one plant species that affect other species in the vicinity, *usually* to their detriment." (We have added the emphasis on "usually.") This is a generally accepted definition of allelopathy. Although Molisch (1937) defined allelopathy to include both beneficial and harmful effects of one plant or microorganism on another, the majority of allelopathy studies are concerned with inhibitory effects. This may in part be due to interest in using allelochemicals as alternatives for synthetic pesticides. Or as pointed out earlier, stimulatory effects are often not as spectacular as inhibitory effects and have been generally ignored (Mallik and Williams 2005). However, there are reports of stimulation of plants by other plants and microorganisms, and visa-versa, which we reviewed earlier (Mallik and Williams 2005). Here we review allelopathic stimulation focusing on rhizosphere microorganisms, and specifically the role of rhizobacteria as biofertilizers.

As the plant root system develops, organic compounds (root exudates) are released into the soil. Root exudates may include passive leakage of low molecular weight compounds (sugars and amino acids), as well as active secretion of high molecular weight compounds across cell membranes (polysaccharides, proteins, fatty and other organic acids, phytohormones and enzymes). The composition of root exudates depends on plant species, growing conditions, plant growth stage, and rooting medium. Exuded compounds are used as nutrients by the numerous microorganisms contained in the rhizosphere, and in turn the compounds released by the microorganisms, either as exudates or metabolic products, affect the quantity and quality of compounds released by the root system (Bolton, Fredrickson and Elliot 1993). The system is highly dynamic and suggests a degree of co-evolution between rhizobacteria and their associated plants (Bolton et al. 1993).

Plant growth-promoting rhizobacteria, a term first used by Kloepper and Schroth (1978), can directly or indirectly promote plant growth (Fig. 1). Some PGPRs may promote plant growth by producing growth regulators that stimulate other beneficial rhizobacteria, stimulate the plant directly, aid in nodulation, or indirectly stimulate nodulation (Fig. 1, 1a–1d). Other PGPRs accelerate mineralization and uptake of certain nutrients (Fe, P, Mn, Zn and Cu) (Tinker 1984) (Fig. 1, 2b). Growth promotion can also occur indirectly when PGPRs function as biocontrol agents of soil-borne plant pathogens and weeds, as promoters of mycorrhizal fungi, provide biological nitrogen fixation (biofertilizer) (Fig. 1, 1e and 2a), or by reducing the negative effect of deleterious rhizobacteria (DRB) (Fig. 1, 3b). However, the major function of PGPR is through the suppression of plant pathogens by releasing antibiotics, cyanide, and enzymes (Kloepper, 1993) (Fig. 1, 3a–3b). Since the rhizosphere is a complex mixture of microorganisms and their numerous interactions, the resulting stimulation of plant growth is probably multifaceted in many cases.

The interest in developing plant growth-promoting rhizobacteria (PGPRs) as crop additives has increased over the past 20 years. What has stimulated the interest in this area? First, the public perception of environmental pollution resulting from the use of synthetic chemicals in agriculture has led to the realization that present agricultural practices should shift from the use of large inputs of fertilizers and pesticides to more environmentally-friendly production practices. Second, if we are to achieve sustainable agriculture, particularly in areas that are resource limited, we must find methods to sustain crop yield and reduce production costs. Beneficial rhizobacteria have potential as part of an overall management system to reduce the use of synthetic compounds and fertilizer, and provide a sustainable agriculture.

This review provides examples of the growth promoting activities of allelopathic rhizobacteria. References cited draw attention to allelopathic stimulation, with the view to exploit the phenomenon where feasible in agriculture and biological research. An extensive review of the allelopathic literature covering this topic is not intended. Production of growth regulators will not be discussed and the reader is referred to Arshad and Frankenberger (1993, 1998), Zahir, Arshad and Frankenberger (2004) and Mallik and Williams (2005).

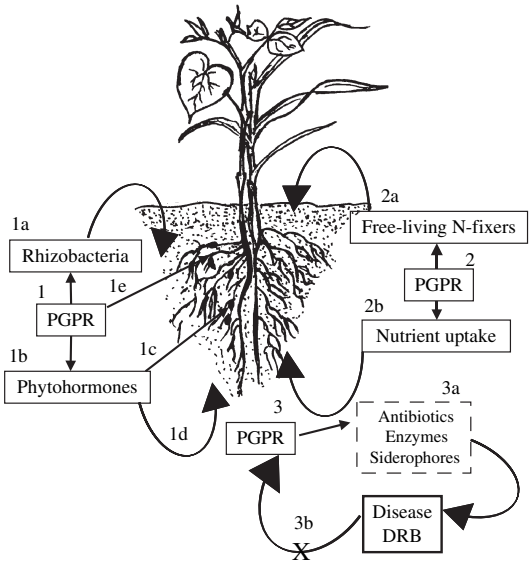

Fig. 1. Possible indirect and direct pathways PGPRs may influence plant growth

17.2 Rhizobacterial Effects on Plant Growth

17.2.1 Plant Disease Control

PGPR-induced systemic disease resistance (ISR) was first reported by Scheffer (1983) when he discovered that prior inoculation of elm trees with four fluorescent pseudomonad strains led to significant reduction in foliar symptoms of Dutch elm disease caused by the fungal pathogen *Ophiostoma ulmi*. Since then, this immunization, or induction of systemic disease resistance, has been reported in a wide variety of plants (Table 1). The inhibition of a phytopathogen by a PGPR can occur via release of a toxic compound, antibiotic or enzyme; or through rapid colonization of the root zone blocking the phytopathogen or DRB development.

While the production of HCN by *Pseudomonas fluorescens* was cited in the suppression of *Thielaviopsis basicola* in tobacco (Keel, Voisard, Berling, Kahr and Defago 1989), suppression of DRB in sugar beet was due to the large population density of the introduced PGPRs (Suslow and Schroth 1982). Potato seed tubers treated with a cell suspension of three fluorescent *Pseudomonas* isolates increased subsequent plant growth and yield, and in this case the authors concluded that PGPR isolates produced a significant amount of siderophores resulting in suppression of DRB by iron deprivation (Geels and Schippers 1983).

PGPR-induced systemic disease resistance may result from biochemical responses in the host plant. Increased phytoalexin levels were reported in carnation inoculated with *Pseudomonas* sp. (van Peer, Niemann and Schippers 1991), while increased levels of protein were found in bean and tomato following seed treatment with a PGPR (Hynes and Lazarovits 1989). In other studies, increased peroxidase

activity localized on the root surface (Albert and Anderson 1987) and lignification of stems/leaves in bean (Anderson and Guerra 1985) and potato (Frommel, Nowak and Lazarovits 1991), after colonization by an introduced PGPR, were related to suppression of the phytopathogen.

Table 1. Examples of growth-promoting rhizobacteria (PGPR) used in disease control

Crop	PGPR	Result	Reference
Carnation	*Pseudomonas* sp.	Induced resistance to *Fusarium oxysporum*	Duijff et al. 1994
Cucumber	Unknown	Induced resistance to mosaic viruses	Raupach et al. 1996
Cucumber	*Pseudomonas putida* *Serratia marcencens*	Induced resistance to *Fusarium* sp.	Liu et al. 1996
Cucumber	Unknown	Induced resistance to Angular leaf spot	Liu et al. 1995
Bean	*Pseudomonas* sp.	Reduced leaf lesions of *Pseudomonas syringae*	Alstrom 1991
Sugar beet	*Pseudomonas* sp.	General protection against pathogens	Suslow and Schroth 1982
Potato	*Pseudomonas* sp.	Suppression of deleterious rhizobacteria	Geels and Schippers 1983
Cotton	*Pseudomonas fluorescens*	Induced resistance to *P. ultimum* and *Rhizoctonia solani*	Howell and Stipanovic 1979, 1980
Wheat	*Pseudomonas fluorescens*	Resistance to *Gaeumannomyces* sp.	Weller and Cook 1983, 1986

Agrobacterium, *Bacillus*, *Burkholderia*, *Erwinia* and *Pseudomonas* species are known antibiotic producers (Kloepper 1994), and 90% of the antibiotic producers also produce siderophores. An antibiotic producing wild strain of *P. fluorescens,* genetically altered to over-produce pyoluteorin and 2,4 diacetylphloroglucinol, effectively protected cucumber plants against *Pythium ultimum* infection (Schneider and Ullrich 1994). Damping-off caused by *P. ultimum* and/or *Rhizoctonia solani* was controlled in cotton by treating seed with *P. fluorescens* pf-5 that produced the antibiotics pyoluteorin and pyrrolnitrin (Howell and Stipanovic 1979, 1980). In naturally infested fields, wheat take-all disease (*Gauemannomyces graminis*) was suppressed in spring and winter wheat by inoculating the seed with *P. fluorescens*. In this case control was linked to the increased level of 2,4-diacetylphloroglucinol or phenazine-1-carboxylate produced by the pseudomonads (Weller and Cook 1983, 1986).

Fusaric acid is a common compound in *Fusarium* infection. Several PGPRs (*P. cepacia* = *Burkholderia cepacia*, *P. solanacearum*) are capable of hydrolyzing fusaric acid, which controls the *Fusarium* infection (Toydoa, Hashimoto, Utsumi, Kobayashi and Ouchi 1988). Lim, Kim and Kim (1991) isolated a strain of *P. stutzeri* that produces two enzymes (chitinase and laminarinase) that lyse *Fusarium* mycelium preventing the fungus from causing root rot in several plant species. Fridlender, Inbar and Chet (1993) isolated the enzyme β-1,3 glucanase from a strain of *P. cepacia* that injures fungal mycelia and reduces plant damage caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *P. ultimum*.

These examples show direct effects, but suppression of plant diseases may be indirect. For example, suppression of *P. ultimum* on sugar beet is probably due to the ability of the introduced pseudomonad to utilize sugar beet exudates to produce compounds inhibitory to the pathogen (Stephens 1994). Part of this suppression may also be due to a reduction in the nutrients available for the pathogen. Ferric ion (Fe^{3+}), the predominant form of iron, is barely soluble. Since available iron is too low to directly support bacterial growth, soil microorganisms secrete low molecular weight siderophores that bind ferric ions and transport them back to the cell membrane, which forms an appropriate receptor compound and makes the iron available for microbial growth (Volk and Wheeler 1980). This process binds most of the available iron in the rhizosphere and prevents the pathogens from developing (O'Sullivan and O'Gara 1992; Tate 2000).

Most plants can grow at low concentrations of available iron, and several plants can bind iron with their own siderophores (Wang, Brown, Crowley and Szaniszlo 1993). Fourteen *Burkholderia cepacia* strains were isolated from a corn rhizosphere and tested for siderophore production and antibiosis against two species of *Fusarium* corn-root pathogen (Bevivino, Sarrocco, Dalmastri, Tabacchioni, Cantale and Chiarini 1998). Hydroxamate-like and thiazole-like siderophores were detected in the culture medium of each strain. Several of the isolates inhibited in vitro growth of *F. moniliforme* and *F. proliferatum*. Antibiosis was more evident in an iron-deficient medium, which suggested the Fe^{3+} deficiency might have enhanced siderophores production and antibiosis (Bevivino et al. 1998). Siderophore production is an effective mechanism in disease suppression. Although the producing agent is affected by several biotic factors (the pathogen, PGPR, type of siderophores produced and the target plant), the use of siderophore-producing PGPR as biocontrol agents for plant pathogens has potential and should be evaluated further.

Many of the examples provided involved crop plants, but PGPRs are also used in forestry to inhibit pathogens. Fungal root disease causes considerable seedling loss in conifer nurseries and reduces seedling survival and growth in reforestation sites. *Burkholderia cepacia* (strain RAL3) and *P. fluorescens* (strain 64-3) reduced (7–42%) *Fusarium oxysporum* root disease in Douglas fir, improved white spruce seedlings survival when planted in soil inoculated with *Fusarium* sp. and *Pythium* sp. in a nursery, and increased (19–23%) survival of bare-root white spruce seedlings planted on a reforestation site as compared to the control (Reddy, Funk, Covert, He and Pedersen 1997). Further discussion on forestry application is given in Section 7.3 "Biofertilizers in Production."

17.2.2 Promotion of Symbiotic Biological Fixation

A few PGPRs have been used to stimulate nodule formation, growth and number, and nitrogen fixation in several legumes. Of 17 *P. fluorescens* and *P. putida* isolated from the root surface of soybean (Polonenko, Scher, Kloepper, Singelton, Laliberte and Zaleska 1987) nine isolates increased nodule weight, while three isolates increased both nodule number and weight. Several strains also increased soybean shoot and root dry weight, but these effects were not associated with an increase in nodule number or nodule weight. In a field study, nine PGPR strains (seven pseudomonads and two *Serratia* sp.) were tested for their effects on nitrogen fixation in lentil and pea inoculated with *Rhizobium leguminosarum* (Chanway, Hynes and Nelson 1989). Pea growth was unaffected; but growth, nodulation and acetylene reduction in lentil were significantly increased by two *P. putida* strains. These results, verified in the laboratory, suggest that these PGPR strains might be useful as inoculants for lentil, depending on the cultivar and growing conditions (Chanway et al. 1989).

Nodulation and N_2-fixation of soybean plants are hampered by cool soil temperatures. Zhang, Dashti, Hynes and Smith (1996) demonstrated that co-inoculation of soybean a PGPR and *Bradyrhizobium japonicum* increased nodulation at cooler soil temperatures. Bai, Zhou and Smith (2003) isolated three *Bacillus* strains from a nodule of field grown soybeans that displayed growth promoting activity. Soybean was inoculated with these strains and *Bradyrhizobium japonicum* and the plants grown under controlled conditions and in the field. Soybean co-inoculation with *Bacillus thuringiensis* NEB 17 provided the most consistent results and the largest increase in total plant biomass, root and shoot weight, nodulation (total number and weight), total nitrogen and grain yield.

These studies, particularly the last two, indicate that PGPRs and rhizobia co-inoculation could improve nodule formation and N_2-fixation, and that co-inoculation may be of a greater value under stress conditions (temperature, salinity or moisture). However, further screening of PGPR strains and testing under various field conditions needs to be done.

17.2.3 Associative Diazotrophs

Associative diazotrophs have gained importance recently as a source of nitrogen for crop production. Beneficial effects of associative diazotrophs (e.g. *Azotobacter*) have been investigated in Europe, particularly in Russia and, since the report of their discovery in grass roots in the late 1970s, diazotrophs (e.g. *Acetobacter*) have been widely studied. Associative diazotrophs include *Azotobacter, Azospirillum, Azomonas, Herbaspirillum, Spirillum, Acetobacter, Beijarinckia, Azoarcus, Burkholderia, Clostridium* and several genera belonging to the Enterobacteriaceae. Here we provide a few examples.

Azospirilla, micro-aerophyllic, heterotrophic diazotrophs have been investigated as possible nitrogen fixing bacteria for grasses since their discovery on the roots of tropical grasses (Day and Dobereiner 1976). Soil application or seed inoculation of *Azospirillum lipoferum* resulted in a 22% increase in rice grain yield in field experi-

ments (Balandreau 2002) and enhanced P and ammonia uptake by the plants (Murty and Ladha 1988), while a 30% yield increase was reported for wheat inoculated with *A. brasilense* (Okon and Labandera-Gonzales 1994). Although these yield increases can be attributed in part to increased nitrogen availability, it was estimated using ^{15}N dilution technique measurements that the *Azospirillum*-root association in grasses and cereals contributed only 1–10 kg N/ha (Kapulnik, Feldman, Okon and Henis 1985). In other work, 12% of the nitrogen accumulated by corn was contributed by *Azospirillum* (Rennie 1980). Some of the yield increases may be due to indirect effects of *Azospirillum* sp. *Azospirillum* inoculation has enhanced root and root hair growth, resulting in significant increase of nitrogen (Fayez and Daw 1987) and mineral uptake (Lin, Okon and Hardy 1983), as well as the production of antifungal and antibacterial compounds, growth regulators and siderophores by the inoculated plants (Pandey and Kumar 1989; Fallik, Sarig and Okon 1994; Okon and Labandera-Gonzales 1994). Based on 20 years of field application data, Okon and Labandera-Gonzales (1994) concluded that *Azospirillum* can increase crop growth and yield by 5 to 30% depending on soil and climatic conditions.

*Azotobacter*s are aerobic heterotrophic associative N_2-fixers, provided an adequate supply of reduced carbon compounds and low oxygen pressure favorable for nitrogenase activity are available. *A. chroococcum* and *A. vinelandii* have been used widely in various studies, and the genus has been reported to increase the yield in rice (Yanni and Abd El-Fattah 1999), and replaced up to 50% of the inorganic nitrogen fertilizer requirements for wheat (Hegazi, Faiz, Amin, Hamza, Abbas, Youssef and Monib 1998). *A. paspali* was first isolated from a grass, *Paspalum notatum* (Dobereiner and Pedrosa 1987). Boddey, Chalk, Victoria, Matsui and Dobereiner (1983) calculated that 11% of the nitrogen accumulated by the grass was contributed by *A. paspali*.

Acetobacter (Gluconacetobacter) diazotrophicus is an endophytic, acid tolerant biological nitrogen fixer (BNF). Boddey, Urquiaga, Ries and Dobereiner (1991) calculated, based on ^{15}N dilution studies, that 60–80% of sugar cane plant nitrogen (equivalent to 200 kg N/ha) is derived from BNF, and that *Acetobacter diazotrophicus* was the principal contributor. Because of this, seedling inoculation with an effective *Acetobacter* strain has become a standard practice in sugarcane cultivation (Lee, Pierson and Kennedy 2002).

Inoculation of rice seedlings with *Burkholderia Vietnamiensis* increased grain yield in field studies (Tran Van, Berge, Ke, Balandreau and Huelin 2000), and this bacterium is capable of contributing 25–30 kg N/ha. Under gnotobiotic conditions this species can fix 19% of the nitrogen required by the rice plant, while another *Burkholderia* sp. was reported to fix 31% of the nitrogen the rice plant required and increase plant biomass by 69% (Baldani, Baldani and Dobereiner 2000).

17.2.4 Interaction with Mycorrhiza

Vesicular-arbuscular mycorrhizal (VAM) fungi are characterized by limited growth within the roots and extensive growth of the hyphae beyond the root zone. VAM fungi can improve plant vigor, nutrient and water uptake, disease resistance and

drought tolerance. The principal contribution of the fungi is assistance in phosphorous acquisition, particularly in phosphorous-depleted soil, and other trace elements (Boddington and Dodd 1998, 1999; Clark 1997). Depending upon soil phosphorous content and crop plant the VAM inoculant application can reduce 25–50% P-fertilization cost (Tiwari, Adholeya and Prakash 2004). Some rhizobacteria have been identified that promote VAM development by enhancing receptivity of the root to VAM fungi and triggering germination of the VAM fungal propagules (Garbaye 1994). VAM improved nodulation of several legumes (Barea, Escudero and Azcon-G de Aguilar 1980; Smith and Bowen 1979), and enhanced N_2-fixation by rhizobia (Chaturvedi and Kumar 1991; Werner, Berbard, Gorge, Jacobi, Kape, Kosch, Muller, Parniske, Scenk, Schmidt and Streit 1994), *Azotobacter* (Alnahidh and Gomah 1991), and *Frankia* (Sempavalan, Wheeler and Hooker 1995). Further information about the synergy between VAM and beneficial rhizobacteria and their potential for stimulating plant growth is given in a recent review of Arturrson, Finlay and Jansson (2006).

17.3 Biofertilizers in Crop Production

Positive effects of PGPR, typically referred to as biofertilizers, seed inoculation have been reported in a variety of crops (Table 2) and have been shown to reduce plant stress (Table 3).

The use of biofertilizers in rice production has been extensively studied. Diazotrophic rhizobacteria that are commonly associated with rice include *Azospirillum, Herbaspirillum* and *Burkholderia* (Baldani et al. 2000; Balandreau 2002; Malik, Mirza, Hassan, Mehnaz, Rasul, Haurat, Bslly and Normand 2002). These diazotrophs, including cyanobacteria, can substantially contribute to the nitrogen requirements of rice plants. Watanabe, Yoneama, Padre and Ladha (1987), and Roger and Ladha (1992) concluded that BNF can provide up to 25% of the nitrogen requirement of rice.

In Vietnam a biofertilizer consisting of *Ps. fluorescens*/*Ps. putida* (BNF), *Klebsiella pneumoniae* (anaerobic BNF, PO_4-solubilizer) and *Citrobacter freundii* (BNF) is used in rice production. *Citrobacter freundii* is also antagonistic to 50% of the common rice rhizospheric bacteria, but not to the other components of biofertilizer, which aids in the establishment of the inoculum (Nguyen, Kennedy and Roughley 2002). This biofertilizer significantly increased grain yield (21% over control) and nitrogen accumulation (Nguyen, Deaker, Kennedy and Roughley 2003). In another field study, a biofertilizer containing two cyanobacteria (*Anabaena* and *Nostoc*), *Azospirillum* sp. and *Azotobacter* sp. applied with a third of the recommended amount of urea fertilizer produced greater rice grain yield than any single component of biofertilizer and/or nitrogen fertilizer (Yanni and Abd El-Fattah 1999). Other multi-strains biofertilizers were used in Pakistan (Malik et al. 2002) and Egypt (Hegazi et al. 1998). Overall, the reported increased rice grain yield due to biofertilizers was about 20%.

Table 2. Selected examples of growth-promoting rhizobacteria (PGPR) on plant growth and production

Plant	PGPR	Response	Reference
Bean	*Pseudomonas putida*	Increased overall performance	Anderson and Guerra 1985
Canola	Unknown	Increased overall performance	Kloepper 1994
Canola	*Pseudomonas putida*	Increased root and shoot length; increased dry weight, chlorophyll and protein content	Glick et al. 1997
Corn	*Pseudomonas aeruginosa, P. fluorescens*	Enhanced seed germination and dry matter accumulation	Hofte et al. 1991
Cotton	*Pseudomonas cepacia*	In the field suppressed *Rhizoctronia soloni*; equivalent to a fungicide treatment; and significantly increased seedling stand	Press and Kloepper 1994
Cotton	*P. fluorescens*	Nematicide against *Rotylenchulus reniformis* and increased growth	Jayakumar et al. 2003
Peanut	*Bacillus subtilis*	Increased yield	Turner and Backmann 1991
Potato	*Pseudomonas* sp.	Increased yield	Geels and Schippers 1983
Rice	*Pseudomonas fluorescens*	Decreased sheath rot	Sakthivel et al. 1986
Rice	*Pseudomonas fluorescens*	Decreased bacterial blight	Velusamy et al. 2003
Spring wheat	*Bacillus* sp.	Increased shoot height and root growth under controlled conditions	Chanway et al. 1988
Spring wheat	*Bacillus* sp.	Increased tiller number and yield	Grayston and Germida 1994

Table 3. Examples of plant growth promoting rhizobacteria (PGPR) reducing plant stress

Plant	Stress	PGPR	Reference
Barley	Heavy metal	*Arthobacter mysorens* *Flavobacterium* sp. *Klebsiella mobilis*	Pishchik et al. 2002
Soybean	Cool soil temperature	*Serratia proteamaculans* *Serratia liquefaciens* *Aeromonas hydrophila*	Zhang et al. 1997
Loblolly pine	Ozone	*Bacillus subtillis* *Paenibacillus macerans*	Estes et al. 2004
Tomato	Salt	*Achromobacter piechaudii*	Mayak et al. 2004
Wheat	Salt	*Azosprillium lipoferum*	Bacilio et al. 2004
Arabidopsis	Water	*Paenibacillus polymyxa*	Timmusk and Wagner 1999

Corn production requires significant amounts of nitrogen. Diazotrophs commonly found in the corn rhizosphere include *Enterobacter, Rahnella aquatilis, Paenibacillus, Azotofixans, Azospirillum, Herbaspirillum seropediacae, Bacillus circulans and Klebsiella* (Chelius and Triplett 2000), and these diazotrophs can contribute significant amounts of nitrogen (Garcia de Salamone, Dobereiner, Urquiaga and Boddy 1996). Application of biofertilizer containing *A. brasilense* increased corn yield 50–95% (0.7–1.0 t/ha) depending on soil nitrogen status. Corn seed inoculation with *H. seropediacae* increased grain yield in greenhouse experiments by 49–82% when nitrogen was added, while only a 16% increase was observed without fertilizer. This indicated that the inoculum improved nitrogen assimilation by the plant (Riggs, Chelius, Iniguez, Kaeppler and Triplett 2001). Application of the inoculant in field experiments at different U.S. locations increased corn yield up to 20% (Riggs et al. 2001). Seed inoculation with a selected strain of *Burkholderia cepacia* enhanced corn yield 6% in field experiments; yield increase in greenhouse test using non-sterile soil varied between 36 and 48% depending on host cultivar and bacterial genotype (Riggs et al. 2001).

Sugarcane, like corn, is a nitrogen-demanding crop. Diazotrophs commonly associated with sugarcane include: *Acetobacter diazotrophicus, Azospirillum brasilense, A. linoleum, A. amazonense, Bacillus brasilensis, Burkholderia tropicalis, Herbaspirillum seropediacae* and *H. rubrisubalbicans* (Ries, Ries, Urquiaga and Dobereiner 2000; Sevilla and Kennedy 2000; Kennedy and Islam 2001). Application of diazotrophic PGPR (in soil or as a settes inoculation) can significantly reduce the amount of fertilizer nitrogen required for sugarcane production (Dobereiner 1997). Boddey, Polidoro, Resende, Alves and Urquiaga (2001), using ^{15}N

natural abundance technique, showed that BNF can contribute 60% of nitrogen assimilated by sugarcane not receiving fertilizer nitrogen. Dobereiner (1997) concluded that BNF can contribute up to 150 kg N/ha. Inoculation of sugarcane settes with biofertilizer (containing diazotrophs *Acetobacter diazotrophicus, Herbaspirillum* sp., *Azospirillum lipoferum* and a vesicular arbuscular mycorrhiza) in field experiments, which received 50% of the recommended nitrogen fertilizer, produced cane yields that were not significantly different from those that received the recommended amount of the fertilizer. It was suggested that the diazotrophs may have contributed the majority of plant's nitrogen requirement, as well as produced appreciable amounts of IAA that promoted rooting and improved growth, and that using biofertilizer could reduce the application of nitrogen fertilizer by 50% without yield loss (Muthukumarasamy, Revathi and Lakshminarasimhan 1999). The examples provided thus far have illustrated the use of PGPRs in crop production; however, there has also been extensive use of PGPRs and mycorrhizal fungi in forestry applications.

Examples of PGPRs used in forestry are provided in Table 4. Several PGPRs have been used to improve container growth and reduce transplant shock. Black oak seedlings inoculated with *Pisolithus tinctorius* improved seedling survival, growth in reforestation sites, and drought tolerance compared to bare root stock (Dixon, Wright, Garrett, Cox, Johnson and Sander 1981, 1983). Even at low colonization levels, American ash inoculated with *Glomus epigaeum* increased the seedling growth and dry weight (Furlan, Fortin and Planchett 1983). Pine seedling inoculated with *Pisolithus tinctorius,* and sawforth oak with *Thelephora terrestris*, enhanced seedling survival and increased plant height and diameter compared with natural inoculation in the field (Anderson, Clark and Marx 1983). Leucaena inoculated with *G. etunicatum* promoted its establishment under low fertility level (Tomar, Shrivastava, Gontia, Khare and Shrivastava 1985), and Thapar and Khan (1985) reported a significant increase in growth and dry weight of hoop pine seedlings grown in soil inoculated with VAM fungi.

As indicated earlier, there is a synergism between VAM and PGPRs. Inoculation of oak seedlings with *Azotobacter* was reported to be beneficial (Panday, Bahl and Rao 1986). Dual inoculation of leguminous trees with rhizobia and VAM fungus improves growth of the trees compared with plants inoculated with either inoculant alone. Significant growth increase of velvet wattle (66%) and of acacia (16%) resulted from seedling inoculation with *Rhizobium* sp. and *Glomus mosseae*, compared with rhizobia inoculation alone (Cornet and Diem 1982). The role of mycorrhiza in trees and the roles their symbioses play in forestry have recently been reviewed (Dahm 2006).

Table 4. Examples of growth-promoting rhizobacteria (PGPR) in forestry

Plant	PGPR	Response	Reference
White spruce, Lodge pole pine	*Bacillus* sp.	Increased seedling emergence, shoot height and weight, root surface area and weight.	Chanway et al. 1991
Lodge pole pine	*Bacillus* sp. and *Wilcoxina miklae* (mycorrhiza) (co-inoculation)	Increased shoot biomass and foliar nitrogen content.	Chanway et al. 1991
Pine, Spruce	Unidentified bacteria	Promoted growth; increased seedling biomass.	Chanway 1997
Loblolly pine	Unidentified bacteria	Reduced fusiform rust infection.	Enebak and Carey 2004
Jeffrey pine	*Pisolithus tinctorius*	Promoted root and shoot growth; increased nutrient uptake.	Walker and Kane 1997
Loblolly pine, Slash pine	Unidentified bacteria	Increased biomass. Promoted root and shoot growth.	Enebak et al. 1998
Loblolly pine	*Bacillus subtilis* *Paenibacillus macerans*	Protected against negative effects of ozone exposure.	Estes et al. 2004

17.4 Inoculum Preparation and Application

The potential of biofertilizers to increase plant growth and yield in controlled environments and the field is well documented. However, examples of inconsistent results are also reported. Inadequate colonization of the host rhizosphere by the introduced agents is probably the principal reason for inconsistencies in the expected results from field application of biofertilizers. Availability of soil nutrients, phosphate in particular, soil pH and moisture content are important factors influencing the survival, proliferation, and host-plant root occupancy. West, Burges, Dixon and Wyborn (1985) reported that soil nutrient availability was the most important factor in the survival of *Bacillus thuringiensis* and *B. cereus.* A better understanding of microbial ecology of the host rhizosphere in the presence of the introduced inoculant is essential before biofertilizers can become regular agriculture practice (Lazarovits and Nowak 1997).

Peat moss has been a popular carrier material for inoculant bacteria, but any suitable locally available material may be used. For example, finely pulverized rice-

husks are used in several Asian countries. The addition of bentonite clay to the carrier material promoted bacterial survival in fine textured soil (England, Lee and Trevors 1993). Chemical polymers for entrapping inoculant bacteria and application for subsequent colonization of the rhizosphere have shown promising results. Addition of other soil amendments may also encourage colonization. In one case, barley straw used as a soil additive promoted survival of the inoculant bacteria and improved root colonization (Stephens 1994).

The physiological status of the bacteria prior to application (mixing with the carrier material) appears to influence the survival and colonization. Application of the bacterium from the late exponential growth phase resulted in higher stabilization and reduced mortality compared to bacteria taken from an earlier growth phase (Vandenhove, Merchx, Wilmots and Vlassak 1991). Heijnen, Hok-A-Hin and van Veen (1992) found that mixing freeze-dried or fresh-grown *R. leguminosarum* cells with 1% bentonite clay prior to introduction to the soil markedly enhanced bacterial survival compared to treatments without the amendment. Starved cells introduced into sandy loam soil significantly enhanced *P. fluorescens* survival and wheat root colonization as compared to fresh cells (Heijnen, Hok-A-Hin and van Elsas 1993). Further research in the area is warranted.

Very few references concerning the delivery of the inoculant and the establishment of an effective population are available. It is known that the population density of the inoculum in the rhizosphere is often proportional to the initial load of inoculum on seed (Milus and Rothrock 1993). Although increasing the amount of inoculum used does increase the potential for a greater population in the rhizosphere, the results are not always consistent (Hebber, Davy, Merrin, McLoughlin and Dart 1992). Introduced bacteria must colonize their new soil-root environment while competing with indigenous microbes. For this reason, competitive ability and greater growth rate of the introduced inoculum in the rhizosphere are considered desirable traits in selecting a strain of inoculant bacteria. The root colonization is a competitive process affected not only by the characteristics of the introduced inoculant and the host, but also soil abiotic and biotic factors in the rhizosphere and their interactions. Few studies have been attempted to develop a screening method for identification of strains of selected bacteria (associative diazotrophs, PGPR, phosphate solubilizer, etc.) capable of establishing and maintaining an effective population density in the host rhizosphere throughout the life cycle of the host (Nijhuis, Maat, Zeegers, Waalwijk and Van Veen 1993). Commercial rhizobial inoculants usually contain multiple strains. Use of multiple strains of an inoculant bacterial species may enhance host plant root colonization; however it can not be recommended prior to field verification.

17.5 Commercial Availability of Biofertilizers

Tiwari et al. (2004) published a list of 35 sources of commercial biofertilizer. Twenty-four of these companies were located in North America. Of the remainder, six were located in Europe, two each in Asia and India, and one in South America. A fairly extensive internet search in 2006 revealed that 16 of these 35 companies were

still actively producing and marketing biofertilizer. Seven of the other 19 had ceased production and sales of biofertilizer, but continued marketing other products. The other 12 companies were either no longer in business or had merged with other corporations.

Results of our 2006 search did, however, consist of a total of 49 sources of biofertilizer in the following locations: 38 in North America, five in Europe, three in India, two in Asia, and one in South America. There may be other sources available that lack an internet site. A representative sample of commercial suppliers is provided in Table 5. Table 6 lists some of the most common uses of biofertilizer, while Table 7 lists some of the typical organisms used. The majority of the products are used for stimulation of growth (23%), insect control (21%), or disease management (14%). Although there appears to be a variety of commercial biofertilizers available, the internet and literature searches did not find many references as to their use in practical applications or recommendations for their use as part of a management practice.

Table 5. Selected biofertilizer companies

Company name	Location	Web address
ABTEC[1]	India	www.abtecbiofert.com
Accelerator Horticulture	USA	www.webberlandscape.com
Advanced Green	Taiwan	itrademarket.com
Aureus Biotech	Singapore	www.aureustech.com
Biocontrol Network	USA	www.biconet.com
BioFertilizer, Inc	Costa Rica	www.biofertilizer.com
BioMax	India	www.indiamart.com
BioOrganics	USA	www.bio-organics.com
BioRize	France	www.biorize.com
Cleary Chemical	USA	www.clearychemical.com
EM America	USA	www.emamerica.com
EuroAgro	Holland	www.euroagroec.com
Horticultural Alliance	USA	www.hortsorb.com
J.H. Biotech	USA	www.jhbiotech.com
Nafed BioFertilizer	India	www.nafed-india.com
Natural Industries	USA	www.naturalindustries.com
PlantWorks, Inc	UK	www.plantworksuk.co.uk
Premier Horticulture	Canada	www.premierhort.com
Prophyta GmbH	Germany	www.prophyta.de
Rhode's Nursery	USA	www.beorganic.com
Rizobacter Argentina S.A.	Argentina	www.rizobacter.com.ar
Roots, Inc	USA	www.rootsinc.com
Sri BioTech	India	www.sribio.com
Verdera	Finland	www.verdera.fi/homeeng.html

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Table 6. Typical uses of biofertilizers

Use	Percentage of products
Disease Control/Resistance/Suppression	14
Establishment/Vigor	13
Fungicide	8
Growth Stimulation	23
Insecticide	21
Nematicide	3
Nitrogen Fixation	5
Nutrient Uptake/Availability	6
Phosphorous Solubility	1
Stress Resistance	3
Yield	3

Table 7. Organisms used in biofertilizers and their typical use

Organism	Use
Acetobacter sp.	Nitrogen Fixation
Aspergillus sp.	Nutrient Uptake/Availability
Athrobacter sp.	Growth, Vigor
Azospirillum sp.	Yield
Azotobacter sp.	Establishment/Vigor
Bacillus sp.	Growth, Insecticide, Fungicide
Beauvaria sp.	Insecticide
Gigaspora sp.	Growth
Gliocladium sp.	Fungicide
Glomus sp.	Growth
Paecilomyces sp.	Nematicide
Phosphobacteria sp.	Phosphorus Solubilization
Pisolithus sp.	Growth
Pseudomonas sp.	Disease Control
Rhizopogon sp.	Disease Suppression
Trichoderma sp.	Fungicide

While biofertilizers are clearly potentially useful, it is apparent that a gap exists between research done by scientists and application in agricultural practices. Part of this may be due to the inconsistencies of the results between laboratory and field studies. It may be that we lack sufficient field studies to determine the beneficial effect of biofertilizers, or that our understanding of rhizosphere dynamics is too limited to understand the conditions required to establish a PGPR. It might be helpful if we evaluated commercially available biofertilizers in the field to establish the range of soils, environments, and management practices that limit their practical application. However, we may find that PGPRs are more useful during stress conditions or marginal production conditions. For example the use of PGPRs to enhance soybean seedling growth and nodulation under cool-soil temperature conditions (see Table 3). The use of biofertilizers in marginal or stress conditions needs to be evaluated further. Their use in resource-limited applications (reduced fertility, minimum input

systems) may be of greater benefit than when biofertilizers are used in conjunction with best management practices.

The gap between discovery of PGPRs, development of biofertilizers and their application may also be the result of inadequate technology transfer and limited farmer education. The Forum for Nuclear Cooperation in Asia held a technical meeting in June 2005 to evaluate the status of biofertilizer use in several Asian countries. In the meeting's summary, Thailand reported a problem with public relations and technology transfer as limiting biofertilizer use, while Indonesia cited lack of education for farmers as a primary problem. However, the countries outline plans to increase biofertilizer education for farmers and public relation efforts to try to make biofertilizer a more attractive option to the local agriculture community. Adequate efforts must be made to translate this research into forms easily adapted to and adopted by farmers in order for biofertilizer to be a viable long-term aspect of the agriculture industry.

17.6 Conclusions

The potential of PGPRs for enhancement of plant growth and yield, and their role in weed and disease suppression is well documented. However, inconsistencies in the effectiveness of PGPR inoculants between laboratory and field studies are a major impediment to their application in agricultural practices (Schroth and Becker 1990; Burdman, Vedder, German, Itzigsohn, Kigel, Jurkevitch and Okon 1998). The complexities of the plant-soil interactions and the dynamics of the rhizosphere organisms need to be more fully understood before the potential of PGPRs can be exploited. Further field studies with known PGPRs and commercial biofertilizers are needed to determine their effectiveness. Encapsulation, product shelf-life, and application methods need further evaluation. Finally, management practices incorporating PGPRs need to be designed and demonstrated as useful in crop production. When nitrogen fixing bacteria were introduced in legume production it took over 30 years to develop the technology to its present level. Effective strains, host compatibility, commercial preparation, and the transfer of the technology require time. We can use this experience to develop biofertilizers and established their use in achieving a sustainable agriculture.

Acknowledgments

The authors thank Crys Davis for her help in the internet and literature searches and assembling the commercial sources of biofertilizers, and Justin Cash for his technical help in literature searches and assistance in maintaining the reference database. In the final stages of preparing the chapter the authors located the following reference that might be of interest – Mulongoy, K., Gianinazzi, S., Rogers, P.A. and Dommergues, Y. (1992) Biofertilizers: agronomics, environmental impacts and economics. In: E.J. DaSilva, C. Ratledge and A. Sasson (Eds.). *Biotechnology: Economic*

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