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Review Article

Plant Microbe Interaction in Rhizosphere

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Abstract

The present review describes the status of rhizosphere of agricultural plants where microbial community play important role by interacting with each other and also with the plants. Plant roots release various substances which provide suitable environment for the colonization of microorganisms around rhizosphere. Beneficial and harmful microorganisms for the growth of plants effect on the growth of the plants has been critically analysed whereas this review gives more emphasis of plant growth promoting rhizobacteria and benefit achieved to plants. N_2 fixation, IAA production, P solubilisation, siderophore production and ACC deaminase activity by the rhizobacteria provide better conditions for the plants growth; therefore, these bacteria are popularly known as plant growth promoting bacteria (PGPR).

Keywords: Rhizosphere; Microbe; Plant; PGPR; IAA; P-solubilization; Siderophore production, ACC deaminase activity

The Living Soil

Soil is a structured, heterogenous and discontinuous system, generally poor in nutrients and energy sources (Nannipierri *et al.*, 2003). It is composed of organic and inorganic matrices formed by the combined action of biotic and abiotic processes (Liesack *et al.*, 1997; Gobat *et al.*, 2004). Organic carbon found in the soil is mainly plant derived (plant remains and rhizodeposition). A carbon source can come from plant remains that are degraded by the macro and microflora into organic matter through the process of humification (Gobat *et al.*, 2004). The soil organic matter (SOM) is composed of non-degraded or partially degraded litter and the humus fractions (Gobat *et al.*, 2004). SOM accounts for as much as one third of the cation exchange capacity of surface soils and is responsible for stability of soil aggregates (Sparling *et al.*, 2006). Eighty to ninety percent of the reactions in soils are mediated by microbes (Coleman and Crossley, 1996). In agro-ecosystems, bacteria are responsible for diverse metabolic functions that affect soil fertility and plant health including nutrient cycling, organic matter formation and deposition, soil structure and plant growth promotion (Kennedy, 1999). The presence of microorganisms in the soil depends on the number and volume of available microhabitats and bacterial activity to the amount of available metabolic substances found in those microhabitats (Nannipieri *et al.*, 2003). The mineral composition, salinity, pH, nutrient availability, organic input, temperature and water content determine which ecological niches are available (Liesack *et al*, 1997). These soil properties in turn depend not only on the fauna and vegetation but also on the geographical, geological, hydrological climate, and anthropogenic influences (Liesack *et al.*, 1997). Soil contains many different microhabitats thus increasing the bacterial diversity. Indeed, several thousand (~ 1.5 X 10^{10}) bacterial species can be found in one gram of soil (Torsvik *et al.*, 1990). The bacterial predation by bacteriophages, protozoans or nematodes enables to re-mineralize the nutrients immobilized by the bacterial biomass (Grifiths and Bargett, 1997). Zones in the soils where microbial activity is increased are defined as hot spots (Hesselsoe *et al.*, 2001). In soils, the rhizosphere is probably the greatest hot spots and offers different habitats and more resources for soil microorganisms than the bulk soil (Kuzyakov, 2002).

Soil is a solid matrix permeated to some degree by an aqueous solution. The soil varies from one place to another place due to the variation in clay content and texture, its water content, and the nature and concentration of solutes in the soil water. These variables influence the osmotic properties of the rhizosphere as well as the osmolarity of the rhizosphere soil. The osmolarity of the rhizosphere is modified due to the activities of roots and rhizobacteria. Osmoadaptive mechanisms are exerted both in rhizosphere bacteria and their interactions with plant roots for their survival. Generally, the osmolarity of rhizosphere water exceeds to that of bulk-soil water. In order to survive and proliferate in the rhizosphere, rhizobacteria are therefore likely to possess mechanisms designed for adaptation to environments of high osmolarity. In most cases, these bacteria have been examined because of interest in their interactions with plants rather than their degree of salt tolerance. When, as is common, NaCl is used as the sole extracellular osmolyte in studies of osmoadaptation, effects of osmolarity, ionic strength, Na^+, and Cl^- are not clearly differentiated. The mechanisms of osmoadaption have been

examined in greatest detail in the relatively salt-tolerant species viz., *Rhizobium meliloti* (Miller and Wood, 1996).

The Rhizosphere

The term "rhizosphere" was first coined by Hiltner in 1904 (Khan, 2005). The narrow zone of soil surrounding the root is called rhizosphere which is influenced by the root system. This zone is rich in nutrients in comparison to the bulk soil, due to the accumulation of a variety of organic compounds released from roots by exudation, secretion, and deposition (Ligaba *et al.*, 2004). These organic compounds are used by microorganisms growing in this region as carbon and energy sources therefore, microbial growth and activity are intense in the rhizosphere. Due to rich amount of carbon compounds in this region, the bacterial populations found around the roots of plants are generally 10 to 1000 times higher than the bulk soil (Lugtenberg and Kamilova, 2009). It is a site with complex interactions between the root and associated microorganisms (Sylvia *et al.*, 1998).

Successful colonization of the rhizosphere environment by microbial inoculants depends on the rhizosphere competence (Jjemba and Alexander, 1999). Indeed, the bacterial strains need to possess particular traits such as chemotaxis towards root exudates, compounds mediating attachment (adhesions, fimbriae, pilli, cell surface proteins and polysaccharides) and a capacity to metabolise root exudates (de Weert *et al.*, 2002). Because they possess numerous root competence traits, the bacteria of the genus *Pseudomonas* are used as models of rhizobacteria (Sorensen *et al.*, 2001). They constitute up to 10% of the culturable rhizospheric microflora (Tarnawski *et al.*, 2003) and are used extensively as PGPR (Lucy *et al.*, 2004).

The activities of roots modify the physico-chemical properties of soil which in turn affects the soil microflora in different ways. Absorption of water and minerals induces a modification of pH and redox potential as well as loss of nutrients in the environment which creates stress to the microbial communities (Baath and Anderson, 2003). The O_2 and CO_2 partial pressure vary during root respiration. There is a negative O_2 gradient from the root surface to its surrounding soil (Hojberg *et al.*, 1999). Due to decreased O_2 level in the vicinity of the root, anaerobic microbial communities such as denitrifying bacteria could be favoured (Ghiglione *et al.*, 2000). The process of rhizodeposition also creates favourable conditions of microbial communities.

The below-ground plant biomass (BGP) can be subdivided into visible fibrous macro-roots and rhizodeposits (Hogh-Jensen and Schjoerring, 2001). Rhizodeposition is a process of release of volatile, non-particulate and particulate compounds from living plant roots. These compounds contain a wide range of organic compounds (Gobat *et al.*, 2004). These rhizodeposits can be subdivided according to their origin. The particulate compounds include root border and root cap cells, sloughed epidermal root cells and root hairs, root fragments and fine roots (Whipps 2001). Root border cells easily separate from plant roots as these moves through the soil (Gobat *et al.*, 2004). They are often still viable and beside secretory cells, contribute large amounts of non-particulate compounds and are therefore sometimes included in the term root exudates (Gobat *et al.*, 2004). Non-particulate compounds include passive or controlled diffused root exudates (Nguyen, 2003) and secretions such as mucilage (a secretion released by cells), lysates and ions released from roots (Wichern *et al.*, 2008). In a wider definition of rhizodeposition, Wichern *et al.*, (2008) described rhizodeposition as the release of all kinds of compounds lost from living plant roots, including ions and volatile compounds. This definition should be used when investigating the rhizodeposition of N, because plant roots release ions, such as NH_4^+ and NO_3^- (Merbach *et al.*, 1999). Beside categorising rhizodeposits by their origin, they can also be distinguished by solubility or extractability with water into water-soluble exudates (e.g. sugars, amino acids, organic acids, hormones, and vitamins), and water insoluble materials, such as decaying fine-roots, root hairs, cell walls, sloughed cells, and mucilage (Merbach *et al.*, 1999). Close to the root, the water-soluble root-borne compounds are dominated by carbohydrates and organic acids, with only small amounts of amino acids (Merbach *et al.*, 1999; Hutsch *et al.*, 2002). Nevertheless, these amino acids are probably the main source of water soluble N lost from roots (Rroco and Mengel, 2000). Moreover, root exudates are a complex mixture with a wide range of substances and can be divided into low-molecular-weight (LMW) and high-molecular weight (HMW) substances (*Bais et al.*, 2006). LMW root exudates are usually dominated by simple sugars, but also include organic acids, amino sugars, phenolics, and inorganic compounds, whereas HMW compounds comprise exoenzymes and in some definitions root border cells (Nguyen, 2003), as well as those root exudates passively diffusing into the soil solution (Nguyen, 2003) which are actively secreted in response to metal toxicity, nutrient stress and the presence/absence of plant and microbial taxa (Bais *et al.*, 2006). This flux is believed to range between 1% and 10% of net assimilated C (Jones *et al.* 1998). Due to their chelating properties and their role in stimulating microbial activity root exudates are believed to play an important role in mediating soil nutrient availability in ecosystems (*Bais et al.*, 2006). Furthermore, exudates are primarily derived from recently-assimilated photosynthate (*Bais et al.*, 2006) and thus, may represent a semi-continuous input of labile C to soil in contrast to transient inputs of C resulting from leaf litter inputs (Kuzyakov and Cheng, 2001).

Rhizosphere microbial communities can significantly influence phytopathogens development (Nehl *et al.*, 1997; Glick, 1995), nutrient acquisition (Lynch, 1990), heavy

metal resistance (Kuffner *et al.*, 2008), and ecological fitness of plants (Barriuso *et al.*, 2008). Moreover, root-induced microbial activity increases the bacterial mucilage production thus modifying the soil structure by the formation of aggregates (Killham and Yeomans, 2001). Rhizobacteria can affect the plant development either negatively (deleterious rhizobacteria) or positively (plant growth promoting rhizobacteria). Deleterious rhizobacteria have been defined as minor pathogens that affect plant by their metabolites without parasiting plant tissues (Nehl *et al.*, 1997). These rhizobacteria can produce phytotoxins, pectinolytic enzymes and/or phytohormones and compete with the plant or with the beneficial microorganisms for the uptake and assimilation of nutrients (Nehl *et al.*, 1997).

Plant Growth Promoting Rhizobacteria

Plant-associated bacteria that are able to colonize roots are called rhizobacteria and can be classified into beneficial, deleterious, and neutral groups on the basis of their effects on plant growth. Beneficial rhizobacteria that stimulate plant growth are usually referred to as Plant Growth Promoting Rhizobacteria or PGPR (Glick, 1995). Plant growth-promoting rhizobacteria enhance plant growth either by direct or indirect mechanisms (Glick, 1995). Several PGPR that have been successful in promoting the growth of crops such as canola, soybean, lentil, pea, wheat and radish have been isolated (Glick *et al.*, 1997; Timmusk *et al.*, 1999). The enhancement of plant growth by PGPR indicates their potential as biofertilizers in the field of agriculture. Bertrand *et al.* (2001) identified bacteria belonging to the genera *Pseudomonas*, *Varivorax*, *Agrobacterium* and *Phyllobacterium* as the most efficient PGPR associated with canola. PGPR may show beneficial effects on plant growth by some of the following key metabolic features.

Nitrogen fixation

Nitrogen is one of the most common nutrients required for plant growth and productivity as it forms an integral part of proteins, nucleic acids and other essential biomolecules (Bockman, 1997). More than 78 % of N_2 is present in the atmosphere, but is unavailable to plants. It needs to be converted into ammonia, a form available to plants and other eukaryotes. Atmospheric N_2 is converted into forms utilized by plants by three different processes, (i) conversion of atmospheric nitrogen into oxides of nitrogen in the atmosphere (ii) industrial nitrogen fixation useing catalysts and high temperature (300-500ºC) to convert nitrogen to ammonia, and (iii) biological nitrogen fixation involves the conversion of N_2 to ammonia by microorganisms using a complex enzyme system identified as nitrogenase (Kim and Rees, 1994). Biological nitrogen fixation contributes about 60% of the earth's available nitrogen and represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha *et al.*, 1997). The availability of nitrogen often limits plant growth in terrestrial ecosystem. It affects the productivity and the species composition of plant communities and ecosystem processes at all scales. In agriculture, nitrogen is one of the most widely used fertilizers, with a still increasing global input. The only biological reaction counterbalancing the loss of N from soils or ecosystems is biological nitrogen fixation.

Any bacterium can be considered as a rhizospheric diazotroph if: (i) it can be isolated from rhizosphere of the plant, and (ii) it does fix nitrogen, as demonstrated by acetylene reduction and/or ^{15}N-enrichment. The most useful methods for examining N_2 fixation in the field and in large greenhouse experiments are still the ^{15}N isotope dilution and ^{15}N natural abundance techniques (James, 2000). Using these methods it was reported that certain Brazilian sugar cane varieties can derive 50 to 80% of plant N from BNF, equivalent to 150 to 170 kg N ha^{-1} y^{-1} (Boddey *et al.*, 1991, 1995). However, the amount of nitrogen fixed is highly variable and depends on plant genotype and environmental conditions (Boddey *et al.*, 1991). In large experiments involving up to 70 rice varieties, it was estimated that the amount of nitrogen derived from air (Ndfa) ranged from 0 to 20.2%, other experiments showed that an equivalent of 16 to 70 kg N ha^{-1} $crop^{-1}$ was fixed (Shrestha and Ladha, 1996). Extensive studies conducted at the International Rice Research Institute, Manila, suggest that on the whole approx. 20 to 25% of the total nitrogen needs of rice can be derived from associative N_2 fixation (Roger and Ladha, 1992). Using the ^{15}N isotope dilution technique, it was estimated that Kallar grass may fix up to 26% of its N content (Malik *et al.*, 1997). For the batatais cultivar of *Paspalum notatum*, this was nearly 11% (Boddey *et al.*, 1983).

Since the first report of N_2 fixation by *Clostridium* sp., the list of N_2-fixing microbes has increased tremendously (Ladha and Reddy, 2000). In general the well authenticated N_2-fixing organisms are all prokaryotes and can, for convenience, be divided into 3 main groups; (1) the free-living forms: these include heterotrophs, chemoautotrophs and phototrophs; (2) the symbiotic forms such as *Rhizobium* and *Frankia* where the presence of the host is usually essential for nitrogenase activity to occur; and (3) the associative symbiosis- those between cyanobacteria and eukaryotes, and between bacteria and tropical grasses where the symbiotic prokaryotes can also fix N_2 in the free-living state (Baldani *et al.*, 1997). Notably much information has been gathered from all three groups, nevertheless, emphasis has always been focused on legume-*Rhizobia* symbiosis, the reason being agriculturally important crops. Several attempts have been made to transfer N_2-fixing ability from *Rhizobia* to other non-leguminous plants but little, if any, success has been attained so far (Ladha and Reddy, 2000).

Certain bacteria fixing N_2 react to a supply of ammonium rapidly by inactivation of nitrogenase activity. The so-called nitrogenase switch-off by ammonium depends on two different mechanisms. In some diazotrophs, such as *Azoarcus* sp strain BH72, *Rhodospirillum rubrum* (Egener *et al*., 2001), *R. capsulatus* and *Azospirillum brasilense*, the iron protein of nitrogenase (NifH) is subject to post-translational modification, which results from a reversible mono-ADP-ribosylation at a specific arginine residue (Halbleib and Ludden, 2000; Egener *et al*., 2001). The basic model for ADP-ribosylation system is based on studies made with *R. rubrum*, where the roles of the NAD^+-dependent enzyme, dinitrogenase reductase ADP-ribosyl transferase (DRAT), and its partner, dinitrogenase reductase-activating glycohydrolase (DRAG) have been implicated (Halbleib and Ludden, 2000). Additionally, a physiological switch-off mechanism, which does not involve this covalent modification of nitrogenase, exists in some bacteria (Pierrard *et al*., 1993). However the mechanism is still unknown. In contrast, nitrogenase switch-off in *Rhodospirillum rubrum* occurs more slowly and is incomplete (Zhang *et al*., 1995). In *R. rubrum*, ammonium-induced switch-off was shown to be absolutely dependent on ADP ribosylation of NifH (Zhang *et al*., 1995), whereas in *R. capsulatus* and *Azospirillum brasilense*, modification of NifH is not absolutely required, indicating a second mechanism of regulation. It has been experimentally shown that a ferredoxin (FdxN) can be essential for the physiological nitrogenase inactivation as detected in the *Azoarcus* sp. (Egener *et al*., 2001).

A potential drawback of associations between non-legume crop and diazotrophs is that the bacteria are expected to use a major portion of their fixed nitrogen for their own metabolism and growth. This leaves little, if any, nitrogen to directly support plant growth and perhaps only after the catabolic breakdown of the bacterial cells. To solve this problem, efforts have been made to identify and isolate bacterial mutants that excrete ammonia for absorption by plants. Wood and Kennedy (1996) reported the existence of a stable ammonia-excreting mutant of *A. brasilense* Sp7S. An initial trial with this ammonia excreting mutant in McCartney bottles showed a significant transfer of newly fixed nitrogen after 72 h of exposure to $^{15}N_2$ (Kennedy *et al*., 1997). However there was only a little transfer of newly fixed nitrogen to the tops of the wheat plant. Using this laboratory model, preliminary information for its improvement with respect to bacterial genotype and the potential role of ammonia excretion in wheat roots has been achieved. Further, auxin analogue, 2,4-D is known to induce nodule-like structures (para-nodules) in a variety of plants including wheat and rice. These structures in wheat and rice showed preferential colonization and N_2 fixation by *Azospirillum brasilense* in comparison to other parts of the roots (Ladha and Reddy, 2000).

Indole- 3-acetic acid (IAA) production

Most of the attention has been focused on the role of the auxin, a best characterized phytohormone. Indole- 3-acetic acid (IAA) is most common and physiologically most active auxin in plants, which is known to stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants (Cleland, 1990; Hagen, 1990). The capacity to synthesize IAA is widespread among soil- and plant-associated bacteria. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce the plant growth regulator IAA (Patten and Glick, 1996). Tryptophan (Trp) is generally considered to be the precursor of IAA. Several IAA biosynthetic pathways (Fig. 1) such as indole-3-acetamide pathway, indole-3-pyruvate pathway, tryptamine pathway, tryptophan side-chain oxidase pathway, indole-3-acetonitrile pathway and tryptophan-independent pathways have been reported in bacteria (Spaepen *et al.*, 2007), although, the best-characterized pathways in bacteria for the conversion of Trp to IAA are the indole-3-acetamide pathway and the indole-3-pyruvate (IPyA) pathway (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996). IAA biosynthesis was studied extensively in the case of *Azospirillum* (Costacurta *et al.*, 1994; Lambrecht *et al.*, 2000). A survey of the IAA biosynthesis pathways utilized by plant-associated bacteria reveals that pathogenic bacteria such as *Pseudomonas syringae*, *Agrobacterium tumefaciens*, and *Erwinia herbicola* synthesize IAA predominantly via the indole-3-acetamide (IAM) pathway. Synthesis by this route is generally constitutive. PGPR such as *Rhizobium*, *Bradyrhizobium*, and *Azospirillum* synthesize IAA mainly via the indole-3- pyruvic acid (IPyA) pathway, which may be subject to more stringent regulation by plant metabolites (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996). In the IPyA pathway, Trp is first transaminated to IPyA, subsequently decarboxylated to indole-3-acetaldehyde, which can be oxidized to IAA. A gene (*ipdC*) encoding IPyA decarboxylase (IPDC) was isolated from *Enterobacter cloacae*, and a homologous gene has also been found in *A. brasilense* (Costacurta *et al.*, 1994). By using HPLC and/or GC-MS, the presence of IAA and related compounds in the growth medium could be demonstrated for many diazotrophs, including *Acetobacter diazotrophicus* (Fuentes-Ramirez *et al.*, 1993; Bastián *et al.*, 1998), *Azospirillum* sp. (El-Khawas and Adachi, 1999), *Azotobacter* (Pati *et al.*, 1995), *Herbaspirillum seropedicae* (Bastián *et al.*, 1998), *Klebsiella pneumoniae* (El-Khawas and Adachi, 1999), *Bradyrhizobium elkanii* (Minamisawa *et al.*, 1996), *Rhizobium leguminosarum* bv. *viciae* LPR1105 (Camerini *et al.*, 2008), and *Paenibacillus polymyxa* (Lebuhn *et al.*, 1997). *ipdC* gene has been identified and cloned from various sources (Costacurta *et al.*, 1994; Patten and Glick, 2002). In *Azospirillum brasilense* Sp245, the *ipdC* gene has been cloned

(Costacurta *et al.*, 1994) and purified transaminase converted L-Trp to IPyA thereby confirming the existence of the IPyA pathway (Malhotra and Srivastava, 2008).

DNA sequence and transcriptional analysis of the *ipdC* region in this strain identified another gene, *iaaC*, in a bicistronic operon with *ipdC* which is also transcribed monocistronically (Vande Broek *et al.*, 2005). Expression analysis of *ipdC* revealed a strict regulation. The gene is expressed only in the late exponential phase and is induced by auxins (Vande Broek *et al.*, 2005). Somers *et al.* (2005) demonstrated that the *ipdC* gene product is also involved in the biosynthesis of phenyl acetic acid (PAA), an auxin with antimicrobial activity (Somers *et al.*, 2005). An *A. brasilense ipdC*-knockout mutant showed significantly lower production of PAA (Somers *et al.*, 2005).

The indole-3-acetamide (IAM) pathway has been well characterized in plant growth promoting bacteria. This pathway is completed in two steps; tryptophan is converted to IAM by the enzyme tryptophan- 2-monooxygenase (IaaM) in the first step which is encoded by the *iaaM* gene. In the second step IAM is converted to IAA by an IAM hydrolase (IaaH) which is encoded by *iaaH*. The genes *iaaM* and *iaaH* have been cloned and characterized from various bacteria, such as *Agrobacterium tumefaciens*, *Erwinia herbicola*, *Pseudomonas syringae*, *Pantoea agglomerans*, *Rhizobium* and *Bradyrhizobium* (Theunis *et al.*, 2004).

The first evidence for the role of IAA in plant growth promotion was observed by attempts to mimic the effect of the bacterium on root growth by the direct application of IAA onto the roots. When wheat was inoculated with *A. brasilense* Cd and simultaneously, the pure IAA was applied to the roots both increased root length, number of lateral roots, and number of root hairs (Martin *et al.*, 1989). Similar experiments with *A. brasilense* (Morgenstern and Okon, 1987b) and *P. polymyxa* (Holl *et al.*, 1988) provided strong evidence for IAA production by these diazotrophs and the responsibility of this hormone for the observed effects on plants.

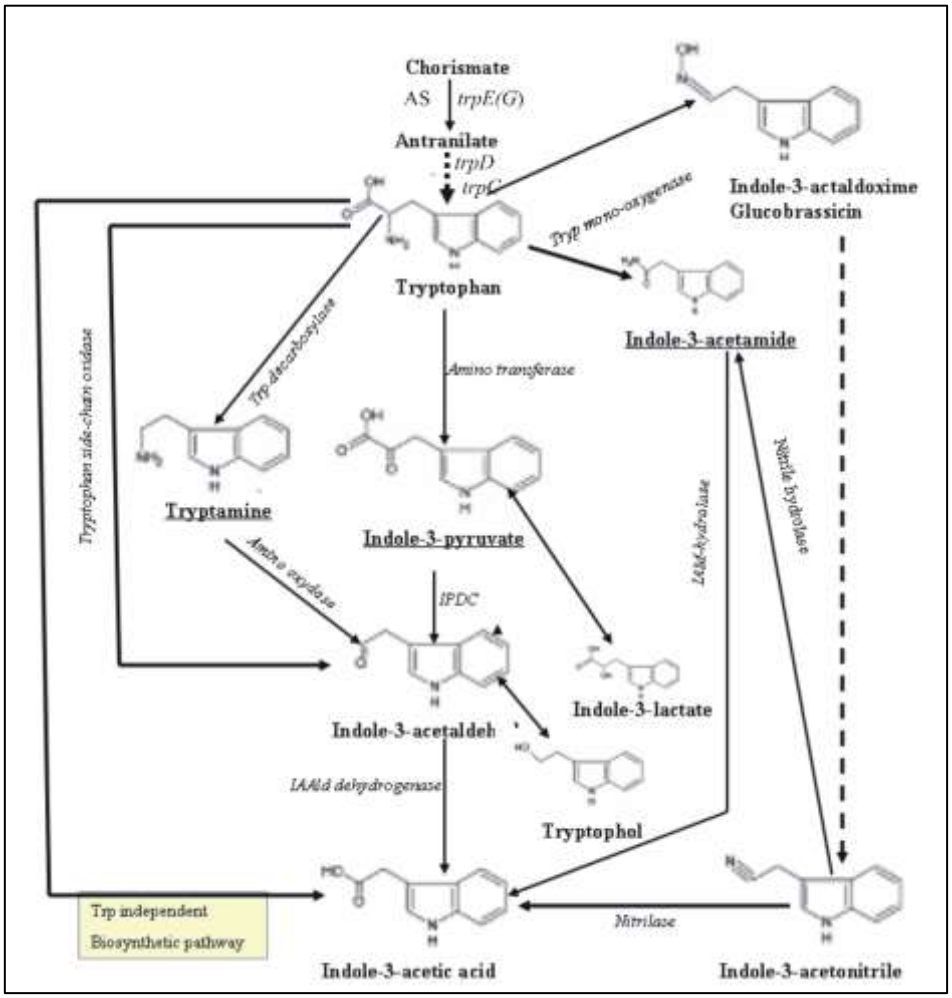

Fig. 1: Overview of the different pathways of IAA biosynthesis in bacteria. The intermediate referring to the name of the pathway or the pathway itself is underlined with a dashed line. IAAld, indole-3-acetaldehyde; IAM, indole-3-acetamide; IPDC, indole-3-pyruvate decarboxylase; Trp, tryptophan (modified from Spaepen *et al.*, 2007).

In addition to IAA, bacteria such as *P. polymyxa* and Azospirilla also release other compounds like indole-3-butyric acid (IBA), Trp and tryptophol or indole-3-ethanol (TOL) in the rhizosphere that could indirectly contribute to plant growth promotion (El-Khawas and Adachi, 1999). IBA is widely used in agriculture as a commercial promoter of root initiation in cuttings (Lebuhn *et al*., 1997). Trp may enhance endogenous IAA synthesis in the plant root. Direct uptake of applied Trp by plant roots followed by conversion into IAA within their tissues has already been proposed by Martens and Frankenberger (1994). In addition to the synthesis of IAA, plant growth promotion by *P. polymyxa* strains was proposed to be due to its production of TOL (Lebuhn *et al*., 1997).

P solubilization

Solubilization of P has been postulated as a possible mechanism of plant growth promotion by PGPR (Rodríguez and Fraga, 1999; (Richardson *et al.,* 2009). P solubilization is important for plant growth because P is an essential nutritional element but at the same time it is one of the least soluble nutrient ions in the environment. It is estimated that usually less than 9-20% of total soil phosphate is being available to plants (Hinsinger, 2001) and this bioavailability depends upon the pH and available metal cations of Fe, Mg, Ca and Al present at the rhizosphere of plants (Hinsinger, 2001). Phosphorus exists in nature in a variety of organic (derived from microorganisms and plants) and inorganic (originating from applied P fertilizer) forms that are either insoluble or very poorly soluble (Schefe *et al.,* 2008). Therefore, the addition of phosphate fertilizers has become a common practice in modern agriculture. However, a large portion of the soluble inorganic phosphate applied to soil as fertilizer is rapidly immobilized by the iron and aluminium in acid soils and by calcium in calcareous soils soon after application, thus becoming unavailable to plants (Holford, 1997). Soil microorganisms are able to solubilize insoluble mineral phosphate by producing various organic acids (Illmer *et al.*, 1995; Jones, 1998). This results in acidification of the surrounding soil, releasing soluble orthophosphate ions ($H_2PO_4^{-1}$ and HPO_4^{-2}) that can be readily taken up by the plants. Furthermore, they are able to solubilize organic P compounds by means of phosphatase enzymes (Garcia *et al.*, 1993). A large number of P-solubilizing bacteria (PSB) have been isolated from the rhizosphere of several crops. It has been reported that P-solubilizing microorganisms may constitute 20 to 40% of the culturable population of soil microorganisms and that a significant proportion of them can be isolated from rhizosphere (Chabot *et al.*, 1996a). Although there is good evidence for P solubilization by these microorganisms in pure culture (Chabot *et al.*, 1996a), it is difficult to demonstrate solubilization in plant-microorganism systems. It is argued that the increased P uptake often observed in plants treated with PGPR is an indirect by product of that interaction and actually reflects a better developed root system and an overall healthier plant. Indeed, in many cases there was a stimulation of plant growth by these PSB, but no effect on P uptake could be observed (Singh and Kapoor, 1998), indicating that other mechanisms than P solubilization could be responsible for plant growth promotion. The production of other metabolites (phytohormones, antibiotics, or siderophores) beneficial to the plant by the phosphate solubilizing strains created confusion about the specific role of phosphate solubilization in plant growth and yield stimulation (Kloepper *et al*., 1989). Experiments performed with P-solubilizing diazotrophs are few, and the results obtained are quite diverse, in fact varying according to plant or bacterial species. *Bacillus megaterium* and *P. polymyxa* are able to enhance growth and yield but not the P uptake of canola, indicating that P solubilization is not the main mechanism responsible for positive growth response (Defreitas *et al*., 1992). Kumar and Narula (1999) used chemically induced mutants of *Azotobacter chroococcum*, isolated from the wheat rhizosphere, with higher phosphate solubilization activity to inoculate wheat and found significantly positive effects of inoculation on percent germination and growth emergence, the mutant strains performing better than the parent strain. However, the mutant strains also produced significantly higher amounts of IAA, suggesting that the effect of inoculation on seed emergence is caused primarily by growth regulators. In addition to fixing nitrogen, *A. chroococcum* is known to produce growth hormones, vitamins, and antifungal substances (Revillas *et al*., 2000). It is felt that in addition to P solubilization, above factors might have contributed to the better growth and early seed emergence of wheat. Similarly, inoculation with two strains of *R. leguminosarum* bv. *phaseoli*, strain P31 and R1, selected for their P solubilization ability, increased lettuce dry matter yield at certain fertilizer levels but had no significant effect on P uptake (Chabot *et al*., 1996a). Also in this case, other mechanisms than P solubilization may be possibly involved since both strains produce siderophores and IAA and strain P31 also produces HCN. To further investigate the role of P solubilization activity of strain R1 in maize root colonization, Chabot *et al.* (1996b) produced two mutants with reduced P solubilization activity by random Tn*5*–mutagenesis. In a rich soil with high available-P content, dry matter of maize was significantly increased by bacterial inoculation, compared to the uninoculated control. However, both mutant strains did not increase dry matter production in the same way, with one mutant performing better than the wild type strain, the other worst, depending on the P fertilization (Chabot *et al*., 1998). Unfortunately no data on P content were presented. The mutants were not completely negative for P solubilization (40% less P solubilization after 16 h incubation) and were not characterized as to which gene was mutated.

The mineral phosphate solubilization by PSB occurs due to production of organic acid (Rodriguez and Fraga, 1999). According to Goldstein (1995) glucose is oxidized to gluconic acid which in turn seems to be a major mechanism for mineral phosphate solubilization (MPS) in gram-negative bacteria. Biosynthesis of gluconic acid is through the enzyme activity of glucose dehydrogenase (GDH) in the presence of pyrroloquinoline quinone (PQQ) as a co- factor. Some genes involved in MPS from different species have been isolated. A gene (*mps*) responsible for MPS has been cloned by Goldstein and Liu (1987) from the gram negative bacterium *Erwinia herbicola*. This gene has been expressed in *E. coli* HB101 which produced gluconic acid and conferred the ability to solubilize hydroxyapatite. *E. coli* is able to synthesize GDH, but not PQQ, thus it is unable to produce gluconic acid. The cloned 1.8 kb locus encodes a protein similar to the gene III product of a *pqq* synthesis gene complex from *Acinetobacter calcoaceticus*, and *pqqE* of *Klebsiella neumonie* (Liu *et al.*, 1997). Another type of gene (*gabY*) involved in the production of gluconic acid and MPS has been cloned from *Pseudomonas cepacia* (Babu-Khan *et al.*, 1995). There is no homology with previously cloned *mps* gene to the *gabY* gene when the sequence is deduced into amino acid, direct oxidation pathway (gluconic acid synthesis) genes but was similar to histidine permease membrane-bound components. In the presence of *gabY*, gluconic acid is produced only if the *E. coli* strain expresses a functional glucose dehydrogenase (*gcd*) gene. According to Babu-Khan *et al.* (1995), this ORF could be related to the synthesis of PQQ by an alternative pathway, or the synthesis of a *gcd* co-factor is different from PQQ. The reported synergistic effect of exogenous PQQ and this gene support alternative pathway (Rodriguez *et al.*, 2006). In another report, a DNA fragment from *Serratia marcenses* cloned in *E. coli* induces gluconic acid synthesis, but showed no homology to *pqq* or *gcd* genes (Krishnaraj and Goldstein, 2001). It was suggested that this gene acted by regulating gluconic acid production under cell-signal effects. A genomic DNA fragment cloned from *Enterobacter agglomerans* showed MPS expression in *E. coli* JM109, although the pH of the medium did not change (Kim *et al.*, 1997).

Siderophore Production

The name siderophore was coined by Lankford in 1973 as a term to describe low molecular weight molecule that bind ferric iron with an extremely high affinity (Lankford and Byers, 1973). Siderophore was derived from a Greek term meaning iron carrier (Ishimaru, 1993). This is an appropriate term because the siderophore binds iron with an extremely high affinity and is specifically recognized by a corresponding outer membrane receptor protein, which in turn actively transports the complex into the periplasm of the cell. The molecular weight of siderophores range from approximately 600 to 1500 daltons, and because passive diffusion does not occur for molecules greater than 600 daltons, siderophores must be actively transported (Ishimaru, 1993). Many bacteria and fungi are capable of producing more than one type of siderophore or have more than one iron-uptake system to take up multiple siderophores (Pérez-Miranda *et al.*, 2007; Dimkpa *et al.*, 2008). Siderophores are classified on the basis of the chemical functional groups they use to chelate iron. Catecholate-type (phenolate) siderophores bind Fe^{3+} using adjacent hydroxyl groups of catechol rings. Enterobactin, also known as enterochelin, is produced by a number of bacteria including *E. coli* and is the classic example of a catechol-type siderophore (Höfte, 1993). It possesses the highest known affinity for Fe^{3+} with a stability constant (Kf) of 1052 (Höfte, 1993). Enterobactin production has been demonstrated in some nitrogen-fixing bacteria, including *Klebsiella pneumoniae* and *K. terrigena* (Höfte, 1993). Fe^{3+} is chelated using nitrogen atoms of thiazoline and oxazoline rings in hydroxamate-type siderophores (Crosa and Walsh, 2002). Ferrichrome is the classic hydroxamate-type siderophore. It is produced by a number of fungi including *Ustilago sphaerogena*. Although produced by fungi, ferrichrome is used by a number of bacterial species with the appropriate receptor protein (Höfte, 1993). Aerobactin is another hydroxamate-type siderophore that is produced by many bacteria including *E. coli* (Buyer *et al.*, 1991). A third class of siderophore utilizes N-hydroxy amino side chains with an oxygen atom as one of the ligands for Fe^{3+}. Anguibactin, produced by *Vibrio anguillarum* incorporates this functional group, but it is also a combination of all three siderophore types in that it is made up of all three functional groups, with three different methods of binding Fe^{3+} (Crosa and Walsh, 2002). A combination of functional groups is not uncommon to find in many siderophores (Crosa and Walsh, 2002).

The transport of iron must be strictly regulated due to its potential toxicity in living systems. In the majority of gram-negative organisms, '*Fur*' (ferric uptake regulator) is considered to be the key regulator for expression of genes involved in iron transport (Höfte, 1993) (Fig. 2). *Fur* is a transcriptional repressor of more than 90 genes, many of which are involved in siderophore synthesis and uptake (Wexler *et al.*, 2003). In environments where iron is abundant, the Fur protein (in the presence of ferrous iron) binds *fur* boxes, blocking access of RNA polymerase to the promoter region and in turn, preventing transcription of certain genes (Masse and Gottesman, 2002). Fur was identified over 30 years ago and it is a part of the Fur superfamily, which also includes PerR, Zur, and Irr (Wexler *et al.*, 2003). In addition to Fur, other Fe-responsive regulators can mediate iron regulation. For example, iron transport in many gram-negative bacteria is mediated by DtxR and related family members. This repressor was identified in *Corynebacterium diptheriae* (Boyd *et al.*,

1990). This protein also uses ferrous iron as a co-repressor (Qian *et al.*, 2002) but shows no sequence homology to Fur (Wexler *et al.*, 2003).

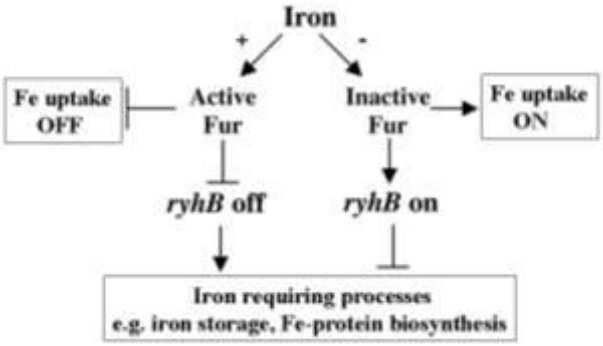

Fig. 2: Roles of *Fur* and *RyhB* in mediating Fe-dependent gene regulation in *E. coli* (modified from Masse and Gottsman, 2002).

Siderophores are produced by PGPR under iron-limited conditions. Leeman *et al.* (1996) reported that lipopolysaccharides (LPS) of *P. yuorescens* strains WCS 374 and WCS 417 are the major determinants of induced systemic resistance (ISR) under iron-replete conditions but under iron-limited conditions, LPS of these bacteria were not involved in ISR in radish against *Fusarium* wilt. They also found that pyoverdin-type pseudobactin, siderophore, produced by these bacteria was responsible for ISR. Application of purified pseudobactin alone, isolated from strain WCS 374, to the roots of radish induced resistance. Thus, different bacterial determinants in inducing systemic resistance in radish vary depending upon iron availability. Induction of ISR by LPS and siderophores seems to be complementary rather than additive and full induction of resistance by one determinant masks contributions by other(s).

Fluorescent pseudomonads form a line of siderophores comprised of a quinoline moiety, responsible for the fluorescence, and a peptide chain of variable length bearing hydroxamic acid and a-hydroxy acid functions. Capacity to form these pseudobactin or pyoverdine type siderophores has been associated with improved plant growth either through a direct effect on the plant, through control of noxious organisms in the soil, or via some other route. Nitrogenase can be said to be an iron-intensive enzyme complex and the symbiotic variety, as found in *Rhizobium* spp., may require an intact siderophore system for expression of this exclusively prokaryotic catalyst upon which all life depends.

Role of 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase)

First report for an enzyme capable of hydrolyzing ACC, the immediate precursor of ethylene was published in 1978, which was isolated from *Pseudomonas* sp. strain ACP (Honma and Shimomura, 1978). The ACC deaminase has been detected in the fungus, *Penicillium citrinum* and in a number of bacteria (Ma *et al.*, 2003; Blaha *et al.*, 2006; Madhaiyan *et al.*, 2006; Belimov *et al.*, 2007). The gene responsible for ACC deaminase activity (*acdS*) has been recently found in *Azopirillum*., *Burkholderia* and *Burkholderia cepacia* genomovars (which include PGPR, phytopathogens and opportunistic human pathogens), and *Agrobacterium* genomovars (Blaha *et al.*, 2006). These microorganisms were identified by their ability to grow on minimal medium containing ACC as its sole nitrogen source (Honma and Shimomura, 1978; Belimov *et al.*, 2007; Ma *et al.*, 2003).

The plant growth-promoting bacterium, *Pseudomonas putida* GR12-2, which contains the enzyme ACC deaminase, stimulates root elongation (Glick *et al.*, 1994) and significantly reduces the level of ACC in emerging roots and shoots. Three separate mutants of *Pseudomonas putida* GR12-2, deficient in ACC deaminase activity, were reported to loose the ability to promote canola root elongation under gnotobiotic conditions (Glick *et al.*, 1994)**.** An IAA over -producing mutant of *Pseudomonas putida* GR12-2 was found to be inhibitory to root elongation (Xie *et al.*, 1996). It is likely that the increased level of IAA secreted by this mutant is taken up by the plant and interacts with the enzyme ACC synthase. Thus, the "excess" bacterial IAA stimulates the synthesis of ACC which is in turn converted to ethylene (Patten and Glick, 1996). In fact, the inhibitory effect of high exogenous IAA levels on root length has been known for quite some time and is generally attributed to the stimulation of ethylene synthesis by IAA (Patten and Glick, 1996). Glick *et al.* (1997) proposed a model by which plant growth promoting bacteria can lower plant ethylene levels and in turn stimulate plant growth. The plant growth promoting bacteria bind to the surface of either the seed or root of a developing plant in response to tryptophan and other small molecules present in the seed or root exudates (Shah *et al.*, 1998)**,** they synthesize and secrete IAA (Fallik *et al.*, 1989), some of which may be taken up by the plant. This IAA, together with endogenous plant IAA, can stimulate plant cell proliferation, plant cell elongation or induce the synthesis of ACC synthase which is able to convert SAM to ACC (Patten and Glick, 1996). It is postulated that much of the ACC produced by this latter reaction may be exuded from seeds or plant roots along with other small molecules normally present in seed or root exudates (Shah *et al.*, 1998). The ACC in the exudates may be taken up by the bacteria and subsequently hydrolyzed by the enzyme, ACC deaminase, to ammonia and a-ketobutyrate. The uptake and cleavage of ACC by plant growth-promoting bacteria decrease the amount of ACC outside the plant. Increasing amounts of ACC are exuded by the plant in order to maintain the equilibrium between

interna1 and external ACC levels. As a result, the presence of the bacteria induces the plant to synthesize more ACC than it would otherwise need and as well, stimulates the exudâtion of ACC from the plant. Thus**,** plant growth-promoting bacteria are supplied with a unique source of nitrogen in the form of ACC that enables them to proliferate under conditions in which other soi1 bacteria may not flourish. As a result of lowering the ACC level within the plant, either the endogenous level or the IAA-stimulated level, the amount of ethylene in the plant is also reduced**.** It is proposed that plant growth-promoting bacteria that possess the enzyme ACC deaminase and are bound to seeds or roots of seedlings can reduce the amount of plant ethylene and thus cause inhibition of root elongation. Thus, these plants should have longer roots and possibly longer shoots as well, in as much as stem elongation, except in flooding resistant plants, is also inhibited by ethylene (Abeles *et al*., 1992). If the model that was previously proposed to explain the role of ACC deaminase in plant growth promotion (Glick *et al.*, 1995; Hall *et al.*, 1996; Glick *et al*., 1997) has any validity, a mutation in a pre-existing gene that generated the first ACC deaminase gene was fortuitous (Shah *et al.*, 1998). Such a mutation could allow a soil bacterium to utilize a unique nutrient source, i.e., ACC, which, under conditions of low nitrogen availability, would allow that bacterium to proliferate. Soil bacteria that have ACC deaminase activity should then have a selective advantage over other soi1 bacteria in situations in which the main bacterial nutrients are from exudates of plants (Shah *et al.*, 1998). It should also be borne in mind that soil bacteria may acquire ACC deaminase genes by mechanisms other than fortuitous mutation-transfer of such a gene from another soil bacterium is another possible mechanism. The regulation of ethylene production in plants, especially to prevent increased ethylene production and accumulation, may reduce many of the inhibitory effects of this hormone (Jacobson *et al.*, l994). Many agricultural and horticultural crops are particularly sensitive to ethylene levels which regulate fruit ripening and control the deleterious effects of senescence in vegetables and flowers (Sisler and Serek, 1997). The bacterial enzyme, ACC deaminase, is potentially a valuable tool for controlling the levels, and hence the effects of ethylene in plants. ACC deaminase has already been used to substantially reduce ethylene levels in transgenic tomato plants which have exhibited a prolonged ripening phase (Klee *et al.*, 1991) and to lower stress ethylene levels following infection by bacterial and fungal pathogens (Lund *et al.*, 1998). Strains of plant growth-promoting bacteria that contain ACC deaminase are known to reduce ACC, and hence ethylene, levels in canola seedlings (Penrose and Glick, 2003), promote root elongation in a variety of plants (Hall *et al.*, 1996), decrease the deleterious effects of flooding on tomato plants, and prolong the shelf-life of ethylene sensitive cut flowers (Klee *et al.*, 1991).

Root Colonization of Rhizobacteria

The colonization of rhizobacteria inside or around roots is determined by many different selection factors that influence the growth and size of different bacterial populations. Plant factors that have an influence upon microbial communities include plant age (Herschkovitz *et al.*, 2005), plant species or even plant genotype (Latour *et al.*, 1996; Dalmastri *et al.*, 1999) and root exudates (de Weert *et al.*, 2002). Biological processes in the rhizosphere are strongly influenced by plant root exudates, which consist of easily degradable organic carbon compounds that attract and stimulate microbial growth (Lynch, 1990). There is a quantitative 'rhizosphere effect' closer to the roots depending on microbial population sizes and activities in that place (Marschner *et al.*, 2004). It is more important that the distribution of microbial populations can be affected by location in the root system (Yang *et al.*, 2001; Marschner *et al.*, 2004); otherwise, it can be difficult to disentangle who contributes what to whom as root-associated microorganisms alter root exudation. For example, flavonoids, whose role in rhizobia–legume interactions is well described, may alter the shape of the rhizosphere microbial community's structure by acting on, and by being altered by 'nontarget' rhizobacteria (Shaw *et al.*, 2006). The population sizes of bacteria are larger in the bulk soil, but in the rhizosphere the phylogenetic diversity is more restricted (Berg *et al.*, 2005). The high concentration of exudates, which are recognized as easily metabolizable resources in the rhizosphere, sustains microbial populations that are more active, denser but less diverse than those present in bulk soil (Inbar *et al.*, 2005). Diversity is further made to seem smaller in the rhizoplane at the plant–soil interface (Herschkovitz *et al.*, 2005). There are a few reports that soil physical parameters (the soil matrix) are also considerably altered by exudation (Walker *et al.*, 2003) and may further select for rhizosphere-competent traits. It is obvious that reciprocal interaction between soil, plant and microorganisms occurs and that they are complex and should be accounted for (Walker *et al.*, 2003; Marschner *et al.*, 2004). However complex this is to achieve, beneficial outcomes of understanding such tripartite interactions on rhizosphere communities may be reaped when data become available. For example, in a study by Berg *et al.* (2005, 2006), it was shown that naturally occurring rhizobacterial and fungal communities antagonistic to *Verticillium dahliae* were further enhanced in certain plant species and at determined sites of cultivation. In summary, current data show that while the microbial diversity may appear labile, cause and effect relationships linking community structure to environmental, biological and agro-technical parameters are only just emerging. This will be very important knowledge when applied to microbial inoculants on a large scale.

Modifications due to activities and their partnership among soil, plants and microorganisms show intricate reaction mechanisms. There is a question; what is the fate of rhizosphere microorganisms when a PGPR is introduced at high levels in the rhizosphere? Undoubtedly, the answer is: it depends on the conditions. This dependence may affect interactions within and between indigenous populations. In relation to the soil–plant–environment background, certain groups may be enhanced, while others may be inhibited, or the introduced PGPR may not affect population structure (Bacilio- Jimenez *et al.*, 2001; Dobbelaere *et al.*, 2003). PGPR enhance more roots in the soil volume, as was shown in maize inoculated with *Azospirillum brasilense* (Dobbelaere *et al.*, 2003) most probably due to direct mechanism of phytohormone excretion. Applying the PCR-denaturing gradient gel electrophoresis (DGGE) (a fingerprinting technique based on the detection of polymorphism in a marker gene common to the targeted populations) for the analysis of bacterial population with general and group-specific primers, respectively, neither dominant nor specific bacterial populations were detected, though, the bacterial populations were affected upon inoculation but the plants developed a denser root system (Herschkovitz *et al.*, 2005; Lerner *et al.*, 2006). Following the inoculation with such PGPR the environment can be affected as a consequence of higher microbial densities and higher metabolic (enzymatic) activity more specifically C, P and N turnover is increased in the rhizosphere (Mawdsley and Burns, 1994; Johansen and Binnerup, 2002). A more developed root system means the ratio between rhizosphere and bulk soil is higher, it is a characteristic of a more active soil and a characteristic thought to be positive (Winding, 2004). There is a logical hypothesis for the indirect action of PGPR which secrete an antibiotic compound may alter the existing rhizobacterial communities. However, fluorescent pseudomonads producing 2,4-diacetylphoroglucinol, phenazine or other antibiotics showed temporally and spatially limited and transient effects on rhizobacterial or fungal populations in colonization with rhizosphere of various plants (Naseby and Lynch, 2001; Bakker *et al.*, 2002). However when soils were systematically replanted in cycling experiments showed the overall small effects. Although large fractions of isolated rhizobacteria may be sensitive to the antibiotic produced by the PGPR *in vitro*, these populations are seldom affected by the presence of the inoculated bacteria *in vivo*, even under gnotobiotic conditions (Johansen and Binnerup, 2002). Niche overlap between an inoculant and resident bacteria appears to be limited even with resident organisms that are phylogenetically closely related to the inoculant. Spatial separation and nutrient versatility are certainly important dimensions contributing to this restricted overlap. Whereas, 2,4-diacetylphoroglucinol producing *Pseudomonas fluorescens* F113Rif appeared to cause a reduction in rhizobial diversity in some studies but produced longer-term residual effects of antibiotic-producing PGPR on resident bacteria (Walsh *et al.*, 2003). Some other studies reported that there are strong shifts in the community structure of some specific bacterial groups. For example, the production of trifolitoxin by *Rhizobium etli* strongly reduced the proportion of trifolitoxin-sensitive bacteria of the α-proteobacteria (Robleto *et al.*, 1998).

Conclusions

On the basis of above evidences described above, it is clear that much more going on around rhizosphere, Due to biological N_2 fixation by bacteria, plant growth becomes faster. Similarly IAA production, P solublization, siderophore production, ACC deaminase activity by PGPR provide suitable environment for plant growth.

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