Chapter 9
Growth Promotion of Legumes by Inoculation of Rhizosphere Bacteria

Satyavir S. Sindhu, Seema Dua, M.K. Verma, and Aakanksha Khandelwal

Abstract Most plants grown in fields are colonized by diverse groups of rhizosphere bacteria that form beneficial or pathogenic relationships with their hosts. The root exudates encourage the development of beneficial bacterial communities in the root zone capable of producing secondary metabolites that improve plant growth and crop yield. These beneficial associations facilitate plant growth either by enhancing crop nutrition, releasing plant growth stimulating hormones, reducing damages caused by pathogens/pests by producing antibiotics, bacteriocins, siderophores, hydrolytic enzymes and other secondary metabolites or by improving resistance to environmental pollutants. Rhizosphere bacteria also supply biologically fixed nitrogen, solubilize bound phosphorus and may provide other nutrients, such as, potassium, iron and sulfur to plants. These beneficial associations hence, reduce the requirement of chemical fertilizers used for crop productivity. Moreover, some rhizobacteria are used to relieve the toxicity of metals and organic toxicants, either through stimulation of microbial degradation of pollutants in the rhizosphere, or by uptake of pollutants/toxicants by the plant. The inoculation of the legumes with such rhizosphere bacteria has often been found to increase symbiotic properties, plant biomass and yields under green house or field conditions. Tremendous progress has been made recently in characterizing the process of rhizosphere colonization, identification and cloning of bacterial genes involved in nitrogen fixation, phosphorus solubilization, production of plant growth regulators and in suppression of plant diseases. The interactions/relationships of rhizosphere bacteria with their hosts and performance of wild-type and genetically manipulated beneficial bacterial populations are discussed for their efficient utilization in legume production under sustainable agriculture systems.

S.S. Sindhu (✉), S. Dua, M.K. Verma, and A. Khandelwal
Department of Microbiology, CCS Haryana Agricultural University, Hisar 125004, India
e-mail: sindhuss@hau.ernet.in

M.S. Khan et al. (eds.), *Microbes for Legume Improvement,*
DOI 10.1007/978-3-211-99753-6_9,

9.1 Introduction

The rhizosphere around the growing plant roots is a very dynamic environment and harbors a large number of total microorganisms, especially bacteria, greater than root-free soil. The heterogenous microbial populations interact with each other and with the plant through symbiotic, associative, neutralist or antagonistic effects. The outcome of colonization and penetration of the plant tissue with a microorganism varies from asymptomatic to disease and from associative to symbiosis, depending upon the mutual perception or recognition between the interacting cells. Such interactions are influenced greatly by the environment. The microbes that penetrate and colonize plants have evolved an elaborate system for subverting the plant defense system. The group of beneficial, root associative bacteria that stimulate the growth of a plant is termed as plant growth-promoting rhizobacteria (PGPR). Fluorescent pseudomonads and bacilli comprise the major group among PGPR along with other bacteria, like, *Acetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Cellulomonas, Clostridium, Enterobacter, Erwinia, Flavobacterium, Pasteuria, Serratia* and *Xanthomonas*. The beneficial rhizosphere microorganisms also include rhizobia and bradyrhizobia, which establish symbiotic relationship with leguminous plants. In the absence of appropriate microbial populations in the rhizosphere, plant growth may be impaired (Sturz et al. 2000).

Legumes are widely used for food, fodder, fuel, timber, green manure, and as cover crops in different agricultural systems. In developing countries, legumes are often an integral part of forest, pastures and agricultural ecosystems. On global scale, nitrogen-fixing legumes are the major source of soil N pool. Legume crops meet their N requirement through symbiotic N_2-fixation by forming nodules with rhizobia. Legumes and the rhizosphere provide most of the nutritional requirements of nodule bacteria and enhances the *Rhizobium* population several folds during plant growth. *Rhizobium*–legume associations are usually host specific, and a given rhizobial strain can infect only a limited number of hosts. Most of the characterized rhizobial strains have been isolated from the limited range of cultivated legume species.

The high-input agricultural practices of the more industrialized nations of temperate zones are rarely suitable for tropical conditions in most developing countries. Therefore, emphasis on biological processes which are able to improve agricultural productivity, while minimizing soil loss and ameliorating adverse edaphic conditions, are essential. A better understanding of rhizobial ecology, optimization of N_2-fixing conditions in legume-*Rhizobium* symbiosis and selection of rhizosphere bacteria having synergistic interactions with *Rhizobium* leading to growth-promoting effects on legumes are crucial for improving and sustaining agricultural ecosystems. The inoculation effects of diverse bacterial groups possessing plant growth-promoting traits on the performance of legumes are discussed. The fundamentals of the different processes involved in plant growth promotion are briefly introduced. The different strategies or biotechnological approaches adopted for enhancing biological N_2-fixation (BNF), P-solubilization, auxins production

and improving biocontrol activity are also described. Various constraints involved in crop improvement following inoculation with genetically engineered bacterial strains and the possibilities of deriving desired benefits by ensuring the establishment and survival of introduced microbial inoculants in soil are explored.

9.2 Mechanisms Involved in Plant Growth Promotion

Microbial ecology of the rhizosphere includes the study of the interactions of microorganisms with each other and the environment surrounding the plant root (Weyens et al. 2009). Rhizosphere microorganisms are of major interest due to their beneficial or detrimental effects on plant growth. It is therefore, important to understand the mechanism by which rhizosphere microorganisms impact plant growth in order to develop technologies that could enhance their activities. Microbial populations present in the rhizosphere of legumes have shown substantial effects on nodulation by *Rhizobium* spp. and on subsequent growth and yield of leguminous crops (Kloepper et al. 1989; Glick 1995). Microorganisms inhabiting rhizosphere of legumes may benefit plants in a variety of ways, like increased recycling, mineralization and uptake of nutrients; synthesizing vitamins, amino acids, auxins, gibberlins and plant growth regulating substances; reducing metal toxicity (bioremediation) in contaminated soils; antagonism with potential plant pathogens through competition and development of amensal relationships based on production of antibiotics, siderophores, and/or hydrolytic enzymes (Stockwell and Stack 2007; Sindhu et al. 2009c).

9.2.1 Increased Recycling, Mineralization and Uptake of Nutrients

Microorganisms in the rhizosphere influence the availability of mineral nutrients to the plants, sometimes by increasing the availability of inorganic nutrients to the plant, and in other cases, using limiting concentrations of inorganic nutrients before they could reach plant roots. Some rhizosphere bacteria, i.e., rhizobia, azotobacters, and azospirilla have the ability to fix atmospheric N into plant utilizable form, ammonia (Franche et al. 2009). Other microorganisms help plants by solubilizing bound P (Vessey 2003) and potassium or by providing iron and sulfur (Crowley et al. 1991; Scherer and Lange 1996; Crowley and Kraemer 2007).

9.2.1.1 Biological Nitrogen-Fixing Bacteria and Inoculation Responses

Sustainable agriculture involves the successful management of agricultural resources to satisfy the changing human needs, while maintaining or enhancing

the environmental quality and conserving natural resources. Consequently, sustainability considerations demand that alternatives to nitrogen fertilizers are sought. In this context, BNF offers an alternative in farming practices as it exploits the capacity of certain N_2-fixing bacteria to reduce atmospheric nitrogen into a compound (ammonia) mediated by enzyme nitrogenase (Bohlool et al. 1992; Burris and Roberts 1993). Legume crops meet their N requirement through symbiotic nitrogen fixation by forming root nodules with rhizobia (Brewin 2002; Gage 2004) which in turn reduce the dependency of agricultural crops on fossil fuel-derived nitrogenous fertilizers. Additionally, biologically fixed N is bound in soil organic matter and thus is much less susceptible to soil chemical transformations and physical factors that lead to volatilization and leaching. Therefore, BNF has an important role in sustaining productivity of soils.

Only some prokaryotes, a few bacteria and cyanobacteria, have acquired the ability to reduce atmospheric dinitrogen and add this essential nutrient to agricultural soils. Biological N_2 fixation occurs in a free-living state, in association with or in symbiosis with plants. Different N_2-fixing bacteria have been used to improve the supply of fixed N as nutrient to crop plants. Among the nitrogen-fixing systems, the legume-*Rhizobium* symbiosis alone accounts for 70–80% of the total N fixed biologically on global basis per annum and one-third of the total N input needed for world agriculture. The symbiotic rhizobia have been found to fix N ranging from 57 to 600 kg ha^{-1} annually (Elkan 1992). Annual inputs of fixed nitrogen are calculated to be 2.95 million tonnes (Tg) for the pulses and 18.5 Tg for the oilseed legumes (Herridge et al. 2008).

Rhizobium includes the fast-growing species, *Bradyrhizobium* includes slow-growing species and *Azorhizobium* includes those fast-growing species capable of forming both stem and root nodules on tropical water-logged legume, *Sesbania*. Chen et al. (1995) proposed a separate genus, *Mesorhizobium*, to indicate a growth rate intermediate between that of *Bradyrhizobium* strains and typical fast-growing *Rhizobium* strains. Subsequently, it was used to denote a phylogenetic position for rhizobia intermediate between these two genera. According to current taxonomic classification of root-nodule bacteria, 11 genera and 45 species have been defined (Sahgal and Johri 2006; Wiliems 2006).

In the rhizosphere of legumes and cereals, other diazotrophic bacteria could also contribute N to plants. Free-living diazotrophic bacteria contribute upto 15 kg ha^{-1} $year^{-1}$ fixed N and the root-associative bacteria fix N to a level of 15–36 kg ha^{-1} $year^{-1}$. Similarly, cyanobacteria, the free-living nitrogen fixers contribute about one-third of the N requirement of the crop and add about 15–80 kg ha^{-1} $year^{-1}$ to the rice cropping system (Elkan 1992) (Table 9.1). The free-living/associative diazotrophs, although have limited potential in terms of average N input on acreage basis but inhabit almost all ecological environments and contribute more in nutrient use efficiency and improvement in crop physiology (Pandey and Kumar 1989; Wani 1990; Fujiata et al. 1992).

The symbiotic effectiveness of different legume species and their microsymbionts has been found to be variable. In general, faba bean (*Vicia faba*), and

Table 9.1 Estimated average rates of biological nitrogen fixation by diazotrophs and associations

Organism/system	N_2 fixed (kg ha^{-1} $year^{-1}$)
Free living microorganisms – *Azotobacter, Clostridium* and *Derxia*	0.1–15
Associative symbioses – *Azoarcus* and *Azospirillum*	5–25
Cyanobacteria – *Nostoc*, *Anabaena,* and *Oscillatoria*	15–80
Azolla–Anabaena symbiosis	313
Rhizobium–legume symbiosis	57–600
Nodulated non-legumes – *Casuarina–Frankia, Parasponia–Rhizobium*	2–300

pigeonpea (*Cajanus cajan*) have been found to be very efficient; soybean (*Glycine max*), groundnut (*Arachis hypogaea*) and cowpea (*Vigna sinensis*) to be average; common bean and pea poor in fixing atmospheric N (Hardarson 1993). Among the legumes, soybean is the dominant crop legume, representing 50% of the global crop legume area and able to fix 16.4 million tones N annually, representing 77% of the N fixed by the legumes (Herridge et al. 2008). Inoculation of legumes with efficient strains of rhizobia has often resulted in significant increases in yields of various legume crops (Thies et al. 1991; Wani et al. 2007; Franche et al. 2009). Elsiddig et al. (1999) studied the inoculation effect of *Bradyrhizobium* strains TAL 169 and TAL 1371 (introduced) and strains ENRRI 16A and ENRRI 16C (local) on five guar (*Cyamopsis tetragonoloba*) cultivars in a field experiment. Most of the *Bradyrhizobium* strains significantly increased yield, protein, crude fiber and mineral content. The locally-isolated strains affected these parameters more than the introduced ones. Karasu et al. (2009) observed that inoculation of chickpea (*Cicer arietinum*) seeds with *R. ciceri* isolate had a significant effect on seed yield, plant height, first pod height, number of pods per plant, number of seeds per plant, harvest index and 1,000 seed weight. But, nitrogen doses (applied at 0, 30, 60, 90, and 120 kg ha^{-1} level as ammonium nitrate) had no significant effect on yield and yield components. Local population genotype as crop material gave the highest yield (2,149.1 kg ha^{-1}) among three chickpea genotypes used.

Sindhu et al. (1992) compared the potential of N fixed by *Rhizobium* strains in chickpea using non-nodulating genotype PM233 derived from normal nodulating genotype ICC640. The N fixed by the *Rhizobium* strains Ca534 and Ca219 in parent cultivar gave the plant dry weights more than those obtained by applying urea (80 kg N ha^{-1}) in the non-nodulating mutant PM233, suggesting that in chickpea effective symbiosis with rhizobia provides more than 80 kg N ha^{-1}. The benefits of N fixed in legumes to subsequent cereal crops are substantial and persist for several years due to progressively slow mineralization. The benefits obtained were of higher magnitude with green manuring crops and upto 532 kg N could be incorporated by 60 days green manuring crops where the rate of N accumulation were rapid upto 10.8 kg N ha^{-1} day^{-1} (Peoples and Herridge 1990).

In coinoculation experiments of N_2-fixing *Azotobacter vinelandii* with *Rhizobium* spp., it was found that coinoculation increased the number of nodules on the

roots of soybean, pea (*Pisum sativum*) and clover (*Trifolium pratense*) (Burns et al. 1981). Increased nodulation of soybean also occurred in field trial. Similarly, coinoculation of *Azospirillum brasilense* with *Rhizobium* strains showed synergistic effect on soybean and groundnut (Iruthayathas et al. 1983; Raverkar and Konde 1988). Compared to single *Rhizobium* inoculation, coinoculation of *Rhizobium* spp. and *Azospirillum* spp. was found more effective in enhancing the number of root hairs, the amount of flavonoids exuded by the roots and the number of nodules (Itzigsohn et al. 1993; Burdman et al. 1997; Remans et al. 2007, 2008b). The effect of *Azospirillum* on the legume-*Rhizobium* symbiosis was found to depend on the host genotype used. It was observed that *Azospirillum–Rhizobium* coinoculation increased the amount of fixed N and the yield of DOR364 genotype of common bean (*Phaseolus vulgaris* L.) across all sites on-farm field experiments, whereas a negative effect of *Azospirillum–Rhizobium* coinoculation on yield and N_2-fixation was observed in BAT 477 genotype on most of the sites as compared to sole application of *Rhizobium* (Remans et al. 2008b).

Field and greenhouse data indicated that increased nodulation of beans (*Phaseolus vulgaris*) by *R. phaseoli* occurred with coinoculation of *Pseudomonas putida* (Grimes and Mount 1984). However, bean yield and shoot weight were not significantly affected by coinoculation, demonstrating that increasing nodule number or infection by *Rhizobium* spp. may not affect plant productivity. Bolton et al. (1990) also demonstrated that nodulation of pea increased following the inoculation of mixtures of *R. leguminosarum* and a deleterious toxin-releasing *Pseudomonas* sp. However, nodules and dry matter accumulation in shoots were the same whether or not the *Pseudomonas* sp. was coinoculated. On the other hand, enhancement in nodulation, root length, plant biomass and yield by mixed inoculation of rhizobia with other rhizobacteria in different legumes have been reported. For example, Chanway et al. (1989) tested nine PGPR strains against single cultivar of lentil and pea in the field. None of the strains stimulated the growth of pea, but in lentil plots inoculated with one or more rhizobacterial strains, there were significant increase in emergence, vigor, nodulation, acetylene reduction activity and root weight. The enhanced nodulation and growth of chickpea along with reduction in wilt incidence was observed on coinoculation of rhizobacteria obtained from chickpea rhizosphere when these strains were coinoculated with an effective *R. ciceri* strain Ca181 (Khot et al. 1996).

Coinoculation of the five plant growth-promoting fluorescent pseudomonad strains, isolated from Indian and Swedish soils, and *R. leguminosarum* bv. *viceae* strains, recovered from Swedish soils, improved growth of pea cv. Capella (Dileep Kumar et al. 2001). In a similar study, Goel et al. (2002) observed that coinoculation of chickpea with *Pseudomonas* strains MRS23 and CRP55b, and *Mesorhizobium* sp. *cicer* strain Ca181 increased the formation of nodules by 68.2–115.4%, at 80 and 100 days after planting as compared to single inoculation of *Mesorhizobium* strain under sterile conditions. The shoot dry weight ratios of coinoculated treatments at different stages of plant growth varied from 1.18 to 1.35 times that of *Mesorhizobium*-inoculated and 3.25–4.06 times those of uninoculated plants. Similar synergistic effects on nodulation and plant growth have also been observed

for other legumes by dual inoculation of *B. japonicum* and *P. fluorescens* in soybean (Li and Alexander 1988; Nishijima et al. 1988; Dashti et al. 1998), *R. meliloti* with *Pseudomonas* in alfalfa (Li and Alexander 1988; Knight and Langston-Unkeffer 1988), *R. leguminosarum* with an antibiotic-producing *P. fluorescens* strain F113 in pea (Andrade et al. 1998) and *Mesorhizobium*/*Bradyrhizobium* strains with *Pseudomonas* sp. in greengram [*Vigna radiata* (L.) wilczek] and chickpea (Sindhu et al. 1999; Goel et al. 2000, 2002).

Similarly, bacterization of *Bacillus* species to seeds or roots altered the composition of rhizosphere, leading to increase in growth and yield of different legume crops (Holl et al. 1988). For instance, Halverson and Handelsman (1991) observed that seed treatment with *B. cereus* UW85 had 31–133% more nodules than untreated soybean plants after 28 and 35 days of planting in the field. In the growth chamber, in sterilized soil-vermiculite mixtures, UW85 seed treatments enhanced nodulation by 34–61% at 28 days after planting. It was suggested that UW85 affected the nodulation process soon after planting by stimulating bradyrhizobial infections or by suppressing the abortion of infections. In a follow up study, Turner and Backman (1991) reported that coating of peanut seeds with *B. subtilis* improved germination and emergence, enhanced nodulation by *Rhizobium* spp., enhanced plant nutrition, reduced levels of root cankers caused by *Rhizoctonia solani* AG-4 and increased root growth. In a similar study, Srinivasan et al. (1997) reported enhanced nodulation in *Phaseolus vulgaris* when coinoculated with *R. etli* strain TAL182 and *B. megaterium* S49. The mixed inoculation increased root hair proliferation and lateral root formation. The potential of *Bacillus* sp. to enhance nodulation, plant dry matter and grain yield on coinoculation with rhizobia has also been reported for pigeonpea (Podile 1995) and white clover (Holl et al. 1988). Sindhu et al. (2002a) found that coinoculation of *Bacillus* strains with effective *Bradyrhizobium* strain S24 caused enhancement in shoot dry mass of green gram ranging from 1.28 to 3.55 at 40 days of plant growth. Nodule promoting effect and increase in nitrogenase activity was also observed with majority of *Bacillus* strains at 40 days of plant growth.

Mishra et al. (2009a) showed that plant growth-promoting bacteria (PGPB) strain *B. thuringiensis*-KR1, originally isolated from the nodules of *Kudzu* vine (*Pueraria thunbergiana*), promoted plant growth of field pea and lentil (*Lens culinaris* L.) when coinoculated with *R. leguminosarum*-PR1 under Jensen's tube, growth pouch and non-sterile soil, respectively. Coinoculation with *B. thuringiensis*-KR1 (at a cell density of 10^6 c.f.u. ml^{-1}) had the highest and most consistent increase in nodule numbers, shoot weight, root weight, and total biomass, over rhizobial inoculation alone. The enhancement in nodulation due to coinoculation was 85 and 73% in pea and lentil, respectively, compared to *R. leguminosarum*-PR1 treatment alone. The shoot dry-weight gains on coinoculation with variable cell populations of *B. thuringiensis*-KR1 varied from 1.04 to 1.15 times and 1.03–1.06 times in pea and lentil, respectively to those of *R. leguminosarum*-PR1 inoculated treatment at 42 days of plant growth. The cell densities higher than 10^6 c.f.u. ml^{-1} had an inhibitory effect on nodulation and plant growth whereas lower inoculum levels resulted in decreased cell recovery and plant growth performance. Similarly,

enhanced nodule number and biomass yield were achieved after coinoculation of soybean with the *B. japonicum* SB1 and the plant growth-promoting *B. thuringiensis*-KR1 (Mishra et al. 2009b).

Inoculation of legumes with different rhizobial strains in general results in a 10–15% increase in yield of legumes. However, the desired impact of biofertilizer on legumes is usually not achieved under certain field conditions. The inoculation with commercial inoculants often fails to improve crop productivity (van Elsas and Heijnen 1990) probably due to the inability of rhizobial species to compete with the indigenous, ineffective and built in populations, which presents a competitive barrier to the introduced strains (Sindhu and Dadarwal 2000). In contrast, production of bacteriocins by rhizobia have been shown to suppress growth as well as nodulation by the indigenous non-producer strains, thus improving nodulation competitiveness of bacteriocin-producing inoculant strains (Goel et al. 1999; Sindhu and Dadarwal 2000). Transfer and expression of *tfx* genes (involved in trifolitoxin production) in various rhizobia showed stable trifolitoxin production and restricted nodulation by indigenous trifolitoxin-sensitive strains on many leguminous species (Triplett 1988, 1990). However, attempts to manipulate certain rhizobial genes in specific legume rhizosphere niches for improving competition have not been impressive (Nambiar et al. 1990; Sitrit et al. 1993; Krishnan et al. 1999).

Biotechnological approaches used to enhance N_2 fixation and crop productivity (Pau 1991; Hardarson 1993; Sindhu et al. 2009a) under field conditions have been of limited use. For example, recombinant constructs of *R. meliloti* and *B. japonicum* having increased expression of *nif*A and *dct*A genes although showed increase in the rate of N fixed but under field conditions, the same constructs did not show any significant increase in N_2-fixation or yields (Ronson et al. 1990). Manipulations of common nodulation genes to improve the bacterial competition have usually resulted in either no nodulation, delayed nodulation or inefficient nodulation (Devine and Kuykendall 1996). Mendoza et al. (1995) enhanced NH_4^+ assimilating enzymes in *R. etli* through genetic engineering, by inserting an additional copy of glutamate dehydrogenase (GDH), which resulted in total inhibition of nodulation on bean plants. However, nodule inhibition effect was overcome when *gdh*A expression was controlled by NifA and thereby, delaying the onset of GDH activity after nodule establishment (Mendoza et al. 1998). Similarly, attempts to engineer hydrogen uptake (Hup^+) ability by cloning hydrogenase genes into Hup^- strains of *Rhizobium* resulted in experimental successes only in areas where soybeans are cultivated and where the photosynthetic energy is limited (Evans et al. 1987). Attempts to develop self-fertilizing crops for N have also been a failure, mainly because of the complexity of the nitrogenase enzyme complex to be expressed in the absence of an oxygen protection system in eukaryotes (Dixon et al. 1997). Moreover, induction of nodule-like structures or pseudonodules using lytic enzymes or hormones treatment in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) though showed nitrogenase activity and $^{15}N_2$ incorporation, but the activity expressed was $>1\%$ of the value observed for legumes (Cocking et al. 1994).

9.2.1.2 Phosphate Solubilization and Mobilization and its Agronomic Significance

In agriculture, phosphorus (P) is second only to N in terms of quantitative requirement for crop plants (Goldstein 1986; Fernandez et al. 2007). It is found in soil, plants and microorganisms in both organic and inorganic forms. However, the total P content in an average soil is 0.05% and only a very small fraction (~0.1%) of the total P present in the soil is available to the plants because of its chemical fixation and low solubility (Stevenson and Cole 1999). The pool of immediately available P is thus, extremely small and must be supplied regularly to offset plant demands (Bieleski 1973). Phosphorus may be added to soil either as chemical fertilizers or as leaf litter, plant residues or animal remains. The P fertilizers are the world's second largest bulk chemicals used in agriculture and therefore, the second most widely applied fertilizer (Goldstein et al. 1993; Goldstein 2007). However, 75% of phosphate fertilizers applied to soil are rapidly immobilized and thus become unavailable to plants (Rodriguez and Fraga 1999). Therefore, P deficiency is a major constraint to crop production and under such conditions, the microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil to make it available to plants and thus maintain the soil health and quality (Rodriguez and Fraga 1999; Richardson 2001; Deubel and Merbach 2005; Chen et al. 2006; Khan et al. 2007).

Phosphate solubilizing (PS) and mobilizing microorganisms include bacteria, actinomycetes as well as the fungi. The most important PS bacteria (PSB) belong to genera *Bacillus* and *Pseudomonas*, though species of *Achromobacter, Alcaligenes, Brevibacterium, Corynebacterium, Serratia* and *Xanthomonas* have also been reported as active P solubilizer (Venkateswarlu et al. 1984; Cattelan et al. 1999; Khan et al. 2007). In a study, Naik et al. (2008) screened 443 fluorescent pseudomonad strains for the solubilization of tricalcium phosphate(TCP) and reported that 18% formed visible dissolution halos on Pikovskaya agar medium plates. Based on phenotypic characterization and 16S rRNA gene phylogenetic analyses, these strains were identified as *P. aeruginosa, P. mosselii, P. monteilii, P. plecoglosscida, P. putida, P. fulva* and *P. fluorescens*. The P-solubilizing *Bacillus* species isolated from the rhizosphere of legumes and cereals included *B. subtilis, B. circulans, B. coagulans, B. firmus, B. licheniformis, B. megaterium* and *B. polymyxa* (Gaind and Gaur 1991; Rajarathinam et al. 1995). Other PSB include species of bacteria like, *A. chroococcum, Burkholderia cepacia, Erwinia herbicola, Enterobacter agglomerans, E. aerogenes, Nitrosomonas, Nitrobacter, Serratia marcescens, Synechococcus* sp., *Rahnella aquatilis*, *Micrococcus, Thiobacillus ferroxidans* and *T. thiooxidans* (Banik and Dey 1983; Kim et al. 1998; Bagyaraj et al. 2000). *Rhizobium* and *Bradyrhizobium* strains have also been found to solubilize rock phosphate (RP) or organic P compounds effectively through the production of organic acids and/or phosphatases (Halder et al. 1991; Abd-Alla 1994). The various fungi having efficient PS ability belong to genera *Aspergillus, Fusarium, Penicillium* and *Trichoderma* (Rashid et al. 2004; Khan et al. 2010). Ahmad et al. (2008) reported that out of 72 isolates obtained from rhizosphere soil and root

nodules, solubilization of P was commonly detected in *Bacillus* (80%) followed by *Azotobacter* (74%), *Pseudomonas* (56%) and *Mesorhizobium* (17%). The principal mechanism of increasing P availability is the microbial production of organic acids that may dissolve appetite, releasing soluble forms of P through acidification of rhizosphere soil. Additionally, the acidification of the rhizosphere environments through metabolic production of hydrogen ions alters the pH sufficiently to mobilize soil minerals (Rodriguez et al. 2006).

Phosphatic biofertilizers were first prepared in USSR, using *B. megaterium* var. *phosphaticum* as PSB and the product was named as "phosphobacterin." It was extensively used in collective farming for seed and soil inoculation, and reported to give 5–10% increase in crop yields. Subsequently, inoculation experiments using phosphobacterin and other PSM for legumes like, groundnut, peas, and soybean showed an average 10–15% increase in yields in about 30% of the trials (Agasimani et al. 1994; Dubey 1997; Vessey 2003). The variations under field conditions are expected due to the effect of various environmental conditions and survival of the inoculant strains in soil. The inoculation of PSB along with Rock phosphate (RP) also resulted in increased availability of P for plant utilization (Jisha and Alagawadi 1996). It was observed that inoculation of mineral phosphate solubilizing bacteria (MPSB) along with 17.5 kg P ha^{-1} as Massourie rock phosphate (MRP) increased dry matter in chickpea and was as effective as single super phosphate (Prabhakar and Saraf 1990). Saraf et al. (1997) showed that PSB inoculation increased seed yield (10.3 q ha^{-1}) of chickpea as compared to control (8.8 q ha^{-1}). Increased grain yield (14%) and uptake of N and P was reported in chickpea by inoculation of PSB along with P fertilizers. The grain and straw yield of chickpea was found to increase with increasing levels of P (0–60 kg P_2O_5 ha^{-1}) which further improved by inoculation of PSB (Sarawgi et al. 1999, 2000). Plant growth-promoting fluorescent pseudomonad isolate PGPR1, which produced siderophore and indole acetic acid, and solubilized TCP under in vitro conditions, significantly enhanced the pod yield (23–26%, respectively), haulm yield and nodule dry weight over the control during 3 years.

Phosphorus deficiency has a negative effect on BNF and the impaired BNF in P-deficient plants is usually explained by an effect of the low P supply on the growth of the host plant, on the growth and functioning of the nodule, or on the growth of both plant and the nodule (Christiansen and Graham 2002). Some particular strategies have been adopted for the adaptation of nodulated legumes to limited P supply, such as the maintenance of concentrations of P in nodules much higher than in other organs (Pereira and Bliss 1987), higher absorption of P from the solution directly by the nodules and bacteroids (Al-Niemi et al. 1998), increased N_2-fixation per unit of nodule mass to compensate for reduced nodulation, (Almeida et al. 2000) and higher accumulation of soluble sugars in nodules than in roots and shoots (Olivera et al. 2004). Araujo et al. (2008) observed an increase in the activities of acid phosphatases and phytases in nodules of common bean genotypes at different levels of P supply indicating that this increase in activities may constitute an adaptive mechanism for N_2-fixing legumes to tolerate P deficiency. Similarly, plants grown at limited P supply can increase the activities of

phosphatases and phytases in roots to hydrolyze organic-P compounds in the soil, thus improving plant P acquisition.

Synergistic effect was observed after coinoculation of N_2-fixing bacteria with PSB. For example, the composite application of *P. putida* and *R. phaseoli* increased P availability to common bean plants and enhanced nodulation of common bean (Grimes and Mount 1984). The seed inoculation with thermo-tolerant PSB, viz. *B. subtilis, B. circulans* and *A. niger* was found to improve nodulation, available P_2O_5 content of soil, root and shoot biomass, straw and grain yield, P and N uptake by mungbean (Gaind and Gaur 1991). High pod yield and P uptake in groundnut due to inoculation of *P. striata* were also recorded (Agasimani et al. 1994). Increased nodulation, yield attributes, seed index and seed yield of rainfed soybean were also reported with combined inoculation of *P. striata* and *B. japonicum* (Dubey 1997). Similarly, a significant increase in nitrogenase activity, plant growth and grain yield of pea was found following dual inoculation of *R. leguminosarum* and PSB (Srivastava et al. 1998).

Attempts to express the mineral phosphate solubilization (MPS) genes in a different host were found to be influenced by the genetic background of the recipient strain, the copy number of the plasmids present and metabolic interactions. Thus, genetic transfer of any isolated gene involved in MPS to improve P-dissolving capacity in PGPR strains, is an interesting approach. An attempt to improve mps in PGPR strains, using a PQQ synthase gene from *E. herbicola* was carried out (Rodriguez et al. 2000). This gene was subcloned in a broad-host range vector pKT230. The recombinant plasmid was expressed in *E. coli* and transferred to PGPR strains of *Burkholderia cepacia* and *P. aeruginosa*, using tri-parental conjugation. Several of the exconjugants showed a larger clearing halo on medium plates containing TCP as the sole P source. This experiment indicated the heterologous expression of this gene in the recombinant strains and improved MPS ability of PGPR.

9.2.1.3 Mineralization of Potassium, Iron and Sulfur Nutrients in the Rhizosphere

Potassium (K) is the third major essential nutrient for plant growth. It plays an essential role in enzyme activation, protein synthesis and photosynthesis. Potassium in soil is present in water-soluble (solution K), exchangeable, non-exchangeable and structural or mineral forms. Of these, water-soluble and exchangeable pools are directly available for plant uptake. At low levels of exchangeable K in certain soils, non-exchangeable K can also contribute significantly to the plant uptake (Memon et al. 1988). India ranks fourth after USA, China and Brazil in terms of total consumption of K-fertilizers. Some microorganisms in the soil are able to solubilize "unavailable" forms of K-bearing minerals, such as micas, illite and orthoclase, by excreting organic acids which either directly dissolves rock K or chelate silicon ions to bring the K into solution (Barker et al. 1998; Bennett et al. 1998). These microorganisms are commonly known as potassium-solubilizing bacteria (KSB) or potassium-dissolving bacteria or silicate-dissolving bacteria whose application is

termed as "biological potassium biofertilizer (BPF)". It was shown that KSB increased K availability in soils and increased mineral uptake by plant (Sheng and Huang 2002; Sheng et al. 2002, 2003). Therefore, application of KSB holds a promise for increasing K availability in soils.

In Egypt, some studies were conducted on potassium-dissolving bacteria which were mainly concentrated on their K releasing capacity along with their effects on growth and K uptake of the treated plants. In a trial conducted by Balabel-Naglaa (1997), there were positive responses of broad bean to inoculation with some species of *Bacillus* (K releasing bacteria). These positive responses were obvious on dry weight of shoot and root, nodule number and dry mass of nodules, nitrogenase activity, N, P, K contents of foliage, number as well as dry weight of pods, seed and straw yields. Hu et al. (2006) isolated two phosphate-and potassium-solubilizing strains, KNP413 and KNP414 from the soil of Tianmu Mountain, Zhejiang Province (China). Both isolates actively dissolved mineral P and K, while strain KNP414 showed higher dissolution capacity even than *Bacillus mucilaginosus* AS1.153, the inoculants of potassium fertilizer widely used in China. In another study, Lian et al. (2008) studied the mechanism for the release of mineralic potassium using a thermophilic fungus *Aspergillus fumigatus*. The thermophilic fungus *A. fumigatus* promoted potassium release by means of at least three likely routes, firstly, by complexing soluble organic ligands, secondly, appealing to the immobile biopolymers such as the insoluble components of secretion and thirdly, involving mechanical forces in association with the direct physical contact between cells and mineral particles.

Iron is yet another essential nutrient and is abundant in soil but most of it is found in the insoluble form, ferric hydroxide. Thus, iron is only available to organisms at concentrations at or below 10^{-18} M in soil solutions at neutral pH. To cope up with its solubility, many microorganisms synthesize extracellular, low molecular weight, high affinity Fe^{3+} chelators commonly referred to as siderophores, in response to iron stress (Neilands 1981; Neilands and Nakamura 1991) that transport iron into bacterial cells. Fuhrmann and Wollum (1989) detected a decrease in the number of taproot nodules and in seedling emergence of soybean and altered nodulation competition among *B. japonicum* strains when coinoculated with *Pseudomonas* spp. Iron availability was implicated as a factor involved in the plant-*B. japonicum*-rhizosphere microflora interactions. Thus, rhizobacteria help plants in absorbing iron from the soil. The metal-chelating agents produced by rhizobacteria also play an important role in the acquisition of heavy metals (Leong 1986). These organic substances scavenge Fe^{3+} and significantly enhance the bioavailability of soil bound iron (Kanazawa et al. 1994) and regulate the availability of iron in the plant rhizosphere (Bar-Ness et al. 1992; Loper and Henkels 1999). The competition for iron in the rhizosphere is controlled by the affinity of the siderophore for iron, and the probable availability of iron to the microorganisms ultimately decides the rhizosphere population structure. The concentration of various types of siderophores, kinetics of exchange and availability of Fe-complexes to microbes as well as plants has been found to control the binding affinity of siderophore (Loper and Henkels 1999). Interestingly, the binding affinity of phytosiderophores for iron

is less than the affinity of microbial siderophores, but plants require a lower iron concentration for normal growth than microbes do (Meyer 2000).

Masalha et al. (2000) reported that plants grown under non-sterile soil systems were better in terms of iron nutrition than those grown under sterile conditions. Their data emphasized the role of microbial community on the iron nutrition of plants. It has been demonstrated that plants grown in metal-contaminated soils are often iron deficient and the production of siderophores by plant growth-promoting bacteria may help plants to obtain sufficient iron (Wallace et al. 1992; Burd et al. 2000). In fact, there is evidence that at least part of the toxic effects of some heavy metals in plants results from an induced iron deficiency and since bacterial siderophores could provide iron to various plants (Bar-Ness et al. 1991; Wang et al. 1993), therefore, siderophores produced by rhizobacteria may reduce nickel toxicity by supplying the plant with iron and hence reduce the severity of nickel toxicity (Bollard 1983; Bingham et al. 1986).

Another plant nutrient, sulfur (S), is the ninth and least abundant essential macronutrient. Its uptake and assimilation is crucial because of the key role played by the S containing aminoacids, methionine and cysteine in maintaining protein structure and because of its role in plant defense (Rasch and Wachter 2005). Sulfur atoms are widely distributed in soil and are found in a wide variety of organic and inorganic forms. These atoms are an integral component of soil humus, plants, microbial biomass and minerals. Scherer (2009) reported that sulfate (SO_4^{2-}), which is a direct source of sulfur for plants, contributed up to 5% of total soil S; generally more than 95% of soils S are organically bound. Sulfur containing minerals include pyrite (FeS_2) which occurs in ingenious rocks, gypsum ($CaSO_4.2H_2O$) and epsonite ($MgSO_4.7H_2O$). Despite its abundance in the earth's crust, S is often present in suboptimal quantities in soil or either in unavailable states. Moreover, the decrease of S input from atmospheric depositions has led to S deficiency of crops over the past two decades on a worldwide scale that reduced yield and affected the quality of harvested products. Especially in Western European countries, incidence of S deficiency has increasingly been reported in oilseed rape, which is an S demanding plant (Fismes et al. 2000). Therefore, more attention should be paid to the optimization of S fertilizer application, in order to cover plant S requirements whilst minimizing environmental impacts.

Sulfur turnover involves both biochemical and biological mineralization (Gharmakher et al. 2008). Biochemical mineralization, which is the release of SO_4^{2-} from the ester sulfate pool through enzymatic hydrolysis, is controlled by S supply, while the biological mineralization is driven by the microbial need for organic C to provide energy. The biological oxidation of elemental S and inorganic S compounds such as H_2S, sulphite and thiosulphate is brought about by chemo-autotrophic bacteria and photosynthetic bacteria. Sulfur-oxidizing bacteria include *Beggiotoa*, *Chromatium, Chlorobium, Thiobacillus*, *Sulfolobus, Thiospira* and *Thiomicrospira*. The species of *Bacillus, Pseudomonas, Arthrobacter* and *Flavobacterium* are also reported to oxidize elemental S or thiosulphate to sulfate. Under anaerobic conditions, sulfate is reduced to H_2S by sulfate-reducing micro-organisms, mostly the bacteria. Many bacteria including species of *Bacillus* and

Pseudomonas are known to reduce S or sulfate to H_2S, but among these *Desulphovibrio desulfuricans* and *Desulfotomaculum* spp. are important.

Nitrogen fixation appears to be affected by S fertilization in faba bean, lucerne, pea and in red clover (DeBoer and Duke 1982; Scherer and Lange 1996; Habtemichial and Singh 2007). An important link between S and N nutrition was found in white clover, lucerne and pea (Zhao et al. 1999; Varin et al. 2009) and sulfur fertilization was found to stimulate N_2 fixation strongly. Scherer et al. (2008) observed that the amount of leghaemoglobin was reduced by S deficiency in peas and alfalfa, when no S was added and nodules devoid of leghaemoglobin were more numerous. Varin et al. (2008) analyzed a set of functional traits in three white clover lines along a gradient of N and S fertilization on a poor soil. Nitrogen was found to be the most limiting factor for the VLF (very low fixation) line. S was the element that modulated the most traits for the nitrogen fixing lines NNU (normal nitrate uptake) and LNU (low nitrate uptake). Nitrogen fertilization was found to inhibit N_2 fixation in clover but N_2 fixation was enhanced when S was added. S fertilization also increased nodule length, as well as the proportion of nodules containing leghaemoglobin. Thus, sensitivity of white clover to S nutrition would be a disadvantage for competition in a situation of sulfur impoverishment.

9.2.2 Synthesis of Auxins, Cytokinins, Gibberlins and Vitamins

Microbial communities of soil and rhizosphere have been found to synthesize auxins, cytokinins, vitamins and gibberellin-like compounds (Arshad and Frankenberger 1991; Derylo and Skorupska 1993; Patten and Glick 1996; Gutierrez-Manero et al. 2001). These compounds increase the rate of seed germination and stimulate the development of root tissues leading to an increase in the capacity of the root system to provide nutrients and water to above ground organs of plants (Arkhipova et al. 2007), and also help the plants to tolerate abiotic stress (Yang et al. 2009). Derylo and Skorupska (1993) reported that stimulation of clover plant growth under gnotobiotic conditions resulted from the secretion of water-soluble B vitamins by fluorescent *Pseudomonas* sp. strain 267. This was demonstrated by enhancement of clover growth by naturally auxotrophic strains of *R. leguminosarum* bv. *trifolii* in the presence of the *Pseudomonas* sp. strain 267 supernatant. The addition of vitamins to the plant medium increased symbiotic N_2-fixation by the clover plants.

Indole acetic acid (IAA) is known as the main auxin in plants and has been implicated in all aspects of plant growth and development (Taele et al. 2006). The exposure of plant roots to exogenous microbially produced IAA can affect plant growth in diverse ways, varying from pathogenesis and growth inhibition to plant growth stimulation (Spaepen et al. 2007). In fact, low levels of IAA released by rhizobacteria has been found to promote primary root elongation, whereas, high levels of IAA stimulated lateral and adventitious root formation (Glick 1995) but inhibited primary root growth (Xie et al. 1996). Thus, plant growth-promoting bacteria can facilitate plant growth by altering the hormonal balance within the

affected plant (Lambrecht et al. 2000; Kamnev 2003). Such relationships of rhizobacteria between different crop species could be cultivar or genotype-specific (Cattelan et al. 1998). For example, the rhizosphere of wheat seedlings harbors a significant proportion of bacteria that produce phytohormone, indole acetic acid (IAA), known to increase root growth (Patten and Glick 2002). Moreover, differential response of inoculation was observed in two genotypes of common bean with *A. brasilense* Sp245 mutant strain having reduced auxin biosynthesis or to addition of increasing concentrations of exogenous auxin (Remans et al. 2008a). Genetic analysis of recombinant inbred lines revealed two quantitative trait loci (QTLs) associated with basal root responsive to auxin in common bean.

Although significant and consistent yield increases of rhizobia-inoculated crops have been attributed to N_2 fixation, plant growth regulators may also be involved (Mayak et al. 1999; Malik and Sindhu 2008). For instance, the rhizobial species are known to produce IAA in vitro (Bandenoch-Jones et al. 1982; Wang et al. 1982; Boiero et al. 2007) and nodulated roots often contained substantially greater auxin concentrations than non-nodulated roots (Dulhart 1967, 1970). Inoculation of soybeans with spontaneous mutants of *Rhizobium japonicum* that overproduced IAA (30-fold more auxin than the wild-type strain) showed a three-fold increase in the number of root nodules (Kaneshiro and Kwolek 1985). Mutants of *B. elkanii* strain deficient in IAA production induced fewer nodules on soybean roots in comparison to the parental strain and the normal numbers of nodules were reestablished following application of exogenous IAA (Fukuhara et al. 1994). IAA derived from *B. elkanii* has been implicated as a causative agent in the swelling of outer cortical cells of soybean roots and was suggested to provide a competitive advantage for nodulation (Yuhanshi et al. 1995). However, enlargement of cortical cells was not observed after inoculation with either IAA-deficient mutants of *B. elkanii* (Yuhanshi et al. 1995) or wild-type *B. japonicum* strains that do not produce IAA (Minamisawa and Fukai 1991). Prinsen et al. (1991) demonstrated that flavonoids released from legume plant roots, which also act as inducers of *Rhizobium* nodulation genes, stimulated the production of IAA, suggesting that nodule morphogenesis could be controlled by the highly specific nodulation signal in combination with phytohormones such as auxins, released by rhizobia.

Coinoculation of legumes with *Rhizobium* and free-living IAA-producing bacteria such as *Azospirillum brasilense* (Yahalom et al. 1990) and several *Bacillus* species (Srinivasan et al. 1996) significantly increased the number of nodules on the host roots and increased nodule fresh weight and nitrogenase activity, compared to inoculation with *Rhizobium* alone. Zhang et al. (1996) reported that *Serratia* stimulated soybean growth through the production of a plant growth-regulating compound, which stimulated overall plant vigor and growth, resulting in subsequent increase in nitrogen fixation. Mayak et al. (1999) showed that an IAA over producing mutant of *P. putida* caused extensive development of adventitious roots on mung bean cuttings. It was suggested that inoculation with these free-living bacteria increase the number of infection sites on roots for attachment and nodulation by *Rhizobium*. In addition, enhanced production of flavonoid-like compounds or phytoalexins in roots of several crop plants by inoculation of *Pseudomonas* sp.

(Parmar and Dadarwal 1999; Goel et al. 2001) could induce the transcription of nodulation (*nod*) genes (Peter and Verma 1990), leading to increase in nodulation. In contrast, similar experiments using mutants of *B. megaterium* with altered IAA production levels had a negative effect on symbiotic parameters (Srinivasan et al. 1996). Mutants of *Pseudomonas* strains altered in IAA production were derived by Tn5 mutagenesis (Malik 2002; Malik and Sindhu 2008). Coinoculation studies of wild-type *Pseudomonas* strains with *Bradyrhizobium* strain S24 and IAA overproducer *Pseudomonas* mutants resulted in more nodules in green gram compared to wild type *Bradyrhizobium* strain at 50 days of growth. Camerini et al. (2008) introduced *iaa*M gene (involved in IAA biosynthesis) from *Pseudomonas savastanoi* and the *tms*2 gene from *A. tumefaciens* into *R. leguminosarum* bv. *viciae* LPR1105. Free-living bacteria harboring the promoter *iaa*M-*tms*2 construct (strain RD20) released 14-fold more IAA in the growth medium than the wild-type parental strain and elicited the development of vetch root nodules containing up to 60-fold more IAA than nodules infected by the wild-type strain LPR1105. The root nodules elicited in vetch by RD20, were heavier and had an enlarged and more active meristem, and showed a significant increase in acetylene reduction activity (ARA).

9.2.3 *Effect of Rhizobacteria on Phytoremediation in Metal Stressed Soil*

Pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution (Kabata-Pendias and Pendias 1989). Heavy metal pollution of soil is a significant environmental problem and has its negative impact on human health and agriculture. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Ni. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth (Bingham et al. 1986). In addition, excessive metal concentrations in contaminated soils resulted in decreased soil microbial activity and soil fertility, and yield losses (McGrath et al. 1995). Phytoremediation has been reported to be an effective, in situ, non-intrusive, low-cost, ecofriendly, socially accepted technology to remediate polluted soils (Garbisu et al. 2002). Another alternative is to provide them with an associated plant growth-promoting rhizobacteria, which also is considered an important component of phytoremediation technology (Glick 2003; Jing et al. 2007). Therefore, the use of rhizobacteria to enhance phytoremediation of soil heavy metals pollution has recently received more attention (Weyens et al. 2009).

The functioning of associative plant-bacterial symbioses in heavy-metal-polluted soil can be affected from the side of both the micropartner (plant-associated bacteria) and the host plant (Glick 1995). Chaudri et al. (1992) found that *Rhizobium* populations were reduced at concentrations >7 mg kg^{-1} soil in their Cd treatments. Field studies of metal contaminated soils have similarly

demonstrated that elevated metal loadings can result in decreased microbial community size (Chander and Brookes 1991). Some rhizobacteria can exude a class of rhizobacterial secretion, such as IAA, siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which increased bioavailability and facilitated root absorption of heavy metals, such as Fe (Crowley et al. 1991), enhanced tolerance of host plants by improving the P absorption (Liu et al. 2000) and promoted plant growth (Burd et al. 2000; Ellis et al. 2000). Rajkumar et al. (2005) isolated *Pseudomonas* sp. strain RNP4 from tannery waste contaminated soil which tolerated concentrations up to 450 mg Cr^{6+} L^{-1} on a Luria-Bertani (LB) agar medium and reduced a substantial amount of Cr^{6+} to Cr^{3+} in LB liquid medium. The strain also produced substantial amount of IAA, exhibited the production of siderophores and solubilized phosphorus. The strain was found to promote the growth of black gram, Indian mustard and pearl millet in the presence of Cr^{6+}, suggesting the innate capability of the *Pseudomonas* isolate for parallel bioremediation and plant growth promotion. In another study, Safronova et al. (2006) found that pea plants inoculated with root-associated bacteria containing ACC deaminase activity produced longer roots, greater root density and improved nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Inoculation of pea plants with a poplar endophyte that degraded 2,4-dichlorophenoxyacetic acid (2,4-D) resulted in increased removal of 2,4-D from the soil (Germaine et al. 2006). Moreover, the plants did not show toxic responses and did not accumulate 2,4-D in their tissues.

Liu et al. (2007) demonstrated that inoculation of alfalfa with *Comamonas* sp. strain CNB-1 not only removed 4-chloronitrobenzene (4-CNB) completely within 1 or 2 days from soil but also eliminated the phytotoxicity of 4-CNB to alfalfa plants. Tank and Saraf (2009) selected five plant growth-promoting bacterial strains based on their P solubilization ability, IAA production and biocontrol potentials. These isolates were also able to grow and produced siderophores in presence of heavy metals like Ni, Zn, and Cd. A positive response of bacterial inoculants was observed in chickpea plants towards toxic effect of nickel present in soil at different concentrations (0, 1 and 2 mM) and bacterial inoculants enhanced fresh and dry weight of chickpea plants even at 2 mM nickel concentration. The accumulation of nickel $plant^{-1}$ was just 50% in *Pseudomonas*-inoculated plants as compared to uninoculated plants with 2 mM nickel concentration along with increased biomass. The development of engineered endophytic bacteria that improved the phytoremediation of volatile organic compound trichloroethylene (TCE) was found to protect host plants against the phytotoxicity of TCE and contributed to a significant decrease in TCE evapotranspiration (Barac et al. 2004). Similarly, the genetic modification of the polychlorinated biphenyls (PCB)-degrading bacteria *Pseudomonas fluorescens* F113, to improve its performance in the rhizosphere, could be manipulated by improving symbiotic microorganisms. Thus, the rhizoremediation of PCBs by *P. fluorescens* was improved in which biphenyl degradation is regulated using a system that responds to signal from alfalfa roots (Villacieros et al. 2005).

9.2.4 Rhizobacteria as Biocontrol Agents

The suppression of growth of soil-borne plant pathogens by the use of microorganisms, natural or modified, genes or gene products to reduce the effects of undesirable organisms (pests) is referred to as biocontrol. Rhizobacteria inhibit the growth of various pathogenic bacteria and fungi resulting in suppression of the diseases caused by such pathogens (Weller 1988; Thomashow and Weller 1996). Disease suppression by biocontrol agents involves a sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment (Pierson and Weller 1994). Strains of *Pseudomonas fluorescens, P. putida, P. aureofaciens, P. cepacia* and *P. aeruginosa* have been found to antagonize the growth of pathogens leading to substantial disease control (Chandra 1997; Weller 2007). Different strains of *Bacillus thruingiensis, B. sphaericus, B. cereus* and *B. subtilis* are also used as biocontrol agents (Asaka and Shoda 1996; Hervas et al. 1997).

Trapero-Casas et al. (1990) reported that coating of chickpea seeds with the *P. fluorescens* (strain Q29z-80) increased the yield which was comparable to those obtained with any of the fungicide seed treatments used to control seed rot and preemergence damping-off disease caused by *P. ultimum* in the field. Hervas et al. (1997) observed that treatment of *B. subtilis,* nonpathogenic *F. oxysporum* and/or *T. harzianum, when* applied alone or in combination, to chickpea cultivars "ICCV 4" and "PV 61" could effectively suppress the disease caused by the highly virulent *F. oxysporum* f. sp. *ciceris*. In comparison with the control, the final disease incidence was reduced by *B. subtilis* (18–25%) or nonpathogenic *F. oxysporum* (18%). The extent of disease suppression was higher and more consistent in cultivar "PV 61" than in "ICCV 4" whether colonized by *B. subtilis,* non pathogenic *F. oxysporum* or *T. harzianum.* Nautiyal (1997) found that among 478 bacteria obtained from roots of chickpea rhizosphere by random selection, 44 rifampicin resistant strains showed biocontrol activity against *F. oxysporum* f. sp. *ciceri*, *R. bataticola* and *Pythium* sp. under in vitro studies. In a greenhouse test, seed bacterization of chickpea with *P. fluorescens* NBRI 1303 increased the germination of seedlings by 25% reduced the number of diseased plants by 45% as compared with non-bacterized controls. Significant growth increases in terms of shoot length, dry weight, and grain yield, averaging 11.6, 17.6, and 22.61%, respectively, above untreated controls were obtained in field trials.

Plant growth-promoting fluorescent pseudomonad isolate PGPR1, which produced siderophore and IAA, and solubilized TCP under in vitro conditions, also suppressed the soil-borne fungal diseases like collar rot of peanut (caused by *A. niger*) in field trials (Dey et al. 2004). Jamali et al. (2004) studied effect of seven antagonistic bacteria on control of Fusarium wilt under green house conditions. Isolates B-120, B-32, B-28 and B-22 were identified as *B. subtilis* and isolates Pf-100, Pf-10 and CHAO were identified as *P. fluorescens*. Results revealed that only isolate B-120 reduced *Fusarium* wilt of chickpea in both seed and soil treatments. Soil treatment of bacteria showed better effects on plant growth than

that of bacterial seed treatment. Statistically significant biocontrol effects were observed when lettuce seedlings were inoculated into naturally *Rhizoctonia solani*-infested lettuce fields with bacterial suspensions of two endophytic strains, *Serratia plymuthica* 3Re4-18 and *P. trivialis* 3Re2-7 with rhizobacterium *P. fluorescens* L13-6-12, 7 days before and five days after planting in the field (Scherwinski et al. 2008). Usually, no general relationship was observed between the ability of a bacterium to inhibit a pathogen under in vitro and in situ disease suppression (Schroth and Hancock 1982; Wong and Baker 1984). Bacterial strains producing the largest zones of inhibition on agar media do not always make the best biocontrol agents. Therefore, some in vitro conditions have been modified to more closely simulate natural conditions (Randhawa and Schaad 1985). Among the numerous examples of biocontrol agents reported for disease control of soil-borne pathogens, only few studies provide mechanistic information for the activities of these agents. Recently, the use of mutants that lack certain in vitro and in situ activities have provided strong evidence for the involvement of specific molecules in biocontrol.

9.2.4.1 Mechanisms Involved in Biocontrol

For effective biocontrol of plant disease, the rhizobacteria must establish and grow in an ecological habitat that includes indigenous pathogenic microorganisms. Thus, root colonization by rhizobacteria appears to be an important factor in biological control and plant growth promotion. In recent years, tremendous progress has been made in characterizing the process of root colonization by biocontrol agents, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to pathogen suppression (Benizri et al. 2001; Sindhu et al. 2009b). Rhizobacteria inhibit the growth of phytopathogenic microorganisms by various mechanisms.

Competition for Nutrients and Infection Sites

The rhizosphere microflora directly or indirectly inhibit the invasion of pathogen on plant tissue. Root-inhabiting microorganisms and plant pathogens could compete for space, nutrients or even for binding sites on the root surface. Space and nutrients competition could result in failure of the pathogen to develop critical population densities for disease initiation, whereas, the competition for specific binding sites would reduce the capability of plant pathogen to initiate the infection process. Pseudomonads possess the capacity to catabolize diverse nutrients and have fast generation time in the root zone, and hence, they are logical candidates for competition for nutrients against the slow growing pathogenic fungi and could result in biological control of pathogens (Weller 1985). Elad and Chet (1987) carried out a study to evaluate the antagonistic mechanism of rhizobacteria against damping off disease caused by *Pythium*. The competition for nutrients between

germinating oospores of *Pythium aphanidermatum* and biocontrol rhizobacteria was unique and was correlated significantly with disease suppression.

Interference in Chemotactic Attraction

Crop rotations and tillage management have been shown to influence specific microbial populations (Sturz et al. 1997). Rhizobacteria could spur a root exudation response in plants that is species specific (Merharg and Killham 1995). The close interactions between plants and rhizobacteria encourage the establishment of specific and beneficial rhizosphere, and such associations between different crop species could be cultivar-specific. Thus, certain cultivars of clover can foster the development of rhizo- and endophytic bacteria that favor the growth and development of specific cultivars of potatoes (Sturz and Christie 1998). An additional role of rhizosphere microbes in reducing root disease incidence is in interfering with chemotactic attraction of the pathogen to root receptor sites. Scher et al. (1985) suggested that chemotaxis might be the first step in root colonization. A variety of compounds as components of root exudates may serve as attractants for plant pathogens. Growth of root inhabitants (including mycorrhizal fungi) necessarily reduced both the quantity and diversity of organic compounds diffusing from the root, thereby, diminishing the probability of encounter by a plant pathogen (Davis et al. 1979).

Antibiotic Production

Antibiotic production by rhizobacteria is one of the major mechanisms postulated for antifungal activity to suppress pathogens in the rhizosphere and to promote plant growth. The role of antibiotics in disease suppression has been demonstrated in many biocontrol systems by mutant analyses and biochemical studies using purified antibiotics (Stockwell and Stack 2007). These antimicrobial compounds may act on plant pathogenic fungi by inducing fungistasis, inhibition of spore germination, lysis of fungal mycelia or by exerting fungicidal effects. A large number of antibiotics including phenazine carboxylic acid, diacetyl phloroglucinol, oomycin A, pyocyanine, pyrroles, pyoluteorin, pyrrolnitrin, iturin A, surfactin, etc. are produced by rhizobacteria (Bender et al. 1999; Sindhu et al. 2009b), which help in the suppression of pathogen growth. The first antibiotic clearly implicated in biocontrol by fluorescent pseudomonads was the phenazine derivative that contributed to disease suppression by *Pseudomonas fluorescens* strain 2-79 and *P. chlororaphis* strain 30–84 (formerly *P. aureofaciens*), which were suppressive to the take-all disease of wheat roots caused by *Gaeumannomyces graminis* var. *tritici* (Gurusiddaiah et al. 1986). The antibiotic was found active against several fungi including *G. graminis* var. *tritici*, *R. solani* and *P. aristesporum. Pseudomonas fluorescens* strain CHAO was found to produce a variety of secondary metabolites, i.e., 2,4-diacetyl phloroglucinol, pyoluteorin, hydrogen cyanide, salicylic acid,

pyochelin and pyoverdine, and protected various plants from diseases caused by soil borne pathogenic fungi (Stutz et al. 1986). Anjaiah et al. (2003) found that an isolate of *P. aeruginosa* PNA1, obtained from chickpea rhizosphere, protected the plants from *Fusarium* wilt until maturity in moderately tolerant genotypes of pigeonpea and chickpea. Root colonization of pigeonpea and chickpea, which was measured using a *lac*Z-marked strain of PNA1, showed ten-fold lower root colonization of susceptible genotypes than that of moderately tolerant genotypes, indicating that this plant-bacteria interaction could be important for disease suppression in this plant. Its Tn5 mutants (FM29 and FM13), which were deficient in phenazine production, caused a reduction or loss of wilt disease suppression in vivo. Similarly, *B. cereus* strain UW85 suppressed the diseases caused by the oomycetes. Analysis of *B. cereus* mutants showed a significant quantitative relationship between disease suppressiveness and the production of two antibiotics, zwittermicin A and kanosamine (Silo-Suh et al. 1994; Milner et al. 1996). The purified antibiotics suppressed the disease and inhibited the development of oomycetes by stunting and deforming germ tubes of germinating cysts. *Bacillus subtilis* RB14, which produced antibiotics iturin A and surfactin, was found to suppress damping off disease caused by *Rhizoctonia solani* (Asaka and Shoda 1996).

Production of Siderophores

Iron is an essential element for all living organisms and most of it is found in the insoluble form at neutral pH. To cope up with low solubility of iron, many microorganisms synthesize extracellular Fe^{3+} chelators i.e., siderophores, in response to low iron stress (Neilands and Nakamura 1991) that transport iron into bacterial cells. Plant growth promoting rhizobacteria (PGPR) produced different types of siderophores, which were involved in disease suppression and plant growth promotion (Leong 1986). The various categories of siderophores produced by PGPR include catechol, hydroxamate, pyoverdine, pyochelin, cepabactin, schizokinen and some other types like azotochelin, rhizobactin, anthranilic acid and azotobactin.

Kloepper et al. (1980) were the first to demonstrate the importance of siderophores production in biocontrol of plant pathogens with pseudobactin, a siderophore produced by *Pseudomonas* strain B10. The addition of 1.0 μM ferric chloride to an iron-deficient medium abolished the antagonism under in vitro conditions and the fluorescence by the PGPR were not observed. Studies with various siderophore-negative Tn5 mutants showed that pseudobactin of either pyoverdine and pyochelin type was necessary to achieve wild-type levels of protection against *Pythium*-induced damping off disease (Buysens et al. 1996). Goel et al. (2000) isolated pigment overproducer mutant MRS16M-1 from *Pseudomonas* strain MRS16, that was more inhibitory to the fungal pathogens, whereas, non-producer mutant MRS16M-5 was less inhibitory on nutrient agar medium. Addition of 100 μM ferric chloride to the medium decreased inhibition of fungal growth, suggesting the involvement of siderophores and other antifungal

secondary metabolites. Dileep Kumar et al. (2001) found that both the fluorescent pseudomonads and *Rhizobium* strains exhibited a wide range of antifungal activity against pathogens specific to pea. In a synthetic culture medium, all the plant growth promoting fluorescent pseudomonad strains produced siderophores, which expressed antifungal and antibacterial activity. Seed bacterization with plant growth-promoting strains, alone and together with a *Rhizobium leguminosarum* biovar *viceae* isolate, 361–27 reduced the number of infected peas grown in *Fusarium oxysporum* infested soils. Seed bacterization with siderophore-producing *P. fluorescens* isolates, *viz.* PGPR1, PGPR2 and PGPR4, suppressed the soil-borne fungal diseases like collar rot of peanut caused by *A. niger* and isolate PGPR4 also suppressed stem rot caused by *S. rolfsii* (Dey et al. 2004).

Production of Hydrolytic Enzymes

Some cell wall lysing enzymes produced by rhizobacteria have been found to cause the destruction of pathogens. For example, Chet et al. (1990) cloned the gene encoding chitinase enzyme from *S. marcescens* and transferred it into *E. coli*. The partially purified chitinase caused extensive bursting of the hyphal tips. This chitinase preparation was effective in reducing disease incidence caused by *R. solani* under greenhouse conditions. In other study, Chet et al. (1993) isolated three different chitinase genes from *Serratia*, *Aeromonas*, and *Trichoderma*. The cloned genes were expressed in *E. coli* and subsequently introduced into *R. meliloti*, *P. putida*, and *Trichoderma* strains resulting in increased chitinolytic activity of transformants against *Sclerotium rolfsii* and *R. solani*. Recombinant strains of *R. meliloti* were constructed which carried *chi*A genes to produce chitinase. The recombinant strain expressed chitinase during symbiosis in alfalfa roots (Sitrit et al. 1993). Khot et al. (1996) reported that certain isolates of *Pseudomonas* and *Bacillus* produced chitinase, β-1,3 glucanase (laminarinase) and siderophores. Seed inoculation of these bacteria or application of cell free extract on seed resulted in 48.6 and 31.6% reduction of the wilt incidence of chickpea under field conditions in a wilt sick nursery. *Pseudomonas* strains isolated from the rhizosphere of chickpea and green gram were also found to produce chitinases and cellulases in culture-free supernatants and inhibited growth of *P. aphanidermatum* and *R. solani* on potato dextrose agar medium plates (Sindhu and Dadarwal 2001).

Production of Secondary Metabolites

Among other metabolites, hydrogen cyanide (HCN) is produced by many rhizosphere bacteria and has been demonstrated to play a role in biological control of pathogens (Voisard et al. 1989). HCN over-producing bacterial strains resulted in small but statistically significant increase in the suppression of symptoms caused by *Mycophaerella graminicola* and *Puccinia recondita* f. sp. *tritici* on wheat seedling

leaves. *Pseudomonas aeruginosa* strain zag2 was reported to produce pyocyanin, siderophore and hydrogen cyanide (Hassanein et al. 2009). The minimum inhibitory concentration of the extracted pigmented compound against *Candida albicans* was 40.69 μg ml^{-1} and the antifungal activity of the compound was remarkable at 100°C for 20 min. The toxic volatile compound HCN produced by the bacteria was found to reduce the growth of both *F. oxysporum* and *Helminthosporium* sp. whereas *A. niger* was not affected.

The fungal pathogens also cause the plant to synthesize stress ethylene (van Loon et al. 2006) and much of the damage sustained by plants infected by phytopathogens occurs as a result of the response of the plant to the increased levels of ethylene (van Loon 1984). It is well known that exogenous ethylene often increases the severity of fungal infection, while some ethylene synthesis inhibitors significantly decrease the severity of a fungal infection (Elad 1990; Robinson et al. 2001). A number of PGPR, which stimulated root growth of different plant species were found to contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolysed the ethylene precursor ACC to ammonia and α-ketobutyrate, and as a result, decreased ethylene biosynthesis by plants (Glick et al. 1994; Hall et al. 1996; Belimov et al. 2001). The ACC deaminase-containing biocontrol bacterial strains were also found more effective than biocontrol strains that did not possess this enzyme (Wang et al. 2000).

Induction of Systemic Resistance

Some biocontrol agents induced a sustained change in the plant and increased its tolerance to infection against fungal and bacterial pathogens (Maurhofer et al. 1998), a phenomenon known as induced systemic resistance (ISR). Various non-pathogenic rhizobacteria have the ability to induce a state of systemic resistance in plants, which provided protection against a broad spectrum of phytopathogenic organisms including fungi, bacteria and viruses (Bakker et al. 2007). Induced resistance brought about by prior inoculation of the host by a pathogen, avirulent or incompatible forms of a pathogen, or heat killed pathogens has been attributed to induce physiological response of the host plant against subsequent inoculation by the virulent pathogens (Hoffland et al. 1996). Induced systemic resistance in plants has been demonstrated in over 25 crops, including legumes, cereal crops, cucurbits, solanaceous plants and trees against a wide spectrum of pathogens. The mechanism of ISR has also been studied in plant growth-promoting *Bacillus* spp. (Kloepper et al. 2004). Bacterial production of the volatile 2,3-butanediol triggered the expression of *Bacillus*-mediated ISR in *Arabidopsis*. The signaling pathway that is activated in this case depended on ethylene but was independent of salicylic acid and jasmonic acid signaling (Ryu et al. 2004). Whether or not biocontrol agents suppress disease by inducing resistance, it is essential that ISR and biocontrol strategies be compatible because future agricultural practices are likely to require the integration of multiple pest control strategies.

9.2.5 Selection of Rhizobacteria with Multiple Plant Growth-Promoting Traits

The use of beneficial soil microorganisms as agricultural inputs for improved crop production requires selection of rhizosphere-competent microorganisms with plant growth-promoting attributes. The selection of PGPR strains depends largely on their growth promoting activities, such as, production of IAA and siderophores, P solubilization and inhibition of pathogenic microorganisms. However, the presence of one or all of these traits does not qualify them to be a PGPR for one particular crop or spectrum of crops. For example, Cattelan et al. (1999) reported one rhizobacterial isolate which did not share any of the PGPR traits tested in vitro except antagonism to *Sclerotium rolfsii* and *Sclerotinia sclerotium*, but it promoted soybean growth. This indicates that besides such growth promoting traits, there are unexplained mechanisms, which also influence the growth of plants and requires the close proximity of PGPR to the roots of plants. Hence, there is no clear separation of growth promotion in plants and biological control induced by bacterial inoculants (Lugtenberg et al. 1991; Bloemberg and Lugtenberg 2001). Bacterial strains selected initially for in vitro antibiosis as part of evaluating biological control activity frequently demonstrated growth promotion in the absence of target pathogen (Sindhu et al. 1999; Goel et al. 2002). Similarly, PGPR selected initially for growth promotion in the absence of pathogens, may demonstrate biological control activity when challenged with the pathogens, presumably by controlling deleterious microorganisms or non-target pathogens.

Direct growth promotion occurs when a rhizobacterium produces metabolites that directly promote plant growth without interactions with native microflora (Kloepper et al. 1991). Dileep Kumar and Dube (1992) reported that fluorescent siderophore-producing *Pseudomonas* strain RBT13, originally isolated from the tomato roots, enhanced seed germination of chickpea and soybean, and resulted in increased root and shoot weight as well as yield of the crops. Sindhu et al. (2002b) reported plant growth promoting effects of fluorescent *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions in chickpea. The coinoculation resulted in enhanced nodulation by *Mesorhizobium* sp. and increased shoot dry weight by 3.92–4.20 times in comparison to uninoculated controls. Under indirect growth promotion mechanism, the production of antibiotics, siderophores and HCN by microorganisms decreased the population and activities of pathogens or deleterious microorganisms and thereby, increased the plant growth (Pierson and Weller 1994).

Nine different isolates of PGPR (*Pseudomonas* sp.) were selected from a pool of 233 rhizobacterial isolates obtained from the peanut rhizosphere based on ACC-deaminase activity (Dey et al. 2004). Four of these isolates, *viz*. PGPR1, PGPR2, PGPR4 and PGPR7 produced siderophore and IAA. In addition, *P. fluorescens* PGPR1 also possessed the properties like, P-solubilization, ammonification and inhibited *A. niger* and *A. flavus* under in vitro conditions. In addition to the traits exhibited by PGPR1, the strain PGPR4 showed strong in vitro inhibition to

S. rolfsii. In field trials on peanut, plant growth-promoting fluorescent pseudomonad isolates, *viz*. PGPR1, PGPR2 and PGPR4, significantly enhanced the pod yield (23–26, 24–28, and 18–24%, respectively), haulm yield and nodule dry weight over the control in 3 years. Inoculation with plant growth-promoting *P. fluorescens* isolates, *viz*. PGPR1, PGPR2 and PGPR4, was found to suppress the soil-borne fungal diseases like collar rot of peanut caused by *A. niger* and isolate PGPR4 also suppressed stem rot caused by *S. rolfsii*. Hynes et al. (2008) screened 563 bacteria originating from the roots of pea, lentil and chickpea for the suppression of legume fungal pathogens and for plant growth promotion. Screening of bacteria showed that 76% isolates produced siderophore, 5% isolates showed ACC deaminase activity and 7% isolates were capable of indole production. Twenty-six isolates (5%) suppressed the growth of *Pythium* species strain p88–p3, 7% suppressed the growth of *Fusarium avenaceum* and 9% suppressed the growth of *R. solani* CKP7. Four isolates promoted the growth of lentil and one isolate promoted the growth of pea. Fatty acid profile analysis and 16S rRNA sequencing of the isolates showed that 39–42% were the members of Pseudomonadaceae and 36–42% of the Enterobacteriaceae families.

In search of efficient PGPR strains, 72 bacterial isolates were obtained from different rhizospheric soil and plant root nodules (Ahmad et al. 2008). Of these, more than 80% of the isolates belonging to genera *Azotobacter*, *Pseudomonas,* and *Mesorhizobium* produced IAA, whereas, only 20% of the *Bacillus* was IAA producer. Solubilization of P was commonly detected in the isolates of *Bacillus* (80%) followed by *Azotobacter* (74%), *Pseudomonas* (56%) and *Mesorhizobium* (17%). All tested isolates produced ammonia. Siderophore production and antifungal activity of these isolates except *Mesorhizobium* were exhibited by 10–13% isolates. HCN production was more common trait of *Pseudomonas* (89%) and *Bacillus* (50%). *Pseudomonas* Ps5 and *Bacillus* B1 isolates showed broad-spectrum antifungal activity against *Aspergillus*, *Fusarium* and *Rhizoctonia bataticola*.

9.3 Biotic and Abiotic Factors Affecting Rhizosphere Colonization

It is well established that root colonization by biocontrol agent and beneficial microorganisms is a prerequisite to suppress the plant disease and to enhance plant growth. Root colonization by introduced bacteria could be improved by increasing the population size, distribution or survival of bacteria, along with manipulation of soil factors that may positively or negatively affect colonization. Bacterial traits such as growth rate, cell surface properties, motility (Boelens et al. 1994), chemotaxis to root exudates, production of secondary metabolites and tolerance to stressed environment (e.g., dehydration and temperature) also contributes to rhizosphere competence. Plant characteristics, like root structure, age and plant genotype as well as physico-chemical properties of soil, application of pesticides etc. were

found to affect rhizosphere colonization by the beneficial rhizobacteria. Use of green fluorescent protein (*gfp*) and in situ monitoring based on confocal laser scanning microscope (CLSM) could be used to understand the rhizosphere competence and root colonization (Johri et al. 2003). Using this technique, it was found that the *Pseudomonas* (biocontrol strains) colonized the seed and root surface at the same position, as did the pathogenic fungi that they controlled (Bloemberg et al. 2000). Another promising option considered important for understanding colonization is to screen mutants directly. Mutants of *Pseudomonas* strains of both phenotypes have been identified and analysis of these mutants indicated that prototrophy for amino acids and vitamins, rapid growth rate, utilization of organic acids and lipopolysaccharide properties contributed to colonization ability. Modification of genes involved in the biocontrol activity of biological control agents also played a key role in improving the potential rhizosphere competence as well as antifungal activity of biological control agents (Carroll et al. 1995). Moreover, biocontrol activity of *P. fluorescens* carrying PCA coding mini-Tn5 vector was enhanced by introducing *phz*H gene from *Pseudomonas chlororaphis* PCL1391 (Timmis-Wilson et al. 2000).

9.4 Development of Bacterial Inoculants and Constraints in Their Use

Rhizobium and *Bradyrhizobium* inoculants have been marketed with success for over a century. Releases of these nodule-forming microorganisms into soils have been successful. Inoculation with such inoculants has resulted in their establishment into soil and onto plant roots to a level sufficiently higher for the intended purpose. However, the desired impact of biofertilizer application under field conditions has been variable and inoculation of legume plants with commercial inoculant strains often fails to improve crop productivity (van Elsas and Heijnen 1990; Akkermans 1994). The problem is of the survival of inoculant diazotrophic bacteria under field conditions. For each introduction, abiotic soil factors such as texture, pH, temperature, moisture content and substrate availability need critical assessment since these factors largely determine the survival and activity of the introduced microorganisms (van Veen et al. 1997). In addition, the response of the inoculant to the prevailing soil conditions also depends on its genetic and physiological constitution (Brockwell et al. 1995). The use of genetic markers like resistance to antibiotics or introduction of metabolic markers from other bacterial species could help in tracing the introduced strains, whether it is rhizobia, cyanobacteria, azotobacters or azospirilla (Wilson et al. 1995). Another important reason for the inconsistency observed due to inoculation of PGPR could be the coating of seeds by low number of rhizobial cells. Higher or lower dosage of PGPR may have a detrimental effect on nodulation and growth of plant as demonstrated by Plazinski and Rolfe (1985). On the commercial front, approximately 20 bacterial biocontrol products based on *Pseudomonas*, *Bacillus*, *Streptomyces* and *Agrobacterium* strains have been

marketed. The discovery of many traits and genes involved in the beneficial effects of PGPRs has resulted in a better understanding of the performance of bioinoculants in the field.

Some of these strains may provide effective control of diseases in certain soils, in certain geographic regions or on particular crops**.** Generally, microorganisms isolated from the rhizosphere of a specific crop are better adapted to that crop and may provide better control of disease than organisms originally isolated from other species (Cook 1993). Despite the extensive research where biological agents have been used to control plant diseases, there have been limited commercial success. Many biological agents do not perform better in the field due to the complexity and variability of physical, chemical, microbiological and environmental factors in the field. Therefore, applications of a mixture of biocontrol agents may be a more ecologically sound approach because it may result in better colonization and enhance the level and consistency of disease control by providing multiple mechanisms of action, a more stable rhizosphere community and effectiveness over a wide range of environmental conditions occurring throughout the growing season. In addition, the genetic diversity of these strains may be tapped by combining them in mixed inoculants. Certain mixtures of fluorescent pseudomonads suppressed disease more effectively than did single-strain inoculants (Pierson and Weller 1994; Duffy et al. 1996).

Spadro and Cullino (2005) concluded that the use of genetically modified microorganisms could play an important role in crop production and protection. Genetic manipulation could result in new biocontrol strains with increased production of toxic compounds or lytic enzymes, improved space or nutrient competence, wider host range or enhanced tolerance to abiotic stress (Glick and Bashan 1997). Thus, biocontrol performance of soil pseudomonads may be improved by the introduction of antibiotic biosynthetic genes (Maurhofer et al. 1992; Haas and Keel 2003). Recombinant DNA strains with greatly increased diacetyl phloroglucinol (DAPG) and phenazine-1-carboxamide (PCN) antibiotics production have been constructed (Mavrodi et al. 1998, 2001). The production of DAPG and PCN could be placed under the control of strong promoters or of exudate-induced or rhizosphere-induced promoters (Mavrodi et al. 2006). Genes and enzymes involved in the biocontrol mechanism could also be applied directly or transferred to crops.

From the perspective of developing nations, these are exciting strategies that may help to increase yield while reducing the inputs and environmental problems. However, most of the microbial biodiversity in soil remains unexplored and much work remains to be done to first identify and then characterize microorganisms that could be used in such applications. Furthermore, such approaches require a detailed knowledge of the molecular signaling that takes place between plants and microbes to drive expression of desirable traits and to suppress unwanted effects in a controlled manner. Future strategies are required to clone genes involved in the production of antibiotics, siderophores and other metabolites, and to transfer these cloned genes into the rhizobacterial strains having good colonization potential along with other beneficial characteristics such as N_2 fixation, P-solubilization and/or hormone production. Exploiting plants and microbes by using such an

integrated approach requires a coordinated strategy to understand the degree and complexity of plant-microbe interactions employing modern "genomics/proteomics" technologies. The generation of complete genomic sequences for plant-associated bacteria, including pathogens and symbionts is already increasing our knowledge of these organisms. The increasing amount of genomic data available for the model plant species and their associated microorganisms, will assist in determining the most suitable beneficial bacterial strains for inoculation. In the near future, the molecular tools adopted in manipulation of bacterial traits are likely to improve the availability of nutrients, efficiency of phytoremediation and enhancement of biocontrol activity that will consequently improve the crop productivity and also protect the food chain by reducing levels of agrochemicals in food crops.

9.5 Conclusion

Although striking advances have been made in understanding the molecular and biochemical mechanism regulating N_2 fixation, P solubilization and hormone production, this has yet to be translated into applied environments. To overcome the problem of establishment of inoculated microbes, the beneficial bacteria intended for inoculation should be selected from local ecological niches and reinoculated into the same environment to ensure the desired benefits. The effects of soil and environmental factors on the physiology and ecology of introduced microorganisms are still poorly understood. Research is therefore, needed to understand the in situ physiology of inoculant cells and strategies must be developed as to how such microbes could be manipulated for desired performance. For example, the use of reporter genes inserted either randomly or directly into the bacterial genome may allow the specific detection and possible enhancement of in situ gene expression in inoculant cells.

The complex interactions among the PGPR, the pathogen, the plant and the environment are responsible for the variability observed in disease suppression and plant growth promotion. However, genetic manipulation of PGPR has the potential to construct significantly better strains with improved biocontrol efficacy (Trevors et al. 1990; Chet et al. 1993). Further, the efficacy of biocontrol bacteria can be improved by developing better cultural practices and delivery systems that favor their establishment in the rhizosphere. From the application point of view, consortia of ecologically diverse strains for N_2 fixation, P-solubilization, root growth promotion among others, should be practiced instead of single strain. In near future, both traditional and biotechnological approaches could be employed to increase rates of N_2 fixation, P solubilization, hormone production and increase in efficiency of biocontrol activity along with bioremediation of contaminants, leading to increase in crop yield under sustainable agricultural crop production system.

References

Abd-Alla MH (1994) Use of organic phosphorus by *Rhizobium leguminosarum* biovar *viceae* phosphatases. Biol Fertil Soils 18:216–218

Agasimani CA, Mudalagiriyappa MV, Sreenivasa MH (1994) Response of groundnut to phosphate solubilizing microorganisms. Groundnut News 6:5–7

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Akkermans ADL (1994) Application of bacteria in soils: problems and pitfalls. FEMS Microbiol Rev 15:185–194

Almeida JPF, Hartwig UA, Frehner M, Nosberger J, Luscher A (2000) Evidence that P deficiency induced N feedback regulation of symbiotic N_2 fixation in white clover (*Trifolium repens* L.). J Exp Bot 51:1289–1297

Al-Niemi TS, Kahn ML, McDermott TR (1998) Phosphorus uptake by bean nodules. Plant Soil 198:71–78

Andrade G, De Leij FAAM, Lynch JM (1998) Plant mediated interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. Lett Appl Microbiol 26:311–316

Anjaiah V, Cornelis P, Koedam N (2003) Effect of genotype and root colonization in biological control of fusarium wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. Can J Microbiol 49:85–91

Araujo AP, Plassard C, Drevon JJ (2008) Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. Plant Soil 312:129–138

Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentieve AI, Kudoyarova GR (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315

Arshad M, Frankenberger WT Jr (1991) Microbial production of plant hormones. Plant Soil 133:1–8

Asaka O, Shoda M (1996) Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. Appl Environ Microbiol 62:4081–4085

Bagyaraj DJ, Krishnaraj PU, Khanuja SPS (2000) Mineral phosphate solubilization: agronomic implications, mechanism and molecular genetics. Proc Indian Natn Sci Acad (PINSA) B66:69–82

Bakker PAHM, Pieterse CMJ, van Loon LC (2007) Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97:239–243

Balabel-Naglaa MA (1997) Silicate bacteria as biofertilizers. MSc Thesis, Agriculture Microbiology Department, Faculty of Agriculture, Ain Shams University, Egypt

Bandenoch-Jones J, Summons RR, Djordjevic MA, Shine J, Letham DS, Rolfe BG (1982) Mass spectrometric quantification of indole-3-acetic acid in *Rhizobium* culture supernatants: relation to root hair curling and nodule initiation. Appl Environ Microbiol 44:275–280

Banik S, Dey BK (1983) Phosphate solubilizing potentiality of microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. Zentralbl Microbiol 138:17–23

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Barker WW, Welch SA, Chu S, Banfield JF (1998) Experimental observations of the effects of bacteria on aluminosilicate weathering. Am Mineral 83:1551–1563

Bar-Ness E, Chen Y, Hadar Y, Marchner H, Romheld V (1991) Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant Soil 130:231–241

Bar-Ness EB, Hadar Y, Chen Y, Shanzer A, Libman J (1992) Iron uptake by plants from microbial siderophores. Plant Physiol 99:1329–1335

Belimov AA, Safronova VI, Sergeyeva TA, Egorova TN, Matveyeva VA, Tsyganov VE, Borisov AY, Tikhonovich IA, Kluge C, Preisfeld A, Deitz KJ, Stepanok VV (2001) Characterization of

plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 47:642–652
Bender CL, Rangaswamy V, Loper J (1999) Polyketide production by plant-associated pseudomonads. Annu Rev Phytopathol 37:175–196
Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci Technol 11:557–574
Bennett PC, Choi WJ, Rogera JR (1998) Microbial destruction of feldspars. Min Manag 8: 149–150
Bieleski RL (1973) Phosphate pools, phosphate transport and phosphate availability. Annu Rev Plant Physiol 24:225–252
Bingham FT, Pereyea FJ, Jarrell WM (1986) Metal toxicity to agricultural crops. Met Ions Biol Syst 20:119–156
Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Cur Opin Plant Biol 4:343–350
Bloemberg GV, Wijijes AHM, Lamers GEM, Sluurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different auto fluorescent proteins in the rhizosphere; new perspectives for studying microbial communities. Mol Plant-Microbe Inter 13:1170–1176
Boelens J, Woestyne MV, Verstraete W (1994) Ecological importance of motility for the plant growth-promoting *Rhizopseudomonas* strain ANP15. Soil Biol Biochem 26:269–277
Bohlool BB, Ladha JK, Garrity DP, George T (1992) Biological nitrogen fixation for sustainable agriculture: a perspective. Plant Soil 141:1–11
Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V (2007) Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl Microbiol Biotechnol 74:874–880
Bollard EG (1983) Involvement of unusual elements in plant growth and nutrition. In: Lauchli A, Bielsky RL (eds) Inorganic plant nutrition: encyclopedia of plant physiology, vol 15B, Springer-Verlag KG. Berlin, Germany, pp 695–744
Bolton H Jr, Elliott LF, Turco RF, Kennedy AC (1990) Rhizoplane colonization of pea seedlings by *Rhizobium leguminosarum* and deleterious root colonizing *Pseudomonas* sp. and effects on plant growth. Plant Soil 123:121–124
Brewin NJ (2002) Pods and nods: a new look at symbiotic nitrogen fixing. Biologist 49:1–5
Brockwell J, Bottomley PJ, Thies JE (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143–180
Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245
Burdman S, Kigel J, Okon Y (1997) Effects of *Azospirillum brasilense* on nodulation and growth of common bean (*Phaseolus vulgaris* L.). Soil Biol Biochem 29:923–929
Burns TA Jr, Bishop PE, Israel DW (1981) Enhanced nodulation of leguminous plant roots by mixed cultures of *Azotobacter vinelandii* and *Rhizobium*. Plant Soil 62:399–412
Burris RH, Roberts GP (1993) Biological nitrogen fixation. Annu Rev Nutr 13:317–335
Buysens S, Heungens K, Poppe J, Hofte M (1996) Involvement of pyochelin and pyoverdine in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl Environ Microbiol 62:865–871
Camerini S, Senatore B, Lonardo E, Imperlini E, Bianco C, Moschetti G, Rotin GL, Campion B, Defez R (2008) Introduction of a novel pathway for IAA biosynthesis to rhizobia alters vetch root nodule development. Arch Microbiol 190:67–77
Carroll H, Moenne-Loccoz Y, Dowling DN, O'Gara F (1995) Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2, 4-diacetyl phloroglucinol does not influence the ecological fitness of Pseudomonas fluorescens F113 in the rhizosphere of sugar beets. Appl Environ Microbiol 61:3002–3007
Cattelan AJ, Hartel PG, Furhmann JJ (1999) Screening of plant growth promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Cattelan AJ, Hartel PG, Fuhrmann JJ (1998) Bacterial composition in the rhizosphere of nodulating and non-nodulating soybean. Soil Sci Soc Am J 62:1549–1555

Chander K, Brookes PC (1991) Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulaiton in a sandy loam soil and silty loam UK soil. Soil Biol Biochem 23:927–932

Chandra SN (1997) Selection of chickpea-rhizosphere-competent *Pseudomonas fluorescens* NBRI1303 antagonistic to *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Pythium* sp. Curr Microbiol 35:52–58

Chanway CP, Hynes RK, Nelson LM (1989) Plant growth promoting rhizobacteria: effect on the growth and nitrogen fixation of lentils (*Lens esculenta* Moench) and pea (*Pisum sativum* L.). Soil Biol Biochem 21:511–512

Chaudri AM, McGrath SP, Giller KE (1992) Survival of the indigenous population of *Rhizobium leguminosarum* biovar *trifolii* in soil spiked with Cd, Zn, Cu and Ni salts. Soil Biol Biochem 24:625–632

Chen WX, Wang ET, Wang SY, Li YB, Chen XQ, Li Y (1995) Characteristics of *Rhizobium tianshanense* sp. nov., a moderately and slowly growing root nodule bacterium isolated from an arid saline environment in Xinjiang. Int J Syst Bacteriol 45:153–159

Chen YP, Rekha PD, Arun AB, Shen FD, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chet I, Borak Z, Oppenheim A (1993) Genetic engineering of microorganisms for improved biocontrol activity. Biotechnology 27:211–235

Chet I, Ordentlich A, Shapira R, Oppenheim A (1990) Mechanism of biocontrol of soil borne plant pathogens by rhizobacteria. Plant Soil 129:85–92

Christiansen I, Graham PH (2002) Variation in dinitrogen fixation among Andean bean (*Phaseolus vulgaris* L.) genotypes grown at low and high levels of phosphorus supply. Field Crops Res 73:133–142

Cocking EC, Webster G, Batchelor CA, Davey MR (1994) Nodulation of non-legume crops: a new look. Agro-Industry Hi-Tech. pp 21–24.

Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31:53–80

Crowley DE, Kraemer SM (2007) Function of siderophores in the plant rhizosphere. In: Pinton R et al (eds) The rhizosphere, biochemistry and organic substances at the soil-plant interface. CRC Press, FL, pp 73–109

Crowley DE, Wang YC, Reid CPP, Szansiszlo PJ (1991) Mechanism of iron acquisition from siderophores by microorganisms and plants. Plant Soil 130:179–198

Dashti N, Zhang F, Hynes RK, Smith DL (1998) Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycine max* (L.) Merr.] under short season conditions. Plant Soil 200:205–213

Davis RM, Menge JA, Erwin DE (1979) Influence of *Glomus fasciculatum* and soil phosphorus on Verticillium wilt of cotton. Phytopathology 69:453–456

DeBoer DL, Duke SH (1982) Effects of sulphur nutrition on nitrogen and carbon metabolism in lucerne (*Medicago sativa* L.). Physiol Plant 54:343–350

Derylo M, Skorupska A (1993) Enhancement of symbiotic nitrogen fixation by vitamin-secreting fluorescent *Pseudomonas*. Plant Soil 54:211–217

Deubel A, Merbach W (2005) Influence of microorganisms on phosphorus bioavailability in soils. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer-Verlag, Berlin, Heidelberg, pp 177–191

Devine TE, Kuykendall LD (1996) Host genetic control of symbiosis in soybean (*Glycine max* L.). Plant Soil 186:173–187

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394

Dileep Kumar BS, Berggren I, Maartensson AM (2001) Potential for improving pea production by coinoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant Soil 229:25–34

Dileep Kumar BS, Dube HC (1992) Seed bacterization with a fluorescent *Pseudomonas* for enhanced plant growth, yield and disease control. Soil Biol Biochem 24:539–542

Dixon R, Cheng Q, Shen GF, Day A, Day MD (1997) *Nif* genes and expression in chloroplasts: prospects and problems. Plant Soil 194:193–203

Dubey SK (1997) Coinoculation of phosphate solubilizing bacteria with *Bradyrhizobium japonicum* to increase phosphate availability to rainfed soybean on vertisol. J Indian Soc Soil Sci 45:506–509

Duffy BK, Simon A, Weller DM (1996) Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all disease on wheat. Phytopathology 75:774–777

Dulhart J (1967) Quantitative estimation of indole acetic acid and indole carboxylic acid in root nodules and roots of *Lupinus luteus* L. Acta Bot N 16:222–230

Dulhart J (1970) The bioproduction of indole-3-acetic acid and related compounds in root nodules and roots of *Lupinus luteus* L. by its rhizobial symbiont. Acta Bot N 19:573–615

Elad Y (1990) Production of ethylene in tissues of tomato, pepper, French bean and cucumber in response to infection by *Botrytis cinerea*. Physiol Mol Plant Pathol 36:277–287

Elad Y, Chet I (1987) Possible role of competition for nutrients in biocontrol of *Pythium* damping off by bacteria. Phytopathology 77:190–195

Elkan GH (1992) Biological nitrogen fixation systems in tropical ecosystems: an overview. In: Mulongoy K, Gueye M, Spencer DSC (eds) Biological nitrogen fixation and sustainability of tropical agriculture. John Wiley, Chichester, UK, pp 27–40

Ellis RJ, Timms-Wilson TM, Bailey MJ (2000) Identification of conserved traits in fluorescent pseudomonads with antifungal activity. Environ Microbiol 2:274–284

Elsiddig AE, Elsheikh, Ibrahim KA (1999) The effect of *Bradyrhizobium* inoculation on yield and seed quality of guar (*Cyamopsis tetragonoloba* L.). Food Chemistry 65:183–187

Evans HJ, Harker AR, Papen H, Russell SA, Hanus FJ, Zuber M (1987) Physiology, biochemistry and genetics of the uptake hydrogenase in rhizobia. Annu Rev Microbiol 41:355–361

Fernandez LA, Zalba P, Gomez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43:805–809

Fismes J, Vong PC, Guckert A, Frossard E (2000) Influence of sulfur on apparent N-use efficiency, yield and quantity of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. Eur J Agron 12:127–141

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

Fuhrmann J, Wollum AG II (1989) Nodulation competition among *Bradyrhizobium japonicum* strains as influenced by rhizosphere bacteria and iron availability. Biol Fertil Soils 7:108–112

Fujiata K, Ofosu-Budu KG, Ogata S (1992) Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant Soil 141:155–175

Fukuhara H, Minakawa Y, Akao S, Minamisawa K (1994) The involvement of indole-3-acetic acid produced by *Bradyrhizobium elkanii* in nodule formation. Plant Cell Physiol 35:1261–1265

Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 68:280–300

Gaind S, Gaur AC (1991) Thermotolerant phosphate solubilizing microorganisms and their interaction with mungbean. Plant Soil 133:141–149

Garbisu C, Hernandez-Allica J, Barrutia O, Alkorta I, Becerril JM (2002) Phytoremediation: a technology using green plants to remove contaminants from polluted areas. Rev Environ Health 17:173–188

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte enhanced phytoremediation of the organochlorine herbicide 2, 4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310

Gharmakher HN, Machet JM, Beaudoin N, Recous S (2008) Estimation of sulfur mineralization and relationships with nitrogen and carbon in soils. Biol Fertil Soils 45:297–304

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnol Adv 15:353–378

Glick BR, Jacobson CB, Schwarze MMK, Pasternak JJ (1994) 1-aminocyclopropane-1-carboxylic acid deaminase mutants of the plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2 do not stimulate canola root elongation. Can J Microbiol 40:911–915

Goel AK, Sindhu SS, Dadarwal KR (1999) Bacteriocin producing native rhizobia of green gram (*Vigna radiata*) having competitive advantage in nodule occupancy. Microbiol Res 154:43–48

Goel AK, Sindhu SS, Dadarwal KR (2000) Pigment diverse mutants of *Pseudomonas* sp.: inhibition of fungal growth and stimulation of growth of *Cicer arietinum*. Biol Plant 43:563–569

Goel AK, Sindhu SS, Dadarwal KR (2001) Application of plant growth-promoting rhizobacteria as inoculants of cereals and legumes. In: Yadav AK, Raychaudhary S, Motsara MR (eds) Recent advances in biofertilizer technology. Society for promotion and utilization of resources and technology, New Delhi, pp 207–256

Goel AK, Sindhu SS, Dadarwal KR (2002) Stimulation of nodulation and plant growth of chickpea (*Cicer arietinum* L.) by *Pseudomonas* spp. antagonistic to fungal pathogens. Biol Fertil Soils 36:391–396

Goldstein AH (1986) Bacterial solubilization of microbial phosphates: historical perspective and future prospects. Am J Altern Agric 1:51–57

Goldstein AH (2007) Future trends in research on microbial phosphate solubilization: one hundred years of insolubility. In: Velazquez E, Roderguez-Barrueco C (eds) Proceedings of first international meeting on microbial phosphate solubilization. Springer-Verlag, Berlin, pp 91–96

Goldstein AH, Rogers RD, Mead G (1993) Separating phosphate from ores via bioprocessing. Biotechnology 11:1250–1254

Grimes HD, Mount MS (1984) Influence of *Pseudomonas putida* on nodulation of *Phaseolus vulgaris*. Soil Biol Biochem 16:27–30

Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ (1986) Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. Antimicrob Agents Chemother 29:488–495

Gutierrez-Manero FJ, Ramos-Salono B, Probanza A, Mehouachi J, Tadeo FR, Talon M (2001) The plant growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce a high amounts of physiologically active gibberllins. Physiol Plant 111:206–211

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu Rev Phytopatol 41:117–153

Habtemichial KH, Singh BR (2007) Wheat response to N_2 fixed by faba bean (*Vicia faba* L.) as affected by sulphur fertilization and rhizobial inoculation in semi-arid northern Ethiopia. J Plant Nutr Soil Sci 170:412–418

Halder AK, Mishra AK, Chakrabarty PK (1991) Solubilizing of inorganic phosphates by *Bradyrhizobium*. Indian J Expt Biol 29:28–31

Hall JA, Peirson D, Ghosh S, Glick BR (1996) Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Isr J Plant Sci 44:37–42

Halverson LJ, Handelsman J (1991) Enhancement of soybean nodulation by *Bacillus cereus* UW85 in the field and in a growth chamber. Appl Environ Microbiol 57:2767–2770

Hardarson G (1993) Methods for enhancing symbiotic nitrogen fixation. Plant Soil 152:1–17

Hassanein WA, Awny NM, El-Mougith AA, El-Dien SH (2009) The antagonistic activities of some metabolites produced by *Pseudomonas aeruginosa* Sha8. J Appl Sci Res 5:404–414

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hervas A, Landa B, Jiménez-Díaz MR (1997) Influence of chickpea genotype and *Bacillus* sp. on protection from Fusarium wilt by seed treatment with nonpathogenic *Fusarium oxysporum*. Eur J Plant Pathol 103:631–642

Hoffland E, Hakulinen J, Van-Pelt JA (1996) Comparison of systemic resistance induced by avirulent and non-pathogenic *Pseudomonas* species. Phytopathology 86:757–762

Holl FB, Chanway CP, Turkington R, Radley RA (1988) Response of crested wheatgrass (*Agrepyron cristatum* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. Soil Biol Biochem 20:19–24

Hu XF, Chen J, Guo JF (2006) Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain. World J Microbiol Biotechnol 22:983–990

Hynes RK, Leung GC, Hirkala DL, Nelson LM (2008) Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil and chickpea grown in western Canada. Can J Microbiol 54:248–258

Iruthayathas EE, Gunasekaran S, Vlassak K (1983) Effect of combined inoculation of *Azospirillum* and *Rhizobium* on nodulation and N_2-fixation of winged bean and soybean. Sci Hort (Amsterdam) 20:231–240

Itzigsohn R, Kapulnik Y, Okon Y, Dovrat A (1993) Physiological and morphological aspects of interaction between *Rhizobium meliloti* and alfalfa (*Medicago sativa*) in association with *Azospirillum brasilense*. Can J Microbiol 39:610–615

Jamali F, Sharifi-Tehrani A, Okhovvat M, Zakeri Z, Saberi-Riseh R (2004) Biological control of chickpea *Fusarium* wilt by antagonistic bacteria under greenhouse condition. Commun Agric Appl Biol Sci 69:649–651

Jing Y, He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B 8:192–207

Jisha MS, Alagawadi AR (1996) Nutrient uptake and yield of sorghum (*Sorghum bicolor* L. Moench) inoculated with phosphate solubilizing bacteria and cellulolytic fungus in cotton stalk amended vertisol. Microbiol Res 151:1–5

Johri BN, Sharma A, Virdi JS (2003) Rhizobacterial diversity in India and its influence on plant health. In: Ghose TK, Ghosh P (eds) Advances in biochemical engineering. Springer, Berlin, pp 84–89

Kabata-Pendias A, Pendias H (1989) Trace elements in the soil and plants. CRC Press, Boca Raton, FL

Kamnev AA (2003) Phytoremediation of heavy metals: an overview. In: Fingerman M, Nagabhushanam R (eds) Recent advances in marine biotechnology, vol 8, Bioremediation. Science Publishers Inc, Enfield (NH), USA, pp 269–317

Kanazawa K, Higuchi K, Nishizawa NK, Fushiya S, Chino M, Mori S (1994) Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient conditions in Gramineae. J Exp Bot 45:1903–1906

Kaneshiro T, Kwolek WF (1985) Stimulated nodulation of soybeans by *Rhizobium japonicum* mutant (B-1405) that catalyzes the conversion of tryptophan to indole-acetic acid. Plant Sci 42:142–146

Karasu A, Öz M, Doğan R (2009) The effect of bacterial inoculation and different nitrogen doses on yield and yield components of some chickpea genotypes (*Cicer arietinum* L.). African J Biotechnol 8:59–64

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi - current perspective. Arch Agron Soil Sci 56:73–98

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture - a review. Agron Sustain Dev 27:29–43

Khot GG, Tauro P, Dadarwal KR (1996) Rhizobacteria from chickpea (*Cicer arietinum* L.) rhizosphere effective in wilt control and promote nodulation. Indian J Microbiol 36:217–222

Kim KY, Jordan D, McDonald GA (1998) *Enterobacter agglomerans*, phosphate solubilizing bacteria and microbial diversity in soil: effect of carbon sources. Soil Biol Biochem 30:995–1003

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:883–884

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–44

Kloepper JW, Mahaffee WF, McInroy JA, Backman PA (1991) Comparative analysis of five methods for recovering rhizobacteria from cotton roots. Can J Microbiol 37:953–957

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94:1259–1266

Knight TJ, Langston-Unkeffer PJ (1988) Enhancement of symbiotic dinitrogen fixation by a toxin-releasing plant pathogen. Science 241:951–954

Krishnan HB, Kim KY, Krishnan AH (1999) Expression of a *Serratia marcescens* chitinase gene in *Sinorhizobium fredii* USDA191 and *Sinorhizobium meliloti* RCR2011 impedes soybean and alfalfa nodulation. Mol Plant-microbe Interact 12:748–751

Lambrecht M, Okon Y, Vande Broek A, Vanderleyden J (2000) Indole-3-acetic acid: a reciprocal signalling molecule in bacteria-plant interactions. Trends Microbiol 8:298–300

Leong J (1986) Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. Annu Rev Phytopathol 24:187–209

Li DM, Alexander M (1988) Co-inoculation with antibiotic-producing bacteria to increase colonization and nodulation by rhizobia. Plant Soil 108:211–219

Lian B, Wang B, Pan M, Liu C, Teng HH (2008) Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigates*. Geochim Cosmochim Acta 72:87–98

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Liu L, Jiang CY, Liu XY, Wu JF, Han JG, Liu SJ (2007) Plant-microbe association for rhizoremediation of chloronitroaromatic pollutants with *Comamonas* sp. strain CNB-1. Environ Microbiol 9:465–473

Loper JE, Henkels MD (1999) Utilization of heterologous siderophore enhances levels of iron available to *Pseudomonas putida* in rhizosphere. Appl Environ Microbiol 65:5357–5363

Lugtenberg BJJ, de Weger LA, Bennett JW (1991) Microbial stimulation of plant growth and protection from disease. Cur Opin Microbiol 2:457–464

Malik DK (2002) Influence of indole acetic acid producing Pseudomonas strains on symbiotic properties in green gram (*Vigna radiata*) and chickpea (*Cicer arietinum*). PhD thesis, submitted to CCS HAU, Hisar

Malik DK, Sindhu SS (2008) Transposon-derived mutants of *Pseudomonas* strains altered in indole acetic acid production: effect on nodulation and plant growth in green gram (*Vigna radiata* L.). Physiol Mol Biol Plants 14:315–320

Masalha J, Kosegarten H, Elmaci O, Mengal K (2000) The central role of microbial activity for iron acquisition in maize and sunflower. Biol Fertil Soils 30:433–439

Maurhofer M, Keel C, Schnider U, Voisard C, Hass D, Defago G (1992) Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHAO on its disease suppressive capacity. Phytopathology 82:190–195

Maurhofer M, Reimmann C, Schmidli-Sacherer P, Heeb S, Haas D, Defago G (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88:678–684

Mavrodi DV, Blankenfeldt W, Thomashow LS (2006) Phenazine compounds in fluorescent *Pseudomonas* spp.: biosynthesis and regulation. Annu Rev Phytopatol 44:417–445

Mavrodi DV, Bonsal RF, Delaney SM, Soule MJ, Phillips G (2001) Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. J Bacteriol 183:6454–6465

Mavrodi DV, Ksenzenko VN, Bonsal RF, Cook RJ, Boronin AM (1998) A seven gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. J Bacteriol 180:2541–2548

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18:49–53

McGrath SP, Chaudri AM, Giller KE (1995) Long-term effects of metals in sewage sludge on soils, microorganisms and plants. J Ind Microbiol 14:94–104

Memon YM, Fergus IF, Hughes JD, Page DW (1988) Utilization of non-exchangeable soil potassium in relation to soil types, plant species and stage of growth. Aust J Soil Res 26:489–496

Mendoza A, Leija A, Martinez-Romero E, Hernandez G, Mora J (1995) The enhancement of ammonium assimilation in *Rhizobium elti* prevents nodulation of *Phaseolus vulgaris*. Mol Plant-Microbe Interact 8:584–592

Mendoza A, Valderrama B, Leija A, Mora J (1998) NifA-dependent expression of glutamate dehydrogenase in *Rhizobium etli* modifies nitrogen partitioning during symbiosis. Mol Plant-Microbe Interact 11:83–90

Merharg AA, Killham K (1995) Loss of exudates from roots of perennial rye grass inoculated with a range of microorganisms. Plant Soil 170:345–349

Meyer JM (2000) Pyoverdines: pigments siderophores and potential taxonomic markers of fluorescent *Pseudomonas* species. Arch Microbiol 174:135–142

Milner JL, Stohl EA, Handelsman J (1996) Zwittermicin A resistance gene from *Bacillus cereus*. J Bacteriol 178:4266–4272

Minamisawa K, Fukai K (1991) Production of indole-3-acetic acid by *Bradyrhizobium japonicum*: a correlation with genotype grouping and rhizobitoxine production. Plant Cell Physiol 32:1–9

Mishra PK, Mishra S, Selvakumar G, Bisht JK, Kundu S, Gupta HS (2009a) Coinoculation of *Bacillus thuringiensis* -KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.). World J Microbiol Biotechnol 25:753–761

Mishra PK, Mishra S, Selvakumar G, Kundu S, Gupta HS (2009b) Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thruingiensis*-KR1. Acta Agric Scand B Soil Plant Sci 59:189–196

Naik PR, Raman G, Narayanan KB, Sakthivel N (2008) Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. BMC Microbiol 8:230–243

Nambiar PTC, Ma SW, Iyer YN (1990) Limiting insect infestation of nitrogen fixing root nodules of the pigeon pea (*Cajanus cajan* L.) by engineering an entomocidal gene in its root nodules. Appl Environ Microbiol 56:2866–2869

Nautiyal CS (1997) Selection of chickpea rhizosphere-competent *Pseudomonas fluorescens* NBRI 1303 antagonistic to *Fusarium oxysporum* f. sp. *ciceris*, *Rhizoctonia bataticola* and *Pythium* sp. Curr Microbiol 35:52–58

Neilands JB (1981) Microbial iron compounds. Annu Rev Biochem 50:715–731

Neilands JB, Nakamura K (1991) Detection, determination, isolation, characterization and regulation of microbial iron chelators. In: Winkelmann G (ed) Microbial iron chelators. CRC Handbook of CRC Press, London, UK, pp 1–14

Nishijima F, Evans WR, Vesper SJ (1988) Enhanced nodulation of soybean by *Bradyrhizobium* in the presence of *Pseudomonas fluorescens*. Plant Soil 111:149–150

Olivera M, Tejera N, Iribane C, Ocana A, Lluch C (2004) Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris* L.): effect of phosphorus. Physiol Plant 121:498–505

Pandey A, Kumar S (1989) Potential of azospirilla as biofertilizers for upland agriculture – a review. J Sci Ind Res 48:134–144

Parmar N, Dadarwal KR (1999) Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. J Appl Microbiol 86:36–44

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Patten CL, Glick BR (2002) The role of bacterial indoleacetic acid in the development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Pau AS (1991) Improvement of *Rhizobium* inoculants by mutation, genetic engineering and formulation. Biotechnol Adv 9:173–184

Peoples MB, Herridge DF (1990) Nitrogen fixation by legumes in tropical and subtropical agriculture. Adv Agron 44:155–223

Pereira PAA, Bliss FA (1987) Nitrogen fixation and plant growth of common bean (*Phaseolus vulgaris* L.) at different levels of phosphorus availability. Plant Soil 104:79–84

Peter NK, Verma DPS (1990) Phenolic compounds as regulators of gene expression in plant-microbe interaction. Mol Plant-Microbe Interact 3:4–8

Pierson FA, Weller DM (1994) Use of mixtures of fluorescent pseudomonad to suppress take-all and improve the growth of wheat. Phytopathology 84:940–947

Plazinski J, Rolfe BG (1985) *Azospirillum-Rhizobium* interaction leading to a plant growth stimulation without nodule formation. Can J Microbiol 31:1026–1030

Podile AR (1995) Seed bacterization with *Bacillus subtilis* AF1 enhances seedling emergence, growth and nodulation of pigeonpea. Indian J Microbiol 35:199–204

Prabhakar M, Saraf CS (1990) Dry matter accumulation and distribution in chickpea as influenced by genotype, P source and irrigation level. Indian J Agric Sci 60:204–206

Prinsen EN, Chauvaux J, Schmidt M, John U, Wieneke J, De Greef J, Schell O, Onckelen van H (1991) Stimulation of indole-3-acetic acid production in *Rhizobium* by flavonoids. FEBS Lett 282:53–55

Rajarathinam K, Balamurugan T, Kulasekarapandian R, Veersami S, Jayabalan M (1995) Isolation and screening of phosphate solubilizers from soil of Kamarajar district (Tamil Nadu). J Exotoxic Environ Monit 5:155–157

Rajkumar M, Nagendran R, Lee KJ, Lee WH (2005) Characterization of a novel Cr^{6+} reducing *Pseudomonas* sp. with plant growth-promoting potential. Curr Microbiol 50:266–271

Randhawa PS, Schaad NW (1985) A seedling bioassay chamber for determining bacterial colonization and antagonism on plant roots. Phytopathology 75:254–259

Rasch T, Wachter A (2005) Sulphur metabolism: a versatile plateform for launching defence operations. Trends Plant Sci 10:503–509

Rashid M, Khalil S, Ayub N, Alam S, Latif M (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak J Biol Sci 7:187–196

Raverkar KP, Konde BK (1988) Effect of *Rhizobium* and *Azosprillum lipoferum* inoculation on the nodulation, yield and nitrogen uptake of peanut cultivars. Plant Soil 106:249–252

Remans R, Beebe S, Blair M, Manrique G, Tover E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, Vanderleyden J (2008a) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant Soil 302:149–161

Remans R, Croonenborghs A, Torres-Gutierrez R, Michiels J, Vanderleyden J (2007) Effects of plant growth promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant nutrition. Eur J Plant Pathol 119:341–351

Remans R, Ramaekers L, Schalkens S, Hernandez G, Garcia A, Reyes JL, Mendez N, Toskano V, Mulling M, Galvez L, Vanderleyden J (2008b) Effect of *Rhizobium-Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. Plant Soil 312:25–37

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–906

Robinson MM, Shah S, Tamot B, Pauls KP, Moffatt BA, Glick BR (2001) Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase. Mol Plant Pathol 2:135–145

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodriguez H, Fraga R, Ganzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth promoting bacteria. Plant Soil 287:15–21

Rodriguez H, Gonzalez T, Selman G (2000) Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J Biotechnol 84:155–161

Ronson CW, Bosworth A, Genova M, Gudbrandsen S, Hankinson T, Kwaitowski R, Ratcliffe H, Robie C, Sweeney P, Szeto W, Williams M, Zablotowicz R (1990) Field release of genetically engineered *Rhizobium meliloti* and *Bradyrhizobium japonicum* strains. In: Gresshoff PM, Roth LE, Stacey G, Newton WE (eds) Nitrogen fixation: achievements and objectives. Chapman and Hall, New York, USA, pp 397–403

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Safronova VI, Stepanok VV, Enggvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Sahgal M, Johri BN (2006) The changing face of rhizobial systematic. Cur Sci 84:43–48

Saraf CS, Shivkumar BG, Patil RR (1997) Effect of phosphorus, sulphur and seed inoculation on performance of chickpea (*Cicer arietinum*). Indian J Agron 42:323–328

Sarawgi SK, Tiwari PK, Tripathi RS (1999) Uptake and balance sheet of nitrogen and phosphorus in gram (*Cicer arietinum*) as influenced by phosphorus biofertilizers and micronutrients under rain-fed conditions. Indian J Agron 44:768–772

Sarawgi SK, Tiwari PK, Tripathi RS (2000) Growth, nodulation and yield of chickpea as influenced by phosphorus, bacterial culture and micronutrients under rain-fed conditions. Madras Agric J 86:181–185

Scher FM, Kloepper JW, Singleton CA (1985) Chemotaxis of fluorescent *Pseudomonas* spp. to soybean root exudates in vitro and in soil. Can J Microbiol 31:570–574

Scherer HW (2009) Sulfur in soils. J Plant Nutr Soil Sci 172:326–335

Scherer HW, Lange A (1996) N_2 fixation and growth of legumes as affected by sulphur fertilization. Biol Fertil Soils 23:449–453

Scherer HW, Pacyna S, Spoth KR (2008) Low levels of ferrodoxin, ATP and leghaemoglobin contribute to limited N_2 fixation in peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. Biol Fertil Soils 44:909–916

Scherwinski K, Grosch R, Berg G (2008) Effect of bacterial antagonists on lettuce: active biocontrol of *Rhizoctonia solani* and negligible, short-term effects on nontarget microorganisms. FEMS Microbiol Ecol 64:106–116

Schroth MN, Hancock JG (1982) Disease-suppressive soil and root-colonizing bacteria. Science 216:1376–1381

Sheng XF, He LY, Huang WY (2002) The conditions of releasing potassium by a silicate-dissolving bacterial strain NBT. Agric Sci China 1:662–666

Sheng XF, Huang WY (2002) Mechanism of potassium release from feldspar affected by the strain NBT of silicate bacterium. Acta Pedol Sinica 39:863–871

Sheng XF, Xia JJ, Chen J (2003) Mutagenesis of the *Bacillus edphicaus* strain NBT and its effect on growth of chili and cotton. Agric Sci China 2:410–412

Silo-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J (1994) Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Appl Environ Microbiol 60:2023–2030

Sindhu SS, Dadarwal KR (2000) Competition for nodulation among rhizobia in legume-*Rhizobium* symbiosis. Indian J Microbiol 32:175–180

Sindhu SS, Dadarwal KR (2001) Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. *Cicer* in chickpea. Microbiol Res 156:353–358

Sindhu SS, Dadarwal KR, Davis TM (1992) Non-nodulating chickpea breeding line for the study of symbiotic nitrogen fixation potential. Indian J Microbiol 40:211–246

Sindhu SS, Gupta SK, Dadarwal KR (1999) Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of growth of green gram (*Vigna radiata*). Biol Fertil Soils 29:62–68

Sindhu SS, Gupta SK, Suneja S, Dadarwal KR (2002a) Enhancement of green gram nodulation and plant growth by *Bacillus* species. Biol Plant 45:117–120

Sindhu SS, Jangu OP, Sivaramaiah N (2009a) Genetic engineering of diazotrophic bacteria to improve nitrogen fixation for sustainable agriculture. In: Sayyed RZ, Patil AS (eds) Biotechnology emerging trends. Scientific Publishers, Jodhpur, India, pp 73–112

Sindhu SS, Rakshiya YS, Malik DK (2009b) Rhizosphere bacteria and their role in biological control of plant diseases. In: Sayyed RZ, Patil AS (eds) Biotechnology emerging trends. Scientific Publishers, Jodhpur, India, pp 17–52

Sindhu SS, Rakshiya YS, Sahu G (2009c) Biological control of soilborne plant pathogens with rhizosphere bacteria. Pest Technol 3:10–21

Sindhu SS, Suneja S, Goel AK, Parmar N, Dadarwal KR (2002b) Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions. Appl Soil Ecol 19:57–64

Sitrit Y, Barak Z, Kapulnik Y, Oppenheim AB, Chet I (1993) Expression of *Serratia marcescens* chitinase gene in *Rhizobium meliloti* during symbiosis on alfalfa roots. Mol Plant Microbe Interact 6:293–298

Spadro D, Cullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Prot 24:601–613

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbial Rev 31:425–428

Srinivasan M, Petersen DJ, Holl FB (1996) Influence of indole acetic acid producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnotobiotic conditions. Can J Microbiol 42:1006–1014

Srinivasan M, Petersen DJ, Holl FB (1997) Nodulation of *Phaseolus vulgaris* by *Rhizobium etli* is enhanced in the presence of *Bacillus*. Can J Microbiol 43:1–8

Srivastava TK, Ahalwat LPS, Panwar JDS (1998) Effect of phosphorus, molybdenum and biofertilizers on productivity of pea. Indian J Plant Physiol 3:237–239

Stevenson FJ, Cole MA (1999) Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients, 2nd edn. Wiley, London

Stockwell VO, Stack JP (2007) Using *Pseudomonas* spp. for integrated biological control. Phytopathology 97:244–249

Sturz AV, Carter MR, Johnston AW (1997) A review of plant disease, pathogen interactions and microbial antagonism under conservation tillage in temperate humid agriculture. Soil Till Res 41:169–189

Sturz AV, Christie BR (1998) The potential benefits from cultivar specific red clover-potato crop rotations. Ann Appl Biol 133:365–373

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Stutz EG, Defago G, Kern H (1986) Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. Phytopathology 76:181–185

Taele WD, Paponov IA, Palme K (2006) Auxin in action: signaling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859

Tank N, Saraf M (2009) Enhancement of plant growth and decontamination of nickel-spiked soil using PGPR. J Basic Microbiol 49:195–204

Thies JE, Singleton PW, Bohlool BB (1991) Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field grown legumes. Appl Environ Microbiol 57:19–28

Thomashow LS, Weller DM (1996) Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stacey G, Keen N (eds) Plant-microbe interactions, vol 1, Chapman and Hall. New York, USA, pp 187–235

Timmis-Wilson TM, Ellis RJ, Renwick A, Rhodes DJ, Mavrodl DV, Weller DM, Thomashow LS, Bailey MJ (2000) Chromosomal insertion of phenazine-1-carboxylic acid biosynthesis pathway enhances efficacy of damping off disease control by *Pseudomonas fluorescens*. Mol Plant-Microbe Interact 13:1293–1300

Trapero-Casas A, Kaiser WJ, Ingram DM (1990) Control of *Pythium* seed rot and preemergence damping-off of chickpea in the U.S. Pacific Northwest Spain. Plant Dis 74:563–569

Trevors JT, Van Elsas JD, Van Overbeek LS, Starodub ME (1990) Transport of genetically engineered *Pseudomonas fluorescens* strain through a soil microcosm. Appl Environ Microbiol 56:401–408

Triplett EW (1988) Isolation of genes involved in nodulation competitiveness from *Rhizobium leguminosarum* bv. *trifolii* T24. Proc Natl Acad Sci USA 85:3810–3814

Triplett EW (1990) Construction of a symbiotically effective strain of *Rhizobium leguminosarum* bv. *trifolii* with increased nodulation competitiveness. Appl Environ Microbiol 56:98–103

Turner JT, Backman PA (1991) Factors relating to peanut yield increased following *Bacillus subtilis* seed treatment. Plant Dis 75:347–353

van Elsas JD, Heijnen CE (1990) Methods for the introduction of bacteria in soil: a review. Biol Fertil Soils 10:127–133

van Loon LC (1984) Regulation of pathogenesis and symptom expression in diseased plants by ethylene. In: Fuchs Y, Chalutz E (eds) Ethylene: biochemical, physiological and applied aspects. Martinus Nijhoff, the Hague, The Netherlands, pp 171–180

van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci 11:184–191

van Veen JA, van Overbeek LS, van Elsas JD (1997) Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol Rev 61:121–135

Varin S, Leveel B, Lavenant SL, Cliquet JB (2008) Does the white clover response to sulphur availability correspond to phenotypic or on congenetic plasticity. Acta Oecologica 35:452–457

Varin S, Lemauviel-Lavenant S, Cliquet JB, Diquelou S, Padraic T, Michaelson-Yeates T (2009) Functional plasticity of *Trifolium repens* L. in response to sulphur and nitrogen availability. Plant Soil 317:189–200

Venkateswarlu B, Rao AV, Raina P (1984) Evaluation of phosphorus solubilization by microorganisms isolated from arid soils. J Indian Soc Soil Sci 32:273–277

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255: 571–586

Villacieros M, Whelan C, Mackova M, Molgaard J, Sanchez-Contreras M, Lloret J, de Carcer DA, Oreuzabal RI, Bolanos L, Macek T, Karlson U, Dowling DN, Martin M, Rivilla R (2005) PCB rhizoremediation by *Pseudomonas fluorescens* F113 derivatives using a *Sinorhizobium meliloti nod* system to derive *bph* gene expression. Appl Environ Microbiol 71:2687–2694

Voisard C, Keel C, Hass D, Defago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358

Wallace A, Wallace GA, Cha JW (1992) Some modifications in trace elements toxicities and deficiencies in plants resulting from interactions with other elements and chelating agents: The special case of iron. J Plant Nutr 15:1589–1598

Wang TL, Wood EA, Brewin NJ (1982) Growth regulators, *Rhizobium* and nodulation in peas: the cytokinin content of a wild type and Ti-plasmid containing strain of *R. leguminosarum*. Planta 155:350–355

Wang Y, Brown HN, Crowley DE, Szaniszlo PJ (1993) Evidence for direct utilization of a siderophore, ferroxamine B, in axenically grown cucumber. Plant Cell Environ 16:579–585

Wang C, Knill E, Glick BR, Defago G (2000) Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHAO and its

gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities. Can J Microbiol 47:642–652

Wani PA, Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with nitrogen fixing and phosphate-solubilizing rhizobacteria on the performance of field grown chickpea. J Plant Nutr Soil Sci 170:283–287

Wani SP (1990) Inoculation with associative nitrogen fixing bacteria: role in cereal grain production improvement. Indian J Microbiol 30:363–393

Weller DM (1985) Application of fluorescent pseudomonads to control root diseases. In: Parker CS, Rovira AD, Moore KJ, Wong PTW, Kollmorgan JF (eds) Ecology and management of soilborne plant pathogens. American Phytopathological Society, St. Paul, pp 137–140

Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407

Weller DM (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97:250–256

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe parternerships to improve biomass production and remediation. Trends Biotechnol 27:591–598

Wiliems A (2006) The taxonomy of rhizobia: an overview. Plant Soil 287:3–14

Wilson KJ, Peop MB, Jefferson RA (1995) New techniques for studying competition by rhizobia and for assessing nitrogen fixation in the field. Plant Soil 174:241–253

Wong PTW, Baker R (1984) Suppression of wheat take-all and ophiobolus patch by fluorescent pseudomonads from a *Fusarium*-suppressive soil. Soil Biol Biochem 16:397–403

Xie H, Pasternak JJ, Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indole acetic acid. Curr Microbiol 32:67–71

Yahalom E, Okon Y, Dovrat A (1990) Possible mode of action of *Azospirillum brasilense* strain Cd on the root morphology and nodule formation in burr medic (*Medicago polymorpha*). Can J Microbiol 36:10–14

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yuhanshi KI, Akao S, Fukuhara H, Tateno E, Chum JY, Stacey G, Hara H, Kubota M, Asami T, Minamisawa K (1995) *Bradyrhizobium elkanii* induces outer cortical root swelling in soybean. Plant Cell Physiol 36:151–157

Zhang F, Dashti N, Hynes RK, Smith DL (1996) Plant growth-promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann Bot 77:453–459

Zhao FJ, Wood AP, McGrath SP (1999) Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). Plant Soil 212:209–219