Review

Plant growth promoting rhizobacteria and their potential for biocontrol of phytopathogens

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Growth promotion and disease control by rhizobacteria are complex interrelated processes that involve direct and indirect mechanisms. The mechanisms include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, production of siderophore, antibiotics, hydrogen cyanide hydrogen cyanide (HCN) and volatile compounds. They also include mineral solubilization competition, and induction of systemic resistance. These bacteria are suitable as soil inoculants because they have the potential for rapid and aggressive colonization. This feature alone is characterised as a disease control mechanism, which prevents the invasion of detrimental soil microorganisms onto the root surface. Inoculant-based plant growth-promoting rhizobacteria (PGPR) is applied extensively on agricultural crops to improve plants' growth and simultaneously reduce chemical inputs like fertilizer and pesticide which can cause environmental degradation. The structure of the rhizobacterial community is affected by several factors including plant genotype and is determined by the amount and composition of root exudates. In addition, soil type and fertility are the contributing factors that shape the community. This form of communication can affect plants' growth, nutrient status and also susceptibility to stress and pathogens in the host plant. PGPR inoculants cause diverse beneficial interactions among plants, which leads to sustainable and environment-friendly agriculture. The application of rhizosphere soil of agricultural crops with desirable bacterial populations is considered promising in both laboratory and greenhouse experiment. Further, a clearer understanding of the way PGPRs promote plants' growth can lead to expanded exploitation of these 'biofertilizers' in order to reduce the potential negative environmental effects associated with food and fiber production.

Key words: Rhizobacteria, plant growth-promoting rhizobacteria (PGPR), root microbione, phytohormones, biocontrol, soil-borne phytopathgen, fluorescent pseudomonads.

INTRODUCTION

Members of the genus *Pseudomonas* are rod-shaped Gram-negative bacteria that are characterized by metabolic versatility, aerobic respiration, motility owing to one or several polar flagella, and a high genomic G+C content (59–68%). The classification method divides all *Pseudomonas* spp. into five groups based on the relatedness of their rRNA genes, which undergo fewer changes than most other DNA sequences in the course of evolution (Von Graevenitz, 1977). Bacteria belonging to the genus *Pseudomonas* are effective root colonizers

and biocontrol agents. Growth promotion and disease control by *Pseudomonas* spp*.* are complex interrelated processes involving direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of ACC deaminase, production of siderophore, antibiotics, hydrogen cyanide HCN and volatile compounds. Others include mineral solubilization competition, and induced systemic resistance (Lucy et al., 2004; Adesemoye et al., 2008). These bacteria are used to improve vegetable crops yield and to reduce economic and environmental costs with mineral fertilizers (Dias et al., 2013).

This large and heterogeneous group comprises, most notably, *Pseudomonas aeruginosa*, *Pseudomonas putida, Pseudomonas fluorescens* and *Pseudomonas syringae*. They are found in soils, foliage, fresh water, sediments, and seawater (Von Graevenitz, 1977). Among PGPR, fluorescent pseudomonads are good rhizosphere colonizers, although they have also been found inside tissues of flowers and fruits (Compant et al., 2010). They possess high rhizosphere competence and they are recognized as one of the main groups of PGPR or plant-probiotic bacteria (Höfte and Altier, 2010). Whereas most studies have focused on suppression of fungi and oomycetes, others have shown that plant-pathogenic nematodes may also be suppressed by the production of various inhibitory compounds (Glick, 2010). Inoculant-based PGPRs are applied extensively on agricultural crops to improve plant growth and at the same time to reduce chemical inputs including fertilizer and pesticide which can cause environmental degradation.

The interaction or communication between plants and rhizobacteria occurs through chemical signals released by both partners. The structure of the rhizobacterial community is affected by several factors including plant genotype and is determined by the amount and composition of root exudates (Marschner et al., 2004). In addition, soil type and fertility are contributing factors that also shape the community (Innes et al., 2004). The rhizobacterial community may influence this interaction by exuding compounds as a means of communication recognized by neighbouring bacteria and root cells of host plants (Bais et al., 2004; Gray and Smith, 2005). This form of communication can affect plant growth, nutrient status and also susceptibility to stress and pathogens in the host plant (Morgan et al., 2005).

ROOT MICROBIOME

Rhizosphere is the thin layer of soil adjacent to plant roots that are influenced by root activities. This term was first introduced by Lorenz Hiltner, a soil microbiologist, in the early 1900's after years of studying the role of different plant (legumes and non-legumes) root exudates in attracting different bacterial communities surrounding the root zone. He also studied how the bacteria colonizing the root surface and epidermis influence plants' nutrient availability (Hartmann et al., 2008). Hiltner's original definition of rhizosphere now extends to the larger proportion of the soil around plant roots that are also affected by root growth and the physical, chemical and biological properties of the soil (McCully, 2005). The rhizosphere is an intense interactive zone as the root releases sugars, amino acids and other organic compounds that can be utilised by soil microorganisms, including bacteria, for their viability (Dobbelaere et al., 2003; Singh et al., 2004; Lambers et al., 2009).

This nutritious environment results in a much higher population of bacteria in the rhizosphere but lower diversity/species richness than the bulk soil (van Loon and Bakker, 2003; Lugtenberg and Kamilova, 2009). The bacteria that occupy the rhizosphere are collectively termed rhizobacteria. Rhizobacteria can have profound effects on plant health and nutrition.

Antoun and Prévost (2006) classified rhizobacteria as being neutral, deleterious or beneficial. The presence of the neutral group might be insignificant to the host plant, while deleterious rhizobacteria produce metabolites that are adverse to plant health. The concept of deleterious rhizobacteria is debatable because previous studies were mostly done in gnotobiotic and soil-less conditions without any challenge from native soil bacteria (Antoun and Prevost, 2006), and these conditions are unlikely to exist naturally. Glick et al. (1999) stated that more destructive effects on agronomically important crops are mostly caused by phytopathogenic fungi, such as *Fusarium* and *Phytium* genera; thus the negative effects of deleterious rhizobacteria on plant growth are rarely discussed in relation to this topic. The beneficial categories of rhizobacteria are able to promote plant growth and development, and are generally further grouped according to their physical interaction with the host plant (Glick et al., 1999). Beneficial rhizobacteria may form symbiotic interactions which involve modification of the morphology of the host plant root through nodule formation. Other beneficial rhizobacteria are free-living in the soil and employ associative relationships with the host plant. These free-living rhizobacteria are defined as PGPR and form associations with many different plant species (Kloepper et al., 1989).

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by both partners. The structure of the rhizobacterial community is affected by several factors including plant genotype and is determined by the amount and composition of root exudates (Marschner et al., 2004).

Ashrafuzzaman et al. (2009) reported that PGPRs are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Plant growth promoting rhizobacteria (PGPR) can be applied to a wide range of plant and would promote growth and disease control (Yan et al., 2003). Biocontrol effects are not only characterized by reductions of pathogen level, but also by an increase of tolerance and/or resistance, growth and yield of inoculated plants. To increase agricultural efficiency, increase in plant growth by using eco-friendly alternatives is essential for sustainable agricultural production. Thus, the use of natural processes to improve the quantity and quality of agronomics can result in development of expanded food production system, which will ultimately bring sustainability to the ecological systems (Avis et al., 2008; Berg, 2009).

Currently, there is increasing interest in the introduction of bacterial biocontrol agents for managing soil-borne pathogens, partly as a response to public concerns about deleterious effect of synthetic fungicides and also because of the lack of effective control for soil-borne pathogens (Cook, 1993; Schmiedeknecht et al., 2001). In this direction, biological control of soil-borne diseases and plant growth promotion by an application of specific microorganisms to seed or planting materials has been studied over the last years (Thomashow and Weller 1996; Kilian et al., 2000; Bochow et al., 2001; Schmiedeknecht et al., 2001; Swelim et al., 2003).

PLANT GROWTH PROMOTING RHIZOBACTERIA

This group of rhizobacteria is mostly Gram-negative and rod-shaped; a lower proportion is Gram-positive rods, cocci and pleomorphic. Examples include *Allorhizobium undicola* (de Lajudie et al., 1998a), *Azorhizobium caulinodans* (Dreyfus et al., 1988), *Bradyrhizobium japonicum* (Guerinot and Chelm, 1984), *Mesorhisobium chacoense* (Velazquez et al., 2001), *Mesorhizobium pluriforium* (de Lajudie et al., 1998b), *Rhizobium ciceri* (Nour et al., 1994), *Rhizobium etli* (Segovia et al., 1993), *Rhizobium fredii* (Scholla and Elkan, 1984), *Rhizobium galegae* (Lindstrom, 1989), *Rhizobium gallicum*, *Rhizobium giardinii* (Amarger et al., 1997), *Sinorhizobium arboris* (Nick et al., 1999), *Sinorhizobium fredii* (Chen et al., 1988) and *Sinorhizobium medicae* (Rome et al., 1996).

PGPRs were first defined by Kloepper and Schroth (1978) as root-colonizing bacteria that are beneficial for plant growth. Due to their importance in increasing seedling emergence, vigor, biomass, proliferation of root systems, and crop yield in many species, several studies have focused on identifying PGPR in natural systems and the development of these bacterial strains for commercial use (Podile and Kishore, 2006).

PGPRs inhabit the rhizosphere, the volume of soil under the immediate influence of the plant root system, and favor the establishment of a large amount of active microbial population. Plants release metabolically active cells from their roots and deposit as much as 20% of the carbon allocated to roots in the rhizosphere, suggesting a highly evolved relationship between the plant and rhizosphere microorganisms (Handelsman and Stabb, 1996). Rhizosphere is subject to dramatic changes, which create interactions that lead to biocontrol of diseases (Rovira, 1965, 1969, 1991; Hawes, 1991; Waisel et al., 1991). *Streptomyces* spp. have been described as rhizosphere-colonizing bacteria and antifungal biocontrol agents useful in controlling fungal root diseases, and able to work *in vitro* as producers of siderophore and plant growth-promoting hormones (Rothrock and Gottlieb, 1984; Miller et al., 1990). PGPRs are free-living bacteria that have beneficial effects on plants. PGPRs enhance emergence of seedlings, colonize roots and stimulate overall plant growth. They also improve seed germination, root development, mineral nutrition and water utilization. They can also suppress diseases of plants. The manipulation of the crop rhizosphere by inoculation with PGPR for biocontrol of plant pathogens has shown considerable promise (Handelsman and Stabb, 1996; Siddiqui and Mahmood, 1999; Berg et al., 2002; Nelson, 2004).

A diverse array of bacteria, including species of *Pseudomonas*, *Bacillus*, *Azospirillum* and *Azotobacter* has been shown to promote plant growth. The mechanism by which these rhizobacteria enhance plant growth are not clear, but it is postulated that they may produce phytohormones, suppress plant pathogens, fix nitrogen, mineralize organic phosphorus and/or enhance mineral uptake (Grayston et al., 1990; Joo et al., 2004).

The search for PGPRs and their mode of action is increasing at a rapid rate in order to use the best PGPR strains as commercial biofertilizer. Investigations into the mechanisms of plant growth promotion by PGPR strains indicated that effective PGPRs increased plant growth basically by changing the whole microbial community structure in rhizosphere (Kloepper and Schroth, 1981). According to Glick et al. (1999), the general mechanisms used for plant growth promotion by PGPR include associative nitrogen fixation, lowering of ethylene levels, production of siderophores and phytohormones, induction of pathogen resistance, solubilization of nutrients, promotion of mycorrhizal functioning, decreasing of pollutant toxicity etc. Castro et al. (2009) suggested that PGPR strains can promote plant growth and development

either directly and indirectly. Direct stimulation includes biological nitrogen fixation, producing phytohormones like auxins, cytokinins and gibberellins, solubilizing minerals like phosphorus and iron, production of siderophores and enzymes and induction of systemic resistance, while indirect stimulation is basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for niches within the rhizosphere (Zahir et al., 2004; van Loon 2007). PGPR strains, especially *P. fluorescens* and *Bacillus subtilis* are best recorded as the most promising candidates for indirect stimulation (Damayanti et al., 2007). Besides, nitrogen transformation, increasing bioavailability of phosphate, iron acquisition, exhibition of specific enzymatic activity and plant protection from harmful pathogens with the production of antibiotics can also successfully improve the quality of crops in agriculture (Spaepen et al., 2007). Thus, based on their mechanism of action, PGPRs can be categorized into three general forms: biofertilizer, phytostimulator and biopesticide. The phenomenon of quorum regulation can affect the expression of each of these traits as PGPRs are reported for their regular interactions with the resident microbial community in rhizosphere (Lugtenberg and Kamilova, 2009). PGPR may use more than one of these mechanisms to enhance plant growth as experimental evidence suggests that the plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously (Martinez-Viveros et al., 2010).

Biochemical and molecular approaches are providing new insight into the genetic basis of these biosynthetic pathways, their regulation and importance in biological control (Joshi and Bhatt, 2011). However, to be more effective in the rhizosphere, PGPR must maintain a critical population density for a longer period, although inoculation of plants with PGPR can temporarily enhance the population size. Regarding *Pseudomonas* role in producing PGPs, Lifshitz et al. (1987) found that inoculation of canola (*Brassica compestris* seeds with a nitrogen-fixing strain of *P. putida* (GR122) drastically increased the root length of seedlings grown in sterile growth pouches. El-Khawas (1995) and Forlani et al. (1995) identified several bacteria strains of genera *Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsilla, Sarcina* and *Pseudomonas* isolated from the rhizosphere of various crops as auxins producer strains. El-Khawas et al. (2000) reported that the ability of *Pseudomonas* sp. and other isolates to produce auxins for different incubation periods was measured using spectrophotometer and determined as ug/ml minimal media supplemented with glucose and tryptophan. The amounts of auxins produced were greater after 72 h than 24 or 48 h. The amounts of auxins ranged from 33 to 75 ug/ml after 72 h.

Filamentous actinobacteria are also considered as one

of the important community in rhizosphere microbiota. Garcia de Salamone et al. (2001) stated that five plant growth promoting rhizobacteria (PGPR) strains produced the cytokinin dihydrozeatin riboside (DHZR) in pure culture.Cytokinin produced by *P. fluorescens* G20-18.a rifampicin resistance mutant (RIF), and two *TnphoA*-derived mutants (CNT1, CNT2), with reduced capacity to synthesize cytokinins, were further characterized in pure culture. G20-18 produced higher amounts of isopentenyl adenosine (IPA), trans-zeatin ribose (ZR), and DHZR than the three mutants during stationary phase. IPA was the major metabolite produced, but the proportion of ZR and DHZR accumulated by CNT1and CNT2 increased with time. No differences were observed between strain G20-18 and the mutants in the amounts of indole acetic synthesized; gibberellins were not detected in supernatants of any of the strains. Schmiedeknecht et al. (2001) studied the effect of different environmental conditions on plant growth promotion of two *B subtilis* strains. In these studies, culture solution and soil under different ecological factors were used. They pointed out that these bacterial strains may produce substances that enhance plant growth and yield of maize and sunflower.

Patten and Glick (2002) reported that indole acetic acid accumulates in the culture medium of the plant growth promoting bacterium *P. putida* Gr12-2 only when grown in the presence of oxogenous tryptophan. This suggests that the expression of indole pyruvate decarboxylase, a key enzyme in the IAA biosynthesis pathway in this bacterium, may be regulated by tryptophan. Bai et al. (2002) isolated plant growth promoting *Bacillus* sp. from surface sterilized soybean root nodules. Three isolates were found to increase soybean weight when soybean seedlings were co-inoculated with one of the isolates and *Bradyrhizobium japonicum* and / or nitrogen – free condition compared with plants inoculated with *B. japonicum*.

Bacillus is a Gram- positive aerobic organism that can resist environmental stress by forming endospores (Kumar et al., 2011); many strains of *Bacillus* and *Paenibacillus* are known to stimulate plant growth. Emmert and Handelsman (1999) highlighted the endospore forming character of *Bacillus* as important for a potential biocontrol inoculant. This is because the spore can endure heat and desiccation ensuring the formulation will be stable over time. This genus is considered non-rhizosphere competent, unlike *Pseudomonas*; but given that rhizospheric competency is strain-dependent, some strains of *Bacillus* may be rhizosphere competent (Kumar et al., 2011).

Nasr (2002) revealed that the highest levels of auxin were produced by *Bacillus cereus* and *P. fluorescens*, grown on shaker as a batch culture of 8.3 and 4.4 mg/L, respectively. Regarding the effect of different concentrations of tryptophan (TRP) and zinc (Zn) added to the culture media for maximizing the biosynthesis of

auxin, *P. fluorescens* recorded the highest amount of auxin among tested microorganisms. The higher auxin excreted was 8.3, 11.5, 10.3, and 13 mg/L on King's medium, medium supplemented with tryptophan, medium supplemented with zinc (on orbital shaker) and medium containing 0.1 mg/ml TRP + 0.001 mg/ml Zn (in fermentor), respectively.

The inoculation of *Pinus pinea* plants with plant growth promoting rhizobacteria of the genus *Bacillus* (*B. licheniformis* CECT5001 and *B. pumilus* ECT501s) promoted the growth of *P. pinea* seedling. This is probably caused by gibberellin production (Probanza et al., 2002). Joo et al. (2004) isolated *B. cereus* Mj-1, *B. macroides* Cj-29, and *B. pumilus* from the rhizosphere of red pepper which promoted the growth of seedlings.

Gibberellins (GAs), a well-known plant growth promoting hormone, were detected in the culture broth of their rhizobacteria. Khaled et al. (2003) noted that the inoculation of 4 cultivars of wheat with plant growth promoting rhizobacteria (*Pseudomonas* sp.) significantly increased plant height (up to 9.9%), number of tillers (up to 32.3%) and spike, spike length (up to 6.8%), straw and yields (up to 16.1, 29.0%), respectively in all tested cultivars of wheat with different degrees of efficiency. Lucas-Garcia et al. (2003) reported that the inoculation of seedling (*Capsicum annuum cv. Roxy*) with *P. fluorescans* Aur 6 as a plant growth promoting bacteria (PGPB) significantly enhanced all biometric parameters measured such as fresh weight, height, neck root diameter and slender index (height/neck root diameter). *P. fluorescans* strain Aur 6 effects could be related to auxin and siderophore production.

The colonization of plant rhizosphere by *Azospirillum* sp., *B. subtilis* sp., and *Pseudomonas* sp., has been well studied (Steenhoudt and Vanderleyden, 2000; Trivedi et al., 2005). Moreover, immobilized form of PGPR inoculants in comparison to free forms has greater ability of survival and plant root colonization. It has been reported that soil microorganisms, including free-living as well as associative and symbiotic rhizobacteria belonging to the genera like *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Proteus, Pseudomonas, Rhizobium, Serratia, Xanthomonas* in particular, are the integral parts of rhizosphere biota (Glick, 1995; Kaymak, 2011) exhibiting successful rhizosphere colonization. Lugtenberg et al. (2001) reported a large number of cell surface molecules as responsible for effective rhizosphere colonization. Rhizospheric colonization is thus considered as a crucial step in the application of microorganisms for beneficial purposes such as biofertilization, phyto-stimulation, biocontrol and phytoremediation; although the colonization of rhizosphere by PGPRs is not a uniform process. For example, *Kluyvera ascorbata* colonized the upper two-thirds of the surface of canola roots but no

bacteria were detected around the root tips (Ma et al., 2001).

Phosphorus is one of the most essential nutrient requirements in plants. Ironically, soils may have large reservoir of total phosphorous (P) but the amounts available to plants are usually a tiny proportion of this total. This low availability of phosphorous to plants is because of the vast majority of soil P found in insoluble forms; and the plants can only absorb it in two soluble forms: mono-basic ($H_2PO_4^-$) and diabasic (HPO_4^{2-}) ions (Glass, 1989). Several phosphate solubilizing microorganisms (PSMs) are now recorded to convert the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids or protons (Richardson et al., 2009) and chelation and exchange reactions (Hameeda et al., 2008). Saprophytic bacteria and fungi are reported in the chelation-mediated mechanisms (Whitelaw, 2000) for solubilizing phosphate in soil. Release of plant root exudates such as organic ligands can also alter the concentration of P in soil solution (Hinsinger, 2001).

According to Nahas (1996), phosphate solubilization takes place through various microbial processes including organic acid production and proton extrusion. In certain cases, phosphate solubilization is induced by phosphate starvation (Gyaneshwar et al., 1999). Bacterial genera like *Azospirillum, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are the most significant phosphate solubilizing bacteria (Sturz and Nowak, 2000; Sudhakar et al., 2000; Mehnaz and Lazarovits, 2006). Rhizobacteria can solubilize inorganic P sources and enhance growth and yield of crop plants. Besides, examples of some widely reported P solubilising microbial species intimately associated with a large number of agricultural crops like potato, tomato, wheat, radish, pulses etc., are *Azotobacter chroococcum, Bacillus circulans* and *Cladosporium herbarum* (Singh and Kapoor, 1999), *B. japonicum* (Antoun et al., 1998), *Enterobacter agglomerans* (Kim et al., 1998), *Pseudomonas chlororaphis* and *P. putida* (Cattelan et al., 1999) and *Rhizobium leguminosarum* (Chabot et al., 1998).The ability of PGPRs to solubilize mineral phosphate, therefore, has been of immense interest to agricultural microbiologists since it can enhance the availability of phosphorus for effective plant growth. PGPRs have been recorded to solubilize precipitated phosphates to plants, representing a possible mechanism of plant growth promotion under field conditions. Synthesis of organic acids by rhizosphere microorganisms could be the possible reason for solubilization of inorganic P sources (Verma et al., 2001).

Sumera et al. (2004) revealed that 12 plant growth promoting rhizobacteria *Bacillus* strains were isolated from rice. Nine isolates produced indole acetic acid

ranging from 20.0 - 90.8 mg/L. Most of the isolates showed resistance against environmental stresses like 10 - 40°C, 0.2 - 1.0 M salt concentration and 4.0 - 8.5 pH range. Inoculation with these bacterial isolates resulted in higher plant biomass, root area and total N and P contents in Tanzannian rice variety BKWPRAT 3036B under controlled condition. Concurrently, the bacterial enzyme ACC deaminase acts as an extracellular sink for plant-produced ethylene precursor, ACC. It metabolizes it into the inert byproducts ammonia and "ketobutyrate, reduces the amount of ACC available for conversion into ethylene and minimizes the stress response that is a result of increased ethylene concentration in the plant (Gamalero et al., 2009). Several direct and indirect mechanisms for growth-promotion have been documented.   Direct mechanisms include nitrogen-fixation (Bashan et al., 2004), production of phytohormones such as the auxin indole-3-acetic acid (IAA), which stimulates cell growth and proliferation at low concentrations (Vessey, 2003), metabolism of the ethylene precursor 1, aminocyclepropane-1-carboxylate (ACC) through the enzyme ACC deaminase (Glick et al., 1998), and increased availability of iron through bacterial production of siderophores (Kloepper et al., 1991).

Ashrafuzzaman et al. (2009) observed that isolates PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induced the production of IAA, whereas only PGT3 isolate was able to solubilize phosphorus. Most of the isolates resulted in a significant increase in plant height, root length, and dry matter production of shoot and root of rice seedlings. Furthermore, PGPR isolates remarkably increased seed germination of rice. Among the ten isolates, PGB4 and PGG2 were found almost equally better in all aspects such as dry matter production, plant height and root length of rice, and IAA production. Isolate PGT3 was also found to be promising in IAA production having an additional property of phosphate solubilization. Growth promoting substances are likely to be produced in large quantities by these rhizosphere microorganisms that influence indirectly the overall morphology of the plants. Recent progress in our understanding on the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action should facilitate their application as a reliable component in the management of sustainable agricultural system (Bhattacharyya and Jha, 2012). There are some PGPRs that can exert a positive plant growth by direct mechanisms such as solubilization of nutrients, nitrogen fixation, production of growth regulators, etc., or by indirect mechanisms such as stimulation of mycorrhizae development, competitive exclusion of pathogens or removal of phytotoxic substances (Bashan and de-Bashan, 2010). However, in accordance with their degree of association with the plant root cells, PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth

promoting rhizobacteria (iPGPR) (Martinez-Viveros et al., 2010). The ePGPRs may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex; on the other hand, iPGPRs locate generally inside the specialized nodular structures of root cells. Potential role of PGPRs in conferring resistance to water stress in tomatoes and peppers has been investigated (Mayak et al., 2004).

Fluorescent pseudomonads and species of *Bacillus* were reported with very high efficiency in host root colonization and production of growth metabolites resulting in improved strategic crop yield (Khalid et al., 2004). Plant-root interactions in rhizosphere may include root–root, root-insect and root-microbe interactions, resulting in the production of more root exudates that ultimately favour maximum microbial populations (rhizosphere engineering) in this ecologically significant region. Changes in rhizobacterial community structure have been reported with the application of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), resulting in significant alterations in plant–microbes interactions (Herschkovitz et al., 2005). However, successful root colonization and persistence of PGPRs in plant rhizosphere are required in order to exert their beneficial effect on the plant (Elliot and Lynch, 1984).

The intimacy between the plants and environment in rhizosphere is thus essential for better acquisition of water and nutrients by plants as well beneficial interactions of plants with soil-borne microorganisms (Ryan et al., 2009). According to Cardoso and Freitas (1992) the rhizosphere microbial communities are vigorously associated with the biogeochemical cycling of nutrients like C, P, N and S, removal of toxins and production of phytohormones or antibiotics etc. Rhizobacteria may depend on other microbes for nutrient sources as one microbe may convert plant exudates into a form that can be used by another microbe. Thus, rhizosphere has a versatile and dynamic ecological environment of intense plant-microbe interactions (Mayak et al., 2004) harnessing essential micro and macro-nutrients affecting plant growth; although, the process of root colonization is under the influence of various parameters such as bacterial traits, root exudates and several other biotic and abiotic factors (Benizri et al., 2001). PGPR can alter root architecture and promote plant development with the production of different phytohormones like IAA, gibberellic acid and cytokinins (Kloepper et al., 2007). Several PGPRs as well as some pathogenic, symbiotic and free living rhizobacterial species are reported to produce IAA and gibberllic acid in the rhizospheric soil and thereby play a significant role in increasing the root surface area and number of root tips in many plants (Han et al., 2005).

Recent investigations on auxin synthesizing rhizobacteria (Spaepen et al., 2007) as phytohormone

producer demonstrated that rhizobacteria can synthesize IAA from tryptophan by different pathways; although the general mechanism of auxin synthesis was basically concentrated on the tryptophan-independent pathways. Phytopathogenic bacteria rather use the indole acetamide pathway to synthesize IAA that has been implicated earlier in the tumor induction in plants. Swain et al. (2007) reported a positive effect of IAA producing strains of *B. subtilis* on *Dioscorea rotundata* L. They applied a suspension of *B. subtilis* on the surface of the plant, which resulted in an increase in the root: stem ratio as well as number of sprouts compared to the non-inoculated plants. Potentiality of *Azotobacter* spp. to produce high amount of IAA (7.3–32.8 mg/ml) in agriculture was reported by Ahamad et al. (2005). Similarly, significant shoot growths in maize and rice dwarf mutants were promoted by gibberellins-like substances excreted by *Azospirillum* spp. (Boiero et al., 2007).

Ribaudo et al. (2006) represented some of the efficient PGPR strains as the producer of different plant growth regulators. IAA-mediated ethylene production could increase root biomass, root hair number and consequently the root surface area of PGPR inoculated tomato plants. Involvement of PGPR formulated cytokinins was also observed in root initiation, cell division, cell enlargement and increase in root surface area of crop plants through enhanced formation of lateral and adventitious roots (Werner et al., 2003). It has been established that the working pathways of these phytostimulators leading to overall development in crop plants are differently regulated by catabolite repression as physiological regulator of biofilm formation (Zaied et al., 2009).

The discovery of rhizobacterial-produced volatile organic compounds (VOCs) constitutes an important mechanism for the elicitation of plant growth by rhizobacteria. Ryu et al. (2003) recorded some PGPR strains namely *B. subtilis* GB03, *B. amyloliquefaciens* IN937a and *Enterobacter cloacae* JM22 that released a blend of volatile components, particularly, 2, 3-butanediol and acetoin, which promoted growth of *Arabidopsis thaliana.* This suggests that synthesis of bioactive VOCs is a strain-specific phenomenon. Acetoin-forming enzymes have been identified earlier (Forlani et al., 1999) in certain crops like tobacco, carrot, maize and rice, although their possible functions in plants were not properly established in that period. It has now been established that the VOCs produced by the rhizobacterial strains can act as signaling molecule to mediate plant–microbe interactions as volatiles produced by PGPR colonizing roots are generated at sufficient concentrations to trigger the plant responses (Ryu et al., 2003). Farmer (2001) identified low-molecular weight plant volatiles such as terpenes, jasmonates and green leaf components as potent signal molecules for living

organisms in different trophic levels. However, to acquire a clear appreciation on the mechanisms of VOCs in signaling plants to register plant defence more investigations into the volatile components in plant-rhizobacteria system should follow.

PGPRs comprise a broad range of soil bacterial taxa (Vessey, 2003; Lucy et al., 2004). Some common and well identified genera are *Azospirillum, Pseudomonas, Azotobacter*, and *Bacillus. Azospirillum* is a Gram negative, motile vibrio or *spirillum*, 1 µm in diameter, and is one of the most well studied genera since it is a free-living beneficial root associated bacterium (Morgan et al., 2005).The Bashan foundation, a non-profit scientific organization in Oregon, USA, has extensively studied and dedicated one of its major research programs to PGPR especially *Azospirillum*. The foundation provides a number of comprehensive papers on this particular genus, from the effective isolation and quantification methods from wheat roots, root colonization characteristics in different plant species, detailed PGP mechanisms, ecology, agricultural applications, physical and molecular studies and also the future challenges and potential use of *Azospirillum* as a commercial PGP inoculant (Bashan and Levanony, 1985; Bashan et al., 2004; Mayak et al., 2004; Bashan and de-Bashan, 2010).

Iron is abundant in the Earth's crust but most of it is in the highly insoluble from of ferric hydroxide and thus unavailable to organisms in soil solution. Some bacteria have developed iron uptake systems (Neilands and Nakamura, 1991). These systems involve a siderophore – an iron binding ligand – and an uptake protein, needed to transport iron into the cell. It has been suggested that the ability to produce specific siderophores, and/or to utilize a broad spectrum of siderophores, may contribute to the root colonizing ability of *Pseudomonas* strains. The production of siderophores that chelate, and thereby scavenge, the ferric iron in the rhizosphere, may result in growth inhibition of other microorganism whose affinity for iron is lower (Kloepper et al., 1988). Siderophore mechanisms will only be relevant under conditions of low iron availability. As soil pH decreases below 6, iron availability increases and siderophores become less effective (Neilands and Nakamura, 1991). Optimal suppression of pathogens occurred at levels between 10^{-9}-10^{-24} M. The critical level of iron at which a siderophore-producing strain of *P. putida* suppressed the growth of a fungal pathogen, *Fusarium oxysporum*, was found to be $< 10^{-16}$ M (Neilands and Nakamura, 1991). Since the synthesis of each siderophore generally requires the activity of several gene products (Mercado-Blanco et al., 2001), it is difficult to genetically engineer bacteria to produce modified siderophores. Complementation studies of siderophore-deficient mutants of *P. fluorescens* M114 indicated that at least five separate genetic loci are needed to encode the enzymes involved in the synthesis of the siderophore pseudobactin M114 (O'Sullivan et al.,

1990).

Zulfitri (2012) has shown the importance of selecting the most effective plant-microbe interactions by screening desired PGP traits and the most responsive host plant to ensure the most beneficial effects on plant growth. Sp245 culture grown with tryptophan and *L. stoechas cutting* was the most effective combination and showed comparable root growth parameters compared to commercial rooting hormone; it was superior to the control treatment. The IAA concentration and Sp245 cells contained in the immersion solution most likely contributed to the improved root growth. Peat cultures of Sp245 as possible inoculants formulation were ineffective for *L. stoechas* cutting-based propagation due to the low concentration of IAA. This study has contributed information to the application of IAA-producing rhizobacteria in plant propagation, specifically ornamental cutting production, and also has attempted to explore the possibility of reducing the synthetic root growth hormone in ornamental propagation.

ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IN BIOLOGICAL CONTROL

Biological control plays an important role in suppression of soil-borne plant pathogens. It is defined as, the reduction of inoculum density in its active or dormant state, by one or more organism. Biological control and biological preparations occupy a very tiny place on the map of plant production (Harman, 1991). The rhizosphere of plants is the habitat of a community comprising many different organisms. Soil bacteria often possess traits that enable them to act as antagonists by suppressing soil-borne plant diseases, for example, by excreting antifungal metabolites that directly or indirectly support plant growth (Haas and Defago, 2005).

Rhizobacteria as biocontrol agents

Soil-borne pathogens are well known for their devastating effects on plant health and yield. For successful disease management, it is important to find the most effective and economical ways to protect the plant from various pests or diseases. The use of PGPR as inducers of systemic resistance in crop plants against different pathogens has been demonstrated under field conditions (Wei et al., 1996). The use of natural PGPR strains in plant frontline defense may offer a practical way to deliver immunization. PGPRs have been reported to increase plant resistance to fungal, bacterial and viral diseases (Maurhofer et al., 1998), insects (Zehnder et al., 1997) and nematodes (Sikora, 1992). Mode of action studies has revealed that biological control by PGPR involves production of bacterial metabolites that reduce the

population or activities of pathogens or deleterious rhizosphere microflora (Glick, 1995; Kloepper, 1996). These metabolites may include siderophores that bind Fe, making it less available to certain members of the native pathogenic microflora (Berthelin et al., 1991; Subba Rao, 1993).

Ghonim (1999) reported that *B. subtilis* reduced the harmful effect of *F. oxysporum*, the causative agent of tomato wilt disease. Tomato seeds treated with biocontrol agent, *B. subtilis* and sown in soil infested with *F. oxysporum* produced less infected plants compared to those treated with the pathogen only; and also had improved some growth parameters such as fresh and dry weights of shoots and roots. Kazmar et al. (2000) stated that *B. cereus* had beneficial effects on crop health including enhancement of soybean yield and nodulation, suppression of damping-off of tomato and suppression of damping-off alfalfa disease (Benizri et al., 2001), being able to influence the plant development as well to protect the plant roots against phytopathogens. Ezzat et al. (2001) studied microbial communities of the rhizosphere of peto-86, Pritchard and Super-Marmande tomato *(Lycopersicon esculentum Mill)* cultivars. The identified antifungal substances produced by rhizobacteria belonged to genera, *Bacillus, Enterobacter* and *Pseudomonas*. The most active antifungal producer was *Bacillus* sp. which was identified as *B. subtilis*. King's broth medium was the most suitable one for antifungal substances produced by *B. subtilis*.

Sarhan et al. (2001) observed that cell-free culture filtrate of *B. subtilis* inhibited the mycelial growth, radial growth, spore germination germ-tubes length of *F. oxysporum* LSP *lycopersici* and also fusaric acid decreased. They also found that treatment of tomato seedlings with *B. subtilis* spore suspension reduced tomato wilt disease index and fusaric acid content in tomato plants. On the other hand, treatment with *B. subtilis* spore suspension enhanced the growth parameters of tomato plants and inhibited the disruption of parenchymatous tissues of cortex of crown region of tomato seedlings. Volatile of *Stenotrophomonas*, *Serratia*, and *Bacillus* species inhibited mycelial growth of many fungi and *A. thaliana* (40 to 98%), and volatile of *Pseudomonas* species and *Burkholderia cepacia* retarded the growth to lesser extents. *Aspergillus niger* and *Fusarium* species were resistant, and *B. cepacia* and *Staphylococcus epidermidis* promoted the growth of *Rhizoctonia solani* and *A. thaliana*. Bacterial volatiles provide a new source of compounds with antibiotic and growth-promoting features (Berg et al., 2005; Vespermann et al., 2007).

Numbers of reports (Gomes et al., 2000; Sousa et al., 2008; Köberl et al., 2013; Köberl, 2013) are available on the potential of actinomycetes as plant growth-promoting agent. Actinomycetes strains like *Micromonospora* sp.*, Streptomyces* spp.*, Streptosporangium* sp.*,* and

Thermobifida sp *.,* are recorded as best to colonize the plant rhizosphere, showing an immense potentiality as biocontrol agent against a range of root pathogenic fungi (Franco-Correa et al., 2010). Rhizosphere *streptomycetes* as potential biocontrol agent of *Fusarium* and *Armillaria* pine rot and as PGPR of *Pinus taeda* were reported (de Vasconcellos and Cardoso, 2009). Evidences are now available on actinobacteria used in the control of *R. solani and Pseudomonas solanacearum* in tomato and *Colletotrichum musae* in banana (Taechowisan et al., 2003). Soil actinomycetes are also an important source of diverse antimicrobial metabolites (Terkina et al., 2006). de Vasconcellos et al. (2010) isolated and screened antagonistic actinobacteria of *Araucaria angustifolia* rhizosphere for the production of active metabolites. The metabolites, especially, IAA and chitinase are recorded as responsible for the degradation of different complex and relatively recalcitrant organic compounds present in soil. Similar antagonistic activity of endophytic *Streptomyces griseorubiginosus* against *F. oxysporum* f. sp. *cubense* has been recorded by Cao et al. (2004).

Mode of action for the suppression of phytopathogens by PGPRs

The growth stimulation in plants by PGPR can be a direct effect of production of secondary metabolites such as auxins, IAA, cytokinins, riboflavin and vitamins (Dakora, 2003). These stimulate growth of plant organs via cell division and expansion (Campanoni et al., 2003) or by improving nutrient availability (Glick, 1995; Chabot et al., 1996; Yanni et al., 1997). They also release organic acids, which help to make available forms of nutrients (Biswas et al., 2000) and often lead to increase plant growth through uptake of water and mineral nutrients or indirectly when the rhizobia inhibits pathogens or deleterious microorganisms by producing siderophores, HCN (Vidhyasekaran and Muthamilan, 1999; Wei et al., 1996) and antibiotics (Glick, 1995) in the rhizosphere.

One of the major mechanisms postulated for the biological control of plant root diseases is the production of antimicrobial compounds by the disease control agent. Hanlon et al. (1994) revealed that *B. subtillus* inhibited phytopathogenic fungi by antibiosis mechanism; it produced a lipopeptide substance. Anjaiah et al. (1998) selected *P. aeruginosa* PNA1 from a total of 98 fluorescent pseudomonads isolated from chickpea rhizosphere in India. This strain was highly and widely effective against a number of phytopathogenic fungi and Oomycetes. Antagonism could be attributed to the production of 1-substituted phenazine, such as phenazine carboxylic acid (PCA) and ixychlororaphin (OCP). Shirifi et al. (1998) stated that the antimicrobial compound 2,4 diacetylphloroglucinol produced by

fluorescent pseudomonads was used for protecting plant roots against fungal pathogens.

Anjaiah et al. (2003) reported that *P. aeruginosa* PNA1, an isolate from chickpea rhizosphere in India, protected pigeonpea and chickpea plants from *Fusarium* wilt disease, caused by *F. oxysporum* f. sp. *ciceris* and *Fusarium udum*. Inoculation with strain PNA1 significantly reduced the incidence of *Fusarium* wilt in pigenopea and chickpea on both susceptible and moderately tolerant genotypes. Root colonization of pigeonpea and chickpea showed ten-fold lower root colonization of susceptible genotypes than that of moderately tolerant genotypes. This indicates that this plant-bacteria interaction could be important for disease suppression in this plant. Strain PNA1 produced two phenazine antibiotics: phenazine-1-carboxiylic acid and oxychlororaphin, *in vitro*. It has been shown before that phenazines, mainly pyocyanin (De Vleesschauwer et al., 2006), and certain lipopeptides (massitolide) (Tran et al., 2007) are able to trigger an immune response in the plant which will lead to systemic disease resistance in leaves. Previous research has made it clear that *Pseudomonas* CMR12a produced two cyclic lipopeptides, one that is related to tolaasin, and another one related to the orfamides that are also produced by the well-known biocontrol agent, *P. fluorescens* Pf5. Phenazines and cyclic lipopeptides produced by *Pseudomonas* CMR12a are involved in biocontrol against *R. solani* on bean and cabbage (D'aes et al., 2011). Mutant analysis has revealed that phenazines can be active alone, while the two cyclic lipopeptides produced by *Pseudomonas* CMR12a act in concert and are both necessary for effective biocontrol. The various modes of action of a *B. subtilis* strain, FZB24 against phytopathogens are examined by Kilian et al. (2000), showing the role of the bacterium in plant vitality (Figure 1).

According to Cakmakci et al. (2006), soil rhizobacterial populations are capable of exerting beneficial effects on many plants like wheat, potato, maize, grasses, pea and cucumber by colonizing rhizosphere. Applications of PGPR increased the nodulation and nitrogen fixation of soya bean (*Glycine max* L. Merr.) over a wide range of root zone temperatures (RZTs). Thus, it has been established that the inoculation of PGPRs can increase nodulation, nitrogen uptake, growth and yield response of crop plants. In addition to this, employing microorganisms as co-culture in biotization is also another important area of research (Sekar and Kandavel, 2010) in recent decade.

Large numbers of PGPR strains of different bacterial classes and genera with multifunctional traits have, therefore, been described for their potent application in boosting plant activities in modern agriculture. However, it is equally important to study in detail the potentiality of this group of rhizospheric microbiota along with their mechanism of action involved in sustainable crop
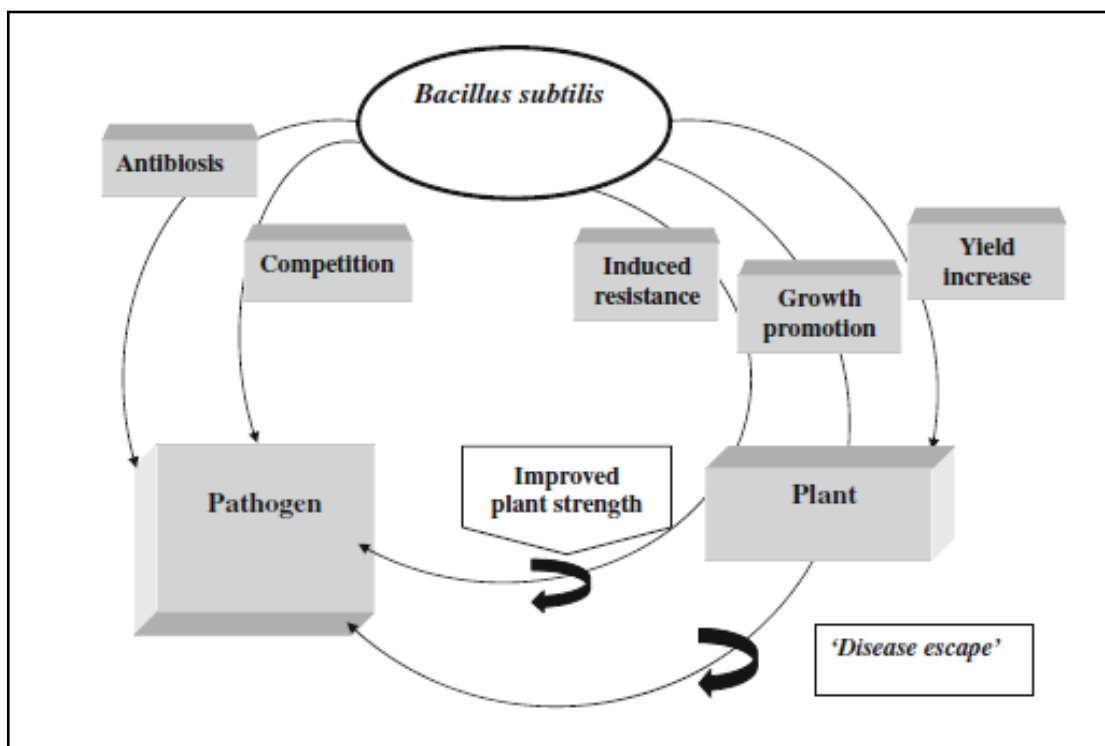
Figure 1. Modes of action of *Bacillus subtilis* strain, FZB24 promting plant growth (Adapted from Kilian et al., 2000).

production. There is need to improve the knowledge for the selection of potent microbial strains colonizing rhizosphere of growing plants for specific restoration programmes. PGPR can promote growth and yield of crop plants by direct and indirect mechanisms. In some PGPR species, plant growth promotion dominates with nitrogen fixation, phosphate solubilization and production of phytohormones like auxin and cytokinin and volatile growth stimulants such as ethylene and 2,3-butanediol (Ryu et al., 2003; Vessey, 2003).

Secretion of inhibitory substances against plant pathogens by PGPR

Production of siderophore compounds

Siderophores play an important role in the biocontrol of some soil-borne plant diseases and in plant iron nutrition (Loper and Buer, 1991). Siderophores are low molecular weight, high affinity iron (III) chelators that transport iron into bacterial cells (Leong, 1986). These systems are composed of ferric-specific ligands (siderophores) and their cognate membrane receptors as chelating agents in bacteria (Neilands, 1989). Subsequently, siderophores have been shown to be involved in the suppression

of *F. oxysporum* (Baker et al., 1986). Because siderophores sequester the limited supply of iron (III) in the rhizosphere, they limit its availability to pathogens and ultimately suppress their growth (Schroth et al., 1984).There are two strategies for acquiring iron (Römheld, 1987). Strategy I is characterised by an increase in the activity of a NADPH-dependent 'reductase' and an increase in H^+ release. Strategy II is characterised by enhanced release of phytosiderophores and by a highly specific uptake system for Fe (III) phytosiderophores. Both activities are thought to enhance the solubilisation of Fe (III).

Numerous bacterial and fungal species have been shown to produce siderophores compounds. Carson et al. (2000) reported that two major types of siderophoric compounds were produced by microorganisms: hydroxamate and catechol compounds. Hydroxamate siderophores usually contain N hydroxyrnithine as the ligand involved in the chelation of iron. De Bellis and Ercolani (2001) determined rootlet elongation and bacterial growth on rootlets after inoculation of cucumber and spinach seedlings with *Pseudomonas* strains; they differ in the production of siderophores and HCN. Siderophore producers grew more profusely on cucumber. Sharma and Johri (2003) bacterized maize seeds with siderophore producing pseudomonads to

develop a system suitable for better iron uptake under iron-stressed conditions. Siderophore production was compared in fluorescent pseudomonads sp. GRP3A, PRS, and *P. chlororaphis* ATCC 9446 in standard succinate (SSM) and citrate (SCM) media. Succinate was more suitable for siderophore production; however, deferentiation of media resulted in increased siderophore production in all the strains. Maximum siderophore level (216. 23 μ g/ml) was observed in strain PRS, in SSM after 72 h of incubation. Strains GRP3A and PRS were also antagonistic against the phytopathogens, *Colletrichum dematium*, *R. solani* and *Sclerotium rolfsii*. Bacterization of maize seeds with strains GRP3A and PRS$_9$ showed a significant increase in germination percentage and plant growth.

Siderophore production for rhizosphere colonization has also been recorded as one of the important mechanism by certain PGPRs (*B. japonicum*, *R. leguminosarum* and Sinorhizobium meliloti) (Carson et al., 2000; El-Tarabily and Sivasithamparam, 2006) with plant growth promoting activity. Besides, iron-chelating siderophores (Schippers et al., 1988), antibiotics (Weller, 1988) and hydrogen cyanides (Stutz et al., 1986) are also likely to be produced by PGPR strains, participating tremendously in the reduction of phytopathogens and deleterious rhizobacteria with a corresponding improvement in plant health. However, regardless of the mechanism of plant growth promotion, PGPR must colonize the rhizosphere or root itself (Glick, 1995).

Production of hydrogen cyanide

Thomashow and Weller (1996) found that biocontrol mechanisms of bacteria, such as certain *Pseudomonas* strains, were usually based on secreted bioactive factors that attack the pathogen, e.g. antibiotics, exoenzymes, or HCN. Dekkers et al. (2000) showed that phenazine-1-carboxamide (oxychlororaphin, or OCP), a phenazine produced by *P. chlororaphis* PCL1391, suppressed tomato root rot caused by *F. oxysporum f. sp. radicis-lycopersici*. Lugtenberg et al. (2001) revealed that fluorescent pseudomonads frequently have been considered effective biological control agents against soil-borne plant pathogens because of their rapid and aggressive colonization of plant roots. They added that competition for nutrients in the rhizosphere at preferred colonization sites was one mechanism, while others include the production of metabolites, such as antibiotics, siderophores, and hydrogen cyanide. Kremer and Souissi (2001) reported that rhizobacteria strains were characterized by the ability to synthesize hydrogen cyanide and having effects on seedling root growth of various plants. They found that approximately 32% of bacteria from a collection of over 2000 isolates were cyanogenic, evolving HCN from trace concentrations to

>30 nmoles/mg cellular protein. Cyanogensis was predominantly associated with pseudomonads and was enhanced when glycine was provided in the culture medium.

Production of antibiotics

In many biocontrol systems, one or more antibiotics have been shown to play a role in disease suppression. Molecular tools have been effective here, because mutants defective in antibiotic production are easily obtained, and in vitro assays are useful tests. The most widely studied group of rhizospheric bacteria with respect to the production of antibiotics is that of the fluorescent pseudomonads. The first antibiotics described as being implicated in biocontrol were phenazine derivatives produced by fluorescent pseudomonads (Weller and Cook, 1983). Their role has been elucidated by transposon insertion mutations which result in a defect in production of phenazine-1-carboxylate, thus reducing disease suppressive activity (Pierson and Pierson, 1996).

The genes encoding the enzymes responsible for synthesis of the metabolites have been isolated and their regulation studied (Bangera and Thomashow, 1996; Pierson et al., 1995). Global regulatory elements have been shown to coordinate the production of these metabolites (Pierson et al., 1994). The presence of other bacteria can influence phenazine production by *P. aureofaciens*, since mutants cannot be produced by other (related) rhizosphere inhabitants (Pierson and Pierson, 1996; Wood and Pierson, 1996). Also, other environmental sensors such as the regulatory proteins GacA and ApdA can influence the production of secondary metabolites involved in pseudomonads biocontrol (Corbell and Loper, 1995; Haas et al., 2002). In addition, sigma factors are important for sigma[70] and the stress-related sigma[s] have critical roles in the production of antibiotic metabolites in disease suppression (Schnider et al., 1995). Paul and Banerjee (1986) mentioned that soluble antibiotics produced by *Streprtomyces galbus* could inhibit spore germination of *Alternaria solani, A. niger, Curvularia pallescense* and *Helminthosporium oryzae.*

Antibiotic production is one of the most intensively studied aspects of biocontrol, but in many cases it is difficult to distinguish between antibiosis and competition. Several studies have demonstrated that production of antibiotics (Pyrrolnitrin, phycocyanin, 2,4-diacetylphloroglucinol) by microbial inoculants can cause suppression of pathogens (Subba Rao, 1993; Glick, 1995). Glick (1995) was of the view that the most effective mechanism that a PGPR can employ to prevent proliferation of phytopathogens is the synthesis of antibiotics.

Streptomyces lydicus WYEC108 showed strong *in vitro*

antagonism against various fungal plant pathogens in plate assays by producing extracellular antifungal metabolites. When *Pythium ultimum* or *R. solani* was grown in liquid medium with *S. lydicus* WYEC108, inhibition of growth of the fungi was observed. When *S. lydicus* WYEC108 spores or mycelia were used to coat pea seeds, the seeds were protected from invasion by *P. ultimum* in an oospore-enriched soil. While 100% of uncoated control seeds were infected by *P. ultimum* within 48 h after planting, less than 40% of coated seeds were infected. When the coated seeds were planted in soil 24 h prior to introduction of the pathogen, 96 h later, less than 30% of the germinating seeds were infected. Plant growth chamber studies were also carried out to test for plant growth effects and suppression by *S. lydicus* WYEC108 of *Pythium* seed rot and root rot. When *S. lydicus* WYEC108 was applied as a spore-peatmoss-sand formulation (108 CFU/g) to *P. ultimum*-infested sterile or nonsterile soil planted with pea and cotton seeds, significant increases in average plant stand, plant length, and plant weight were observed in both cases compared with untreated control plants grown in similar soils. *S. lydicus* WYEC108 hyphae colonized and were able to migrate downward with the root as it elongated. The potential of microbial antagonism was explored in the control of sugar beet disease caused by *Fusarium*. *In vitro* studies showed that 70% concentration of the culture filtrate of *Streptomyces aureofaciens* significantly inhibited the spore germination, mycelial growth and sporulation of *Fusarium solani*. The studies *in vivo* involved different treatments: seed coating treatment was the most effective in controlling *F. solani* at all cultivation periods in all the three-sugarbeet cultivars Raspoly, TOP and Tribel. The former cultivar showed the highest growth response compared to the other two cultivars. Soil pre-inoculation was less effective whereas seed-soaking treatment was the least effective in this respect (Moussa and Rizk, 2002).

Streptomyces spp., isolated from the rhizosphere soils of various crops, were screened by dual culture and cell free culture filtrate techniques against *F. oxysporum* f.sp. *dianthi* and *F. oxysporum* f.sp. *gladioli*, causing wilt in carnation (*Dianthus caryophyllus*) and gladiolus (*Gladiolus hortulanus*), respectively. Results indicated that *Streptomyces* sp. isolate CAAC-Banuri exerted maximum inhibition against *F. oxysporum* f.sp. *dianthi* and GLAC-Kotli was highly effective in inhibiting the growth of *F. oxysporum* f. sp. *gladioli* (Shanmugam et al., 2004). The culture filtrate and crude extract from *S. aureofaciens* CMUAc130 were all inhibitory to *C. musae* and *F. oxysporum*. The culture filtrate and crude extract from this strain were all inhibitory to tested phytopatho-genic fungi. The major active ingredients from the culture filtrate of *S. aureofaciens* CMUAc130 were purified by silica gel-column chromatography and identified to be (i) 5,7-dimethoxy-4-*p*-20 methoxylphenylcoumarin and (ii)

5,7-dimethoxy-4-phenylcoumarin by NMR and mass-spectral data, respectively. Bioassay studies showed that compounds (i) and (ii) had antifungal activities against tested fungi, and their minimum inhibitory concentrations were found to be 120 and 150 µg ml^{-1}, respectively (Thongchai et al., 2005). Cao et al. (2005) also studied the controlling of *F. oxysporium* f.sp *cubense in vitro* of banana plants grown in pots by *Streprtomyces* sp.

Plant hormone production

Plant growth hormones are organic compounds that influence the physiological processes in plants at extremely low concentrations. Production of phytohormones by inoculants has been suggested as one of the most plausible mechanisms of action affecting plant growth. There are five classes of well-known phytohormones, namely auxins, IAA, cytokinins, ethylene and abscisic acid. Soil microbiotas, particularly the rhizosphere microflora, are potential sources of these phytohormones (Frankenberger and Arshad, 1995; Costacurta and Vanderleyden, 1995; Patten and Glick, 1996; Arshad and Frankenberger, 1998). Plant growth regulators help to solubilise nutrients so that they can easily be taken up by plant via activating the roots and stimulating cell division of root tissues. Solubilisation of nutrients such as phosphorus and iron by rhizobia makes them more readily available for plant uptake, as demonstrated by Belimov et al. (1995), Noel et al. (1996), Glick et al. (1998) and Biswas et al. (2000). They suggested that production of organic acids was the major mechanism of action by which insoluble phosphorus compounds were converted to more soluble forms. Other scientists reported that rhizobia can create an acidic environment to promote mineral nutrient solubilisation (Alexander, 1977). The rhizobia influence crop growth and development by changing the physiological status (Glick and Bashan, 1997) and morphological characteristics of inoculated roots (Noel et al., 1996; Yanni et al., 1997), which favours improved nutrient uptake (Okon and Kapulnik, 1986). The ability of rhizobia to solubilise both inorganic and organic phosphate has been the subject of many investigations (Abd-Alla, 1994; Martin et al., 2002).

Other potential mechanisms

Other mechanisms for biological control of disease may include competition for infection sites and nutrients, parasitism on pathogens, that is, destruction of fungal pathogens by the action of lytic enzymes (e.g. chitinase and ß-1, 3-glucanase) that degrade fungal cell walls, and uncharacterised antifungal factors (Fridlender et al., 1993;

Kloepper, 1996). Buchenauer (1998) reported various mechanisms for biological control such as competition for space and nutrients in the rhizosphere and spermosphere, lytic enzymes, HCN and many other metabolites produced by rhizobia. A consortium of PGPR may often have more influence on biological control and plant growth than a single strain (Krishnamurthy and Gnanamanickam, 1998; Bapat and Shah, 2000). However, in some cases, mixtures of different strains had no synergistic effect. Recent work on the broad spectrum of PGPR-mediated induced systemic resistance against different pathogens in different crops has gained importance (Ramamoorthy et al., 2001).

FLUORESCENT PSEUDOMONADS

The genus *Pseudomonas*, firstly described by Migula in 1894, is characterized as straight or slightly bent Gram-negative rods with one or more polar flagella, not forming spores. Its metabolism is chemoorganotrophic and strictly aerobic with a respiratory type in which oxygen is used (Fuchs et al., 2001). *Pseudomonas* is an aerobic Gram negative, fast growing, competitive root colonist, and is commonly found in the rhizosphere (Weller, 2007). Lugtenberg and Dekkers (1999) reviewed molecular based studies on identifying traits responsible for effective colonization of *Pseudomonas* by screening impaired mutants on different plants, then comparing their colonization ability with the wild type. The authors noted that slow growth and an inability to biosynthesize essential amino acids are among factors affecting the rhizosphere competence of PGPR. Kumar et al. (2011) found that effective root colonization and survival in the presence of indigenous soil inhabitants determine the rhizospheric competency of a PGPR. Some *Pseudomonas* strains have been shown to improve plant growth by releasing a wide range of antifungal metabolites that suppress the growth of pathogens of agronomically important crops in both laboratory and field trials (Haas and Keel, 2003). Amein et al. (2008) reported that a strain of *P. fluorescens* provided consistent protection to field grown winter wheat seedlings from blight disease over two growing seasons. A considerable increase in plant survival rate and yield was also reported. *Pseudomonas* is the largest of the groups, and includes both fluorescent and non-fluorescent ones. The most important fluorescent species are *P. aeruginosa*, *P. fluorescens*, *P. putida* and plant pathogenic species (*P. syringae*) (Scarpellini et al., 2004). Several species of rRNA group I pseudomonads have the ability to produce and excrete, under condition of iron limitation, soluble yellow green pigments that fluorescence under UV light named pyoverdines (PVDs) or pseudobactins, which act as siderophores for these bacteria (Meyer, 2000). These molecules are thought to be associated with biocontrol of

fungal pathogens in the biosphere (Fuchs et al., 2001).

The abundance of literature on genus *Pseudomonas* is due to their elevated metabolic versatility capable of utilizing a wide range of simple and complex organic compounds and holding an important position in biosphere ecology (Scarpellini et al., 2004). Consequently, they are isolated from a variety of natural sources including soil, plants and mineral waters and from clinical specimens and they are characterized by a high level of metabolic diversity (Moore et al., 1996). Often, they are able to survive and multiply in poor nutrient conditions. Fluorescent pseudomonads have been considered as an important bioinoculants due to their innate potential to produce plant growth promoting hormones and antimicrobial secondary metabolites (Costa et al., 2006; Dong and Zhang, 2005).

Fluorescent pseudomonads are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases (Gardner et al., 1984; Moeinzadeh et al., 2010). They produce secondary metabolites such as antibiotics (Keel et al., 1992), Phytohormones (Keel et al., 1992), Volatile compound Hydrogen Cyanide (HCN) and siderophores (Defago and Haas, 1990). Plant growth-promoting ability of these bacteria is mainly because of the production of IAA (Patten and Glick, 2002), siderophores (Schippers et al., 1987) and antibiotics (Colyer and Mount, 1984). Production of antibiotics such as phenazine-1-carboxylic acid (PCA), pyocyanin, 2-acetamidophenol, pyrrolnitrin, pyoluteorin, phenazine-1-carboxylic acid, 2,4-diacetylphloro-glucinol, viscosinamide and tensin in different species of pseudomonads has been reported (Sunish Kumar et al., 2005). Production of siderophores has also been linked to the disease suppressing ability of certain fluorescent *Pseudomonas* species (Loper and Buyer, 1991). The control of *Phytophthora* root rot of soybean (Lifshitz et al., 1987), tobacco black root rot (Keel et al., 1989), fungal diseases of orange, lemon citrus roots (Gardner et al., 1984), and ornamental plants (Yuen and Schorth, 1986) has been demonstrated with fluorescent pseudomonads. A soil isolate CV6 was identified according to chemotaxonomic characterizations as well as 16S rDNA gene sequence analysis. The possible growth-promoting and biocontrol potential of the fore mentioned strain has been investigated by determining the secondary metabolites, viz. IAA, siderophore, and HCN production. Mezaache-Aichour et al. (2012) isolated fluorescent Pseudomonads bacteria from rhizosphere of potato plants in Algeria and identified it as *Ps. chlororaphis* and found that this isolate was capable of inhibiting the growth of phytopathogenic fungi *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *albedinis*, *F. solani* and *R. solani* and the oomycete *P. ultimum*. Extracts of supernatants from liquid cultures of this *Ps. chlororaphis* isolate completely inhibited these organisms when incorporated into potato dextrose agar

at a rate equivalent to 0.31 ml culture filtrate/ml, or greater.

Conflict of interest

The authors have not declared any conflict of interests.

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