Chapter

6

Ecology, Diversity and Application of Plant Growth Promoting Rhizobacteria

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ABSTRACT

Interest in rhizosphere and soil ecology is growing among rhizobiologists. Soil microorganisms are essential for nutrient cycling in the biosphere. In the rhizosphere, this is even more important because of the size of this ecosystem. Studies in microbial ecology will be crucial in obtaining specialized microorganisms, which can be used to solve various environmental problems. The future of PGPR ecology research depends on the development of new technologies such as DNA/RNA microarrays to provide a general view of PGPR diversity structure and function.

***Key words*:** Rhizobacteria, PGPR, Diversity, Ecology, Inoculation, Pseudomonads, Bacilli, Rhizobia, AM fungi

1. INTRODUCTION

The term 'rhizosphere' was first defined by German agronomist Hiltner, in 1904, as the effect of the roots of legumes on the surrounding soil, in terms of higher microbial activity because of the organic matter released by the roots. It is the zone of soil surrounding a plant root where the biology and

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chemistry of the soil are influenced by the plant root. It is an area of intense biological and chemical activity influenced by the compounds exuded by the roots and by the microorganisms feeding on the compounds. The interactions taking place in the rhizosphere between the plant roots, soil, microorganisms and other soil organisms have been studied well and reviewed (Pinton *et al*., 2001). These interactions significantly influence plant growth and crop yields. A large number of macroscopic organisms and microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Rhizobacteria are rhizosphere competent bacteria that inhabit plant roots and exert a positive effect ranging from direct influence to indirect effects. Rhizobacteria may have neutral, deleterious or beneficial effects on growth of plants. The neutral rhizobacteria may not influence the plant growth. Deleterious rhizobacteria (DRB) are predominantly saprophytic bacteria that aggressively colonize plant seeds, roots and rhizospheres and readily metabolize organic substances released by plant tissues. Unlike typical phytopathogens, DRB do not invade and parasitize vascular tissues; DRB that inhabit plants endophytically are found intercellularly beneath epidermal cells and in intracellular spaces of root cortical cells without inducing disease symptoms (Kremer, 2006).

2. PLANT GROWTH PROMOTING RHIZOBACTERIA

Bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth-promoting rhizobacteria (PGPR) (Kloepper *et al.,* 1980). Plant growth-promoting rhizobacteria (PGPR) was first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants following inoculation onto seed and that enhance plant growth. Generally, about 2–5% of rhizosphere bacteria are PGPR (Antoun & Prévost, 2005). PGPR are free-living bacteria. However, some authors use a broader definition of PGPR to include symbiotic microorganisms like nitrogen-fixing rhizobia. Vessey (2003) and Gray and Smith (2005) designated rhizobia and *Frankia* species involved in symbiotic associations with higher plants as intracellular PGPR or symbiotic PGPR. Dinitrogen fixing associative symbiotic bacteria which do not cause any morphological modification of the host plant are considered as PGPR.

PGPR may enhance plant growth by direct or indirect mechanisms (Kloepper, 1993; Lazarovits & Nowak, 1997). Direct mechanisms of enhancement in plant growth include production of phytohormones, increased availability of nutrients to plants, stimulation of disease resistance mechanisms, etc. Indirect mechanisms include control of plant diseases, stimulation of other beneficial symbioses and degradation of xenobiotics in contaminated soils and thus protecting the plants (Jacobsen, 1997). Based

on their functions, PGPR may be classified as biofertilizers (increasing availability of nutrients to plants), biopesticides (controlling diseases, insect pests, nematodes, etc. by production of antibiotics, antifungal metabolites, etc.), phytostimulators (production of plant growth hormones) and rhizoremediators (degradation of pollutants) (Somers *et al.,* 2004). In most cases a single PGPR exhibits multiple growth promoting attributes including biocontrol ability (Vessey, 2003). PGPR are commonly used to improve crop yields. In addition to their proven usefulness in agriculture, they possess potential in solving environmental problems. Some PGPR participate in phytoremediation techniques to decontaminate soils and waters. A considerable number of soil and rhizospheric fungi and bacteria collectively known as plant growth promoting microorganisms (PGPM) have demonstrated ability to colonize plant roots and to provide benefits to their hosts. Among these benefits, many authors documented improved root hydraulic conductance and alleviation of abiotic stresses such as drought and salinity. Today, it is accepted that movement through aquaporins represents a quite faster pathway of water movement across biological membranes. Groppa *et al.* (2012) reviewed the state of art in the knowledge of PGPM effects on plant water status and root hydraulic conductance, with special emphasis on the experimental data that prove or suggest an impact of PGPM on root aquaporins under both normal and water limiting conditions.

3. DIVERSITY OF PGPR

In recent years the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere and also because mechanisms of action of PGPR have been deeply studied (Barriuso *et al.,* 2008). The earlier studies on PGPR laid emphasis on biological control of plant diseases and hence bacteria like fluorescent pseudomonads and *Bacillus* spp. were described. During recent years, with the elucidation of many mechanisms of plant growth-promotion involving large number of plant and microbial species, knowledge about very diverse bacterial taxa has been obtained (Lucy *et al.,* 2004). The biodiversity and ecology of some of the most abundant genera of PGPR is mentioned briefly in the following sections.

3.1. Pseudomonads

Among Gram-negative soil bacteria, *Pseudomonas* is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved (Lucas Garcia *et al.,* 2004; Patten & Glick, 2002). Pseudomonads were the first group of bacteria to be described as PGPR.

Seeds, planting materials or roots of plants when treated with pseudomonads were found to be beneficial for plant growth and crop yield. Increase in fresh matter yield of radish (*Raphanus sativus* L.) was obtained by seed inoculation with fluorescent pseudomonads (Kloepper & Schroth 1978). Similarly, growth increases in seedling and mature root weights of sugar beet were obtained by inoculating sugar beet (*Beta vulgaris* L.) with selected strains of fluorescent *Pseudomonas* spp. (Suslow & Schroth, 1982). Several *Pseudomonas* isolates have been reported to solubilise inorganic forms of insoluble phosphates into soluble forms, thereby increasing the availability of phosphates to the plant (Rodriguez & Fraga, 1999). Inoculation with tricalcium phosphate solubilizing *Pseudomonas* sp. 24 caused a significant increase in maize plant height after 60 days of growth and an 18% increase in lettuce shoot fresh matter yield in field trials conducted at Quebec (Canada) (Chabot *et al.,* 1996). Pseudomonads have been known to benefit plants by promoting plant growth and by protecting plants from disease causing pathogens. Production of indole acetic acid (IAA) by *Pseudomonas putida* GR12-2 significantly enhanced the root development in canola (*Brassica rapa*) (Patten & Glick, 2002). GR12-2 produces 1-aminocyclopropane-1-carboxylic acid (ACC) – deaminase, which degrades ACC, the direct precursor of ethylene, thus preventing plant production of inhibitory levels of ethylene (Jacobson *et al.,* 1994). The use of mutants with reduced capacity to produce cytokinins, revealed the importance of cytokinin production in the plant growth promoting ability of *Pseudomonas fluorescens* strain G20-18 (Garcia de Salamone, 2000).

Pseudomonads are well known biocontrol agents. The production of metabolites like antibiotics, siderophores, HCN, etc. is the primary mechanism of biocontrol (Weller & Thomashow, 1993). A heat labile polar substance produced by *Pseudomonas aeruginosa* 78 causes *in vitro* juvenile mortality of *Meloidogyne javanica*, the root knot nematode (Ali *et al*., 2002). Siderophore production under iron limiting conditions has been reported for the antagonism of some strains of *P. aeruginosa* against *Pythium* spp., the causal agents of damping-off and root rot of many crops (Charest *et al.,* 2005). Some strains of fluorescent pseudomonads produce many metabolites and have been studied in great details worldwide. *P. fluorescens* CHAO produces several bioactive compounds such as antibiotics, siderophores, HCN, IAA, etc. and is most widely studied PGPR for its biocontrol and growth-promoting abilities (Weller & Thomashow 1993). *P. fluorescens* CHAO also produces 2,4-diacetylphloroglucinol (DAPG), an important mechanism of suppression of take-all disease of wheat and black root rot of tobacco. Kang *et al.* (1998) reported the production of a novel lipopeptide antibiotic (AFC-BC11) produced by *Burkholderia cepacia* which controlled damping-off of cotton caused by *Rhizoctonia solani* in a gnotobiotic system. Many strains of fluorescent pseudomonads induce systemic resistance

against pests and diseases (Ramamoorthy *et al.,* 2001). There are many reports of the beneficial effects of PGPR on vegetable crops. In a cucumber crop grown in spring season, *P. corrugata* strain 13 and *P. fluorescens* strain 15 produced 88% more marketable fruit, while in a fall crop with severe disease pressure due to higher slab temperatures, both strains significantly increased by 600% the marketable fruit. The strain 15 also increased fruit production in treatments not inoculated with the pathogen (Paulitz & Belanger, 2001). Several strains of fluorescent pseudomonads play important role in making soils naturally suppressive to diseases such as *Fusarium* wilt (Mazzola *et al.,* 2002), and take-all caused by *Gaeumannomyces graminis* var. *tritici* (Weller *et al*., 2002).

Biological control of plant pathogens in disease suppressive soil is due to the existence of mixture of microbial antagonists (Lemanceau & Alabouvette, 1991). Hence, efficiency of biocontrol agents could be increased by the development of compatible strain mixtures of different biocontrol organisms (Raupach & Kloepper, 1998). Application of the mixture of phloroglucinol producers of *P. fluorescens* F113 and a proteolytic rhizobacterium suppressed sugar beet damping-off (Dunne *et al.,* 1998). Combination of iron chelating *Pseudomonas* strains and inducers of systemic resistance suppressed *Fusarium* wilt of radish better than the application of individual strains (de Boer *et al*., 2003).

Soaking of rice seeds in water containing 10g of talc based formulation of *P. fluorescens* consisting mixture of PF1 and PF2 (10^8cfu/g) for 24h controlled rice sheath blight under field condition (Nandakumar *et al.,* 2001). PGPR have been used for the control of diseases of spices. Talc and peat-based formulations of *P. chlororaphis* were prepared and used for the management of rhizome rot of turmeric (Nakkeran *et al.,* 2004). Foliar spray is another method of controlling foliar diseases. Spray application of *P. fluorescens* on to foliage (1 kg of talc based formulation /ha) on 30, 45, 60, 75 and 90 days after sowing reduced leaf spot and rust of groundnut under field conditions (Meena *et al.,* 2002). Viswanathan and Samiyappan (2002) delivered fluorescent pseudomonads through sett treatment. Two budded sugarcane setts were soaked in talc formulation of *P. fluorescens* (20 g/l) for one hour and incubated for 18h prior to planting. Planting of treated setts increased cane growth, sugar recovery and reduced red rot incidence under field conditions. Garcia de Salamone *et al*. (2012) measured the response of three rice cultivars to PGPR inoculation under field conditions with a commercial formulation containing strains of *Pseudomonas fluorescens* and *Azospirillum brasilense*. PGPR inoculation increased aerial biomass production, harvest index, and grain yield of the Supremo 13 cultivar by 4.7%, 16%, and 20.2%, respectively. Inoculation of the Yeruá cultivar increased aerial biomass by 1.9% and grain yield by 11%. The results also

indicate that the combined inoculation with *P. fluorescens* and *A. brasilense* has significant potential when applied to rice.

3.2. Bacilli

Among the gram-positive bacterial genera found in soils under different types of management regimes worldwide, the majority (95%) were putative *Bacillus* species, as well as related taxa such as *Paenibacillus*, *Salibacillus*, *Gracilibacillus*, etc. (Garbeva *et al*., 2003). They developed the PCR-denaturing gradient gel electrophoresis (DGGE) technique to study the diversity of *Bacillus* in agricultural soils under different management regimes.

Bacillus spp. are ubiquitous in soils and because of the ability to produce endospores, they can survive for long periods under adverse environmental conditions. Some species of *Bacillus* are diazotrophs. *Bacillus* species have been reported to promote the growth of plants (Kokalis-Burelle *et al*., 2002) and are very effective biological control agents for plant diseases. *Bacillus* is found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan *et al*., 2006). *Bacillus megaterium* is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint (Kaymak *et al*., 2008).

Bacillus spp. have been widely used as biocontrol agents to control diseases caused by phytopathogens in many crop species. *Bacillus megaterium* KL39, a biocontrol agent of *Phytophthora* blight disease of red pepper, produces an antifungal antibiotic active against a broad range of plant pathogenic fungi (Jung & Kim, 2003). *Bacillus cereus*, *B. lentimorbus*, and *B. licheniformis* were found to be very effective in inhibiting *Fusarium roseum* var. *sambucinum*, the causal agent of dry rot of potato tubers (Sadfi *et al*., 2001). The antifungal activity of the isolates was attributed to the inhibitory volatile substances and lytic chitinases produced by the isolates.

Different formulations and delivery systems have been tried for *Bacillus* cultures. Incorporation of commercial chitosan- based formulations LS254 (comprising of *Paenibacillus macerans* and *B. pumilus*) and LS255 (comprising of *P. macerans* and *B. subtilis*) into soil at the ratio of 1: 40 (formulation: soil) increased bio-matter production by increasing both root and shoot length and yield (Vasudevan *et al*. 2002). Dipping of *Phyllanthus amarus* seedlings in talc-based formulation of *B. subtilis* (BSCBE4) for 30 minutes prior to transplanting reduced stem blight of *P. amarus* (Mathiyazhagan *et al.,* 2004).

3.3. Nitrogen-Fixing PGPR

Many diazotrophic bacteria stimulate plant growth because of their ability to fix atmospheric nitrogen. *Azospirillum* has been studied in much detail for its growth promoting abilities in cereals by the fixation of atmospheric nitrogen and production of growth hormones. Bashan *et al.* (2004) reviewed the advances made in research with *Azospirillum* and its use as a PGPR. This group of free-living rhizobacteria encompasses ten species, each one classified according to its particular biochemical and molecular characteristics: *A. lipoferum, A. brasilense, A. amazonense , A. halopraeferens, A. irakense, A. largimobile , A. doebereinerae , A. oryzae, A. melinis* and recently *A. canadensis* (Mehnaz *et al.,* 2007). Apart from the growth promoting abilities, *A. brasilense* was recently reported to synthesize phenylacetic acid (PAA), an auxin-like molecule with antimicrobial activity (Somers *et al.,* 2005). The growth promoting ability of certain endophytes has been attributed to N_2 fixation. The diazotrophic endophytes such as *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., etc. stimulate plant growth by fixing nitrogen. Besides, free-living N_2 fixers like *Azotobacter* sp. and *Paenibacillus polymyxa* also act as PGPR (Vessey, 2003). The family Azotobacteriaceae comprises of two genera namely, *Azomonas* (non-cyst forming) with three species (*A. agilis, A. insignis* and *A. macrocytogenes*) and *Azotobacter* (cyst forming) comprising of 6 species, namely, *A. chroococcum, A.vinelandii, A. beijerinckii, A. nigricans, A. armeniacus* and *A. paspali.*

Azoarcus spp. belong to the β-subclass of the Proteobacteria. Most species have been isolated from roots or stems of Kallar grass (*Leptochloa fusca*) (Hurek *et al.,* 1997). *Azoarcus* is an aerobic/microaerophilic nitrogen-fixing bacterium and can infect roots of rice plants as well. The genus *Azoarcus* has been identified, with two species, *A. indigens* and *A. communis*, and three additional unnamed groups, which were distinct at species level. Several new genera like *Azovibrio restrictus*, *Azospira oryza* and *Azonexus fungiphilus* have been described which were previously included in the genus *Azoarcus* (Reinhold-Hurek & Hurek, 2000). *Azoarcus* sp. strain BH72 has been described as a model for nitrogen fixing grass endophytes (Hurek & Reinhold-Hurek, 2003). *Gluconacetobacter diazotrophicus* is a gram-negative strictly aerobic bacteria isolated from roots and stems of sugarcane (*Saccharum officinarum*), and from the inner tissues of elephant grass (*Pennisetum purpureum*), finger millet (*Eleusine coracana)* and sweet potato (*Ipomoea batatas*) (Munoz-Rojas & Caballero-Mellado, 2003). Similarly, *Herbaspirillium* is an endophyte, which colonizes rice, maize (*Zea mays*), sorghum (*Sorghum bicolor*), sugarcane, etc. (James *et al.,* 2002). The genus *Burkholderia* is known to fix atmospheric nitrogen and several plant isolates like *B. vietnamiensis* and *B. kururiensis* have been described as nitrogen

fixers (De Los Santos *et al.,* 2001; Coenye & Vandamme, 2003). Luna *et al.* (2012) studied the colonization and yield promotion of tomato by *Gluconacetobacter diazotrophicus*. Inoculation of tomato seedlings with *Gluconacetobacter diazotrophicus* resulted in significant root and stem colonization. Bacteria were found on the junction of emergence of the lateral roots, root hairs and stomata. Inoculation with *G. diazotrophicus* led to increase in fruit production of tomato.

The inoculation effects of these diazotrophs have not always shown growth promotion due to augmentation of biological nitrogen fixation. Like other PGPR, these associative diazotrophs may enhance plant growth through different direct or indirect mechanisms (Dobbelaere *et al.,* 2003).

3.4. Rhizobia

Rhizobia are root nodulating bacteria and microsymbiont in the legume-*Rhizobium* symbiosis. Strains from this genus may behave as PGPR when they colonize roots from nonlegume plant species in a nonspecific relationship. A number of individual species have been reported to release plant growth regulators, siderophores and hydrogen cyanide or may increase phosphate availability, thereby improving plant nutrition (Antoun *et al.,* 1998). Stimulation of growth of maize and lettuce (*Lactuca sativa* L.) was obtained under field conditions by inoculation with dicalcium phosphate solubilizing strains of *Rhizobium leguminosarum* bv. *phaseoli* (Chabot *et al.,* 1996). Many rhizobial isolates from different cross-inoculation groups of rhizobia, isolated from soils in Iran were able to mobilize P from organic and inorganic sources (Alikhani *et al*., 2006). In the presence of the flavonoid naringenin, strain ORS571 of *Azorhizobium caulinodans* was able to colonise the roots of *Brassica napus* (O'Callaghan *et al.,* 2000). There are reports of the endophytic presence of rhizobia in some non-legume plants. Rhizobia have been found as endophytes in non-legume plants in regions where legumes are cultivated in rotation with non-legumes. *Rhizobium etli* is a natural endophyte of maize traditionally cultivated for thousands of years in Mesoamerica, in association with beans (*Phaseolus vulgaris*) (Guttiérrez-Zamora & Martinez-Romero, 2001). The populations of rhizobia were greater in the bulk soil, rhizosphere and rhizoplane of barley, wheat and canola when these crops were grown in rotation after pea as compared to monoculture, and *R. leguminosarum* bv. *viciae* colonized the root interiors of the three plants (Lupwayi *et al*., 2004).

Rhizobia have been reported to have biological control abilities against some plant pathogens. In a field naturally infested with *Pythium* spp. inoculation of pea (*Pisum sativum* L.) and sugar beet with strain R12 of *R. leguminosarum* bv. *viciae*, isolated from lentil (*Lens culinaris*) in Alberta,

Canada, significantly increased seedling emergence four weeks after planting (Bardin *et al.,* 2004). In another field experiment performed with sugar beet, rhizobia R12 was as effective as the fungicide Thiram™ used as seed treatment to control *Pythium* diseases. The lipopolysaccharides of *R. etli* G12 induced the systemic resistance to infection by the cyst nematode *Globodera pallida* in potato roots (Reitz *et al*., 2000).

4. EFFECTS OF PGPR INOCULATION ON THE SOIL-PLANT-MICROBE ECOSYSTEMS

PGPR are inoculated through seed or seedlings with the purpose of introducing a large number of beneficial microorganisms in the rhizosphere of plants. However, these inoculations with seemingly innocuous microorganisms may have non-target effects on plants, other microorganisms and soil fauna like nematodes, protozoa, etc. It was observed that the introduction of bacterial biocontrol agents affected microbial community structures, which were probably of minor importance for soil functioning (Winding *et al.,* 2004).

5. FACTORS INFLUENCING SOIL MICROBIAL STRUCTURE AND ACTIVITY

5.1. The Plant Factor

Plants influence the diversity of microorganisms inhabiting the rhizosphere. Different cultivars of the same plant species respond differently to inoculation with the same introduced organism. Plant genotype affects root colonization by the introduced bacteria, the total population size of microbial communities on plant and the composition of those communities (Smith & Goodman, 1999). The diversity of soil-borne populations of fluorescent pseudomonads in flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.) grown in the same soil was studied by Lemanceau *et al.* (1995). The populations isolated from uncultivated soils were different from those isolated from plants (rhizosphere, rhizoplane or root tissue), and analysis of the bacterial isolates indicated that plant has a selective influence on fluorescent pseudomonads and the selection was more strongly expressed with flax than with tomato plants. The DGGE fingerprints obtained from the rhizosphere of strawberry (*Fragaria ananassa* Dutch.), oilseed rape (*Brassica napus* L.) and potato showed plant dependent shifts in the relative abundance of the rhizosphere populations, which became more pronounced in the second year of growing the same crop (Smalla *et al.,* 2001).

5.2. Soil Organisms

Soil is home to many fauna like protozoa, nematodes, earthworms and other lower groups of minute animals, insects, etc. Bonkowski *et al.* (2000) mentioned the important function of soil fauna in regulating rhizosphere microbial processes and thus affecting plant growth. Protozoa consume the bacterial metabolites and enhance the nutrient cycles and energy flows for the benefit of microorganisms, plants and animals (Foissner, 1999). Ronn *et al.* (2002) observed that grazing by a mixed assemblage of soil protozoa (seven flagellates and one amoeba) had significant effects on the bacterial community structure in a soil microcosm as revealed by the PCR-DGGE as well as the community level physiological profiling determined with the biolog plates. Amoebas are the most important bacterial grazers in soil (Bonkowski, 2004). Earthworms change the microbial habitats by churning out the soil physically (Amador & Gorres, 2005). The vegetative cells of *Bacillus thuringiensis* var. *kurstaki* DMU67R were reported to be found in the gut of earthworm species *Lumbricus rubellus*, *L. terrestris*, and *Apporrectodea caliginosa*. Spore germination of *A. caliginosa* DMU67R was restricted to the gut (Hendriksen & Hansen, 2002). In the presence of nematodes (*Caenorhabditis elegans*, *Acrobeloides thornei* and *Cruznema* sp.), rhizosphere colonization of wheat by the PGPR, *Pseudomonas corrugata*, *P. fluorescens* and *Bacillus subtilis* was substantially increased (Knox *et al.,* 2003).

5.3. Arbuscular Mycorrhizae

More than 80% of all terrestrial plant species form mycorrhizal associations (Sylvia, 2005). The rhizosphere is thus influenced by the plant roots as well as by the mycorrhizal fungus. The mycorrhizosphere is the zone influenced by both the root and the mycorrhizal fungus and it includes the more specific term "hyphosphere" which refers only to the zone surrounding individual hyphae (Johansson *et al*., 2004). Bacterial communities associated with plant roots may be affected by root-colonisation with AM fungi. This may be due to metabolic products of AM fungi and their resultant changes. The hyphal exudates may have detrimental or stimulatory effect on rhizosphere bacteria. Sood (2003) reported greater attraction of the PGPR *Azotobacter chroococcum* and *Pseudomonas fluorescens* towards tomato roots colonized by *Glomus fasciculatum* compared to non-arbuscular mycorrhizal tomato roots. Rhizosphere bacteria remain in close association with AM fungi. Endosymbiotic bacteria closely related to the genus *Burkholderia* have been found in the symbiotic AM fungi *Gigaspora margarita*, *Scutellospora persica* and *Scutellospora castanea* (Bianciotto *et al.,* 2000). PGPR and AM fungi interactions have shown synergistic effects. In a Petri plate system, roots of carrot (*Daucus carota* L.) inoculated with phosphate solubilizing bacteria

Pseudomonas aeruginosa showed substantial increase in P solubilization when infected with *G. intraradices* (Villegas & Fortin, 2001).

5.4. Abiotic Factors

The occurrence and activity of soil microorganisms are affected by a variety of environmental factors as well as plant-related factors (species, age etc.). Abiotic stress factors include high and low temperature, salinity, drought, flooding, ultraviolet light, air pollution (ozone) and heavy metals. The yield losses associated with abiotic stresses can reach 50% to 82%, depending on the crop. The abiotic factors like physico-chemical properties of soil, presence of pesticides, agricultural chemicals, industrial effluents, etc. affect the plant growth and the associated microflora and fauna. These abiotic factors influence the functions of PGPR and other soil bacteria and also their interaction with plant species. The environmental factors modulating the biosynthesis of antibiotic and siderophore by the disease-suppressive strain *P. fluorescens* CHAO was studied by Duffy and Defago (1999). The production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) was stimulated by Zn^{2+}, NH_4MO^{2+} and glucose, and production of pyoluteorin was stimulated by Zn^{2+}, Co^{2+} and glycerol and was repressed by glucose. The production of the siderophore pyochelin was increased by Co^{2+}, fructose, mannitol and glucose. The metal resistant PGPB can serve as an effective metal sequestering and growth-promoting bioinoculant for plants in metal stressed soil (Rajkumar & Freitas, 2008). The deleterious effects of heavy metals taken up from the environment on plants can be lessening with the use of PGP bacteria or mycorrhizal fungi (Belimov *et al*., 2005; Denton, 2007). Cadmium in soil induces plant-stress ethylene biosynthesis and probably contributes to the accumulation of ACC in roots, the PGPR protect the plants against the inhibitory effects of cadmium (Amico *et al*., 2008). PGPR can have positive effects on vigour and productivity, especially under stress conditions. Seed inoculations with PGPR in asparagus (*Asparagus officinalis* L) results in a positive response and enhances plant growth under drought (Liddycoat *et al.,* 2009). Cheng *et al.* (2012) studied the combined effects of the plant growth-promoting bacterium *Pseudomonas putida* UW4 and salinity stress on *Brassica napus* proteome. The effects of salt and bacteria on the canola proteome were shown to be quite diverse, with salinity stress causing more dramatic plant protein expression changes than bacteria. In addition, bacteria were demonstrated to moderate some of the salt effects on plant protein differential expression. This work contributed to the understanding of how plant protein expression is affected by various environmental signals. Rojas-Tapias *et al.* (2012) reported the partial alleviation of salinity stress in maize due to inoculation with *Azotobacter chroococcum* strains C5 and C9. C5 and C9 were able to increase plant

growth and photosynthetic pigments content, besides improving K^+/Na^+ ratio and polyphenol content.

5.5. Root Colonization by Introduced PGPR

For any PGPR to bring about beneficial effects on crop growth and yield it is highly essential to have good root colonization ability. PGPR strains with high rhizosphere competence and root colonizing ability exhibit enhancement of plant growth and yield. Somers *et al.* (2004) reviewed the mechanisms involved in the establishment of a successful interaction between PGPR and plant roots. The rhizosphere competent pseudomonads are particularly efficient in using pyoverdine-mediated iron uptake system and in reducing nitrogen oxides (Latour *et al*., 2003). Guerrero-Molina *et al*. (2012) confirmed effective rhizosphere colonization of strawberry mother-plants and also the colonization of *A. brasilense* to new daughter-plants via stolons. This is the first report about *A. brasilense* colonization from one strawberry plant to another one by colonizing inner tissues of roots and stolons. This means that a single inoculation with selected PGPR would allow the growers to have numerous plant generations at nursery already inoculated and with better conditions to be planted at field, contributing to sustainable strawberry cultivation.

6. INTERACTIONS BETWEEN PGPR AND OTHER SOIL MICROORGANISMS

Soil is a complex ecosystem involving plants, microorganisms, insects, numerous small flora and fauna. The PGPR have interactions with symbiotic and non-symbiotic microorganisms and other small organisms present in soil.

6.1 Interaction Between PGPR and Symbiotic Organisms

6.1.1. *Interaction between PGPR and mycorrhizae*

Mycorrhizal symbioses are important considering the fact that a large number of terrestrial plants are mycorrhizal. This symbiosis helps the plant in the acquisition of water and minerals, besides protection from diseases. The development of mycorrhizae cause changes in the rhizosphere microbial community which results in interaction among rhizosphere microorganisms (Bianciotto & Bonfante, 2002). Bianciotto *et al*. (1996) suggested that bacteria attached to spores or hyphae of AM fungi produced extracellular soluble factors which mediated bacterial-fungal interactions and AM fungi

served as vehicles for colonization of plant roots by rhizobacteria. Some PGPR can promote mycorrhizal functioning while certain interactions like grazing of the external hyphae by soil organisms are detrimental (Hodge, 2000). Rhizobacteria showing a beneficial effect on mycorrhizae are often termed as "mycorrhizae-helper bacteria". Bianciotto *et al.* (2004) observed strong evidence of a vertical transmission of endobacteria through the vegetative generation of AM fungus.

Studies have shown that inoculation with PGPR and diazotrophs along with AM fungi may increase plant growth and yield. Chanway and Hall (1991) estimated that associative nitrogen fixation by *Bacillus* could contribute in part to the growth promotion effect observed with *Pinus contorta* inoculated with the mycorrhizal fungus *Wilcoxina mikolae*. Colonization with AM fungi may modify the root exudates pattern, which may act as chemo- attractants for the soil bacteria. In a dual inoculation study with *Glomus mosseae*, *Bacillus coagulans* was superior to *Azotobacter chroococcum* in enhancing plant biomass of *Simarouba glauca* (Sailo & Bagyaraj, 2003). Wu *et al.* (2005) reported increased growth and nutrient uptake of maize , enhanced root colonization by the AM fungus and improved soil properties when inoculated with a biofertilizer containing N-fixer (*A. chrooccum*), P solubilizer (*B. megaterium*) and K solubilizer (*B. mucilaginous*) and AM fungus (*G. mosseae* or *G. intraradices*). There are certain reports, however, which do not find any beneficial effects of such inoculation. Russo *et al.* (2005) reported that mycorrhization of wheat and maize was not affected by different *Azospirillum* species or by a genetically modified derivative of *A. brasilense* overproducing indole-3-acetic acid.

Studies on interaction between PGPR and ectomycorrhizae also show beneficial and detrimental effects. In a co-inoculation study *Laccaria bicolor* and *P. fluorescens* strain BBc6 significantly inhibited mycorrhizal development in *Eucalyptus diversicolor* (Dunstan *et al.,* 1998). On the contrary, a PGPR effect was also observed in the same study with an unidentified bacterium resulting into 49% more shoot dry weight than the uninoculated control.

6.1.2. *Interaction between PGPR and rhizobia*

Biological nitrogen fixation (BNF) is an important activity of rhizobia residing inside the root nodules of leguminous plants. BNF can be improved by selection of superior and efficient strains of rhizobia, through breeding and through application of rhizobia in combination with PGPR.

Several workers have reported the beneficial effects of co-inoculation of free-living diazotrophs *Azospirillum* and *Azotobacter* in terms of increase

in nodulation and yields of legume crops such as chickpea, alfalfa, clover, soybean, pea, etc. (Iruthayathas *et al.,* 1983; Sarig *et al.,* 1986; Yahalom *et al.,* 1987). Gus-reporter gene was used in a study by Tchebotar *et al.* (1998), in which an equal mixture of *Azospirillum lipoferum-Rhizobium leguminosarum* bv. *trifolii* increased nodulation in clovers, and *Azospirillum* was observed colonizing tap root, root hairs and sites near or on the nodules. This study suggested creation of additional infection sites by *Azosprillum* which resulted in increased nodulation by rhizobia.

Studies have been done with other PGPR as well, which show enhancement in nodulation, nitrogen fixation and eventually crop growth and yield as a result of synergistic effects of PGPR on rhizobia. However, the effects vary from crop to crop and also under the experimental situations. Strains of fluorescent *Pseudomonas* having antifungal activity and producing siderophores when used with *Rhizobium* in pea production, showed reduction in the number of *Fusarium oxysporum* infected peas grown in infested soils and an improvement in plant biomass (Kumar *et al.,* 2001). In another study involving the DAPG-producing *P. fluorescens*, De Leij *et al.* (2002) suggested that DAPG can induce morphological changes in the plant that can lead to enhanced infection and nodulation by *Rhizobium*. *Pseudomonas* strains antagonistic to fungal pathogens (*Aspergillus* sp., *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*) stimulated nodulation and improved plant growth in chickpea (*Cicer arietinum*) when co-inoculated with *Mesorhizobium* (Goel *et al.,* 2002). There are reports of negative influence of PGPR on rhizobial infection. Deleterious strains of *P. putida* producing extracellular metabolites inhibited the growth of *R. leguminosarum* and disrupted the initial pea root infection process (Berggren *et al.,* 2001). Chebotar *et al.* (2001) reported strain dependent effects of PGPR on rhizobia. Co-inoculation with *P. fluorescens* 2137 increased the colonization of *B. japonicum* on soybean roots, nodule numbers and ARA, while coinoculation with *P. fluorescens* WCS365 had the opposite effects. PGPR have been shown to positively influence the competitiveness of inoculants rhizobia against the native ones. In a study involving green gram (*Vigna radiata*) grown in non-sterile soil, *Bradyrhizobium* sp. strain S24 occupied 60% of nodules in single inoculation and this value was increased to 81% in the presence of *Enterobacter* strain EG-ER-1 (Gupta *et al*., 1998).

Inoculation of phosphate solubilizing bacteria (PSB) along with rhizobia and PGPR has also been tried upon to enhance the benefits of inoculation. Gunasekaran *et al.* (2004) tested a inoculants mixture of a PGPR (*Pseudomonas* KB-133), a PSB (*B. megaterium*) and a *Rhizobium* sp. strain (COC 10) for enhancing nodulation and yield in blackgram.

6.1.3. *Interaction between PGPR, rhizobia and AM fungi*

The effects of combined inoculation with PGPR, AM fungi and rhizobia have been tested by many workers trying to reap the benefits of the synergy between these three groups. Extracellular metabolites produced by the organisms could possibly be the reason for the synergistic effects. The addition of cell-free culture filtrate of PGPR to the mycorrhizal and nodulated legume *Hedysarum coronarium* resulted in maximum plant growth and nutrient uptake in comparison to washed cells of PGPR or the whole bacterial cultures (Azcòn, 1993).

The interactive effects of PGPR, AM fungi and rhizobia have also shown bioremediation in heavy metal contaminated and polluted soils (Vivas *et al.,* 2003a; Vivas *et al*., 2003b). In a Pb contaminated soil, co-inoculation with *Brevibacillus* sp., an indigenous PGPR strain, and a mixture of indigenous AM fungal species, enhanced plant growth, mycorrhizal infection, N and P content in clover, along with a decrease in the amount of Pb absorbed (Vivas *et al*., 2003b).

6.1.4. *Interaction of PGPR with other microorganisms*

Plant growth promoting rhizobacteria (PGPR) interact with the microorganisms present in the rhizosphere and influence the rhizosphere microbial community. The changes in the microbial community may be a factor responsible for the growth promotion due to the introduction of PGPR. In a study on European alder, inoculation with the PGPR *Bacillus licheniformis* improved growth of the plant and induced different changes in phospholipids profile and culturable bacteria according to the soil used (Ramos *et al.,* 2002).

PGPR have been mainly studied for their biocontrol role and for inducing systemic resistance against fungal, bacterial, viral diseases as well as against insect and nematode pests. Ramamoorthy *et al.* (2001) discussed the possibility of developing mixed inoculants against various pathogens of a crop. PGPR produce compounds involved in plant defense mechanisms as a result of physiological changes in plants. The compounds like salicylic acid, lipopolysaccharides, siderophores, etc. are produced by PGPR that induce systemic resistance in plants.

6.1.5. *Interaction of PGPR with soil fauna*

Soil fauna along with plant roots and microorganisms play an important role in nutrient cycling and the availability of essential nutrients to the plants. The protozoa and the nematodes are important players in this process. The protozoa and nematodes represent 70 and 15% respectively, of

total respiration of soil animals (Foissner, 1987). These two groups also contribute to nitrogen mineralization in soil (Griffiths, 1994).

6.1.6. *Interaction of PGPR with protozoa*

Protozoa are known to be grazers of rhizosphere bacteria. The protozoa release nutrients from consumed bacterial biomass and thereby increase plant growth. They also influence the root architecture and the rhizosphere microbial community. In an experiment with watercress in the presence of *Acanthamoebae*, the root system was greater and more branched and there was an increase in proportion of IAA producing rhizosphere bacteria, indicating hormonal effect on plant growth (Bonkowski & Brandt, 2002). The selective grazing of rhizosphere bacteria by protozoa might favour certain bacteria capable of promoting plant growth by producing hormones.

6.1.7. *Interaction of PGPR with nematodes*

The majority of the studies on interaction of PGPR with nematodes are on the biocontrol of plant parasitic nematodes. The genera of PGPR widely studied include *Pseudomonas*, *Bacillus, Serratia, Streptomyces, Alcaligenes, Agrobacterium, Clostridium* and *Desulfovibrio* (Siddiqui & Mahmood, 1999). Bacteria producing hydrolytic enzymes, HCN, etc. or having phenol oxidation and antifungal activity such as *Stenotrophomonas maltophilia*, *Bacillus mycoides* and *Pseudomonas* sp. reduced the density of *Trichodorid* nematodes on potato by 56% to 74% (Insunza *et al.,* 2002). Diazotrophs like *Rhizobium* sp., *Azotobacter*, *Azospirillum* and the AM fungus *Glomus* have been reported to reduce the infestation and galling of chickpea by *Meloidogyne javanica* (Siddiqui & Mahmood, 2001). Seed treatment and soil application of *P. aeruginosa* strain 78 reduced root knot incidence of mungbean besides the reduction in the population density of *Meloidogyne javanica* under field conditions (Ali *et al.,* 2002).

Nematodes, especially the non-parasitic types, are known to be vectors for rhizosphere colonization with bacteria (Knox *et al.,* 2003). Three species of nematodes (*Caenorhabditis elegans, Acrobeloides thornei* and *Cruznema* sp.) promote rhizosphere colonization of four strains of beneficial bacteria in sand-based microcosm system.

7. CONCLUSIONS

Increase in public concern about the environment has increased the need to develop and implement effective plant growth promoting and biocontrol agents for crop production and protection. An effective PGPR could be

developed for growth promotion and disease control only after understanding its ecology and performance in the environment in which it is expected to perform. During the last few decades a large number of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium, Serratia,* etc. have been reported to enhance plant growth. The direct promotion of plant growth by PGPR involves either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth takes place when PGPR prevent deleterious effects of one or more phytopathogenic microorganisms. The exact mechanisms by which PGPR promote plant growth are still being unraveled, but are thought to include the ability to produce plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins; asymbiotic N_2 fixation; production of siderophores, antibiotics and cyanide; solubilization of mineral phosphates and other nutrients, etc. Some plant growth promoting rhizobacteria may promote plant growth indirectly by affecting symbiotic nitrogen fixation, enhancing nodulation or nodule occupancy. In addition to these traits, PGPR must be rhizosphere competent, able to survive and colonize the rhizosphere aggressively. Certain environmental factors like climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil affect the performance of the inoculated PGPR strains. To achieve the maximum growth promoting interaction between PGPR and crop plants, it is necessary to understand the mechanisms of the interactions and to understand the ecology of PGPR. It is necessary to develop strains which are highly efficient in field conditions. The soil microbial diversity should be explored for strains having multiple plant growth promoting activities and well adapted to particular soil environments. Plants produce strong selective pressure in the rhizosphere and select bacteria beneficial for their growth and health. A thorough knowledge of the mechanisms and performance related to growth promotion and disease control will help in the selection of promising candidates that suits industries to produce reliable commercial products (Collins *et al*., 2003). The efficacy of PGPR could be improved through the usage of compatible mixed inoculum which could have a consistent performance under diverse environmental conditions (Guetsky *et al*., 2001; Janisiewicz, 1996). Inoculation of PGPR has an impact on the rhizosphere microbial communities and this impact must be further studied because of its influence on the PGPR effect. The application of modern molecular tools is helping us to understand and manage the rhizosphere in a better way and will lead to new products with improved effectiveness.

7.1. Future Prospects

Interest in rhizosphere and soil ecology is growing among rhizobiologists. Soil microorganisms are essential for nutrient cycling in the biosphere. In the rhizosphere, this is even more important because of the size of this ecosystem. Studies in microbial ecology will be crucial in obtaining specialized microorganisms, which can be used to solve various environmental problems. The future of PGPR ecology research depends on the development of new technologies such as DNA/RNA microarrays to provide a general view of PGPR diversity structure and function.

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