Chapter 2
Beneficial Interactions of Plant Growth Promoting Rhizosphere Microorganisms

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2.1 Introduction

The plant rhizosphere is the major soil ecological environment for plant–microbe interactions involving colonization of different microorganisms in and around growing roots which may either result in associative, symbiotic, neutralistic, or parasitic interactions depending upon plant nutrient status in soil, soil environment, plant defence mechanism, and the type of microorganism proliferating in the rhizosphere zone. Finding the microorganisms very close to epidermis, plants secrete signal molecules for protection against invasion of the heterogeneous microbes in the root zone, and at this stage the differentiation takes place between pathogenic, associative, symbiotic, or neutralistic adaptation of microbes with the plant (Hayat et al. 2010). The plant signal molecules produced in response to microbial adhesion are the flavonoids and flavones which are secreted in the rhizosphere bacteria and some remain attached to plant cell walls to act as antimicrobial agents (phytoalexins).

In legume–*Rhizobium* symbiosis, the rod-shaped soil bacterium, *Rhizobium*, induces nitrogen-fixing nodules on the roots of leguminous plants. In this process, dinitrogen which is chemically inert and makes up approximately 80% of the volume present in the earth's atmosphere is reduced to ammonia by the bacterial enzyme nitrogenase. The plants provide a micro-aerobic environment for the effective functioning of the oxygen-sensitive nitrogenase and carbohydrates for bacterial endosymbionts to support their metabolism. In return, the bacteria fix atmospheric nitrogen used by the plant for the synthesis of organic nitrogenous compounds to meet its biological needs. Due to its agricultural importance, this symbiotic association has been the subject of extensive scientific research, and different laboratories world over are trying to increase the effectiveness of symbiosis through genetic manipulation of the host and the bacterium and to extend the

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Rhizobium–host infectivity to other non-leguminous crops (Stacey et al. 1980; Fisher et al. 1985; Fisher and Long 1992).

In the rhizosphere system, plant growth promoting bacteria (PGPR) and nodule promoting rhizobacteria (NPR) capable of producing growth promoting substances have been identified. These organisms induce phytoalexins production by the plant, creating antibiosis in the rhizosphere for pathogenic forms, siderophores production to chelate insoluble cations and associative action with the plant (Lifshitz et al. 1986; Halverson and Handelsman 1991). The rhizosphere bacteria involved in such type of interactions are species of *Pseudomonas* and *Bacillus* (Capper and Higgins 1993; Guaiquil and Luigi 1992; Parmar and Dadarwal 1997). This chapter will focus on the effect of such bacteria and will provide an insight into plant–microbe interactions.

2.2   Interactions Among Diazotrophs

Rhizobium, a gram-negative bacteria, is able to establish symbiosis with leguminous plants such as *Cicer* as well as many other rhizobacterial strains, and develops positive interactions with legumes by inhabiting root nodules. Within these nodules, nitrogen-fixing bacteria reduce atmospheric nitrogen to ammonia. This provides many plants with a sufficient useable nitrogen source (Sessitsch et al. 2002). Studies on legume rhizosphere bacteria have shown that besides indigenous rhizobia interacting and competing for nodulation with an inoculant strain by antagonistic or synergistic interactions, other diazotrophs such as *Azotobacter* and *Azospirillum* as well as rhizosphere fungi and bacteria especially species of *Pseudomonas* and *Bacillus* do interact with *Rhizobium* affecting nodulation and nitrogen fixation (Bolton et al. 1986; El-Mokadem 1989; Ahmad et al. 2006; Gaind et al. 2007; Rodriguez and Frioni 2003). These diazotrophs manage important biological functions by symbiotically interacting with *Rhizobium* populations within the rhizosphere and help create a beneficiary region where interacting microorganisms benefit from additional nutrient resources (Halbleib and Ludden 2000; Gaind et al. 2007).

2.2.1   Interaction of Rhizobium with Azotobacter/Azospirillum

Interactions of *Azotobacter/Azospirillum* with the *Rhizobium* as co-inoculants have been observed to be synergistic in a majority of studies conducted under laboratory, greenhouse or field conditions. Combined inoculation of *Azotobacter* and *Rhizobium* sp. produces a positive response. *Azotobacter* sp. influence *Rhizobium* by significantly increasing nodulation. Increasing N_2 content within roots and shoots of respiring/metabolizing plant cells improves conditions within the rhizosphere and enhances synergistic interactions between host and *Azotobacter* sp. In an open field conditions, *Azotobacter* and *Azospirillum* have both been shown to improve growth yields in various soil mineral compositions. This suggests that a mutualistic

relationship exists between *Azotobacter* and *Azospirillum* where both interact with the *Rhizobium* to improve *Cicer arietinum* (chick pea) yields (Parmar 1995; Parmar and Dadarwal 1997).

The beneficial effects of *Azotobacter* and *Azospirillum* on plants are mainly attributed to improvements in root development, an increase in the rate of water and mineral uptake by roots, the displacement of fungi and plant pathogenic bacteria, and to a lesser extent, biological nitrogen fixation (Okon and Itzigsohn 1995). Associative effect of *Azospirillum lipoferum* and *Azotobacter chroococcum* with *Rhizobium* sp. improved the growth of chick pea grown on both loamy sand and sandy soils (El-Mokadem et al. 1989). Associative effect of *A. chroococcum* on *Bradyrhizobium* strains (BM 42 and BM 43) specific to moong bean (*Vigna radiata*) was also observed (Yadav and Vashishat 1991). The effect was more pronounced when *A. chroococcum* was co-inoculated with both the strains of *Bradyrhizobium*.

Certain species of *Azospirillum* have been used to study the relationship between free-living nitrogen-fixing rhizobacteria and legumes. Abundant in the rhizosphere, *Azospirilla* possesses a versatile metabolic system where carbon and nitrogen are metabolized readily. In an unfavourable arid or nutrient-deficient conditions, *Azospirilla* can morphologically transform into what appears to be enlarged cysts and the development of an outer polysaccharide coat by accumulating poly-L-hydroxybutyrate granules which serve as carbon and energy sources. A phenotypic advantage, such as a flagellum, allows the highly motile *Azospirillum* genus to swim toward nutrients via chemotactic attraction thus enhancing growth and increased yields (Steenhoudt and Vanderleyden 2000). The inoculation of legumes with *Azospirillum* prompts enlarged lateral roots and root hairs. This results in improved water uptake and retention with higher nutrient uptake (Steenhoudt and Vanderleyden 2000).

Some of the studies have shown that a relationship exists between chemotactic behaviour and *Azotobacter's* influence on plant growth such as cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) (Kumar et al. 2007). In the areas of soil where plant root exudates such as sugars, glucose, amino acids and organic acids have been deposited, bacteria mobilize towards these exudates through chemotactic attraction. Increased yields and enhanced growth using *A. chroococcum* indicate a positive response attributed to nitrogen fixation, phosphorus mobilization, bacterial production and the release of phytohormones (Kumar et al. 2007).

2.2.2 Interaction of Rhizobium sp. with Rhizobacteria

Rhizosphere bacteria, especially species of *Pseudomonas* and *Bacillus,* have been identified in the rhizosphere of various leguminous and non-leguminous crops that help in plant colonization and suppression of plant pathogens (Table 2.1). Such characteristics have defined rhizobacteria more recently as PGPR or NPR. Interactions of these rhizobacteria with *Rhizobium* may be antagonistic or synergistic and the beneficial effects of these bacteria have been extensively exploited for

Table 2.1 Important plant growth-promoting rhizobacteria (PGPR)

PGPR	Agricultural crop	References
Pseudomonas fluorescens PCL1606	Avocado	Cazorla et al. (2006)
P. fluorescens CHA0	*Arabidopsis* sp.	Iavicoli et al. (2003)
Bacillus subtilis FB17	*Arabidopsis thaliana*	Rudrappa et al. (2008)
Collimonas fungivorans	Tomato	Kamilova et al. (2008)
Bacillus subtilis, *B. amyloliquefaciens* IN 937, *Enterobacter cloaca*	*Arabidopsis* sp.	Ryu et al. (2003)
P. putida KD	Tomato and cucumber	Rezzonoco et al. (2005)
P. fluorescens WCS 365	Tomato	Kamilova et al. (2006)
Comamonas acidovorans	Kiwi	Erturk et al. (2010)
Bacillus cereus UW 85	Grain legumes	Vessey and Buss (2002)
Bradyrhizobium and PGPR	Mungbean	Shaharoona et al. (2006)
Pseudomonas BA-8, *Bacillus* OSU-142, *Bacillus* M-3	Strawberry	Pirlak and Kose (2009)
Agrobacterium amazonense	Rice	Rodrigues et al. (2008)
Bacillus cepacia strain OSU-7	Stored potatoes	Recep et al. (2009)
Pseudomonas brassicacaerum, *P. Marginali*, *P. oryzihabitans*, *P. putida*, *Alcaligenes xylosoxidans*	Indian mustard and rape	Belimov et al. (2007)

economic gains in the recent years (Bolton et al. 1986; Halverson and Handelsman 1991; Parmar 1995).

Parmar and Dadarwal (1999) studied co-inoculation of the rhizobacteria with effective *Rhizobium* strains of chickpea and observed a significant increase in nodule weight, root and shoot biomass and total plant nitrogen when grown either in sterilized chillum jars or under pot culture conditions. The *Rhizobium* stimulatory *Pseudomonas* sp. "CRP55b" showed maximum increase in all the symbiotic parameters. On co-inoculation with *Rhizobium* strains "Ca181" and "Ca313", *Pseudomonas* sp. "CRP55b" and "CRS68" resulted in significant increases in nodule weight, root and shoot biomass and total plant nitrogen (Fig. 2.1). The nodule-stimulating rhizobacteria enhanced levels of flavonoid-like compounds in roots on seed bacterization. In another study, a greater number of nodules per plant were also produced where *Bradyrhizobia* was used with strains of *Pseudomonas aeruginosa* compared with *Bradyrhizobia* used alone (Izhar et al. 1995).

The influence of PGPR on dry matter accumulation and chick pea (*C. arietinum* L.) yield under field conditions has been thoroughly studied (Rokhzadi et al. 2008). Studies have shown that a combined inoculation of *Azospirillum* spp., *A. chroococcum* 5, *Mesorhizobium ciceri* SWR17 and *Pseudomonas fluorescens* P21 improved nodulation, increased dry matter accumulation in roots and shoots, grain yields, biomass and protein yield of chick-pea by a significant margin. This can be attributed to the cumulative effects of an enhanced supply of nutrients, mainly nitrogen and phosphorus and the production of growth promoting substances. In addition, *P. fluorescens* has been found to synergistically interact with additional rhizobacteria to form interactions within the rhizosphere, attributing to
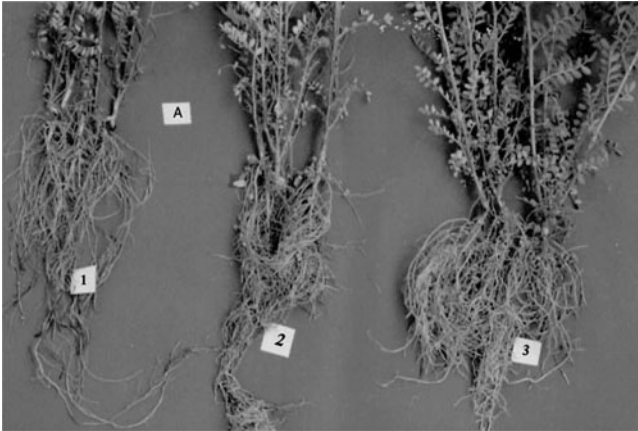
Fig. 2.1 Co-inoculation of *Rhizobium* strain "Ca 313" with *Pseudomonas* sp. "CRS68" showed an increase in nodulation in *Cicer*. (1) Control (no bacteria), (2) Ca 313 alone, (3) Co-inoculated Ca313 + CRS68

phytohormone production, the stimulation of nutrient uptake and the bio-control of deleterious soil bacteria and phyto-pathogenic fungi.

Synergistic effects of plant growth-promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*) were also observed (Tilak and Ranganayaki 2006). Co-inoculation of a variety of PGPR such as *A. chroococcum* and *Pseudomonas putida* with *Rhizobium* sp. (AR-2-2 k) showed increased plant growth, nodulation and improved nitrogenase activity. The association of *Rhizobium* sp. with *P. putida*, *P. fluorescens* and *Bacillus cereus* seems to produce the best agronomical results.

Inoculation of *Rhizobium phaseoli* and PGPR such as *P. fluorescens* P-93 and *A. lipoferum* S-21on bean yield and plant growth parameters yielded promising results (Yadegari et al. 2008). In the dually inoculated plants, there were significant increases in quantity, weight, total dry matter, seed yield, and protein content. All treatment combinations resulted in higher yield; however, *Rhizobium* strain Rb-133 inoculated with *P. fluorescens* P-93 gave the highest number of seeds and pods per plant, seed protein yield, and overall seed quantity.

2.2.3   Interaction of Rhizobium with Actinomycetes

The agonistic and antagonistic effects of soil microbes through various interactions of bacteria, fungi, and *Actinomycetes* on *Rhizobium* have profoundly influenced sustainable annual harvests. *Actinomycetes*, a common antagonistic bacterium is often studied for its inhibitory effects on bacteria within the host rhizosphere. There are various studies in literature suggesting the antagonistic effect of Actinomycetes

under in vitro and in vivo conditions. Out of 60 isolates of Actinomycetes, bacteria and fungi from pasture soil samples, where no nodulation was observed in clover and 25–70% isolates of *Actinomycetes* were antagonistic toward 12 strains of *Rhizobium trifolii* tested (Patel 1974). Nine lysogenic *Streptomyces* sp. NSA4 were isolated from the nodule surface of black gram which was found to inhibit fast- and slow-growing strains of cow pea and soybean rhizobia. The fast-growing strain of *Rhizobium* (both cow pea miscellany and soybean) was more sensitive to antibiosis as compared to slow-growing stains (Jayaraman et al. 1985; Pugashetti et al. 1992). Another study observed that 90% of the *Actinomycetes* sp. isolated from soil obtained from field plots was antagonistic to *Rhizobium japonicum* (Pugashetti et al. 1992). In addition, 70% of other *Actinomycetes* sp. isolated from soybean rhizosphere were antagonistic to its homologous rhizobia. However, few isolates stimulated growth of *Bradyrhizobium japonicum*.

The isolates of *Streptomyces lydicus* WYEC108 from pea plants (*Pisum sativum*) were originally studied for its properties as an antifungal biocontrol agent. This strain is capable of mycoparasitic colonization of fungal root pathogens and the excretion of antifungal metabolites within plant rhizospheres. WYEC108 is a unique *Streptomyces* strain that has the ability to act as a PGPR. It was also hypothesized that root and nodule colonization is one of the several mechanisms by which *Streptomyces* acts as a naturally occurring plant growth-promoting bacterium in pea and possibly other leguminous plants. *Streptomyces* WYEC108 enhanced nodule growth, bacteroid differentiation and act as an aid in bacteroid assimilation of iron and other inorganic nutrients from soils, resulting in enhanced overall growth (Tokala et al. 2002).

There are some specific interactions in plant rhizosphere among different genera of *Actinomycetes*. *Actinomycete* mycelium makes up to 20% of the total bacterial biomass in the rhizosphere. There is significant lytic activity within the rhizosphere. *Actinomycete* mycelium content within the rhizosphere is significantly higher in root systems of healthy plants compared to those of plants suffering from root rot disease. Inoculating winter rye (*Secale cereale* L.) with *Actinomycetes* has beneficiary growth advantages; however, co-inoculation of *Actinomycetes* with the cow clover plants (*Trifolium pretense* L.) had no effect on growth (Merzaeva and Shirokikh 2006).

2.2.4   Interaction of Rhizobium with Mycorrhiza

The role of mycorrhizal fungi, especially the vesicular-arbuscular mycorrhizae (VAM) belonging to the *Zygomycetes* class in phosphorous mobilization in soils having a relatively low level of available phosphorous, is well established for cereals as well as legumes. AM fungi are obligate symbionts, but differ from VAM as they are not host specific.

Associative action of mycorrhizal fungi in legumes has a great impact on root and shoot development and phosphorous uptake which results in the enhancement

of nodulation and nitrogen fixation. There are several studies reporting the interactions between AMF and *Rhizobium* sp. (Adholeya and Johri 1986; Albrecht et al. 1999; Poi et al. 1989; Sivaprasad 1991). Variation in the response of nodulating pigeon pea (*C. cajan*) to different isolates of myccorhizal fungi was also observed (Ianson and Linderman 1993). Inoculation with an effective *Rhizobium* combination with seven VAM fungi (*Glomus* sp.) had a variable effect on plant growth enhancement, nodulation, and N_2 fixation.

There are various studies in the literature describing many significant findings in the synergistic interaction between AMF and asymbiotic N_2-fixing bacteria such as *A. chroococcum*, *Azospirillum* spp. and *Acetobacter diazotrohicus* (Barea et al. 1998; Barea et al. 2002, 2005; Barea and Azcon-Aguilar 1982). The role of AM fungi as P suppliers to legume root nodules is of great relevance when a specific AM fungus, *Rhizobacterium* sp. known for effective nodulation and N_2 fixation was found in a mycotrophic legume *Anthyllis cytisoides* in a Mediterranean semi-arid ecosystem in Spain (Requena et al. 1996, 2001). The strain *Glomus intraaridices* was found to be more effective with *Rhizobium* sp. NR 4, whereas *Glomus coronatum* was effective when co-inoculated with *Rhizobium* sp. NR9 strain. Research has provided evidence that the genetic pathway of AM symbiosis is shared in part by other root–microbe symbioses such as N_2-fixing rhizobia (Peterson and Guinel 2000).

Such specific interactions between AM fungi, *Rhizobium,* and PGPR have provided an insight into specific functional compatibility relationships between AMF and PGPR and their management when used as biofertilizers or biocontrol agents.

2.3 Rhizobacterial Factors in Growth Promotion

Research on the use of rhizobacteria to promote plant growth (legumes as well as non-legumes) has increased dramatically over the last few years due to potential benefits observed in the use of PGPR or NPR, both under cultural conditions as well as under field conditions. A diverse array of bacteria, including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter,* and *Serratia* has been shown to enhance plant growth. The mechanisms by which these rhizobacteria enhance plant growth are multitudinous which include production of plant growth-regulating substances (PGRs), phytohormones, suppression of plant pathogens through antibiosis, bacteriocinogenic action, siderophore production, nitrogen fixation, mineralization of organic phosphorus, production of phytoalexins/flavonoids-like compounds, enhancement of mineral uptake, etc. (Parmar 1995; Parmar and Dadarwal 1997; Mukerji et al. 2006).

The cumulative effect of these complex interactions among plant roots and various microbial populations can result in plant growth promotion and or pathogenesis and decay. This section will focus on plant growth promotion by rhizobacteria either directly or indirectly.

Rhizobacteria can stimulate growth by producing plant growth regulators known as phytostimulators in the absence of pathogens. Many phytostimulators are plant hormone analogues, meaning PGPR produce an identical or nearly identical compound that mimics the action of a plant hormone. These substances are usually light weight volatile organic compounds. Thus far, known phytostimulators include indole-acetic acid (IAA), gibberelic acid, cytokinins, and in some cases ethylene (Ahmad et al. 2008). The plant responses to PGPR have been excellently reviewed elsewhere (Bakker et al. 2007; Van Loon 2007).

2.3.1 Plant Growth Regulators

Many rhizosphere bacteria produce IAA in culture media especially in the presence of tryptophan (Ek et al. 1983; Strzelceyck and Pokjska-Burdzej 1984) in the rhizosphere and rhizoplane of forage grasses and many economically important cereals such as wheat, barley, and pearl millet (Tien 1979), and tomato and bean plants (Barea and Brown 1974). The accumulation of IAA in the cultural filtrate of rhizobacterial isolates from the rhizosphere of sugar beet has been reported previously (Loper and Schroth 1986).

In particular, the production of IAA seems to be one of the most prevalent plant growth promoting traits among PGPR. Higher auxin levels impair plant defence mechanisms making colonization easier. The biosynthesis of IAA in rhizobacteria is affected by several environmental factors. In particular, IAA production increases in conditions of higher pH, limited carbon and higher quantities of tryptophan (Spaepen et al. 2009). Thus far, six pathways for the biosynthesis of IAA have been identified in rhizobacteria, five of which are tryptophan dependant and one which is tryptophan independent. Instead of tryptophan, this pathway depends on the presence of indole-3-glycerolphosphate. Some rhizobacteria have several IAA biosynthesis pathways. In plants, most IAA are found in a conjugated form that allows for storage and prevents degradation (Spaepen et al. 2007).

It is believed that approximately 80% of rhizobacteria produce IAA (Khalid et al. 2004). Arshad and Frankenberger (1988) showed while studying the production of ethylene by the soil fungi *Acremonium falciforms* that microbially produced ethylene can affect plant growth of etiolated pea seedlings. Etiolated pea seedlings presented a classical triple response, which included reduction in elongation, swellings of the hypocotyls and a change in the direction of the growth (horizontal), when *A. falciforms* was used as an inoculant. Further studies showed the production of PGPRs by many soil microorganisms in the presence of suitable precursors (Arshad and Frankenberger 1990).

It has also been well documented that the biosynthesis of auxins with their excretion into soil makes a major contribution to the bacterial plant growth-promoting effect (Lambrecht et al. 2000; Steenhoudt and Vanderleyden 2000). Not as well understood as IAA, cytokinins and gibberellins have also been shown to stimulate root and shoot development in several ways (van Loon 2007). Cytokinins,

for example, have been implicated in cell division and nitrogen fixing nodule development (Murray et al. 2007; Tirichine et al. 2007). They also promote rapid growth of the primary root and enhance branching (Ortiz-Castro et al. 2009). Another class of recently discovered phytostimulating compounds called *N*-acyl-L-homoserine lactones have been shown to modulate gene expression in plants. These molecules are also used by bacteria for cell-to-cell communication (Ortiz-Castro et al. 2009). The cofactor PQQ (pyrroloquinoline quinine) was also identified as a plant growth promoting factor while promoting the growth of tomato and cucumber plants (Choi et al. 2008). The results showed the property of PQQ as an antioxidant; however, the effect is mostly indirect.

These studies suggest that microbially released PGRs in the rhizosphere may affect plant growth and may be subjected to direct uptake by plant roots because of the intimate contact between microbial and plant cells.

2.3.2   Phytoalexins

Phytoalexins are low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms (Dakora 1985; Dakora et al. 1993; Van Peer et al. 1991, 1990). The concept of phytoalexin is expanded because many isoflavonoids (the most widely studied class of phytoalexins) were shown to serve as signal molecules during infection of plant roots by symbiotic microbes (Landa et al. 2002). Phytoalexin synthesis can be used as an indicator of enhanced defence mechanism in bacteria-treated plants. An increase in the production of three phytoalexins, for example, rishitin, lubimin, and solvetivon were observed in potato slices and an inhibition in mycelia growth of *Phytophthora infestans* by culture filtrate of *Streptomyces* (Bochow and Fritzsche 1990). It has also been observed that like other plants, Graminae contain secondary plant metabolites that have been found to be toxic to plant pathogenic fungi and bacteria and are proposed to be responsible for resistance to microbial pathogens (Gross 1991).

Similarly, in another study induction and accumulation of phytoalexins in cow pea roots were observed when infected with myccorhizal fungus and also their resistance to *Fusarium* wilt disease. From the studies in our laboratory (Parmar 1995; Parmar and Dadarwal 1999), it was apparent that the rhizosphere bacteria such as fluorescent Pseudomonads and *Bacillus* sp. produced certain signal molecules which probably enhanced the flavonoid production by plant roots. The enhanced flavonoid production could be an additional factor in nodule promotion. In addition, production of phytoalexins was demonstrated to increase after prior inoculation of chick pea (*C. arietinum* L.) seedlings with non-pathogenic isolates of *Fusarium oxysporum* (inducers) and this was correlated with a delay on the onset of symptom and reduction of *Fusarium* wilt development (Landa et al. 2002).

Indirectly, PGPR can act as biofertilizers via asymbiotic nitrogen fixation and the solubilization of mineral phosphates and other nutrients. Rhizobacteria can also

act as biocontrol agents by producing siderophores that compete with pathogenic organisms for iron, by producing antibiotics and bacteriocins that suppress bacterial pathogens and by producing anti-fungal metabolites (Ahmad et al. 2008).

The optimization of these interactions may lead to improvement in the yields of various leguminous and non-leguminous crops. In particular, the extension of symbiotic biological nitrogen fixation to non-legume crops would be of enormous economical and environmental benefit.

2.3.3   Biocontrol Agents

Biocontrol agents include molecules that induce an immune response within the plant or molecules that in some way suppress plant pathogens either indirectly by competing for essential nutrients or directly inhibiting growth of phytopathogens.

2.3.3.1   Stimulation of Host Defence

When a plant comes into contact with a pathogenic microorganism, it responds with a systemic acquired response (SAR) where the plant's immune system is primed to defend itself against disease. Many phytopathogenic fungi, for example, are known to induce systemic acquired responses in plants. The most common parasitic fungi belong to genera *Pythium* sp., *Rhizoctonia* sp. and *Fusarium* sp. (Mukerji et al. 2006). Some *Fusarium* sp. cause root rots and wilts and some feed on dead plant tissues (Mukerji et al. 2006). The exact mechanisms for how the plant immune system primes itself are still unknown; however, certain molecules in the pathway such as salicylic acid, for example, appear to play a critical role as a plant messenger once the plant is exposed to a pathogen (Wildermuth et al. 2001). Some PGPR can stimulate a plant's defence system without the presence of a pathogen by emitting molecules similar to those in the plant's SAR. This response is called induced systemic resistance (ISR). Some of these molecules include methyl salicylate (MeSA), methyl jasmonate (MeJA) and ethylene. Thus far, evidence of PGPR eliciting ISR has been observed in carnations (Van Peer et al. 1991), the common bean, cucumber (Wei et al. 1991) and grapevine (Verhagen et al. 2004, 2010). In other experiments, the colonization of root systems with PGPR, such as *P. fluorescens*, *P. putida*, *Bacillus pumilus* and *Serratia marcescens* was protected against foliar diseases (Pieterse et al. 2002).

2.3.3.2   Siderophores

Siderophores are small molecules excreted by rhizobacteria when deficient in iron. By complexing with available iron in the rhizosphere it becomes less available to competing phytopathogens which also require iron thus inhibiting competitor growth. Siderophore production by *P. fluorescens* F113 has been shown to play a

role in biocontrol of potato soft rot under iron limiting conditions (Whipps 2001). In addition, the antifungal activity of test isolates was greatly enhanced when both HCN and siderphores were produced indicating that together these two plant growth-promoting activities work synergistically to inhibit pathogenic fungi and protect plant health (Ahmad et al. 2008).

2.3.3.3 Antibiotics

Many rhizobacteria have been shown to produce antibiotics that inhibit the growth of an antagonistic bacterium. *P. fluorescens* (Trevisan) Migula F113, for example, has been shown to control the soft rot potato pathogen *Erwinia carotovora* subspecies *atroseptica* by producing the antibiotic 2,4-diacetylphloroglucinol (DAPG) (Whipps 2001). Three glucanase-producing actinomycetes, when used separately or more effectively in combination, could significantly promote plant growth and therefore inhibit the growth of *Pythium aphanidermatum* (El Tarabily et al. 2009). Other major antibiotics produced by *B. cereus* are phenazine-e-carboxylic acid and phenazine-1-carboxamide; 2,4-diacetyl phloroglucinol (phl) (Dunne et al. 1998), pyoluteorin (Nowak-Thompson et al. 1999), zwittermicin A (Emmert et al. 2004), gluconic acid (Kaur et al. 2006), 2-hexyl-5-propyl resorcinol (Cazorla et al. 2006) and kanosamine (Milner et al. 1996).

Bacteriocins are proteins that normally kill or inhibit the growth of closely related bacterial strains. *Bacteriocin thuricin* 17 was isolated from the PGPR *Bacillus thuringenisis* NEB17 (Gray et al. 2006). Oddly enough, this novel bacteriocin was able to inhibit the growth of not only related gram positive bacterial strains, but also of a gram negative strains of *Escherichia coli* MM294 (pBS42).

2.3.3.4 Antifungal Metabolites

Many antifungal metabolites have been produced and shown to be effective in vitro. These antifungal metabolites are also suspected to have antifungal activity in vivo. These metabolites include ammonia, butyrolactones, 2-4-diacetylphloroglucinol, HCN, kanosamine, Oligomycin A, Oomycin A, phenazine-1-carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (pln), viscosinamide, xanthobaccin and zwittermycin A (Milner et al. 1996; Whipps 2001). In addition, certain fungi have been shown to be sensitive to particular combinations of metabolites.

2.4 Conclusions

The beneficial effects of the rhizobacteria in enhancing root development associated with increase in nodule biomass by native as well as co-inoculated *Rhizobium* strains are well documented. Some degree of specificity was observed with regard

to plant species as the rhizobacteria from *Cicer* rhizosphere were observed as better co-inoculants compared to rhizobacteria isolated from the other crops such as green gram and pigeon pea. Various studies have also provided an evidence of different mechanisms by which there is an increase in crop productivity and the disease suppressive ability of these rhizobacteria. There is still not enough data to suggest the establishment of the newly isolated rhizobacterial strains in the rhizosphere, but further studies using genetically marked strains should make it possible to determine their exact role in rhizosphere establishment.

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