Plant Growth Promoting Rhizobacteria: Fundamentals and Applications

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Abstract Plant growth promoting rhizobacteria (PGPR) have gained worldwide importance and acceptance for agricultural benefits. This is due to the emerging demand for dependence diminishing of synthetic chemical products, to the growing

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necessity of sustainable agriculture within a holistic vision of development and to focalize environmental protection. Scientific researches involve multidisciplinary approaches to understand adaptation of PGPR, effects on plant physiology and growth, induced systemic resistance, biocontrol of plant pathogens, biofertilization, and potential green alternative for plant productivity, viability of coinoculating, plant microorganism interactions, and mechanisms of root colonization. By virtue of their rapid rhizosphere colonization and stimulation of plant growth, there is currently considerable interest in exploiting these rhizosphere bacteria to improve crop production. The main groups of PGPR can be found along with the phyla Cyanobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Therefore, the examples coming up next are related to these microorganisms. Although taxonomic affiliation of validated genera containing PGPR strains described in literature is vast, phenotypic and genotypic approaches are now available to characterize these different rhizobacteria. The progress to date in using PGPR in a variety of applications is summarized and discussed here.

1 Introduction

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 repeatedly reported (Kloepper et al. 1980; Seldin et al. 1984; Chen et al. 1994; Zhang et al. 1996; Amara and Dahdoh 1997; Chanway 1998; Pan et al. 1999; Bin et al. 2000; Gupta et al. 2000; Biswas et al. 2000; Mariano and Kloepper 2000; Asghar et al. 2002; Vessey 2003; Gray and Smith 2005; Silva et al. 2006; Figueiredo et al. 2008; Araújo 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 1998; Gupta et al. 2000; 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 et al. 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 plant growth promoting rhizobacteria – PGPR (Kloepper and Schroth 1978).

Bashan and Holguin (1998) proposed the division of PGPR into two classes: biocontrol-PGPB (plant growth promoting bacteria) and PGPB. This classification may include beneficial bacteria that are not rhizosphere bacteria but it does not seem to have been widely accepted. According to Vessey (2003), numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, and stimulate plant growth by a plethora of mechanisms are collectively known as PGPR. Gray and Smith (2005) have recently shown that the PGPR associations range in the degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular (ePGPR), existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPR), which exist inside root cells, generally in specialized nodular structures.

There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism: suppression of plant disease (bioprotectants), improved nutrients acquisition (biofertilizers), or phytohormone production (biostimulants). Bacteria in the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium* are the biological control agents predominantly studied and increasingly marketed. They suppress plant disease through at least one mechanism, production of antibiotics or siderophores and induction of systemic resistance (Tenuta 2003).

Biofertlilizers are also available for increasing crop nutrient uptake of nitrogen from nitrogen-fixing bacteria associated with roots (Bashan and Holguin 1997), iron uptake from siderophore-producing bacteria (Scher and Baker 1982), sulfur uptake from sulfur-oxidizing bacteria (Stamford et al. 2008), and phosphorus uptake from phosphate-mineral solubilizing bacteria (Chabot et al. 1996). Biofertlilizers, that can cater different needs of growing plant, act as a consortium along with other microorganisms in the rhizosphere. Understanding the interaction between consortium of microbial inoculants and plant systems will pave way to harness more benefits from microbial inoculants for improving plant growth and yield (Raja et al. 2006).

2 Coinoculation of PGPR and Rhizobia: Improving Nodulation

Coinoculation studies with PGPR and Rhizobia have shown increased plant nodulation and N fixation (Li and Alexander 1988; Araújo and Hungria 1999; Vessey and Buss 2002; Silva et al. 2006; Figueiredo et al. 2007). Coinoculation of some *Bacillus* strains with effective *Bradyrhizobium* resulted in enhanced nodulation and plant growth of green gram (*Vigna radiata* L.) (Sindhu et al. 2002). A variety of rhizosphere microorganisms, including *Bacillus* and *Pseudomonas* species, are commonly found in the rhizosphere of leguminous and nonleguminous crops (Li and Alexander 1988). By virtue of their rapid colonization of the rhizosphere and stimulation of plant growth, there is currently considerable interest in exploiting

these rhizosphere bacteria to improve crop production. Application of *Bacillus* and/or *Paenibacillus* species to seeds or roots has been shown to cause alteration in the composition of rhizosphere leading to increase in growth and yield of different crops (Li and Alexander 1988; Vessey and Buss 2002). Disease suppression of alfalfa by *B. cereus* enhanced nodulation and seedling emergence in common bean (Camacho et al. 2001; Figueiredo et al. 2007), soybean (Araújo and Hungria 1999; Vessey and Buss 2002), cowpea (Silva et al. 2006, 2007), and pea (Cooper and Long 1994) have been demonstrated as beneficial effects on plants. Bacilli are also very attractive as potential inoculants in agriculture, as they produce very hardy spores that can survive for prolonged periods in soil and in storage containers (Nelson 2004).

Araújo and Hungria (1999) demonstrated the viability of coinoculating soybean seeds with crude or formulated metabolites, or with cells of *Bacillus subtilis*, to increase the contribution of the biological nitrogen fixation process.

PGPR, in combination with efficient rhizobia, could improve the growth and nitrogen fixation by inducing the occupancy of introduced rhizobia in the nodules of the legume (Tilak et al. 2006). According to Saravana-Kumar and Samiyappan (2007), *Bradyrhizobium* promoted the nodulation and growth of legumes in combination with active ACC deaminase containing PGPR. It has also been established that certain rhizobacteria possess an enzyme ACC-deaminase that hydrolyses ACC into ammonia and α-ketobutyrate (Mayak et al. 1999). ACC-deaminase activity in PGPR plays an important role in the host nodulation response (Remans et al. 2007). PGPR containing ACC-deaminase could suppress accelerated endogenous ethylene synthesis and thus may facilitate root elongation a nodulation and improve growth and yield of plant (Zafar-ul-Hye 2008).

3 Identification and Characterization of Beneficial Bacterial Strains for Agriculture

Identification and characterization of beneficial bacteria involves morphological, physiological and molecular characteristics based on fatty acid analysis, mol (%), G + C contents, DNA–DNA hybridization, and 16S rRNA sequencing. These characteristics help in defining the taxonomy and nomenclature of PGPR.

3.1 Taxonomy of PGPR

Taxonomy is defined as the science dedicated to the study of relationships among organisms and has to do with their classification, nomenclature, and identification (Mayr and Ashlock 1991; Coenye et al. 2005). The accurate comparison of organisms depends on a reliable taxonomic system. Although many new characterization methods have been developed over the last 30 years, the principle of identification

remains the same. Current schemes for identifying different bacterial strains may be roughly divided into four categories effectively based upon (1) traditional biochemical, morphological, and physiological characters, (2) miniaturized versions of traditional biochemical tests (e.g., API kits, VITEK cards, and Biolog plates), (3) chemotaxonomic characters (such as polyacrylamide gel electrophoresis [PAGE], and fatty acid methyl ester [FAME] profiles), and (4) genomic characters (16S rRNA gene sequencing, and DNA–DNA relatedness, and other techniques). Since the fifties, it was becoming clear that no one phenotypic technique would be suitable for identifying all bacterial species. Therefore, the potentials of chemotaxonomic analyses and studies of nucleic acids have been investigated. However, it is impossible to set up standardized conditions to accommodate the growth of all bacterial strains of all species for chemotaxonomic work, and a polyphasic approach is now imperative for a confident classification study. Polyphasic approach refers to the integration of genotypic, chemotypic, and phenotypic information of a microbe in order to perform reliable grouping of the organism (Colwell 1970). Some of the features used for polyphasic characterization of rhizobacteria are presented below. For overviews of modern taxonomy, recent papers can be referred, such as Vandamme et al. (1996), Prakash et al. (2007), Rodríguez-Díaz et al. (2008), and Logan et al. (2009).

3.2 Phenotypic Features

Phenotype includes morphological, physiological, and biochemical properties of the microorganism (de Vos et al. 2009). Traditional phenotypic tests used comprise colony morphology (color, dimensions, form) and microscopic appearance of the cells (shape, endospore, flagella, inclusion bodies), characteristics of the organism on different growth substrates, growth range of microorganisms on different conditions of salt, pH, and temperature, and susceptibility toward different kinds of antimicrobial agents, etc. Even if cell wall composition is analyzed, the Gram reaction is still a valuable diagnostic character. Biochemical tests in bacterial identification include the relationship with oxygen, fermentation reactions, and nitrogen metabolism. Other tests may be performed as appropriate, depending on the bacterial strains studied (Heritage et al. 1996; Rodríguez-Díaz et al. 2008). However, reproducibility of results from phenotypic tests between different laboratories is a great problem, and only standardized procedure should be used during execution of experiment. Other major disadvantage with phenotypic methods is the conditional nature of gene expression wherein the same organism might show different phenotypic characters in different environmental conditions. Therefore, phenotypic data must be compared with similar set of data from type strain of closely related organism(s).

Miniaturized versions of traditional biochemical tests are available for taxonomical studies and mostly contain a battery of dehydrated reagents. Addition of a standardized inoculum initiates the reaction (growth, production of enzymatic activity, etc.). The results are interpreted as recommended by the manufacturer and are readily

accessible with a minimal input of time. The phenotypic fingerprinting systems API 50CH – composed of 49 different carbohydrates and one negative control – have been used to identify *Bacillus* (Logan and Berkeley 1984) and *Paenibacillus* strains (Seldin and Penido 1986), while the API 20NE system has yielded the highest rate of correct identification of *Pseudomonas* species (Barr et al. 1989). In the same way, Biolog assay is considered a much less laborious system for bacterial identification (Miller and Rhoden 1991). This technique is based on the differential utilization of 95 carbon sources and a redox dye, tetrazolium violet, permits colorimetric determination of the increased respiration that occurs when cells are oxidizing a carbon source. The Biolog system was very useful for the identification of PGPR strains belonging to the species *P. azotofixans* (Pires and Seldin 1997).

3.3 Chemotaxonomic Characters

Some chemotaxonomic fingerprinting techniques applied to PGPR identification include FAME profiling, PAGE analysis of whole-cell proteins, polar lipid analysis, quinone content, cell wall diamino acid content, pyrolysis mass spectrometry, Fourier transform infrared spectroscopy, Raman spectroscopy, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Fatty acids are the major constituents of lipids and lipopolysaccharides and have been used extensively for taxonomic purposes. FAME analysis is presently the only chemotaxonomic technique that is linked to a commercial database for identification purposes. Fatty acid profiles showing variability in chain length, double-bond position, and substituent groups are perfectly suitable for taxon description and also for comparative analyses of profiles that have been obtained under identical growth conditions (Suzuki et al. 1993).

Sodium dodecyl sulfate-PAGE of whole-cell proteins requires standardized conditions of growth, combined with a rigorously standardized procedure for analysis, and normalization of the data for computer-assisted comparison of the results. Nevertheless, it has made important contributions to polyphasic taxonomic studies among the aerobic endospore formers (Logan et al. 2009).

Determination of the cell wall composition has traditionally been important in Gram-positive bacteria which contain various peptidoglycan types. The peptidoglycan type of Gram-negative bacteria is rather uniform and provides little information. Preparation of cell wall samples and determination of peptidoglycan structure is usually carried out using the methods described by Schleifer and Kandler (1972).

Isoprenoid quinones occur in the cytoplasmic membranes of most prokaryotes and play important roles in electron transport, oxidative phosphorylation, and, possibly, active transport (Collins and Jones 1981). There are two major structural groups, the naphthoquinones (subdivided into two types: the phylloquinones and the menaquinones) and the benzoquinones. The large variability of the side chains (differences in length, saturation, and hydrogenation) can be used to characterize bacteria at different taxonomic levels (Collins and Jones 1981).

The taxonomic importance of polar lipids has now been demonstrated for some novel genera among the *Bacillaceae*, although many polar lipids detected have not yet been structurally characterized. Likewise, quinones (MK-7, MK-8, and MK-9) have so far been reported for representatives of *Bacillaceae* (Logan et al. 2009).

Finally, pyrolysis mass spectrometry, Fourier transform infrared spectroscopy, and UV resonance Raman spectroscopy are sophisticated analytical techniques which examine the total chemical composition of bacterial cells. These methods have been used for taxonomic studies of particular groups of bacteria, including the members of the family *Bacillaceae* (Vandamme et al. 1996; Logan et al. 2009).

3.4 Genetic Approaches

Genotypic methods are those that are directed toward DNA or RNA molecules. Undoubtedly, these methods have revolutionized the bacterial identification system and taxonomy. Different techniques are now available to subtype bacteria up to strain level, such as restriction fragment length polymorphism (RFLP), plasmid profiling, ribotyping, amplified ribosomal DNA restriction analysis (ARDRA), pulsed field gel electrophoresis (PFGE), and randomly amplified polymorphic DNA (RAPD). Different PGPR have already been characterized by one or more of these methods (Oliveira et al. 2000; von der Weid et al. 2000; Depret and Laguerre 2008; Monteiro et al. 2009; and many others). For a detailed description of these methods, the reviews by Vandamme et al. (1996), Prakash et al. (2007), Rodríguez-Díaz et al. (2008), and Logan et al. (2009) can be referred.

For the description of bacterial taxa, other methods are essentially used. Determination of the moles percent guanosine plus cytosine is one of the classical genotypic methods. Generally, the range observed is not more than 3% within a well-defined species and not more than 10% within a well-defined genus (Stackebrandt and Goebel 1994).

DNA–DNA hybridization or DNA–DNA reassociation technique is based on the fact that at high temperatures DNA can be denatured, but the molecule can be brought back to its native state by lowering down the temperature (reassociation). This technique considers the comparison between whole genome of two bacterial species (Stackebrandt and Liesack 1993). A bacterial species, generally, would include the strain with 70% or greater DNA–DNA hybridization values with 5°C or less ΔTm values, and both the values must be considered. There are many different methods for DNA–DNA hybridization [presented and compared by Mora (2006)], but it is important to state that this technique gives the relative % of similarity but not the actual sequence identity.

DNA microarray is a method which was lined up to overcome the shortcomings of DNA–DNA hybridization. Although DNA microarray also involves hybridization of DNA, it uses fragmented DNA instead of whole genomic DNA. Numerous DNA fragments can be hybridized on a single microarray and gives resolution up to strain level. However, it is still an expensive methodology.

Indeed, taxonomy was revolutionized when the gene sequences of rRNA molecules were introduced to compare evolutionary similarities among strains (phylogenetic comparisons). All the three kinds of rRNA molecules, i.e., 5S, 16S, and 23S and spacers between these can be used for phylogenetic analyses, but 16S rRNA gene (1,650 bp) is the most commonly used marker. It has a universal distribution, highly conserved nature, fundamental role of ribosome in protein synthesis, no horizontal transfer, and its rate of evolution which represents an appropriate level of variation between organisms (Stackebrandt and Goebel 1994). The 16S rRNA molecule comprises of variable and conserved regions, and universal primers for the amplification of full 16S rRNA gene are usually chosen from conserved region while the variable region is used for comparative taxonomy. The 16S rRNA gene sequence is deposited in databases such as Ribosomal Database Project II (http://rdp.cme.msu.edu/) and GenBank (http://www.ncbi.nlm.nih.gov/). Sequences of related species for comparative phylogenetic analysis can also be retrieved from these databases. Thereafter, sequence comparing software packages such as BLAST and CLUSTAL X are used for alignment of 16S rRNA gene sequence. The extent of relatedness between bacterial species can be scrutinized by the construction of phylogenetic trees or dendrograms. The phylogenetic tree ascertains the genus to which the strain belongs and its closest neighbors, i.e., those sharing the clade or showing >97% 16S rRNA gene sequence similarity, are obtained from various culture collections to perform further genotypic, chemotaxonomic, and phenotypic analysis. At present, by correlation with experimental data obtained in the comparison of total genomic DNA (DNA–DNA hybridization), it is proposed that a similarity below 98.7–99% on the 16S rRNA gene sequences of two bacterial strains is sufficient to consider them as belonging to different species. On the other hand, two strains showing similarities above the 98.7% threshold may represent two different species. In these cases, total genome DNA–DNA hybridization must be performed and those strains for which similarities are below 70% are considered to belong to different species (Stackebrandt and Liesack 1993; Stackebrandt and Goebel 1994).

Finally, sequences of other highly conserved housekeeping or other protein-encoding genes, such as *rpoB*, *gyrB*, *recA*, have also great potential for taxonomic analysis at the species level. For example, Mota et al. (2005) obtained clustering patterns for *Paenibacillus* based upon *rpoB* sequence comparisons that were similar to those obtained with 16S rRNA gene sequences. Moreover, Wang et al. (2007) included *gyrB* sequence comparisons in the studies of the *B. subtilis* group and Cerritos et al. (2008) included *recA* sequence comparisons in the work that led to the proposal of a new *Bacillus* species.

4 Prospective Biocontrol Agents of Plant Diseases

Since 1987 in China, PGPR, called yield increasing bacteria (YIB) have been largely applied in 48 different crops over 3.35 millions of hectares (Wenhua and Hetong 1997). In that country, productivity gains as high as 23.1% and 22.5% were

obtained, respectively, in sweet potatoes and potatoes. Additionally, remarkable 85.5% and 80.3% reduction levels of diseases caused by *Xanthomonas oryzae* pv. *oryzae* and *Glomerella cingulata*, respectively, were recorded (Zhang et al. 1996).

Rhizobacteria are effective competitors in the rhizosphere which can establish and persist on roots of agronomically grown plants (Kloepper and Mariano 2000). PGPR may promote plant growth directly on healthy plants or indirectly when controlling phytopathogens or pests in different crops (Kloepper 1993; Medeiros et al. 2005; Zhender et al. 1997; Keel and Maurhofer 2009). They can be isolated from any other plant part besides the roots as well as from the plant surface or interior. According to Hallman et al. (1997), the endophytic bacteria involved in biological control showed advantages of having the same ecological niche of the pathogen and could be protected from diverse abiotic influences.

The PGPR mechanisms for plant growth improvement were already discussed in this chapter. PGPR also exhibit several mechanisms of biological disease control, most of which involve competition and production of metabolites which affect the pathogen directly. Examples of such metabolites include antibiotics, cell wall-degrading enzymes, siderophores, and HCN (Enebak et al. 1998; Kloepper 1993; Weller 1988). It is noteworthy to state that different mechanisms may be found in a single strain and act simultaneously. Some PGPR do not produce metabolites against the pathogens and are spatially separated from them. These two traits suggest that alteration of host defense mechanisms account for the observed disease protection. Induced systemic resistance (ISR) or systemic acquired resistance (SAR) is defined as the activation of chemical and physical defenses of the plant host by an inducer which could be a chemical or a microorganism, leading to the control of several pathogens (Kloepper et al. 1992). Several PGPR strains can act as inducers of ISR (Kloepper et al. 1992), and PGPR-mediated ISR may be an alternative to the use of chemical inducers or pathogens for inducing SAR. This mechanism is discussed separately in this chapter.

Two cases of study will be discussed here: black rot of crucifers, a foliar disease, and Fusarium wilt of banana, a vascular disease. Black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) causes severe economic losses in all developmental crucifer stages (Mariano et al. 2001). *Bacillus* spp. isolated from healthy cabbage, kale, and radish had reduced black rot incidence in kale and cabbage in greenhouse and field experiments (Assis et al. 1996). Monteiro et al. (2005) showed that four of these *Bacillus* strains produced lipopeptides active against *Xcc* during its late growth phase. These peptide antibiotics are amphiphilic compounds with surfactant activity (Zuber et al. 1993). Recently, it was demonstrated that lipopeptides can stimulate ISR in plants, probably by interacting with plant cell membranes and inducing temporary alterations in the plasma membrane which could raise plant defenses (Ongena et al. 2009).

Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* is a very destructive disease in Brazil and other parts of the world. The rhizomes and pseudostems of infected plants used for propagation are the principal sources of inoculum and disease dispersion. Therefore, micropropagated health plantlets are used to prevent or delay the introduction of this pathogen in soils. However, these plantlets

are more susceptible to this and other soilborne pathogens and should be protected before transplanting. PGPR are an alternative for improving this system. In greenhouse studies, endophytic and epiphytic bacteria applied, isolated or in mixtures, as root and substrate treatments, significantly increased the growth of micropropagated banana plantlets and controlled fusarium wilt (Mariano et al. 2004) (Fig. 1). According to Nowak and Shulaev (2003), the production of high-quality propagules with disease resistance may be achieved among others methods by their "in vitro" and "ex vitro" biopriming (priming with beneficial microorganisms).

Commonly, control is based on the use of single biocontrol agents. This strategy must be changed because, from the ecological point of view, the disease is part of a complex agroecosystem. As reported by Fravel (2007), a holistic view of this system can help take correct decisions about management. Therefore, a special approach for improving the PGPR efficiency is the use of mixtures containing different genera or species that presents additive or synergistic effects such as nitrogen-fixing bacteria and mycorrhiza helper bacteria (MHB). Another strategy is to use PGPR, mixed or alternated with fungicides, integrating biological and chemical control.

MHB are those which either assist mycorrhiza formation or promote the functioning of their symbiosis. They exist in arbuscular and ectomycorrhizal systems. MHB present three significant functions: nutrient mobilization from soil minerals, fixation of atmospheric nitrogen, and plant protection against root pathogens (Frey-Klett et al. 2007). According to these authors, PGPR induced increases in mycorrhizal root colonization from 1.1 to 17.5 fold in different interactions. Some of the MHB cited were

Fig. 1 Biocontrol of Fusarium wilt in micropropagated banana plantlet cv. Pacovan treated with *Bacillus pumilus* ENF24 (*right*) compared with plantlet not treated (*left*). Plantlets were vertically sliced to show rhizome discoloration, an internal disease symptom

Pseudomonas fluorescens, *P. monteilii*, *Bacillus coagulans*, *B. subtilis*, *Paenibacillus brasilensis*, *Rhizobium leguminosarum*, and *Bradyrrhizobium japonicum*.

Wheat seeds treated with different mixtures of *Paenibacillus macerans* and difenoconazole showed significant reduced incidences of pathogens (Luz 2003a), and in field all treatments promoted germination and grain yield except for difenoconazole alone that increased only yield. Similar results were obtained when corn seeds were bacterized with the same bioprotector + fludioxonil + metalaxyl M (Luz 2003b). Also *Bacillus*-based treatments have been successfully combined with traditional chemical seed treatments (Bugg et al. 2009). Therefore, the use of such mixtures may lead to a substantial reduction of pesticide use in several crops.

It is also important to focus on the critical stages of commercialization of biocontrol agents. Screening for new agents should consider the biology and ecology of the pathosystem, as well as agricultural practices associated with the crop (Fravel 2007). This knowledge will help prevent variation in field performance which is responsible for lack of wider adoption of biocontrol for disease management. The formulation stage aim is to deliver the biocontrol agent in a physiologically active state to provide the needed control. The formulation must be economical and present good shelf-life and a suitable form for shipping, storage, and application. Risk assessment to human health and to the environment are needed before releasing the new product, and early in the screening; even microorganisms with good biocontrol potential but capable of growing at human body temperature should be eliminated (Fravel 2007). In the United States, organisms currently registered for biocontrol and active compounds isolated from plants or other organisms are listed at http://www.epa.gov/oppbppd1/biopesticides/ingredients/index.htm. A few examples of PGPR and biocontrol products are: *Agrobacterium radiobacter* K1026 (Nogall®), *Bacillus pumilus* QST 2808 (Sonata® TM), *B. pumilus* GB34 (YieldShield®), *B. subtilis* GBO3(Kodiak®), *Pantoea agglomerans* C9-1 (BlightBan C9-1®), *P. agglomerans* E325 (Bloomtime®), *Pseudomonas aureofaciens* Tx-1(Spot-Less®T), *P. syringae* ESC-10 and ESC-11 (Bio-save®), *P. fluorescens* A506 (BlightBan®), *P. chlororaphis* MA 342 (Cedomon®), *Streptomyces griseoviridis* K61 (Mycostop®), and *S. lydicus* WYEC 108 (Actinovate®).

5 Induced Systemic Resistance as a Mechanism of Disease Suppression by Rhizobacteria

The increased level of resistance using external agents, without modifying the genome of the plant, is known as induced or acquired resistance. The expression of induced resistance can be local or systemic when it is expressed at sites not directly exposed to the inducers agent (Stadnik 2000). This agent may be a chemical activator, extracts of cells of living organisms or microorganisms (Romeiro 2000). The event of ISR has been demonstrated in various plants inoculated with different species of rhizobacteria (Liu et al. 1995; Raj et al. 2003; Halfeld-Vieira et al. 2006). This type of induced resistance can occur under

controlled conditions and in the field, and shows advantages such as: effectiveness against various pathogens; stability due to the action of different mechanisms of resistance, systemicity, energy economy; and metabolic utilization of genetic potential for resistance in all susceptible plants (Bonaldo et al. 2005).

The ISR occurs when plants previously exposed to biotic and abiotic agents are induced to defense against pathogens, which are spatially separated from the inducer agent (Pieterse and Van Loon 1999; Stadnik 2000). PGPR that inhabit the soil and are often isolated from the rhizosphere of several plants have been studied as potential biotic agents of ISR (Mariano and Kloepper 2000). *Bacillus* and *Pseudomonas* are among the most studied genera of PGPR.

It is known that susceptible plants have genetic information for efficient mechanisms of resistance to diseases and that these mechanisms can be systematically expressed for long periods of time by prior inoculation with avirulents pathogens, microbial components, and chemical substances (Kuc 1995). The ISR is persistent and presents complex components in different locations which are responsible for the activity of various defense compounds. Consequently, it is more stable when compared with the few pathways arising from the use of chemical pesticides.

Despite the many studies in this area, only in 1961 the induced resistance was first analyzed, by preinoculation of tobacco plants with tobacco mosaic virus (Ross 1961). This procedure protected the plant against other viruses and resulted in the conception of "Systemic Acquired Resistance" (SAR). The activation of defense mechanisms induced by fungi, bacteria, viruses, and nematodes can be achieved by different routes, which may occur alone or concomitantly (Bonaldo et al. 2005).

Problems of variability in the effectiveness of induced resistance to diseases in plants in different soil and climatic conditions may occur, similar to that found in biological control (Kuc 1995). In agriculture, the use of biological products on the induction of resistance in plants has one more benefit that can be added to the already known to reinforce the plant growth promotion. Induction of resistance by the application of chemical inducers has been used in some crops in the integrated management of diseases and pests. The use of biological inducers may be an option in the management of diseases in plants. The positive effects of PGPR on plants usually are included in two categories: promotion of growth and biological control (Mariano and Kloepper 2000). In practice, these effects are often induced by the same strain of PGPR; therefore, some PGPR selected to promote growth also are able to control diseases and vice versa. The presence of the PGPR in the rhizosphere makes the entire plant, including the shoot, more resistant to pathogens.

Induction of resistance promoted by PGPR is active and signaling in the route of salicylic acid with induction of PR-proteins (proteins related to the pathogenesis) or route of the jasmonic acid and ethylene (Hoffland et al. 1995; Pieterse et al. 1998). When the PGPR colonize the root system, constituents of bacterial cell molecules or synthesized by elicitors act as a biochemical signal. This time, the genes that encode for the synthesis of components of the dynamic resistance are activated and ISR is expressed (Romeiro 2000). Wei et al. (1991) working with cucumber and anthracnose caused by *Colletotrichum orbiculare* showed that this plant could be used as a model for ISR.

In addition to the PR-proteins, the plants produce other enzymes of the defense, including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenol-oxidase (PPO). Peroxidase and PPO are catalysts in the formation of lignin. PAL and other enzymes are involved in the formation of phytoalexins. Chen et al. (2000) reported that ISR mediated by PGPR against *Pythium aphanidermatum* in cucumber was associated with an increase of peroxidases, PPO and PAL. Metabolic changes involved in the defense mechanism of plants are correlated with changes in activity of key enzymes in primary and secondary metabolism. The production of enzymes related to pathogenesis (PR-proteins) by strains of rhizobacteria is considered the largest property of the antagonistic strains (Saikia et al. 2004). Among these enzymes can be highlighted chitinases, lipoxygenases, peroxidases, and glucanases. Plants express the activity of peroxidase during pathogen–host interaction (Saikia et al. 2006), where this enzyme has been implicated in the oxidation of phenols (Schmid and Feucht 1980), lignification (Saparrat and Guillen 2005), plant protection (Hammerschmidt et al. 1982), and elongation of plant cells (Goldberg et al. 1986). Increased activity of peroxidase has been correlated with resistance in many plant species, including rice and wheat (Young et al. 1995). The action of lipoxygenase products contributes to the defense reactions involving the inhibition of growth of the pathogen and induction of phytoalexins (Li et al. 1991). The phytoalexins are secondary metabolites, antibiotics, low molecular weight produced by plants in response to physical stress, chemical, or biological. They are able to prevent or reduce the activity of pathogens, the rate of production dependent on the genotypes of host and/or pathogen (Daniel and Purkayastha 1995). The phytoalexin compounds are biocides and are directly related to the defense mechanisms of plants.

In several studies, the quantification of activity of enzymes involved in the induction of resistance has been used as a parameter to assess the induction mechanism (biotic or abiotic) involved (Knorzera et al. 1999; Campos et al. 2004; Nakkeeran et al. 2006; Silva et al. 2004; Halfeld-Vieira et al. 2006; Saikia et al. 2006). The increase in activity and accumulation of these enzymes depend mainly on the inducing agent but also the genotype of the plant, physiological conditions, and the pathogen (Tuzun 2001). Depending of pathosystem studied, a variety of substances are produced by rhizobacteria and has been linked to activation of mechanisms of disease suppression in plants which reduce the damage caused by phytopathogens. Thus, the application of PGPR in agriculture via soil or seed inoculation can be characterized as a beneficial component in the integrated management of diseases.

6 Bacterial Biofertilizers

Before initiating a review of PGPR as biofertilizers, it is necessary to define the term biofertilizer. It is proposed frequently here that biofertilizers designate the biological products which contain microorganisms providing direct and indirect gains in yield from crops. Vessey (2003) defines biofertilizers as a substance which

contains living microorganisms which, when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients the host plant. Rhizobacteria, associated with rhizosphere, can fix nitrogen, and solubilizing phosphorus has been used as inoculum in nonleguminous species such as maize, rice, wheat, and sugar cane (Dobereiner 1997). Biofertilizers have been an alternative to mineral fertilizers to increase the yield and plant growth in sustainable agriculture (Canbolat et al. 2006).

The mechanisms by which PGPR promote plant growth are not fully understood but include among others: ability to produce or change the concentration of plant hormones (Mordukhova et al. 1991); asymbiotic N_2 fixation (Boddey and Dobereiner 1995); and solubilization of mineral phosphate and other nutrients (De Freitas et al. 1997). The production of hormones in PGPR in numerous studies reports the importance of indolacetic acid (IAA) in the roots development (Aloni et al. 2006). The effect of exogenous IAA in the plant can stimulate or inhibit growth and is often a function of hormones concentration available; in addition, the sensitivity of plant tissue changes according to hormones concentration (Persello-Cartieux et al. 2003). It was reported that isolates of *Pseudomonas* (fluorescent) produced exudates in roots of maize in response to IAA (Pan et al. 1999). Gibberellins were detected in several cultures of *B. subtilis*, but were not detected in the presence of auxin (Broadbent et al. 1971). Analyzing the sources of IAA with bacterial origin, Loper and Schroth (1986) found two strains of *Pseudomonas* spp. producing high concentrations of IAA (5–10 mg/ml), which reduced roots elongation and increased shoot/root proportion in sugar beet plants (*Beta vulgaris*) when applied as seed inoculant in this culture. Araújo et al. (2005) detected auxin production in two strains of *B. subtilis* which provided benefits in growth of soybean, in addition to be antagonists of phytopathogenic fungi in culture. Araújo and Hungria (1999) found that *B. subtilis* (AP-3) or its metabolites provided increase in nodulation and yield of soybean in the field.

Gains in nutrition in plants inoculated with rhizobacteria have also been demonstrated as a benefit of the presence of this group of microorganisms in the rhizosphere. In relation to nitrogen for several years has been discovered the potential of bacteria from the genus *Azospirillum*; fixing nitrogen when in free-living (Boddey and Dobereiner 1995), which when associated with the rhizosphere may contribute to nitrogen nutrition of plants. Concerning phosphate nutrition, Rodriguez and Fraga (1999) mention that strain from the genus *Pseudomonas*, *Bacillus* and *Rhizobium* are among the bacteria with the greatest potential of solubilization of phosphorus in the soil.

The solubilization of insoluble phosphates mediated by microorganisms is associated with the detachment of organic acids which are often combined with other metabolites, as found in vitro, that the potential for P solubilization by microorganisms is directly related to production of siderophores, lytic enzymes, and phytohormones (Vassilev et al. 2006). With the increased availability of nutrients in the soil by the action of *B. subtilis*, was shown higher absorption of nutrients such as phosphorus and nitrogen in plants inoculated with rhizobacteria

on seeds (Araújo 2008). Richardson (2000) reported that most soils are poor in available phosphorus and phosphate fertilizer represents a high cost to the farmer; therefore, it is interesting to take advantage of soil microorganisms used as inoculum for the mobilization of phosphorus in poor soils. In addition to phosphorus solubilization, other mechanisms are also related to the microbial metabolism in soil, such as enzymes production (nitrogenase, chitinases, and glucanases) (Cattelan et al. 1999).

Some failures derived from the use of biofertilizers containing PGPR may be related to interspecific genetic interaction by the rhizobacteria and the host plant. Previous studies have documented phenotypic variation within cultivars with respect to health and nutrition of plants from microbial inoculation (Remans et al. 2008). Different cultures and species or cultivars may produce different types of root exudates, which may support the activity of the inoculum or serve as substrate for the formation of biologically active substances by the inoculum (Khalid et al. 2004). Dalmastri et al. (1999) reported that different maize cultivars could provide variation in the rhizosphere colonization by *Burkholderia*. Phenotypic variation among cultivars may be partly due to genetic variation and suggested that the breeding of the host was performed in conjunction with PGPR in biofertilizers (Remans et al. 2008). Another strategy to reduce the effects of phenotypic variation can be the use of biofertilizers with more than two isolates in their composition. Studies conducted for 2 years with the application of biofertilizers originating from a mixture of isolates of *Bacillus* showed increase in plant growth and productivity (Adesemoye et al. 2008).

A major problem for massive use of PGPR has been formulated for its commercial use. These include production in the scale of fermentation microorganisms with management of the quality, stability, and effectiveness of the product. *B. subtilis* has been assessed as of great potential for use in agriculture and has been used in the formulation of commercial products for agricultural use in several countries (Lazzareti and Bettiol 1997). Several substances have been used in experimental formulations such as lactose, peptone, gum arabic and xanthan, cellulose, and others (Schisler et al. 2004). This formulation may require a significant value to determine the effectiveness of the final product based on rhizobacteria such as the *B. subtilis*.

Development of formulations with a potential PGP to ensure survival and activity in the field and compatibility with chemical treatment of seeds has been the focus of researches with application of PGPR in agriculture. The research among other things optimizes growth conditions before the formulation, development of vehicles, and appropriate technology for application (Date 2001). In registration and marketing of products with PGPR, a large number of constraints are found (Mathre et al. 1999).

The U.S. market based on the information of the committee of biological products from the American Phytopatology Society (APS) in 2005 has registered the following products: ten products based on the *Bacillus* (*BioYield*, *Companion*, *EcoGuard*, *HiStick N/T*, *Kodiak* , *Mepplus*, *Serenade*, *Sonata*, *Subtilex*, *YieldShield*), two products with *Burkholderia cepacia* (*Deny* and *Intercept*), and six

products based on *Pseudomonas* (*AtEze*, *Bio-save*, *BlightBan*, *Frostban*, *Spot-Less*). Most of these products has been disposed in powder solubleformulate. Different genera of bacteria have been studied as PGPR; however, investments in research and development of bioproducts have been higher in projects on *Pseudomonas* and *Bacillus*. Works on *Pseudomonas* have been focused on alternatives to improve the survival of this species of bacteria in commercial formulations. Furthermore, bacteria from the genus *Bacillus*, which are tolerant to desiccation and heat, have a longer life in commercial formulations; this explains the greater availability of commercial products based on *Bacillus*.

Currently, biofertilizers with PGPR are still not a reality of extensive commercialization – unlike the agricultural use of legume inoculants using rhizobia already a reality for almost a century – except for *Azospirillum* inoculants that are available for a variety of crops in Europe and Africa (Vessey 2003). There is no doubt that the lack of consistent responses in different host cultivars (Remans et al. 2008) and different field sites (Hilali et al. 2001) are reasons that limit expansion of the marketing of biofertilizers with PGPR. For these, it would be necessary to carry out more studies on ecology and colonization of microorganisms in the rhizosphere at different situations, since the biofertilizers with PGPR are restrictive for certain cultivars, climate, and soil conditions.

7 Concluding Remarks

PGPRs are the potential tools for sustainable agriculture and trend for the future. For this reason, there is an urgent need for research to clear definition of what bacterial traits are useful and necessary for different environmental conditions and plants, so that optimal bacterial strains can either be selected and/or improved. Combinations of beneficial bacterial strains that interact synergistically are currently being devised and numerous recent studies show a promising trend in the field of inoculation technology.

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