Potential Role of PGPR in Agriculture

2

PGPR strains have diverse applications in agriculture, horticulture and forestry. There are several reports in the literature indicating that PGPR could be proved as a boon in sustainable agriculture. PGPR are used as bioinoculants, biofertilizers and biocontrol agents, with practical potential in improved agriculture (Table 2.1). Their beneficial events could be biological control of diseases and pests, plant growth promotion, increasing crop yields and quality improvement that can take place simultaneously and sequentially (Fig. 2.1). Genera of PGPR include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Bacillus* and *Paenibacillus*, and some are members of the *Enterobacteriaceae*. Direct use of microorganisms to promote plant growth and to control plant pests continues to be an area of rapidly expanding research.

2.1 Plant Growth Promotion

PGPR strains can promote plant growth and development mainly by the following means:

- Production of phytohormones
- Biological nitrogen fixation
- Solubilization of mineral phosphates and other nutrients
- Production of siderophores that chelate iron and make it available to plant root
- PGPR as biofertilizer
- Promotion of mycorrhizal functioning

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.

2.1.1 Production of Phytohormones

PGPR produce phytohormones that are believed to be related to their ability to stimulate plant growth. Hormones are organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. They interact with specific target tissues to cause physiological responses, such as growth or fruit ripening. Botanists recognize five major groups of hormones: auxins, gibberellins, ethylene, cytokinins and abscisic acid.

IAA is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement. This hormone is very commonly produced by PGPR and implicated in the growth promotion by PGPR. However, the effect of IAA on plants depends on the plant sensitivity to IAA and the amount of IAA produced from plant-associated bacteria and induction of other phytohormones. The bacterial IAA from *Pseudomonas putida* played a major role in the development of host plant root system. Most commonly, IAA-producing PGPR are believed to increase root growth and root length, resulting in greater root

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Table 2.1 Forms of PGPR and their mechanism of action stimulating plant growth

PGPR forms	Definition	Mechanism of action
Biofertilizer	A substance that contains live microorganisms which, when applied on the seed, plant surface or soil, colonizes the rhizosphere and promote plant growth through increased supply of primary nutrients for the host plant	Biological nitrogen fixation. Utilization of insoluble phosphorus
Phytostimulator	Microorganism, with the ability to produce phytohormones such as indole acetic acid, gibberellic acid, cytokinins and ethylene	Production of phytohormones
Biopesticide	Microorganisms that promote plant growth by controlling phytopathogenic agents	Production of antibiotics, siderophores, HCN
		Production of hydrolytic enzymes
		Acquired and induced systemic resistance

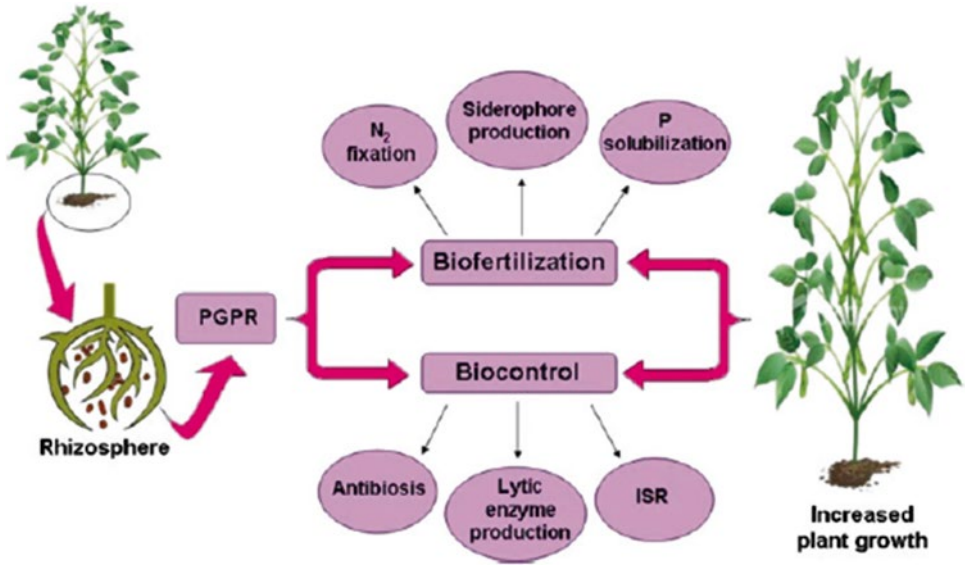

Fig. 2.1 Schematic illustration of important mechanisms (biofertilization and biocontrol of pathogens) known for plant growth promotion by PGPR

surface area which enables the plant to access more nutrients from soil.

Cytokinins are a class of phytohormones which are known to promote cell divisions, cell enlargement, and tissue expansion in certain plant parts (Salisbury 1994). Cytokinin is produced by *Pseudomonas fluorescens* isolated from the rhizosphere of the soybean (De Salamone et al. 2001).

Gibberellins are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury 1994). Evidence of GA production by PGPR is rare; however, Gutierrez-Manero et al. (2001) provide evidence that four different forms of GA are produced by *Bacillus pumilus* and *B. licheniformis*.

Ethylene is the only gaseous phytohormone. It is also known as the wounding hormone because its production in the plant can be induced by physical or chemical perturbation of plant tissues (Salisbury 1994). Glick and Pasternak (2003) put forward the theory that the mode of action of

Table 2.2 Examples of different phytohormone-producing PGPR

Phytohormones	PGPR
Indole-3-acetic acid (IAA)	*Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Aeromonas veronii*, *Enterobacter cloacae*, *Azospirillum brasilense*, *Enterobacter* spp., *Agrobacterium* spp., *Alcaligenes piechaudii*, *Bradyrhizobium* spp., *Comamonas acidovorans*, *Rhizobium leguminosarum*
Cytokinin	*Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Rhizobium leguminosarum*
Zeatin and ethylene	*Azospirillum* spp.
Gibberellic acid (GA_3)	*Azospirillum lipoferum*, *Bacillus* spp.
Abscisic acid (ABA)	*Azospirillum brasilense*
ACC deaminase	*Bacillus pumilus*, *Burkholderia cepacia*

Table 2.3 Efficacy of PGPR formulations on growth promotion

Formulation	Crop	Results	Reference
Talc-based *P. fluorescens*	Potato	Significant plant growth promotion	Kloepper and Schroth (1981)
	Pigeon pea	Significant increase in grain yield	Vidhyasekaran et al. (1997)
B. subtilis strain LS213 (commercial product)	Muskmelon, watermelon	Increased plant growth and improved yield	Vavrina (1999)
Vermiculite and kaolin based *B. subtilis*	Lettuce	Increased fresh weight	Amer and Utkhede (2000)
Chitin based formulation of *B. subtilis* strain GBO3 + *B. pumilus* strain INR7 (LS256) and *B. subtilis* strain GBO3 + *B. subtilis* strain IN937b	Tomato, pepper	Increased yield of pepper and tomato	Burelle et al. (2002)
Talc-based formulation of *B. subtilis* and *P. chlororaphis* (PA23)	Tomato	Increased growth promotion	Kavitha et al. (2003)
Talc-based *P. fluorescens* FP7 supplemented with chitin	Mango	Increased fruit yield and quality	Vivekananthan et al. (2004)
Talc-based *B. subtilis* (BSCBE4) and *P. chlororaphis* (PA23)	Turmeric	Significant yield increase of rhizomes	Nakkeeran et al. (2004)

some PGPR was the production of 1-carboxylate deaminase, an enzyme which could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plant. The signalling pathway that is activated in this case depends on ethylene but is independent of salicylic acid (SA) and jasmonic acid (JA) signalling (Ryu et al. 2004). It would be interesting to investigate the capacity of plant growth-promoting *Pseudomonas* spp. to produce 2, 3-butanediol and its possible involvement in ISR.

Abscisic acid (ABA), also known as abscisin II and dormin, is a plant hormone. ABA is the major hormone that controls plants' ability to survive in a harsh, changing environment. It is a signalling pathway that is conserved in all types of plants and is considered a very early adaptation to the terrestrial environment. ABA participates in the control of root growth, seed desiccation and dormancy, guard cell responses and cellular osmoprotection. ABA functions in many plant developmental processes, including bud dormancy. It can stimulate root growth in plants that need to increase their ability to extract water from the soil.

Tables 2.2 and 2.3 represent some of the efficient PGPR strains as the producers of different plant growth regulators. The enhancement in

various agronomic yields due to PGPR has been reported because of the production of growth stimulating phytohormones such as indole-3-acetic acid (IAA), gibberellic acid (GA_3), zeatin, ethylene and abscisic acid (ABA).

2.1.2 Biological Nitrogen Fixation

PGPR facilitate plant growth and development directly by supply of nitrogen to plants through nitrogen fixation. Nitrogen (N_2) is one of the principal plant nutrients, becoming a limiting factor in agricultural ecosystems due to heavy losses by rainfall or mineral leaching. Nitrogen is an essential element for all forms of life – a basic requisite for synthesizing nucleic acids, proteins and other organic nitrogenous compounds. Regrettably no plant species are capable of fixing atmospheric dinitrogen into ammonia and expend it directly for its growth. Thus the plants depend on biological nitrogen fixation (BNF). PGPR can fix atmospheric N_2 either symbiotically or non-symbiotically.

2.1.2.1 Symbiotic Nitrogen Fixation

Symbiotic N_2 fixation to legume crops with the inoculation of effective PGPR is well known (Esitken et al. 2006). These PGPR live inside the plant cells and produce nodules. Examples include *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium* and *Sinorhizobium* species. The process of symbiotic N_2 fixation is limited only to legume crops and various trees and shrubs that form actinorhizal roots with *Frankia*.

i) *Rhizobium* Inoculants

These help in establishing efficient symbiotic association with leguminous crops (Fig. 2.2) and thus can fix 50–100 kg N/ha. A 10–70 % increase in yield of crops due to inoculation with *Rhizobium* inoculants over uninoculated has been reported. *Rhizobium* is specific to each legume crop and only the recommended inoculant should be used for each leguminous crop such as cowpea, pigeon pea, peas, etc.

Fig. 2.2 Root nodules colonized with rhizobacteria

2.1.2.2 Non-symbiotic Nitrogen Fixation

Non-symbiotic biological N_2 fixation is basically carried out by free-living diazotrophs that belong to the genera like *Azoarcus* (Reinhold-Hurek et al. 1993), *Azospirillum* (Bashan and de-Bashan 2010), *Burkholderia* (Estrada de los Santos et al. 2001), *Gluconacetobacter* (Fuentes-Ramirez et al. 2001), *Pseudomonas* (Mirza et al. 2006), *Azotobacter*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Acetobacter*, etc. associated with the plant rhizosphere and fix atmospheric N_2 into form which is taken up by the plants. These are free-living rhizobacteria and live outside the plant cells and do not produce nodules. These rhizobacteria are reported to fix atmospheric N_2 in soil (Riggs et al. 2001) and make it available to plants.

i) *Azotobacter* Inoculants

Azotobacter is a free-living aerobic nitrogen fixer which is recommended for non-leguminous crop like potato, tomato, mustard etc. *Azotobacter* fixes atmospheric nitrogen in soil and helps in saving chemical fertilizers by 15–20 kg N/ha. The family Azotobacteriaceae

includes species *A. agilis*, *A. insignis*, *A. macrocytogenes*, *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, and *A. paspali*.

ii) *Azospirillum* Inoculants

Azospirillum fix nitrogen under microaerophilic conditions and are frequently associated with root and rhizosphere of a large number of agriculturally important non-leguminous crops. *Azospirillum* fix atmospheric N_2 in soil and help to save chemical fertilizers by 15–20 kg N/ha and include species like *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. halopraeferens*, *A. irakense*, *A. largimobile*, *A. doebereinerae*, *A. oryzae*, *A. melini* and *A. canadensis*.

Recently, Minorsky (2008) reported a PGPR strain, *Pseudomonas fluorescens* B16, exhibiting vigorous colonization in the roots of tomatoes, causing enhancement in plant height, flower number and total fruit weight. A similar investigation on rhizobia to replace the use of nitrogen fertilizer was made by Vessey (2003) and thereby demonstrated a clear picture of improvement in crop yield after the inoculation of available nutrients by rhizobacteria in agricultural soil.

Biological nitrogen fixation contributes 180×10^6 metric tons/year globally, out of which symbiotic associations contribute 80 % and the rest comes from free-living or associative systems. The use of biofertilizer and bio-enhancer such as N_2-fixing bacteria and beneficial microorganisms can reduce chemical fertilizer applications and consequently lower production cost. PGPR retain more soil organic N and other nutrients in the plant soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients. Besides, combined inoculations of rhizobacterial species to improve the quality of soil also seemed to be a potent area of research in present-day agriculture.

2.1.3 Solubilization of Mineral Phosphates and Other Nutrients

PGPR can change the plant physiology and certain nutritional and physical properties of rhizospheric soil and indirectly influence on the colonization patterns of soil microorganisms in that particular region. Inoculation of rhizobacteria increased uptake of nutrient elements like Ca, K, Fe, Cu, Mn and Zn by plants through stimulation of proton pump ATPase (Mantelin and Touraine 2004). Reports are available on the combinations of *Bacillus* and *Microbacterium* inoculants to improve the uptake of the mineral elements by crop plants (Karlidag et al. 2007). This increase in nutrient uptake by plants might be explained through organic acid production by the plants and PGPR, decreasing the soil pH in rhizosphere. There is an ample evidence (Glass et al. 2002) for the maintenance of soil fertility by the rhizobacterial isolates to increase the availability of nutrients to plants. Solubilization of unavailable forms of nutrients is one of the essential criteria in facilitating the transport of most of these nutrients (Glick 1995).

Plant growth-promoting rhizobacteria are actively involved in the solubilization of important minerals such as phosphorous and iron, thereby enhancing the availability of these essential nutrients to plants (Glick 1995). The positive role of PGPR in stimulating the plant growth by improving solubilization (releasing siderophores or organic acid) and nutrient uptake by the plants has been well documented in the literature (Glick 1995).

2.1.3.1 Phosphorous Solubilization

Phosphorus (P) is the second most important essential plant nutrient but most of P remains fixed in soil which is not available to plants. Phosphorus is necessary for plant growth and is taken by the plants from soil as phosphate anions. Phosphate anions are highly reactive and may be trapped via precipitation with cations such as Mg_2^+, Ca_2^+, Al_3^+ and Fe_3^+ depending on the quality of the soil. Phosphorus is extremely insoluble and unavailable to plants in these forms. As a result, the amount available to plants is usually a small proportion of this total. This low availability of phosphorus to plants is because the vast majority of soil P is found in insoluble forms. Many scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds

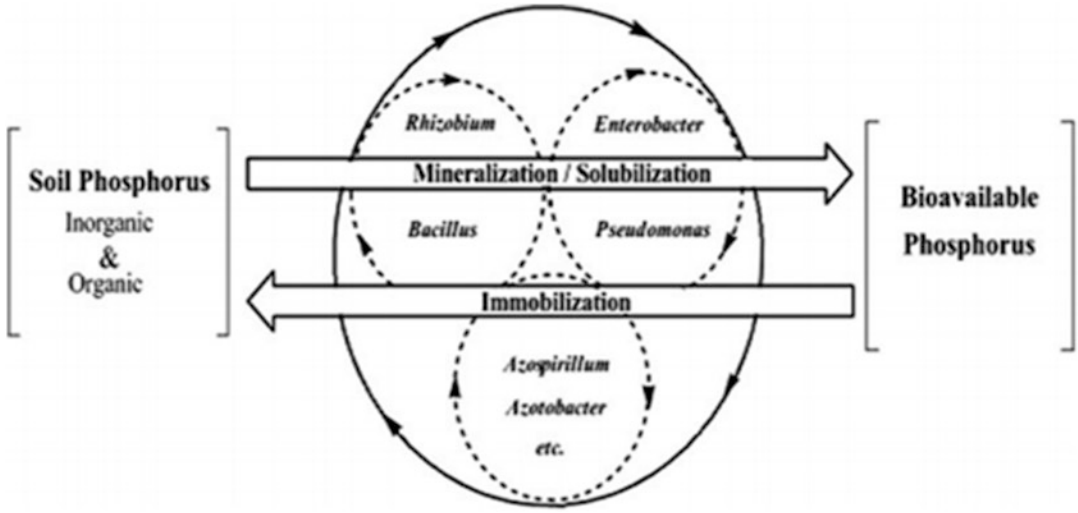

Fig. 2.3 Schematic representation of solubilization of soil phosphorus by rhizobacteria (Khan et al. 2009)

such as dicalcium phosphate, tricalcium phosphate, rock phosphate and hydroxyapatite. These bacteria solubilize phosphate through the production of acids and by some other mechanism and are termed as phosphate-solubilizing bacteria (PSB). Several researchers consequently have isolated PSB from various soils and proved that inoculations of these bacteria increased the plant growth and yield.

The plants can only absorb P in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic (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 for the chelation-mediated mechanisms (Whitelaw 2000) to solubilize phosphate in soil. Release of plant root exudates such as organic ligands can also alter the concentration of P in soil solution (Hinsinger 2001). In certain cases, phosphate solubilization is induced by phosphate starvation (Gyaneshwar et al. 1999). A general sketch of phosphorous solubilization in soil is shown in Fig. 2.3. Inoculation with an efficient P-solubilizing microorganism improves the availability of P from insoluble form of P in soil and enhances use efficiency of phosphatic fertilizer such as super phosphate. There are a number of inoculants which can even degrade rock phosphate and soil fixed P. A number of metabolites are released by these strains which strongly affect the environment and increase nutrient availability for the plants, viz., *B. subtilis*, *B. licheniformis*, *B. megaterium* var. *phosphaticum* and *P. lutea*. Bacterial genera like *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate-solubilizing bacteria (Mehnaz and Lazarovits 2006). Rhizobacteria can solubilize inorganic P sources and enhance growth and yield of crop plants. Examples of some widely reported P-solubilizing microbial species intimately associated with a large number of agricultural crops like potato, tomato, radish, pulses etc. are *Azotobacter chroococcum* (Kumar and Narula 1999), *Bacillus circulans* and *Cladosporium herbarum* (Singh and Kapoor 1999), *Bradyrhizobium*

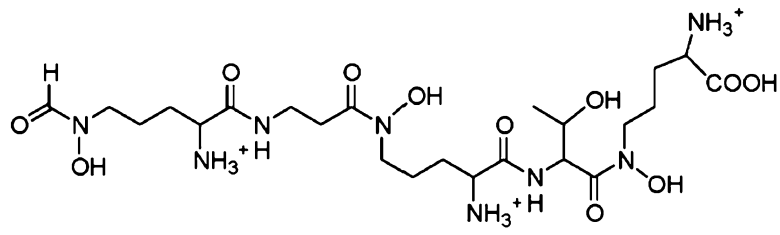

Fig. 2.4 Structure of siderophore

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 PGPR 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. PGPR have been recorded to solubilize precipitated phosphates to plants, representing a possible mechanism of plant growth promotion under field conditions (Verma et al. 2001). Synthesis of organic acids by rhizosphere microorganisms could be the possible reason for solubilization of inorganic P sources.

2.1.3.2 Manganese Oxidation

The availability of manganese (Mn) in the rhizosphere is affected by two major factors: redox condition and pH. In oxidized soils manganese is present in its oxidized form, Mn^{4+}, in the low soluble mineral pyrolusite. Some rhizosphere bacteria (*Bacillus*, *Pseudomonas* and *Geobacter*) can reduce oxidized Mn^{4+} to Mn^{2+}, which is the chemical form that is metabolically useful for plants.

The reaction is as follows:

$$MnO_2 + 4H^+ + 2e^- \leftrightarrow Mn^{2+} + 2H_2O. \quad (2.1)$$

In this reaction two points are important; the reduction of Mn requires electrons and protons. Electrons are supplied by the decomposition of carbonaceous compounds, and protons can be supplied by the proton excretion system of root cells (Marschner 1997). Consequently, the activity of Mn reducers is highly favoured in the rhizosphere. Applications of organic matter also can favour the reduction of Mn (Hue et al. 2001). In alkaline soils where Mn usually is insoluble, the rhizosphere effect is beneficial, but in acidic soils with abundance of Mn minerals, excessive reduction of Mn can induce Mn toxicity in sensitive plants (Hue et al. 1998).

Mn plays an important role in the resistance of plants to disease. Mn, as well as Cu, are required for the synthesis of lignin, which increases the resistance of the root tissues to the penetration of pathogens; consequently Mn-deficient plants are more susceptible to the attack of plant pathogens. *Gaeumannomyces graminis*, like many other soilborne pathogenic fungi, is a powerful oxidizer of Mn that impairs the lignification of roots at infection sites (Graham 1999). Effective rhizosphere Mn reducers (e.g. *Pseudomonas* sp.) could have beneficial effects not only on plant nutrition but also on biocontrol of pathogens. In addition, roots and rhizosphere bacteria can produce chelating agents (phenolic compounds, organic acids) that form soluble complex with Mn and other elements avoiding the reprecipitation of Mn.

2.1.4 Production of Siderophores That Chelate Iron and Make It Available to Plant Root

Iron is one of the bulk minerals present in plenteous amount on Earth, yet it is unavailable in the soil for the plants. This is because Fe^{3+} (ferric ion) is the common form of iron found in nature and is meagrely soluble. To overcome this problem, PGPR secrete siderophores. Siderophores are iron-binding proteins of low molecular mass and have a high binding affinity with ferric ion (Fig. 2.4). Soil Fe is present in oxidized form (Fe^{3+}), as a component of insoluble minerals such

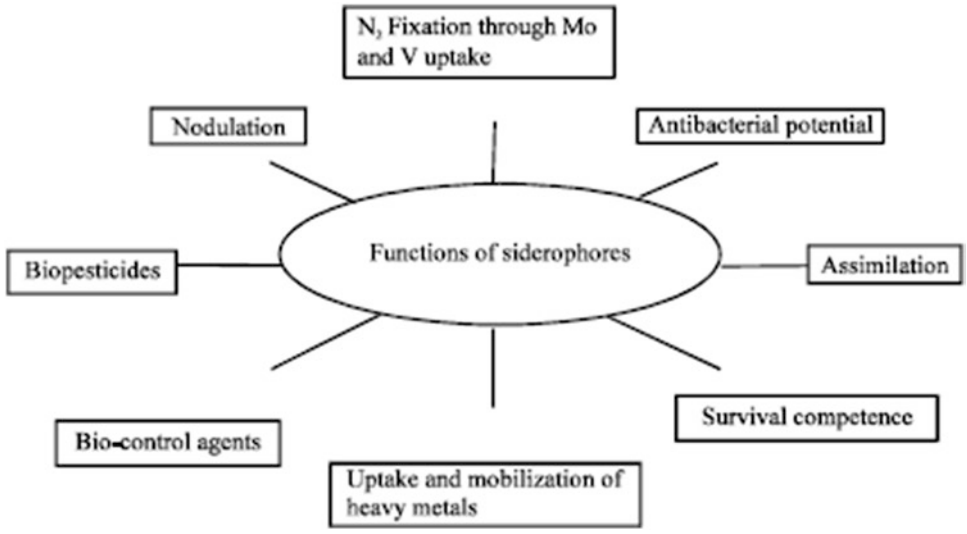

Fig. 2.5 Impact of microbially secreted siderophores on plant growth

as goethite and hematite (Fe_2O_3). Rhizosphere bacteria (*Bacillus*, *Pseudomonas*, *Geobacter*, *Alcaligenes*, *Clostridium* and *Enterobacter*) can reduce Fe^{3+} to Fe^{2+}, the form required by plants. Electrons and protons are available in the rhizosphere and consequently iron is reduced; however it can be reprecipitated. The reactions of reduction are as follows:

$$FeOOH + 3H^+ + e^- \leftrightarrow Fe^{2+} + 2H_2O(1);\ Fe_2O_3 + 6H^+ + 2\ e^- \leftrightarrow 2\ Fe^{2+} + 3H_2O\ (2). \quad (2.2)$$

Siderophores secreted by PGPR improves plant growth and development by increasing the accessibility of iron in the soil surrounding the roots. The plants utilize siderophores secreted by PGPR for sequestering iron. Cucumber has the ability to use microbial siderophores as the sole source of iron than its own siderophores (phytosiderophores). Microbial siderophores are also reported to increase the chlorophyll content and plant biomass in plants of cucumber.

In addition to transporting iron, siderophores have other functions and effects, including enhancing pathogenicity, acting as intracellular iron storage compounds and suppressing growth of other microorganisms. Siderophores can complex other metals apart from iron, in particular the actinides (Fig. 2.5).

2.1.5 PGPR as Biofertilizer

Biofertilizers are the substances, prepared from living microorganisms which, when applied to the seeds or plant surfaces adjacent to soil, can colonize the rhizosphere or the interior parts of the plants and thereby promotes root growth. The term biofertilizer should not be used interchangeably with green manure, manure, intercrop or organic-supplemented chemical fertilizer. Interestingly some PGPR species have appeared to promote plant growth by acting both as biofertilizer and biopesticide. For instances, strains of *Burkholderia cepacia* have been observed with biocontrol characteristics to *Fusarium* spp., and it can also stimulate growth of maize under iron-poor conditions via siderophore production (Bevivino et al. 1998). *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* are reported as the potent PGPR strains for their ability to act as biofertilizers (Vessey 2003). They are an important group of microorganisms used in biofertilizers. Biofertilization accounts for approximately 65 % of the nitrogen supply to crops worldwide.

The relationship between the PGPR and their host can be categorized into two basic

levels of complexity: (i) rhizospheric and (ii) endophytic. In rhizospheric relationship, the PGPR can colonize the rhizosphere, the surface of the root or even the superficial intercellular spaces of plant roots (McCully 2001). It is only due to the changes in different physicochemical properties of rhizospheric soil such as soil pH, water potential and partial pressure of O_2 and plant exudation as compared to the bulk soil that in turn can affect the ability of PGPR strains to colonize the rhizosphere (Griffiths et al. 1999). In endophytic relationship, PGPR resides within the apoplastic spaces inside the host plants. There is a direct evidence of existence of endophytes in the apoplastic intercellular spaces of parenchyma tissue (Dong et al. 1997) and xylem vessel (James et al. 2001). Best examples can be cited from legume–rhizobia symbioses in leguminous plants (Vessey 2003). Thus, the means by which the PGPR enhance nutrient status of host plants and thereby act as biofertilizers can be categorized into five distinct areas such as biological N_2 fixation, increasing the availability of nutrients in the rhizosphere, increasing the root surface area, enhancing beneficial symbioses of the host and finally the combinations of all the above modes of action. However, the degree of intimacy between the PGPR and host plant can vary depending on where and how the PGPR colonizes the plant.

Plant growth-promoting rhizobacteria are beneficial soil bacteria that colonize plant roots and enhance plant growth promotion activity by different mechanisms in various ways. There is an ample evidence in the literature indicating that PGPR can be a best alternative to chemical fertilizer for sustainable and eco-friendly agriculture. They will not only provide nutrients to the plants (direct plant growth promotion) and protect plants against the phytopathogens (indirect plant growth promotion) but also increase the soil fertility. Thus, awareness must prevail among the farmers about the negative impact of chemical fertilizers and positive aspects of PGPR as biofertilizer.

2.1.6 Promotion of Mycorrhizal Functioning

Interactions of arbuscular mycorrhizal fungi (AMF) with PGPR have been described with regard to their effect on mycorrhizal development and functioning. PGPR can promote mycorrhizal functioning. Rhizobacteria showing a beneficial effect on mycorrhizae are often referred to as 'mycorrhizae-helper microorganisms'. Bacteria associated to mycorrhizal fungi adhere to fungal spores and hyphal structures and thus spread to the rhizosphere. There is a strong evidence of a vertical transmission of endobacteria through the AM fungus vegetative generation. However, antagonistic effects are often reported in the AM fungi–PGPR interactions. Positive interactions often result in plant growth improvement.

Inoculation with both free-living nitrogen-fixing bacteria such as *Azospirillum brasilense* or *Azotobacter* and AM fungi increases plant productivity. The nitrogen-fixing bacteria stimulate root colonization by AM fungi and increase their number of internal vesicles; they also alter rhizosphere rhizobial populations. It is not clear whether the enhancement of plant growth is due to free-nitrogen fixation or to the production of plant growth-promoting substances.

Some studies considered free-nitrogen fixers like other PGPR species, without reference to nitrogen fixation activity. For instance, in a study using the nitrogen fixer *A. chroococcum* and *P. fluorescens*, the chemotaxis of these two PGPR towards roots of mycorrhizal tomato plants (*Glomus fasciculatum*) was an important step of communication for root colonization. It was found that *G. fasciculatum* alters the characteristics of root exudates which are chemoattractants specific for each PGPR, amino acids for *P. fluorescens* and sugars for *A. chroococcum*. Different combinations between three PGPR species (*A. chroococcum*, *Azospirillum brasilense* and *Burkholderia cepacia*) and two AM fungi (*Glomus clarum* and *G. fasciculatum*) did not show the same trends on root colonization or on the nutritional status of onion and tomato; the

highest mycorrhizal colonization was achieved by *Azospirillum brasilense* co-inoculated with each AM species on tomato and by single inoculation with *G. fasciculatum* on onion. Ecology of plant growth-promoting rhizobacteria enhanced root colonization by the AM fungus and improved soil properties such as organic matter content and total N. The use of PGPR and AM fungi has been attempted with the aim of protecting plants against pathogens. The interactions of biocontrol PGPR with AM fungi are often contradictory and probably depend on the tested bacterium, the plant species and the environmental factors.

The possibility of optimizing plant growth by managing interactions between AM fungi, PGPR and the *Rhizobium*–legume symbiosis has been considered as a promising avenue, and synergism resulting from these interactions has been demonstrated earlier. Bacterial culture of fluorescent *Pseudomonas* co-inoculated with *Glomus etunicatum* increased root growth, nodulation and N and P uptake in beans (*Phaseolus vulgaris*).

2.2 Production of ACC Deaminase and Regulation of Ethylene Level in Plants

Although ethylene is essential for normal growth and development in plants, at high concentration it can be harmful as it induces defoliation and other cellular processes that may lead to reduced crop performance. Using their 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, PGPR can divert ACC from the ethylene biosynthesis pathway in the root system of *Arabidopsis thaliana* plant (Fig. 2.6) (Desbrosses et al. 2009). Thus, rhizobacteria assist in diminishing the accumulation of ethylene levels and re-establish a healthy root system needed to cope with environmental stress. The primary mechanism includes the destruction of ethylene via enzyme ACC deaminase. The rhizosphere bacteria such as *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium* exhibit ACC deaminase activity

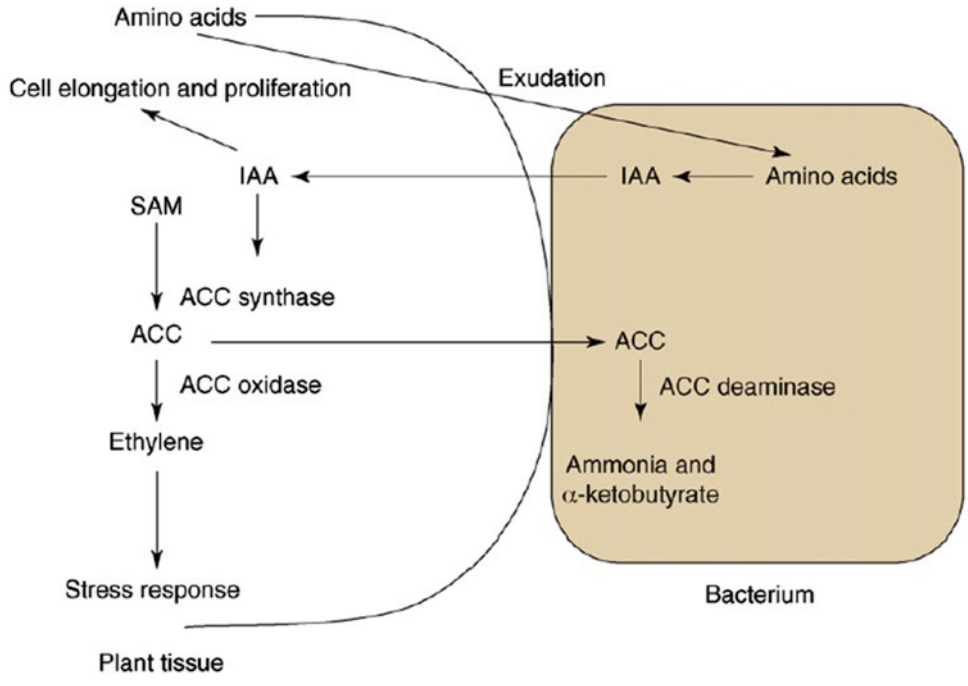

Fig. 2.6 Schematic model to explain how ACC deaminase containing PGPR lowers the ethylene concentration, thereby preventing ethylene-caused inhibition of root elongation (Glick et al. 1999). Key: *IAA* Indole acetic acid, *ACC* 1-aminocyclopropane-1-carboxylic acid, *SAM* S-adenosyl methionine

(Duan et al. 2009). Most of the studies have demonstrated the production of ACC deaminase gene in the plants treated with PGPR under environmental stress. Ghosh et al. (2003) recorded ACC deaminase activity in three *Bacillus* species, namely, *B. circulans* DUC1, *B. firmus* DUC2 and *B. globisporus* DUC3 that stimulated root elongation in *Brassica campestris*. Mayak et al. (2004) observed tomato plants inoculated with the bacterium *Achromobacter piechaudii* under water and saline stress conditions and reported a significant increase in fresh and dry weight of inoculated plants.

PGPR containing ACC deaminase can boost the plant growth particularly under stressed environmental conditions like salinity, drought, waterlogging, temperature, pathogenicity and contaminants in response to a multitude of abiotic and biotic stresses (Saleem et al. 2007). Although efforts have been made to introduce ACC deaminase genes into plants for optimum growth, the genetic modifications for all the plant species are not yet possible due to many handicaps like proprietary rights and international trade agreements on genetically modified (GM) crops and limitations of recombinant DNA technology.

2.3 Production of Volatile Organic Compounds

The discovery of volatile organic compounds (VOCs) produced by rhizobacteria constitute an important mechanism for the elicitation of plant growth by rhizobacteria. Ryu et al. (2003) recorded some PGPR strains namely *Bacillus subtilis* GBO3, *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*, suggesting that synthesis of bioactive VOCs is a strain-specific phenomenon. Acetoin-forming enzymes have been identified earlier (Forlani et al. 1999) in carrot, 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 signalling 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 signalling plants to register plant defence, more investigations into the volatile components in the plant–rhizobacteria system should follow.

2.4 Rhizosphere Engineering

Rhizosphere microbial populations are tremendously affected by the interactions between the plants and the soil environment. Rhizosphere engineering involves the selection of beneficial microbial populations by plant rhizosphere. For instances, some crop species or cultivars select populations of antibiotic-producing strains that play a major role in soils, naturally suppressive to soilborne fungal pathogens (Ryan et al. 2009). Persistent organic pollutants such as polychlorinated biphenyls (PCBs) are a global problem. Using root-associated microbes in rhizospheric engineering approach, the levels of PCBs can be successfully depleted as these microbes can use plant secondary metabolites such as phenylpropanoids (Narasimhan et al. 2003). Similar technology has been developed by Lugtenberg et al. (2001) during their investigation on the growth of microbes with the ability to metabolize exotic nutrients exuded by plants. One of the earliest successes of this technology was based on the favourable partitioning of the exotic nutrient opines, produced by the transgenic plants (Oger et al. 1997) that led to the improved and competitive growth of the metabolizing strains in comparison with the microbes unable to metabolize opines. Rhizosphere engineering ultimately reduces our reliance on agrochemicals by replacing their functions with beneficial microbes, biodegradable biostimulants or transgenic plants (Ryan et al. 2009). It is now possible to create a nutritional bias that may be especially successful

in identifying microbial populations due to the general nutrient-limiting conditions in the rhizosphere.

Molecular microbiological advances have been exploited in order to achieve a complete knowledge of the complex chemical and biological interactions that generally occur in the rhizosphere, ensuring that the strategies to engineer the rhizosphere are safe and eco-friendly to agricultural systems. For example, plants are genetically engineered to modify the rhizosphere pH to release the compounds that could improve nutrient availability, protect plants against biotic and abiotic stresses or encourage the proliferation of beneficial microorganisms. Growth stimulation can be mediated directly through enhanced nutrient acquisition or modulation of phytohormone synthesis, while indirect stimulation involves the induction of plant antagonism (Ryan et al. 2009). Sundheim et al. (1988) observed that an engineered strain of *Pseudomonas* expressing chitinase gene from *Serratia marcescens* more effectively controlled *Fusarium oxysporum* f. sp. *redolens* and *Gaeumannomyces graminis* var. *tritici* in vitro. Recently, experiments targeting on the DAPG-producing PGPR strain *Pseudomonas fluorescens* (phlD?) have demonstrated that plant species can differentially enrich and support different microbial populations (De La Fuente et al. 2006) and genotypes (Landa et al. 2006) in the rhizosphere. Notz et al. (2001) significantly correlated DAPG accumulation by *Pseudomonas fluorescens* CHAO with the expression of DAPG biosynthesis gene phlA and observed that the expression was significantly greater in the rhizosphere of monocots than dicots. Although the exact mechanism is not totally understood, Di Gregorio et al. (2006) noticed a combined application of Triton X-100 and *Sinorhizobium* sp. Pb002 inoculums for the improvement of lead phytoextraction by *Brassica juncea* in EDTA-amended soil.

2.5 PGPR as Biotic Elicitors

Elicitors are chemicals or biofactors of various sources that can trigger physiological and morphological responses and phytoalexin accumulation in plants. It may be abiotic elicitors such as metal ions or inorganic compounds and biotic elicitors, basically derived from fungi, bacteria, viruses, plant cell wall components and chemicals that are released due to antagonistic reaction of plants against phytopathogens or herbivore attack. It has now been observed that the treatment of plants with biotic elicitors can cause an array of defence reactions including the accumulation of a range of plant defensive bioactive molecules such as phytoalexins in the intact plants. Thus, elicitation is being used to induce the expression of genes responsible for the synthesis of antimicrobial metabolites. Rhizosphere microbes are best known to act as biotic elicitors, which can induce the synthesis of secondary products in plants (Sekar and Kandavel 2010). Signal perception is the first committed step towards the biotic elicitor signal transduction pathway in plants. Jasmonic acid and its methyl ester are the signal transducers in a wide range of plant cell cultures that could accumulate rapidly when the suspension cultures of *Rauvolfia canescens* and *Eschscholtzia californica* are treated with a yeast elicitor (Roberts and Shuler 1997). Some of the well-reported PGPR as biotic elicitors have been exemplified in Table 2.4.

Table 2.4 PGPR species as biotic elicitors to elicit plant response

PGPR species	Plant	Metabolite induced	References
Pseudomonas fluorescens	*Catharanthus roseus*	Ajmalicine	Jaleel et al. (2007)
		Serpentine	Jaleel et al. (2009)
P. putida and *P. fluorescens*	*Hyoscyamus niger*	Hyoscyamine and scopolamine	Ghorbanpour et al. (2010)
Bacillus subtilis	*Crocus sativus*	Picrocrocin, crocetin and safranal compounds	Sharaf-Eldin et al. (2008)
B. cereus	*Salvia miltiorrhiza*	Tanshinone	Zhao et al. (2010)

Ajmalicine, serpentine, picrocrocin, crocetin, hyoscyamine and scopolamine, safranal compounds and tanshinone are recorded as the important metabolites produced by PGPR species in eliciting the physiological and morphological responses in crop plants.

2.6 Antagonistic Activity of PGPR

Rhizobacteria can suppress the growth of various phytopathogens in a variety of ways like competing for nutrients and space, limiting available Fe supply through producing siderophores and producing lytic enzymes and antibiosis (Jing et al. 2007). Among PGPR, fluorescent pseudomonads are widely reported for their broad-spectrum antagonistic activity against a number of phytopathogens. Deliveries of microbial antagonists with urban and agricultural wastes are believed to be the most effective means in suppressing root pathogens of avocado and citrus (Sultana et al. 2006). Recently, different PGPR strains of *Rhizobium meliloti* have been reported to produce siderophores (Arora et al. 2001) in iron stress conditions and thereby added an advantage to exclude the pathogen *Macrophomina phaseolina*. Application of *Pseudomonas aureoginosa* in combination with common medicinal plant *Launaea nudicaulis* also holds good promises for effective control of root-infecting fungi (Mansoor et al. 2007).

Competition for nutrients, niche exclusion, induced systemic resistance and production of antifungal metabolites (AFMs) is the probable means responsible for biocontrol activity of PGPR (Bloemberg and Lugtenberg 2001). Most of the PGPR are recorded to produce AFMs, of which phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, viscosinamide and tensin are the frequently detected classes. Among PGPR, *Pseudomonas* is the best-characterized biocontrol agent at the molecular level. *P. fluorescens* strain WCS374 has been recorded to suppress *Fusarium* wilt in radish leading to an average increase of 40 % in yield (Bakker et al. 2007). The individual genes such as phzO and phzH responsible for the presence of functional group on phenazine compound have been detected (Chin-A-Woeng et al. 2001). More recently, information has been generated on the biosynthesis of pyoluteorin in *P. fluorescens* Pf5 and 2, 4-diacetylphloroglucinol in *P. fluorescens* Q2-87 (Kidarsa et al. 2011). Biocontrol activity of *Streptomyces* spp. is reported by Kumar et al. (2009) indicating the tremendous potentiality of PGPR as an alternative in controlling plant diseases in agriculture than that of conventional fungicides. *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Paenibacillus*, *Pseudomonas* and *Streptomyces* are recorded as the potent genera of rhizobacteria acting against the pathogens like tomato mottle virus, *Rhizoctonia bataticola*, *Myzus persicae*, *Acyrthosiphon kondoi* and *Fusarium oxysporum*. Experiments on the dual effect of PGPR and AM fungi on *Fusarium oxysporum* f. sp. *melongenae* causing brinjal wilt have been made by Kalita et al. (2009). PGPR strains such as *Azotobacter* sp., *Azospirillum* sp. and *P. fluorescens* are recorded as the most promising microbes to suppress the wilt disease of brinjal in vitro. The rhizobaceria when inoculated as mixtures exhibited maximum efficiency in the suppression of diseases along with increase in chlorophyll content, total number of leaves and shoot height, thereby increasing overall crop yield than when inoculated singly. However, the application of these PGPR strains did not affect populations of beneficial indigenous rhizosphere bacteria including the fluorescent pseudomonads and the siderophore-producing bacterial strains.

2.7 Rhizoremediation

The application of PGPR in rhizoremediation technologies is now being considered as effective, since inoculation of PGPR strains could aid remarkable enhancement in plant growth and development on contaminated agroclimatic conditions. Rhizobacteria can directly assist rhizoremediation by producing IAA, biological nitrogen fixation, solubilizing P and secreting siderophores (Denton 2007). PGPR strains, pseudomonads and *Acinetobacter* enhance uptake of Fe, Zn, Mg, Ca, K and P by crop plants (Esitken et al. 2006).

PGPR along with AM fungi are now being utilized in the nutrient-poor agricultural soils to increase the solubility of heavy metals, thereby increasing the chances of success in rhizoremediation. Investigations on the application of PGPR strains in decreasing the bioavailability of toxicity resulting in better growth and development in heavy metal contaminated soils through recycling of nutrients, maintaining soil structure, detoxifying chemicals and controlling pests are also well studied (Denton 2007). Studies on certain rhizobacteria in Ni uptake by *Alyssum murale* indicated that this group of bacteria can release the metal from its non-soluble phase by decreasing the pH of the environment (Zhuang et al. 2007). *Azotobacter* spp. facilitate the mobility of heavy metals in the soil and thus enhance bioremediation of soil from heavy metals, such as cadmium, mercury and lead.

Metal contamination of soil has an important bearing on PGPR functions. Metal homeostasis resistance in bacteria is often maintained by sequestration, active efflux, reduced uptake, detoxification and synthesis of binding protein (Choudhary and Shrivastava 2001). In some cases, a few mechanisms may also co-exist. Strain Psd was able to resist Cd, Al and Zn and thus could be able to survive for carrying out its PGPR functions in soil containing high concentrations of these metal ions. Phosphate solubilization by strain Psd is another important property as nonavailability of phosphate can be limiting for plants. Strain Psd could solubilize mineral source of complex phosphate as well as release phosphate from organic sources via two phosphatase enzymes. Mineral phosphate solubilization in bacteria occurs by production of organic acids, and organic phosphate release is aided by acid and alkaline phosphatases (Rodriguez and Fraga 1999). The complete genome sequence analysis of *P. fluorescens* Pf5 and detailed molecular genetic analysis of *P. fluorescens* CHAO have firmly established the biocontrol capabilities and its regulation (Zuber et al. 2003). Phosphate-solubilizing bacteria are common in the rhizosphere (Nautiyal et al. 2000), and secretion of organic acids and phosphatase is the common method of facilitating the conversion of insoluble forms of P to plant available forms (Kim et al. 1998). The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increases nutrient availability to host plants (Richardson 2001). More importantly, increases in root length and root surface area are sometimes reported (Holguin and Glick 2001).

2.8 Resistance to Water Stress

Drought stress causes limitation to the plant growth and productivity of agricultural crops particularly in arid and semi-arid areas. Inoculation of plants with PGPR enhances the production of IAA, cytokinins, antioxidants and ACC deaminase. PGPR are reported as beneficial to the plants like tomatoes and peppers growing on water-deficient soils for conferring resistance to water stress conditions (Aroca and Ruiz-Lozano 2009). More investigations into the mechanisms by which PGPR elicit tolerance to specific stress factors would improve our knowledge on the use of these rhizobacteria in agriculture to provide induced systemic tolerance to water stress.

2.9 Salt Tolerance

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world. Globally, more than 770,000 km^2 of land is salt affected by secondary salinization: 20 % of irrigated land and about 2 % of dry agricultural land. The restriction of plant growth and productivity due to salinity is especially acute in arid and semiarid regions around the world.

PGPR (*Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43) inoculated onto seeds improves emergence performance, growth and nutrient uptake and could induce tolerance to salt stress in radish (Yildirim et al. 2008). Similar findings were reported in the previous studies showing that application of PGPR (*Azospirillum* spp., *Achromobacter* spp., *Serratia* spp., *Rhizobium* spp., *Aeromonas* spp. and *Bacillus* spp.) may

stimulate yield, growth and PNE uptake from soil in different crops such as tomatoes (Mayak et al. 2004) and lettuce (Barassi et al. 2006) under salinity conditions.

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