Chapter 9
Role of PGPR Under Different Agroclimatic Conditions

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9.1 Introduction

- Despite an unprecedented increase in agricultural productivity during the twentieth century, the world faces uncertainty over global food security. The most pressing issue is the predicted increase in global population. Currently, the global population could be fed by the present level of agricultural output, and the global production of food is 145% greater today than it was in 1960 (Pretty 2008). However, it is unlikely that this growth in agricultural productivity can continue to keep pace with the rising population. In addition, increases in productivity over the last 50 years mask significant variations within developing regions that reflect political, economic, and social challenges for the 1.2 billion people who currently live in poverty (Hazell and Wood 2008). Moreover, most developing countries have environmental constraints that will impede the development of agricultural systems able to meet these challenges. These include lack of water, desertification, and insufficient cultivable land. Potentially, such problems could be further exacerbated by climate change. This in turn will place an increased pressure on the available agricultural land and its management (Cummings 2009). During last few decades, agricultural production has increased due to the use of high yielding varieties and enhanced consumption of chemicals, which are used both as fertilizers to provide nutrition and as protection agents to control the damage caused by phytopathogens. Although the use of chemicals has several advantages, such as ease of handling and yielding predictable results, yet several problems related to the continuous export of fertility of the soil, yielding great amount of ecological disastrous soil damage, health problems, and high irrigation demand, etc., came into existence (Harmen 1992). Excessive use

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of chemicals and change in traditional cultivation practices have resulted in the deterioration of physical, chemical, and biological health of the cultivable soil (Paroda 1997). Therefore, the productivity including production of a wide range of agricultural commodities under conditions of shrinking land resources and diminution of both biological potential of soil and biological wealth need to be increased. The objective of agriculture in coming decades is to optimize soil productivity (inclusive of stressed soils) while preserving its capacity to function as a healthy system. In this context, there is a strong case for using microorganisms for improved plant performance in integrated plant management systems. The use of soil microorganisms, which can stimulate plant growth, will be environmentally benign approach for nutrient management and ecosystem functions. This may ensure that nature is not exploited in the production process but is, instead, harmonized so that the entropy of environment decreases and sustainability in agricultural production is promoted (Khan et al. 2007). The management of agricultural soil is fundamental to ensuring a sustainable agricultural system. Consequently, there is increasing interest in developing and implementing the potential contribution of PGPR that are indigenous or inoculated into soils.

9.2 Plant Growth-Promoting Rhizobacteria

The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas et al. 2007). During the past couple of decades, the use of plant growth-promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been reported (Kloepper et al. 1980; Silva et al. 2006; Figueiredo et al. 2008; Arau´jo 2008). Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar, and genotype (Bashan and Holguin 1998; Lucy et al. 2004).

PGPR can affect plant growth by different direct and indirect mechanisms (Glick 1995; Gupta et al. 2000). Some examples of these mechanisms, which can probably be active simultaneously or sequentially at different stages of plant growth, are (1) increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; (2) repression of soilborne pathogens (by the production of hydrogen cyanide, siderophores, antibiotics, and/or competition for nutrients); (3) improving plant stress tolerance to drought, salinity, and metal toxicity; and (4) production of phytohormones such as indole-3-acetic acid (IAA) (Gupta et al. 2000). Moreover, some PGPR have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants (Glick 1995). By lowering ethylene concentration in seedlings and thus its inhibitory effect, these PGPR stimulate seedlings root length (Glick et al. 1999). The bacteria presenting one or more of these characteristics are known as PGPR.

9.3 Rhizosphere Colonization

Plant growth-promoting rhizobacteria (PGPR) colonize the roots of plants following inoculation onto seed and that enhance plant growth. The following are implicit in the colonization process: ability to survive inoculation onto seed, to multiply in the spermosphere (region surrounding the seed) in response to seed exudates, to attach to the root surface, and to colonize the developing root system (Kloepper 1993). The ineffectiveness of PGPR in the field has often been attributed to their inability to colonize plant roots (Bloemberg and Lugtenberg 2001). A variety of bacterial traits and specific genes contribute to this process, but only a few have been identified (Benizri et al. 2001; Lugtenberg et al. 2001). These include motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of specific cell surface components, ability to use specific components of root exudates, protein secretion, and quorum sensing (Lugtenberg et al. 2001). Using molecular markers such as green fluorescent protein or fluorescent antibodies, it is possible to monitor the location of individual rhizobacteria on the root using confocal laser scanning microscopy (Sorensen et al. 2001). This approach has also been combined with an rRNA-targeting probe to monitor the metabolic activity of a rhizobacterial strain in the rhizosphere and showed that bacteria located at the root tip were most active (Lubeck et al. 2000; Sorensen et al. 2001).

An important aspect of colonization is the ability to compete with indigenous microorganisms already present in the soil and rhizosphere of the developing plant. The factors involved in these interactions has been hindered by inability to culture and characterize diverse members of the rhizosphere community and to determine how that community varies with plant species, plant age, location on the root, and soil properties. Phenotypic and genotypic approaches are now available to characterize rhizobacterial community structure. Phenotypic methods that rely on the ability to culture microorganisms include standard plating methods on selective media, community level physiological profiles (CLPP) using the BIOLOG system (Garland 1996), phospholipid fatty acid (PLFA) (Tunlid and White 1992), and fatty acid methyl ester (FAME) profiling (Germida et al. 1998). Culture-independent molecular techniques are based on direct extraction of DNA from soil and 16S-rRNA gene sequence analysis, bacterial artificial chromosome, or expression cloning systems (Rondon et al. 1999). These are providing new insight into the diversity of rhizosphere microbial communities, the heterogeneity of the root environment, and the importance of environmental and biological factors in determining community structure (Smalla et al. 2001). These approaches can also be used to determine the impact of inoculation of plant growth-promoting rhizobacteria on the rhizosphere community (Ciccillo et al. 2002; Steddom et al. 2002).

9.4 Mechanisms of Action

PGPR enhance plant growth by direct and indirect means (Glick 1995). Direct mechanisms of plant growth promotion by PGPR can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Glick 1995). Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bashan and Holguin 1998; Bertrand et al. 2000). PGPR strains may use one or more of these mechanisms in the rhizosphere. Molecular approaches using microbial and plant mutants altered in their ability to synthesize or respond to specific phytohormones have increased understanding of the role of phytohormone synthesis as a direct mechanism of plant growth enhancement by PGPR (Glick 1995; Persello-Cartieaux et al. 2003). PGPR that synthesize auxins and cytokinins or that interfere with plant ethylene synthesis have been identified (Garcia de Salamone et al. 2001).

PGPR that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms. These include the ability to produce siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induce systemic resistance (Bloemberg et al. 2000; Glick 1995). Furthermore, biochemical and molecular approaches are providing new insight into the genetic basis of these traits, the biosynthetic pathways involved, their regulation, and importance for biological control in laboratory and field studies (Bloemberg and Lugtenberg 2001; Bowen and Rovira 1999; Persello-Cartieaux et al. 2003).

9.4.1 Enhancing Phosphorus Availability for Plant Growth by Rhizobacteria

Phosphorus (P) is an essential plant nutrient with low availability in many agricultural soils. Today many agricultural soils have a high total P content due to the application of P fertilizers over long periods of time. On the other hand, much of this P is in mineral forms and is only slowly available to plants (Rodriguez et al. 2006; Richardson et al. 2009). Most of the insoluble P forms are present as aluminum and iron phosphates in acid soils (Mullen 2005) and calcium phosphates

in alkaline soils (Goldstein and Krishnaraj 2007). The ability of rhizosphere bacteria to solubilize insoluble P minerals has been attributed to their capacity to reduce pH by the excretion of organic acids (e.g., gluconate, citrate, lactate and succinate) and protons (during the assimilation of $NH4^+$) (Gyaneshwar et al. 1999; Mullen 2005). These bacteria have been characterized as members of the *Bacillus, Burkholderia, Enterobacter, Klebsiella, Kluyvera, Streptomyces, Pantoea,* and *Pseudomonas* genera (Chung et al. 2005; Hariprasad and Niranjana 2009; Oliveira et al. 2009). These microorganisms grow in media with tricalcium phosphate or similar insoluble materials as the only phosphate source and not only assimilate the element but also solubilize quantities in excess of their nutritional demands, thereby making it available for plants (Martínez-Viveros et al. 2010).

Microorganisms with phosphate-solubilizing potential increase the availability of soluble phosphate and enhance the plant growth (Kucey et al. 1989; Ponmurugan and Gopi 2006). *Pseudomonas* spp. NBRI 4014 enhanced the root and shoot elongation in soybean crop at a significant level in the presence of heavy metals (Gupta et al. 2002). Similarly, phosphate-solubilizing bacteria enhanced the seedling length of *Cicer arietinum* (Sharma et al. 2007), while co-inoculation of PSM and PGPR reduced P application by 50% without affecting corn yield (Yazdani et al. 2009). In another study by Kaur (2008), it has been found that inoculation of spinach with two psychrotolerant stain *Pseudomonas putida* 710 A and *Commamonas aquatica* 710 B resulted an increase P content (Fig. 9.1) in soil as well as in plants (Table 9.1).

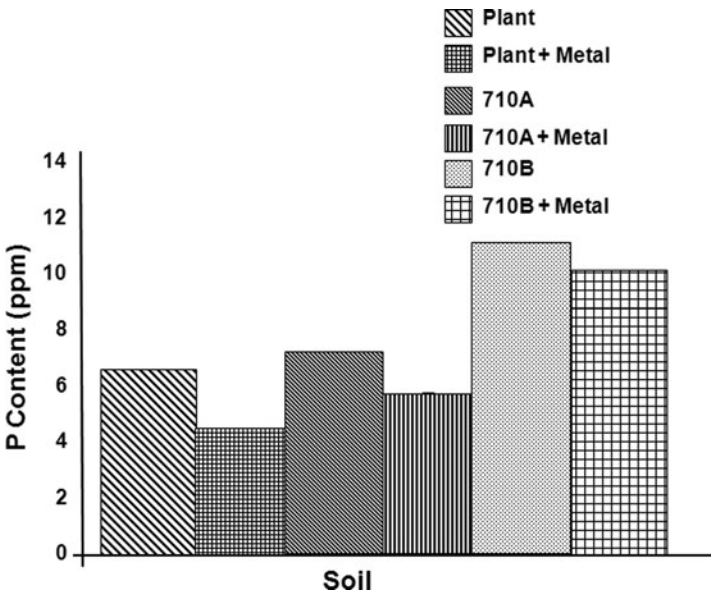

Fig. 9.1 Comparative "P" content in soil in presence of bioinoculants 710A and 710B

Table 9.1 Comparative "P" content in plants and soil in presence of bioinoculants 710A and 710B with respect to control

Strain	P solubilization (in vitro)	Treatments	P content in soil (ppm)	P content in plants (ppm)
–	–	Plants (control)	6.11 ± 0.32	6.62 ± 0.18
Pseudomonas putida 710A	(1.76 μg/ml)	Plants + *P. putida* 710A	6.41 ± 0.12 (4.9%) ↑	15.35 ± 0.20 (131.8%) ↑
Pseudomonas putida 710B	(240 μg/ml)	Plants + *C. aquatica* 710B	11.1 ± 0.52 (81.6%) ↑	18.79 ± 0.44 (183.8%) ↑

↑ % increase with respect to control
± values are SEM

The major limitation today for use of these organisms is the lack of consistent effects in mobilizing P under field conditions. This is likely due to competition with the native microflora and environmental factors that limit either the population size or activity of the PGPR. However, it is now clear that evaluation and ranking of P-solubilizing bacteria under laboratory conditions do not necessarily correspond to the efficacy of the PGPR for enhancing plant P uptake under field conditions (Richardson 2001).

9.4.2 *Facilitated Absorption of Iron by Production of Siderophores*

Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well aerated soil although it is easily precipitated in iron-oxide forms (Verma et al. 2010).

Siderophores are low-molecular-weight iron-binding molecules that are synthesized by many microorganisms (Neilands 1981). These compounds are produced by various types of bacteria in response to iron deficiency which normally occurs in neutral to alkaline pH soils, due to low iron solubility at elevated pH (Sharma and Johri 2003). Iron is essential for cellular growth and metabolism, such that Fe acquisition through siderophore production plays an essential role in determining the competitive fitness of bacteria to colonize plant roots and to compete for iron with other microorganisms in the rhizosphere (Crowley 2006). Siderophore-producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the area around the root (Siddiqui 2006).

Marschner and Römheld (1994) reported that plants may also utilize siderophores synthesized by microorganisms colonizing the rhizosphere; this would be a source of soluble iron for the host plant. Growth of cucumber in the presence of microbial siderophores resulted in increased plant biomass and chlorophyll content (Ismande 1998). Similarly, the growth of mungbean and pigeon pea enhanced in terms of increased root length, shoot length, and chlorophyll content in the presence of siderophore-producing *Pseudomonas putida*

KNP9 and *Proteus vulgaris* KNP3 strain, respectively (Tripathi et al. 2005; Rani et al. 2008). Uptake of microbial siderophores by plants has been attributed to microorganisms living.

9.4.3 Production of Phytohormones

The production of phytohormones by PGPR is now considered to be one of the most important mechanisms by which many rhizobacteria promote plant growth (Spaepen et al. 2007). Phytohormones are signal molecules acting as chemical messengers and play a fundamental role as growth and development regulators in the plants. Phytohormones are organic compounds that in extremely low concentrations influence biochemical, physiological, and morphological processes in plants, and their synthesis is finely regulated (Fuentes-Ramírez and Caballero-Mellado 2006). Numerous fungal and bacterial species can produce phytohormones (Tsavkelova et al. 2006). The phytohormone-producing ability is widely distributed among bacteria associated with soil and plants. Studies have demonstrated that the PGPR can stimulate plant growth through the production of auxins (indole acetic acid) (Spaepen et al. 2008), gibberellins (Bottini et al. 2004), and cytokinins (Timmusk et al. 1999) or by regulating the high levels of endogenous ethylene in the plant (Glick et al. 1998).

9.5 Survival of PGPR Under Different Agroclimatic Conditions

The rhizosphere is a complex habitat: there, the action of a growing root responding to its environment combines with that of the biotic (mostly the resident microorganisms) and abiotic soil components, which also respond to their environments. The introduction of a large amount of exogenous bacteria as an inoculant has the potential to affect these resident microorganisms, and similarly, an inoculant may be affected by them. Such interferences may result in increased, decreased, or no effect on PGPR effectiveness. Other stresses like desiccation, salinity, metals (Fig. 9.2), and temperature have direct effect on microbial population (Rani et al. 2009).

The salt pH and temperature-tolerant phosphate-solubilizing bacteria have been reported to be maximum in the rhizoplane followed by the rhizosphere and root-free soil in alkaline soils. The PSM strains with these stressed properties should therefore serve as an excellent model for studying the physiological, biochemical, and molecular mechanism of phosphate solubilization under stressed ecosystems (Khan et al. 2007).

In a study, screening and selection of cold-tolerant mutants of *Pseudomonas fluorescens* strains GRS_1, PRS_9, and ATCC13525 based on P-solubilization ability and subsequent effect on plant growth promotion under in vitro and in situ

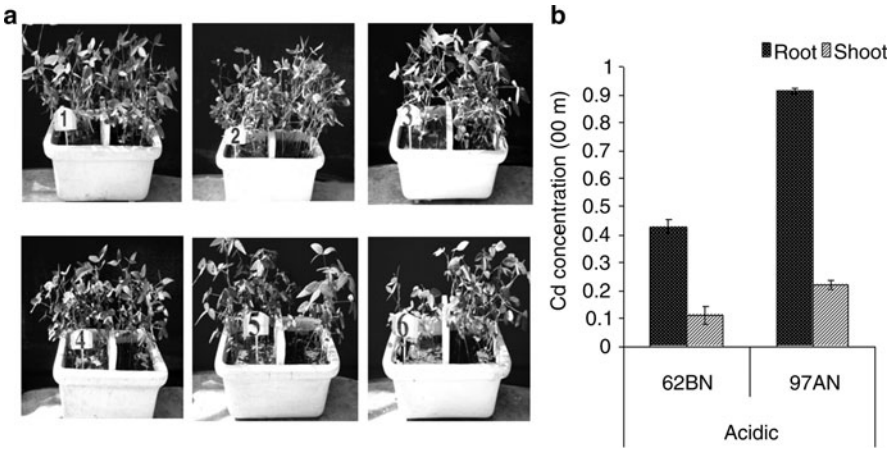

Fig. 9.2 (**a**) Effect of cadmium-resistant *P. putida* 62BN and *P. monteilli* 97AN bioinoculants on soybean growth in acidic soil, wherein (1) plants, (2) plant + cadmium, (3) *P. putida* 62BN, (4) *P. putida* 62BN + cadmium, (5) *P. monteilli* 97AN, and (6) *P. monteilli* 97AN + cadmium, respectively. (**b**) Comparative cadmium accumulation in soybean in the presence of *P. putida* 62BN and *P. monteilli* in acidic soil, respectively ($n = 3$, mean ± SEM)

condition was conducted. It has been found that there was 21-fold increase in $CRPF_2$ (GRS_1 mutant), and subsequent greenhouse trials revealed that $CRPF_2$ was a good rhizosphere colonizer as marked by a significant increase in root and shoot length of mungbean (Katiyar and Goel 2003).

In a screening of 4,800 bacterial isolates from the root-free soil, rhizosphere and rhizoplane of *P. juliflora* growing in alkaline soils, 857 morphotypes solubilized phosphate in agar. Phosphate-solubilizing ability of strain NBRI4 was higher than the control in the presence of salts (NaCl, CaCl2, and KCl) at 30°C, and it further increased at 37°C (Gaur et al. 2004). Strain NBRI2601 (Nautiyal et al. 2000) isolated from the rhizosphere of chickpea and alkaline soils could solubilize phosphorus in presence of 10% salt, pH 12, at 45°C suggesting that extensive diversity searches in appropriate habitats may lead to recovery of effective bacteria. The mechanism of osmotic stress adaptation in *P. aeruginosa* PAO1 was investigated by D'Souza-Ault et al. (1993). By using natural abundance 13C nuclear magnetic resonance spectroscopy, osmotically stressed cultures were found to accumulate glutamate, trehalose, and *N*-acetylglutaminylglutamine amide, an unusual dipeptide previously reported only in osmotically stressed *Rhizobium meliloti* and *P. fluorescens*. The intracellular levels of these osmolytes were dependent on the chemical composition and the osmolality of the growth medium. It was also demonstrated that glycine betaine, a powerful osmotic stress protectant, participated in osmoregulation in this organism (Tilak et al. 2005).

Another problem which is recently increasing is the contamination of soils with heavy metals through a variety of anthropogenic sources such as mining, the combustion of fossil fuels, metal-working factories, and the application of agrochemicals. Heavy metals tend to accumulate in the surface soil layer and can

Table 9.2 Metal-resistant bioinoculants with diverse physiological profile

Strain	Physiological profile	Metal tolerance level	Growth promotory property	Crop used	Reference
NBRI4014	Alkalophile (30°C)	Cd (0.18 mM)	P solubilization (277 μg/ml), siderophore production (143.87 μg/ml)	Soybean	Gupta et al. (2002)
KNP3	Mesophile (30°C)	Cd (1 mM) Pb (1.3 mM) Cu (1.3 mM)	Siderophore production (126.3 μg/ml)	Pigeon pea	Rani et al. (2008)
KNP9	Mesophile (30°C)	Cd (0.5 mM) Pb (1.5 mM)	Siderophore production (96.6 μg/ml)	Mungbean	Tripathi et al. (2005)
710A	Psychrotolerant (10°C)	Cd (1 mM)	P solubilization (1.76 μg/ml)	Spinach	Kaur (2008)
710B	Psychrotolerant (10°C)	Cd (0.5 mM)	P solubilization (240 μg/ml)	Spinach	Kaur (2008)

reach concentrations that are toxic for plants and living organisms (Gupta et al. 2002; Tripathi et al. 2005; Rani et al. 2008). One way to relieve heavy metal toxicity to plants might involve the use of growth-promoting bacteria. Considering our expertise in heavy metal and cold resistance, it was found that many bacterial strains which have shown ability for rescuing plant from metal toxicity and enhanced the plant growth in heavy metal-contaminated microcosm system (Table 9.2).

9.6 Challenges in Selection and Characterization of PGPR

One of the challenges in developing PGPR for commercial application is ensuring that an effective selection and screening procedure is in place, so that the most promising organisms are identified and explored. In the agricultural chemical industry, thousands of prospective compounds are screened annually in efficient high-throughput assays to select the best one or two compounds for further development. Similar approaches are not yet in place for PGPR. Effective strategies for initial selection and screening of rhizobacterial isolates are required. It may be important to consider host plant specificity or adaptation to a particular soil, climatic conditions, or pathogen in selecting the isolation conditions and screening assays (Bowen and Rovira 1999; Chanway et al. 1989). One approach for selection of organisms with the potential to control soilborne phytopathogens is to isolate from soils that are suppressive to that pathogen (Weller et al. 2002). Other approaches involve selection based on traits known to be associated with PGPR such as root colonization (Silva et al. 2003), 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Glick 1995), antibiotic (Giacomodonato et al. 2001),

and siderophore production. Further, the development of high-throughput assay systems and effective bioassays will facilitate selection of superior strains (Mathre et al. 1999).

9.7 Practical Consideration in the Use of PGPR

The concept of PGPR is now well established for both growth promotion as well as biocontrol; still the technology is not commercially successful, mainly because of the lack of reproducibility between trials conducted under controlled conditions (laboratory or glasshouse or greenhouse) and the fields. This happens so as in most cases the microbial inoculants are usually taken from one environment and introduced in another.

A common problem in much research on PGPR has been the failure to monitor the cell density of the introduced bacteria over time to confirm that inoculation was effective. In such cases, it is not possible to determine whether PGPR are responsible for the observed effects or to explain variations in efficacy of the inoculants that may be caused by management or environmental factors (Martínez-Viveros et al. 2010).

Mathematical modeling of the behavior of PGPR soil inoculants has been used to predict how various environmental factors affect the survival and activity of PGPR soil inoculants (Strigul and Kravchenko 2006). Supporting much experimental work, the model by Strigul and Kravchenko illustrates that survival and growth of newly introduced bacteria are strongly limited by competition for organic substrates with the resident microflora. PGPR are predicted to be the most effective in soils with low organic matter or stressed soils where growth of the indigenous population is restricted.

9.8 Rhizoengineering

Rhizoengineering includes strategies for manipulating plants and their root-associated microorganisms to improve plant health and productivity. Some strategies directly target plant processes that impact on growth, while others are based on our knowledge of interactions among the components of the rhizosphere (roots, microorganisms, and soil). For instance, plants can be engineered to modify the rhizosphere pH or to release compounds that improve nutrient availability, protect against biotic and abiotic stresses, or encourage the proliferation of beneficial microorganisms. Rhizobacteria that promote plant growth have been engineered to interfere with the synthesis of stress-induced hormones such as ethylene, which retards root growth, and to produce antibiotics and lytic enzymes active against soilborne root pathogens. Rhizosphere engineering also can involve the selection by plants of beneficial microbial populations. For example, some crop species or cultivars select for and support populations of antibiotic-producing

strains that play a major role in soils naturally suppressive to soilborne fungal pathogens. The fitness of root-associated bacterial communities also can be enhanced by soil amendment, a process that has allowed the selection of bacterial consortia that can interfere with bacterial pathogens. Plants also can be engineered specifically to influence their associated bacteria, as exemplified by quorum-quenching strategies that suppress the virulence of pathogens. New molecular tools and powerful biotechnological advances will continue to provide a more complete knowledge of the complex chemical and biological interactions that occur in the rhizosphere, ensuring that strategies to engineer the rhizosphere are safe, beneficial to productivity, and substantially improve the sustainability of agricultural systems (Ryan et al. 2009).

9.9 Future Prospects

As our understanding of the complex environment of the rhizosphere, of the mechanisms of action of PGPR, and of the practical aspects of inoculant formulation and delivery increases, we can expect to see new PGPR products becoming available. The success of these products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculant formulation and delivery. Genetic enhancement of PGPR strains to enhance colonization and effectiveness may involve addition of one or more traits associated with plant growth promotion. Genetic manipulation of host crops for root-associated traits to enhance establishment and proliferation of beneficial microorganisms is being pursued.

The use of PGPR inoculants in agriculture is already proceeding and offers many opportunities to improve plant nutrition, crop yields, and disease management, while improving sustainability by reducing the need for chemical inputs. Nevertheless, as our understanding of the ecology of these bacteria improves, it should be possible to obtain a more informed explanation of the mechanisms that are involved in plant growth promotion and identify situations in which bioaugmentation with soil inoculants may be useful for increasing crop yields.

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