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Chapter 10

ROLE OF PLANT GROWTH-PROMOTING BACTERIA IN SUSTAINABLE AGRICULTURE

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ABSTRACT

Soil is a dynamic, living, no-renewable resource vital to the production of food and fiber and important to global balance and ecosystem function. In the last decades, it has been given importance to biological processes involving soil microorganisms, contributing to maintenance of soil fertility in relation to N and P and reducing the losses of nutrients, by lixiviation or volatilization. The number of soil microorganisms in a fertile soil can exceed nine billion. Thus, soil microbial has an important role in agriculture, mainly plant growth-promoting bacteria. The most studied genera are still those belonging to the aerobic endospore-forming bacteria, including *Bacillus* spp. and *Paenibacillus* spp., to *Pseudomonas* spp. and to the Rhizobiales order, including *Rhizobium* spp., *Bradyrhizobium* spp., *Mesorhizobium* spp. *Ensifer* spp. and others. In this way, there are several roles of these bacteria on plant growth and yield, mainly in tropical regions. In this chapter, we will show the main characteristics and importance of these bacteria in tropical agriculture.

Keywords: diazotrophic, *Bacillus*, crops science, PGPB, soil microbiology.

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1. BIOLOGICAL NITROGEN FIXATION (BNF)

BNF is the process by which atmospheric nitrogen gas (N_2) is converted into ammonia (NH_3) and it is subsequently available for plants. In agricultural settings, perhaps 79% of this biologically fixed N_2 comes from symbioses involving leguminous plants and bacteria of the family *Rhizobiaceae*. The family *Rhizobiaceae* currently includes six genera: *Rhizobium, Ensifer (Sinorhizobium), Mesorhizobium, Allorhizobium, Azorhizobium*, and *Bradyrhizobium*, which are collectively referred to as rhizobia (van Berkum & Eardly 1998). In recent years, however another α-proteobacteria have been showed to produce nodules in the legume (Moullin et al., 2002) *Methylobacterium* (Sy et al., 2001); *Blastobacter* (Van Berkun & Eardly, 2002), and *Devosia* (Rivas et al., 2002) as well as β- proteobacteria such as *Ralstonia* (Chen et al., 2001) and *Burkholderia* (Chen et al., 2003).

Nodulation is a highly host-specific interaction in which, with few exceptions, specific rhizobial strains infect a limited range of plant hosts. Plants secrete flavonoids that are recognized by the compatible bacteria, resulting in the induction of nodulation genes. These nodulation genes encode enzymes that synthesize a specific lipochitin nodulation signal (Nod signal), which activates many of the early events in the root hair infection process (Oldroyd et al., 2005).

Nitrogen fixation by legumes can also maintain soil fertility and can be of benefit to the following crop. Legumes have considerable potential in crop rotations in sustainable agricultural systems in maintaining soil fertility and thus reducing the need for nitrogen fertilizer. Reduced use of nitrogen fertilizer can decrease nitrate leaching from soils and so can reduce eutrophication, which is a major environmental problem worldwide (Date 1996).

The agronomic implications of this symbiosis have promoted research on biological nitrogen fixation and on the characterization of rhizobia (Fernández-Pascual et al. 2007) as well as success in constructing better inoculants. The inoculants requires: First, strains need to be improved in order to compete successfully with indigenous strains for root nodulation of legumes. Several loci have been identified to date that affect competitiveness for strains nodule occupancy. Usually mutations in these loci affect the ability of a strain to form nodules rapidly and efficiently. Other loci, such as those that confer antibiotic production, can be added to strains to enhance nodulation competitiveness when co-inoculated with antibiotic-sensitive strains. Second, the inoculum strains must be improved with respect to symbiotic nitrogen fixation (Maier & Triplett 1996).

Several environmental conditions are limiting factors to the growth and activity of the N_2-fixing plants. A principle of limiting factors states that "the level of crop production can be no higher than that allowed by the maximum limiting factor" (Brockwell et al., 1995). In the *Rhizobium*-legume symbiosis, which is a N_2-fixing system, the process of N_2 fixation is strongly related to the physiological state of the host plant. Therefore, a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigor of the host legume (Brockwell et al., 1995; Thies et al., 1995).

Figure 1. Cowpea plants (cv IPA 204) without stress and with stress inoculated with strain of *Bradyrhizobium* sp. (EI 6). Water stress treatment was applied by means of a porous cup technique in greenhouse. (Courtesy of Figueiredo, MVB)

2. Co-Inoculation Rhizobia and Plant Growth Promoting Bacteria

Co-inoculation studies with Rhizobia and plant growth promoting bacteria have shown increased plant nodulation and N_2 fixation (Li & Alexander, 1988; Araújo & Hungria, 1999; Vessey & Buss, 2002; Silva, et al. 2006; Figueiredo et al. 2007, 2008). Co-inoculation 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 & 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 & Alexander, 1988; Vessey & Buss, 2002). Disease suppression of alfalfa by *B. cereus* and enhanced nodulation and seedling emergence in common bean (Srinivasan et al. 1996; Camacho et al. 2001) in soybean (Araujo & Hungria, 1999; Lambrecht et al. 2000; Vessey & Buss, 2002); in cowpea (Silva, et al. 2006, 2007); in pea (Cooper & Long, 1994) have been demonstrated as beneficial effects on plants. Bacillus 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 (Araújo, 2008a)

Araújo & Hungria, (1999) demonstrated the viability of co-inoculating soybean seeds with crude or formulated metabolites, or with cells of *Bacillus subtilis*, to increase the contribution of the biological nitrogen fixation process. Silva et al. (2007), found specific nodulation stimulus and increase root dry matter in *Vigna unguiculata* co inoculated with *Bradyrhizobium* sp. and *Paenibacillus polymyxa* (Loutit (L) and *Bacillus sp.* (LBF-410). The data demonstrated a significant positive correlation between specific nodulation and accumulated nitrogen/root dry matter.

According to Dakora (2003), the major contributions made separately by legumes and their microsymbionts that do not relate to root nodule N_2 fixation have been largely ignored. Rhizobia produce chemical molecules that can influence plant development, including phytohormones, lipo-chito-oligosaccharide, Nod factors, lumichrome, riboflavin and H_2 evolved by nitrogenase. When present is soil, Nod factors can stimulate the legume and nonlegume crops by different forms. Very low concentration of lumichrome and H_2 released by bacteroids also promote plant growth and increase biomass in a number of plant species grown under field and glasshouse conditions. Rhizobia are know to suppress the population of soil pathogens in agricultural and natural ecosystems and, in addition to forming nodule symbioses with rhizobia, the legume itself releases phenolics that can suppress pathogens and herbivores, solubilize nutrients, and promote growth of mutualistic microbes. Phytosiderophores and organic acid anions exuded by the host plant can further enhance mineral nutrition in the system. Mycorrhizal infection of roots of legumes has been reported to stimulate both nodulation and N_2 fixation, especially in soils low in available P (Redecker et al., 1997). Actinorhizal interactions (*Frankia*-nonlegume symbioses) are major contributors to nitrogen inputs in forests, wetlands, fields, and disturbed sites of temperate and tropical regions (El-Lakany & Luard, 1982). These associations involve more than 160 species of angiosperms classified among six or seven orders. The contributions of fixed nitrogen to native as well as managed ecosystems by the actinorhizal symbioses are comparable to those of the more extensively studied *Rhizobia*-legume interactions.

3. Action of Plant Growth-Promoting Bacteria in the Rhizospheric Environment

The action of bacteria in processes that affect plant growth is well known and results in increases in seed germination, seedling emergence and plant biomass (Luz, 1996; Kloepper & Schorth, 1981; Araujo & Hungria, 1999; Araujo, 2008b; Turner & Backman, 1991). Additionally, there are many reports on positive effect of growth-promoting bacteria in plants, such increase in plant production and suppression of disease. However, the commercial use of these microorganisms has not always provided positive results. Thus, others studies on microbial ecology in rhizosphere are important (Coelho et al., 2007).

The mechanisms of action of plant growth-promoting bacteria may initially be linked to inhibition of plant pathogens from soil with benefits plant growth indirectly. It is often difficult to recognize the mechanisms and relate directly to promotion of plant growth, since more than one mechanism is produced by bacteria (Araújo et al., 2005).

The identification of compounds produced by *Bacillus subtilis* during their secondary metabolism is important to clarify the beneficial effects of these bacteria on plant growth. The large number of mechanisms involved may be the reason by which *B. subtilis* has been assessed in large spectrum of plants under different conditions (Kilian, 2000). Most recent results suggest that the promotion of plant growth, by bacteria, can be achieved due different pathways and can express differently in plants growing "in vitro" versus "in vivo" (Ryu et al., 2005). Some studies report that the genus *Bacillus* used in agriculture has been related to biological control of plant pathogens (Bettiol, 1991) which has often been attributed to production of antibiotics by bacteria (Phae & Shoda, 1991; Araujo et al., 2005). However, species of *Bacillus* sp can also suppress diseases by other ways of action such as competition for nutrients and induced systemic resistance in plants (Kloepper et al., 1999).

It also has been studied the ways of action of *Bacillus* spp. in control of plant nematodes because many studies have identified some species of this genus with high potential for use in the control of pathogens in soil (Araújo et al., 2002; Neipp & Becker, 1999; Tian et al., 2000). *Bacillus subtilis* have been assessed as interfering in the cycle of nematodes in soil, showing potential for use in the control (Araujo et al., 2002). Neipp & Becker (1999), evaluated the biocontrol activity of rhizobacteria on *Heterodera schachtii*, and found some strains, including *Bacillus megaterium*, which reduced infection of nematodes in sugar beet. Previously, Kloepper et al. (1992) isolated bacteria from rhizosphere of antagonist plants to nematodes and found that species exhibited an antagonism to *Heterodera glycines* and *Meloidogyne incognita* on soybean, with a predominance of isolates from genus *Bacillus*. Other studies have demonstrated the use of these bacteria in the biocontrol of *Heterodera glycines* in soybean (Tian et al., 2000).

According to the results found with *Bacillus* sp. in biological control of plant diseases and the perspectives of use of these species as plant growth promoter, others studies with inoculation of seeds has increased (Liu & Sinclair, 1990, Turner & Backman, 1991; Araujo, 2008a). Thus, some studies identified how these bacteria or their metabolites interact with plant roots in the soil environment (Chanway & Nelson, 1990; Mahaffee & Backman, 1993). The presence of hormones in rhizosphere is an important factor for the regulation of plant growth and the main hormones involved in plant growth are gibberellins, auxins, citoquinines, abscisic acid and ethylene (Taiz & Zeiger, 2004). For example, auxin is important to plant growth in all plants (Brown, 1972). Guerreiro (2008) found production of auxin in various strains of *Bacillus* sampled in agricultural soils from state of São Paulo, Brazil. Araujo et al. (2005) also detected production of auxin in two strains of *Bacillus subtilis* that promoted soybean growth.

Bacteria are also related to processes of phosphorus (P) solubilization in soil. Richardson (2000) reported that in soils with low available P, the use of P fertilization represents high cost to farmers. Thus, there is an advantage by use of bacteria in rock-P solubilization. The high increase in P uptake by plants inoculated with *Bacillus* spp. has been showed due the presence of these bacteria species in rhizosphere. Rodriguez & Fraga (1999) report that *Pseudomonas*, *Bacillus* and *Rhizobium* are among the bacteria with high potential for P solubilization in soil. The main mechanism to P solubilization by bacteria is the release of organic acids that are combined with other metabolites like phytohormones and lytic enzymes (Vassilev et al., 2006).

The association of specific microorganisms with organic wastes can increase the inhibitory potential of rhizobacteria on plant pathogens. In this way, Kloepper et al. (2004)

reported that combination of rhizobacteria with chitin or chitosan enhanced development of seedlings and suppression of disease through the mechanism of induction of resistance mediated by rhizobacteria. Wastes with C and others nutrients provide increases in biocontrol activity of several bacteria (Shaukat & Siddiqui, 2003) by stimulation of growth in *Pseudomonas, Penicillium, Bacillus* and which are involved as potential agents of biological control (Lazarovits, 2001).

4. Concluding Remarks

Plant growth-promoting bacteria can be a true success story in sustainable agriculture. The beneficial events producing biological control of diseases, plant growth promoting, increases in crops yield and quality improvements. *Bacillus* species have been reported to promote the growth of a wide range of plants, however, they are very effective in the biological control of many plant microbial diseases. Co-inoculation, frequently, increase growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients. 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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