Functional Aspects of Plant Growth Promoting Rhizobacteria: Recent Advancements

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Abstract: Background: Numerous bacterial species inhabiting rhizosphere are known to exert beneficial effects upon plant growth. Such bacteria facilitating plant growth are generally referred to as plant growth promoting rhizobacteria. To exert their beneficial effects, rhizobacteria generally must colonize the root surface efficiently. This review therefore, commences with describing the bacterial traits and conditions which are required for the root colonization. **Results:** Several mechanisms by which microbes can act beneficially on plant growth are subsequently described. The beneficial rhizobacteria facilitate the plant growth through N₂ fixation, solubilization of insoluble phosphorus, production of siderophores and production of phytohormones, lowering of ethylene concentration, production of antibiotics and antifungal metabolites and induced systemic resistance. The application of plant growth promoting rhizobacteria as bio-inoculants may be a feasible preference to chemical fertilizers to increase the productivity of various crops. **Conclusion:** In future, the next step should be to explore the rhizobacteria (with multiple plant growth promoting properties) which might flourish well in the geographically diverse niches and to develop appropriate carriers to maintain their viability and plant beneficial activities.

Key words: Plant growth promoting rhizobacteria (PGPR), indole acetic acid, siderophore, phosphate solubilization, rhizosphere

INTRODUCTION

An unanticipated amplification in agricultural practices with the aim to maximize the crop productivity at an unprecedented rate had led to exploitation of the technologies and strategies which are not in favor of the sustainability of soil health (Fox *et al.*, 2007; Kumar *et al.*, 2010). The excessive and ill-advised application of agro-chemicals (fertilizers and pesticides) in agricultural fields is posing grave threats to the soil fertility (Yu *et al.*, 2009). The progressive diminution in the application of plant protectants and agrochemicals in farming practices without affecting yield or quality of the crops can only be possible with the advancement of new generation technologies. During the last couple of decades, the recent biotechnological advancements specifically in agriculture have unlocked new avenues for the augmentation of the agricultural productivity in a sustainable mode and have made possible exploitation of soil microorganisms for improving the crop health (Tank and Saraf, 2010; Khan *et al.*, 2010) and in turn, have presented an economically feasible and ecologically sound alternative to minimize the common agricultural practices which result into decreased soil fertility and also affect the long term stability of soil ecosystem (Oves *et al.*, 2009).

The complexity of the soil ecosystem is established by the numerous and diverse interactions among its physical, chemical and biological components (Buscot, 2005). Especially, the variable genetic and functional activities of the heterogeneous microbial populations have a vital effect on soil functions, as such microbes are considered powerful forces for specific enzyme mediated fundamental metabolic processes (Ahemad *et al.*, 2009). Microorganisms in the soil habitat play key roles in ecosystem functioning through controlling nutrient cycling reactions essential for maintaining soil fertility and also contributing to the genesis and maintenance of soil structure (Kirk *et al.*, 2004). Moreover, these microorganisms affect the biogeochemical cycling of nutrients and consequently help plants to grow better both under conventional and stressed soil environment (Wani *et al.*, 2008; Khan *et al.*, 2009). Microbial communities inhabiting soils interact with plant roots and soil constituents at the root-soil interface (Glick, 1995). And hence, the rhizosphere can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots hairs and plant-produced materials (Dessaux *et al.*, 2009). Largely, three separate but interacting components are recognized in the rhizosphere: the rhizosphere (soil), the rhizoplane and the root itself. Of these, the rhizosphere is the zone of soil

influenced by roots through the release of substrates that affect microbial activity. The rhizoplane, on the other hand, is the root surface, including the strongly adhering soil particles (Fig. 1) while the root itself is a part of the system because certain micro-organisms (like endophytes) colonize root tissues (Bowen and Rovira, 1999). The unique physico-chemical and biological characteristics of the soils that are associated with roots, compared to the soils away from the root and root surface are responsible for the enhanced microbial diversity together with increased numbers and activity of microorganisms in the rhizosphere (Zaidi *et al.*, 2009). During microbes-plant interaction, plant roots exude the organic materials which are used up by root associated microorganisms as nutrients as well as organic material is also supplied to the soil micro biota through the death and decay of plants as either growth substrates and structural components or signal molecules. Microbial activity in the rhizosphere affects the rooting patterns and the supply of available nutrients to plants, in a manner that modify the quality and quantity of root exudates (Gryndler, 2000).

The term rhizobacteria is used to describe a subset of rhizosphere bacteria capable of colonizing the root environment (Kloepper *et al.*, 1991; Kloepper, 1994). Beneficial, root colonizing, rhizosphere bacteria, the PGPR (plant growth promoting rhizobacteria), are defined by three intrinsic characteristics: (i) they must be able to colonize the root (ii) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant growth promotion/protection activities and (iii) they must promote plant growth. Plant growth-promoting rhizobacteria are thus free-living, soil-borne bacteria which when applied to seeds/soils or crops, enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil-borne plant pathogens (Kloepper *et al.*, 1980). The present review enlightens the functional aspects of PGPR, their mechanisms of plant growth promotion and their agricultural importance.

Root colonization by PGPR: The distribution, colonization, multiplication and establishment of introduced PGPR are profoundly affected by biotic and abiotic factors (Khan *et al.*, 2010). The optimal temperature for growth of many PGPR *in vitro* is above 25°C but root colonization is generally greatest below 20°C (Loper *et al.*, 1985). Better root colonization at lower temperature probably reflects the fact that microbial activity in the soil declines with temperature. Further,

Fig. 1: Schematic representation of rhizosphere. Modified from Vega (2007)

slower root growth at lower temperatures may facilitate more effective transport of the bacteria from the inoculum source to the roots. Although PGPR grow best *in vitro* at neutral pH or above, colonization is better at lower pH (Edwards *et al.*, 1998).

The biological composition of the rhizosphere dramatically influences root colonization. Accordingly, the nutrient availability rather than space is the primary determinant of microbial population size in the rhizoplane and rhizosphere (Pal, 1998; Zaidi *et al.*, 2009). Thus, inoculation of PGPR does not lead to a change in the total rhizosphere population but a shift in the composition of the microflora such that introduced bacteria preempts establishment of the normal indigenous strains. The root colonization hence will be greater in sterile or pasteurized soils than in raw soil due to lack of competition, antibiosis and predation from the natural inhabitants of soils. In contrast, as microbial activity increases in unsterilized soils, through inputs of nutrients, the level of colonization by introduced PGPR is reduced (Casida, 1992). Pathogens that are targets of PGPR can influence PGPR populations either positively or negatively (Edwards *et al.*, 1998).

Root colonization is a multistage event involving many bacterial traits and genes. Adhesion of PGPR to roots may be either non-specific resulting from electrostatic forces or involve glycoprotein termed agglutinin (Anderson *et al.*, 1988). For instance, Buell and Anderson (1992) characterized a locus, agg A, from *Pseudomonas* that encodes a 50.5 kDa protein required for agglutinability and adherence. Similarly, several exopolysaccharides (EPS) are reported to be involved in the attachment of rhizobacteria to plant cells and in the nodulation of legumes by *Rhizobium* (Cangelosi *et al.*, 1987; Ahemad and Khan, 2010c). However, one approach to increasing root colonization by PGPR is to increase the level of bacterial inocula applied to the seeds (Bull *et al.*, 1991). Enhancement in colonization by increasing the

initial dose of bacteria on the seeds has, however, limitations (Osburn *et al.*, 1989). Another strategy to increase the colonization involves the use of multiple bacterial strains; PGPR research has focussed primarily on the use of single strains. However, Weller and Cook (1983) reported that the use of *P. fluorescens* 2-79 in combination with *P. fluorescens* 13-79 was superior to either strain when used alone in about 50% of the trials. Furthermore, recombinant DNA technology has provided the most exciting and potentially successful means to improve root colonization and biological control by PGPR (Natsch *et al.*, 1997).

Agricultural importance of PGPR: The PGPR belonging to various bacterial genera are known to participate in many important biological activities, such as the biological control of plant pathogens, nutrient cycling and seedling/plant growth (Persello-Cartieaux *et al.*, 2003; Zahir *et al.*, 2004; Ahemad and Khan, 2010d, e) through the production of various substances (Table 1). Among PGPR, *Pseudomonas* and *Bacillus* are the most commonly described genera possessing plant growth promoting activities but many other taxa are also included in PGPR group. Selected strains of PGPR are being used as seed inoculant (Sahin *et al.*, 2004; Zahir *et al.*, 2004; Rani *et al.*, 2009; Ahemad and Khan, 2010f, Ahemad and Khan, 2009a).

In general, PGPR can be divided into two categories (i) extracellular PGPR (ePGPR), existing in the rhizosphere, on the rhizoplane or in the spaces between cells of the root cortex and (ii) intracellular PGPR (iPGPR) which exist

inside root cells, generally in specialized nodular structures. The latter includes *Rhizobia* and *Frankia* species, both of which fix N in symbiosis with higher plants (Gray and Smith, 2005). Functionally, the PGPR have been separated into two groups: (i) those involved in nutrient cycling and phytostimulation (direct activity) and (ii) those involved in the biocontrol of plant pathogens (indirect activity) (Bashan and Holguin, 1998).

The use of PGPR to augment crop productivity has been limited largely due to the variability and inconsistency of results observed under laboratory, greenhouse and field trials. Soil is an unpredictable environment and an intended result is sometimes difficult to achieve. Climatic variations has also a large impact on the effectiveness of PGPR but sometimes unfavorable growth conditions in the field are to be expected as a normal functioning of agriculture (Zaidi *et al.*, 2009). Despite all these factors, increase in crop yields following PGPR applications in the growth chambers and field trials have also been observed as presented in Table 2.

How PGPR facilitate plant growth?: The PGPR strains facilitate growth of plants either directly or indirectly (Fig. 2) (Glick, 1995). The direct mechanism of plant growth promotion involves the production of substances by bacteria and its transport to the developing plants or facilitates the uptake of nutrients from the recipient environment (Azcon, 1989). The direct growth promoting activity of PGPR includes-(i) N₂ fixation (Wani *et al.*, 2007c) (ii) solubilization of insoluble phosphorus (Khan *et al.*, 2006, 2009) (iii) sequestering of

Table 1: Growth promoting substances released by selected PGPR

PGPR	Plant growth promoting traits	References
<i>Rhizobium leguminosarum</i>	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2009a)
<i>Mesorhizobium</i> sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2010c)
<i>Rhizobium</i> sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2010d)
<i>Azospirillum amazonense</i>	IAA, nitrogenase activity	Elisete <i>et al.</i> (2008)
<i>Mesorhizobium</i> sp.	IAA, siderophores, HCN, ammonia	Wani <i>et al.</i> (2008)
<i>Proteus vulgaris</i>	Siderophores	Rani <i>et al.</i> (2009)
<i>Mesorhizobium ciceri</i> ,	IAA, siderophores	Wani <i>et al.</i> (2007c)
<i>Azotobacter chroococcum</i>		
<i>Pseudomonas</i> , <i>Bacillus</i>	Phosphate solubilization, IAA and siderophores	Wani <i>et al.</i> (2007c)
<i>Klebsiella oxytoca</i>	IAA, phosphate solubilization, nitrogenase activity	Jha and Kumar (2007)
<i>Bacillus</i> spp., <i>Pseudomonas</i> spp.,	IAA, ammonia production	Joseph <i>et al.</i> (2007)
<i>Azotobacter</i> spp., <i>Rhizobium</i> spp.		
<i>Bradyrhizobium</i> sp.	IAA, siderophores, HCN, ammonia	Wani <i>et al.</i> (2007a)
<i>Rhizobium</i> sp.	IAA, siderophores, HCN, ammonia	Wani <i>et al.</i> (2007b)
<i>Pseudomonas fluorescens</i>	Induced systemic resistance, antifungal activity	Saravanakumare <i>et al.</i> (2007)
<i>Pseudomonas chlororaphis</i>	Antifungal activity	Liu <i>et al.</i> (2007)
<i>Bacillus subtilis</i>	Antifungal activity	Cazorla <i>et al.</i> (2007)
<i>Glucanacetobacter diazotrophicus</i>	Zinc solubilization	Saravanan <i>et al.</i> (2007)
<i>Brevibacillus</i> spp.	Zn resistance, IAA	Vivas <i>et al.</i> (2006)
<i>Pseudomonas putida</i>	Siderophores, Pb and Cd resistance	Tripathi <i>et al.</i> (2005)
<i>Pseudomonas fluorescens</i>	IAA, siderophores, antifungal activity	Dey <i>et al.</i> (2004)
<i>Azospirillum brasilense</i> ,		
<i>Azospirillum amazonense</i>	IAA, P solubilization, nitrogenase activity, antibiotic resistance	Thakuria <i>et al.</i> (2004)
<i>Pseudomonas fluorescens</i>	IAA, phosphate solubilization	Jeon <i>et al.</i> (2003)
<i>Kluyvera ascorbata</i>	ACC deaminase, siderophores, metal resistance	Burd <i>et al.</i> (1998)

Table 2: Examples of plant growth promoting rhizobacteria tested for various crop types

PGPR	Plant	Conditions	Results of addition of bacteria to plants	Reference
<i>Pseudomonas</i> sp. PS1	Greengram [<i>Vigna radiata</i> (L.) wilczek]	Pots	Significantly increased plant dry weight, nodule numbers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield and seed protein	Ahemad and Khan (2010d)
<i>Rhizobium</i>	Pea (<i>Pisum sativum</i>)	Pots	When herbicide tolerant <i>Rhizobium</i> strain MRP1 was used with herbicide, it increased the growth parameters at all tested concentrations of herbicides (quizalafop-p-ethyl and clodinafop).	Ahemad and Khan (2009b)
<i>Rhizobium leguminosarum</i> strain MRP1	Pea (<i>Pisum sativum</i>)	Pots	Significantly increased the growth, symbiotic properties (nodulation and leghaemoglobin content), amount of N and P nutrients in plant organs, seed yield and seed protein of pea plants	Ahemad and Khan (2010a)
<i>Mesorhizobium</i> strain MRC4	Chickpea (<i>Cicer arietinum</i>)	Pots	Significantly increased symbiotic properties (nodulation and leghaemoglobin content), root N, shoot N, root P, shoot P, seed yield and seed protein	Ahemad and Khan (2009a), Ahemad and Khan (2010c)
<i>Pseudomonas putida</i> strain R-168, <i>Pseudomonas fluorescens</i> strain R-93, <i>Pseudomonas fluorescens</i> DSM 50090, <i>Pseudomonas putida</i> DSM291, <i>Azospirillum lipoferum</i> DSM 1691, <i>Azospirillum brasilense</i> DSM 1690	Maize (<i>Zea mays</i> L.)	Fields	Plant height, seed weight, number of seed per ear and leaf area, shoot dry weight significantly increased	Gholami <i>et al.</i> (2009)
<i>Azotobacter chroococcum</i> , <i>Azospirillum lipoferum</i>	Cotton (<i>Gossypium hirsutum</i> L.)	Fields	Seed yield (21%), plant height (5%) and microbial population in soil (41%) increased over their respective controls while boll weight and staple length remained statistically unaffected	Anjum <i>et al.</i> (2007)
<i>Pseudomonas putida</i> CC-R2-4, <i>Bacillus subtilis</i> CC-pg104	<i>Lectuca sativa</i> L.	Gnotobiotic conditions	Significant increase in shoot length and root length achieved through encapsulated inoculant	Rekha <i>et al.</i> (2007)
<i>Azospirillum amazonense</i>	Rice (<i>Oryza sativa</i> L.)	Greenhouse	Grain dry matter accumulation (7 to 11.6%), the number of panicles (3 to 18.6%) and nitrogen accumulation at grain maturation (3.5 to 18.5%) increased	Elisete <i>et al.</i> (2008)
<i>Pseudomonas</i> species	Rice (<i>Oryza sativa</i>), maize (<i>Zea mays</i>)	<i>In vitro</i>	<i>Pseudomonas</i> isolated from rice showed a higher ability to control bacterial and fungal root pathogens than that obtained from maize	Lawongsa <i>et al.</i> (2008)
<i>Azospirillum brasilense</i> Sp245	Common bean (<i>Phaseolus vulgaris</i> L.)	Greenhouse	Root growth increased	Remans <i>et al.</i> (2008)
<i>Pseudomonas fluorescens</i> PGPR1, PGPR2, PGPR4	Peanut (<i>Arachis hypogaea</i> L.)	Pots, fields	Significantly enhanced pod yield, haulm yield and nodule dry weight over the control	Dey <i>et al.</i> (2004)
<i>Azospirillum brasilense</i> , <i>Bacillus pantothenicus</i> , <i>Pseudomonas pieketti</i>	Rice (<i>Oryza sativa</i>)	Micro-plots	Increased rice grain yield maximum upto 76.9%	Thakuria <i>et al.</i> (2004)
<i>Bacillus spp.</i>	Barley (<i>Hordeum vulgare</i>)	Greenhouse	Increased root weight upto 16.7% and shoot weight upto 347%	Canbolat <i>et al.</i> (2006)
<i>Bacillus subtilis</i>	<i>Solanum lycopersicum</i> L. (tomato), <i>Abelmoschus esculentus</i> (okra), <i>Amaranthus</i> sp. (African spinach)	Greenhouse	Dry biomass increased 31% for tomato, 36% for okra and 83% for African spinach	Adesemoye <i>et al.</i> (2008)
<i>Pseudomonas aeruginosa</i>	<i>Solanum lycopersicum</i> L. (tomato), <i>Abelmoschus esculentus</i> (okra), <i>Amaranthus</i> sp. (African spinach)	Greenhouse	Dry biomass increased 31% for tomato, 29% for okra and 40% for African spinach	Adesemoye <i>et al.</i> (2008)
Unidentified PGPR isolate	wheat	Gnotobiotic conditions	Increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7%) and shoot dry weight (up to 36.3%) in inoculated wheat seedlings	Khalid <i>et al.</i> (2004)
<i>Bradyrhizobium</i> sp. (vigna) RM8	Greengram	Pots	Enhanced the nodule numbers by 82%, leghaemoglobin by 120%, seed yield by 34%, grain protein by 13%, root N by 41% and shoot N by 37% at 290 mg Ni kg ⁻¹ soil.	Wani <i>et al.</i> (2007a)
<i>Mesorhizobium</i> sp. RC3	Chickpea	Pots	Increased the dry matter accumulation, number of nodules, seed yield and grain protein by 71, 86, 36 and 16%, respectively, compared to noninoculated plants. Nitrogen in roots and shoots increased by 46 and 40%, respectively, at 136 mg Cr/kg.	Wani <i>et al.</i> (2008)
<i>Rhizobium</i> sp. RP5	Pea	Pots	Enhanced the dry matter, nodule numbers, root N, shoot N, leghaemoglobin, seed yield and grain protein by 19, 23, 26, 47, 112, 26 and 8%, respectively, at 290 mg Ni kg ⁻¹	Wani <i>et al.</i> (2007b)

Fig. 2: A general scheme showing how PGPR promote plant growth

iron by production of siderophores (Wani *et al.*, 2008; Rajkumar *et al.*, 2006) (iv) production of phytohormones such as, auxins, cytokinins, gibberellins and (v) lowering of ethylene concentration (Liu *et al.*, 2007; Elisete *et al.*, 2008; Wani *et al.*, 2008). On the contrary, the indirect mechanism of plant growth promotion by PGPR includes (i) antibiotic production (ii) depletion of iron from the rhizosphere (iii) synthesis of antifungal metabolites (iv) production of fungal cell wall lysing enzymes (v) competition for sites on roots and (vi) induced systemic resistance (Saravanakumar *et al.*, 2007; Cazorla *et al.*, 2007). Briefly, the indirect promotion of plant growth takes place when PGPR lessen or prevent the injurious effects of plant pathogens by synthesizing inhibitory substances or by increasing the natural resistance of the host to the pathogens.

Direct mechanisms of action

Nitrogen fixation: Nitrogen (N) is one of the most common nutrients required for optimal plant growth and productivity. Even though, more than 78% of N is present in the atmosphere, yet it remains unavailable to growing plants. It therefore, needs to be converted into ammonia, an available form to plants and other eukaryotes. Atmospheric N is converted into plant utilizable forms by three different processes: (a) conversion of atmospheric N into oxides of N in the atmosphere (b) industrial N fixation that involves the use of catalysts and high temperature (300-500°C) to transform N into ammonia and (c) Biological N Fixation (BNF) which changes the nitrogen to ammonia by microorganisms using a complex enzyme system known as nitrogenase (Kim and

Rees, 1994). Of all the processes, BNF fixes about 60% of the earth's available N and represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha *et al.*, 1997).

Nitrogen fixing organisms can broadly be divided as: (a) symbiotic N₂ fixing bacteria, that includes members of family rhizobiaceae and forms symbiosis with leguminous host plants (e.g., rhizobia) (Zahran, 2001; Ahemad and Khan, 2010a, c, d) and non-leguminous trees (e.g., Frankia). Gram-negative soil bacteria (rhizobia) within the rhizobiaceae phylogenetic family (α -proteobacteria) possess the unique ability to infect and establish a nitrogen-fixing symbiosis with the roots of leguminous plants. The establishment of the symbiosis involves a complex interplay between host and symbiont (Giordano and Hirsch, 2004) resulting in the formation of a novel organ, the nodule which the bacteria colonize as intracellular symbionts (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*, *Gluconoacetobacter diazotrophicus* and *Azococcus* etc. Plant growth-promoting rhizobacteria that fix N₂ in non-leguminous plants are also called as diazotrophs capable of forming a non-obligate interaction with the host plants (Glick *et al.*, 1999). The process of N₂ fixation is carried out by the nitrogenase enzyme coded by nif genes (Kim and Rees, 1994). Nitrogenase was elucidated by Dean and Jacobson (1992) as a two-component metalloenzyme consisting of (i) dinitrogenase reductase which is the iron protein and (ii) dinitrogenase which has a metal cofactor. Based on the metal cofactor three different N fixing systems have been identified (a)

Mo-dinitrogenase (b) V-nitrogenase and (c) Fe-nitrogenase. The existence of the N_2 fixing system differs among bacterial genera (Bishop and Joerger, 1990).

Phytohormones: Another direct mechanism by which PGPR facilitates plant growth is the production of plant growth regulators or phytohormones (Glick, 1995; Wani *et al.*, 2008; Ahemad and Khan, 2009a; Ahemad and Khan, 2010a). Frankenberger and Arshad (1995) have discussed in detail the role of auxins, cytokinins, gibberellins, ethylene and Absciscic Acids (ABA) which when applied to plants, have shown a substantial increase in growth and yield of plants. The production of phytohormones such as, auxins (IAA), cytokinins and gibberellins by natural soil microbial communities have been reported by various workers over the last 20 years (Poonguzhali *et al.*; 2008, Ahemad and Khan, 2010g, h).

As an example, the production of Indole-3-Acetic Acid (IAA) by microorganisms in the presence of the precursor tryptophan or peptone has been reported (Wani *et al.*, 2008; Ahemad *et al.*, 2008). Indole-3-acetic acid, a main auxin in plants is known to control many important physiological processes of plants, such as, cell enlargement, cell division, root initiation, growth rate, phototropism, geotropisms and apical dominance etc., (Zaidi *et al.*, 2009). In plant cells, IAA is largely formed by de novo synthesis from tryptophan which undergoes either oxidative deamination (via the formation of indole-3-pyruvic acid) or decarboxylation (via the formation of tryptamine with indole-3-acetic aldehyde as an intermediate).

The synthesis of IAA by microbes involves one of the three pathways as presented in Fig. 3 (Patten and Glick, 1996): (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in the majority of bacteria like, *Erwinia herbicola*; saprophytic species of the genera *Agrobacterium* and *Pseudomonas*; certain representatives of *Bradyrhizobium*, *Rhizobium*, *Azospirillum*, *Klebsiella* and *Enterobacter* (2) The conversion of tryptophan into indole-3-acetic aldehyde

may involve an alternative pathway in which tryptamine is formed. This pathway is believed to operate in pseudomonads and azospirilla and (3) IAA biosynthesis via indole-3-acetamide formation is reported for phytopathogenic bacteria *Agrobacterium tumefaciens*, *Pseudomonas syringae* and *E. herbicola*; saprophytic pseudomonads like (e.g., *Pseudomonas putida* and *P. fluorescens*). The genes controlling IAA synthesis via this pathway are also reported in symbiotic bacteria (e.g., *Rhizobium* spp., *Bradyrhizobium* spp. and *Azospirillum* spp.), although the activity of the corresponding enzymes is either negligible or not detectable. (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in plants, *Alcaligenes faecalis* and possibly the cyanobacterium (*Synechocystis* sp.) and (5) The tryptophan-independent pathway, more common in plants, is also found in microorganisms (azospirilla and cyanobacteria). However, the synthesis of IAA using this pathway is reported to be insignificant and the mechanisms are largely unknown.

Many bacteria are known to synthesize auxins using such pathways and help the plants to grow better. In this context, rhizospheric microflora colonizing plant roots are of paramount importance in the conversion of tryptophan into IAA. And hence, the removal of tryptophan from the culture medium leads to decrease in the level of IAA by the microorganisms. However, the amount of the auxins formed depends primarily on the composition of the medium and the conditions (e.g., temperature, aeration, etc.) of bacterial growth. Bacteria in general, forms maximum amount of IAA during the steady-state stage of their growth while ammonium ions and glutamine inhibit IAA biosynthesis (Tsavkelova *et al.*, 2006). The genes involved in IAA synthesis in bacterial strains may be plasmid or chromosomal borne. For example, pathogenic bacteria contain Ti plasmids that control the formation of the phytohormone whereas in saprophytic microorganisms, auxin biosynthesis is governed by chromosomal genes (Tsavkelova *et al.*, 2006).

Fig. 3: Biosynthetic pathways of IAA synthesis in bacteria

It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Loper and Schroth, 1986). Of the various PGPR strains, bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium* as well as *Alcaligenes*, *Enterobacter*, *Acetobacter*, *Klebsiella* and *Bradyrhizobium* have been shown to produce auxins which help in stimulating plant growth (Wani *et al.*, 2007a; Poonguzhali *et al.*, 2008; Kumar *et al.*, 2008; Ahemad and Khan, 2010b, g, h). However, the extent of IAA production by bacterial strains could be different due in part to the involvement of biosynthetic pathways, location of the genes, regulatory sequences and the presence of enzymes to convert active free IAA into conjugated forms. Moreover, the synthesis of IAA is also influenced by environmental factors (Patten and Glick, 1996). Synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has also been demonstrated (Wani *et al.*, 2007b). Bent *et al.* (2001) reported that the concentration of indole compounds by three different strains, *Paenibacillus polymyxa* (L6), *P. polymyxa* (Pw-2) and *Pseudomonas fluorescens* (M20) increased with increasing rate of tryptophan (0-200 mg mL⁻¹) at different incubation interval.

Microbial phosphate solubilization: Phosphorus (P), a major plant nutrient is required for various metabolic

processes such as, energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration (Khan *et al.*, 2009). Phosphorus however, is also one of the major nutrients limiting plant growth (Fernandez *et al.*, 2007). Worldwide, 5.7 billion hectares land contain too little available P for sustaining optimal crop production. Phosphorus ion concentration in most soils ranges from 0.1 to 10 µM; P required for optimal growth ranges from 1 to 5 µM for grasses and 5 to 60 µM for high demanding crops such as tomato (*Lycopersicon esculentum*) and pea (*Pisum sativum*) (Raghothama, 1999). Sub-optimal levels of P, can however, lead to a 5 to 15% losses in the yield of crops (Khan *et al.*, 2009). Phosphorus is present in the soils both in organic and inorganic forms (Fig. 4). Of which, organic forms, as found in humus and other organic materials including decayed plant, animal and microbial tissues, is an important reservoir of immobilized P accounting for about 20-80% of total soil P (Richardson, 1994). Phosphorus in labile organic compounds can be slowly mineralized as available inorganic P or it can be immobilized as part of the soil organic matter (McKenzie and Roberts, 1990). Soil inorganic P is however, controlled mainly by solution pH and the concentration of cations and in most soils, maximum P availability occurs between pH 5.5 to 7. Within this pH range, P is fixed by hydrous oxides of Fe, Al and Mn while between pH 6 to 8 and pH 6.5 to 8.5, P is fixed by silicate minerals and Ca, respectively. As a consequence, the most efficient use of P in neutral and calcareous soils occurs between pH 6 to 7.

Fig. 4: Movement of phosphorus in soils

However, the majority of P is unavailable for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils. Therefore, phosphatic fertilizers are applied to soil to replenish the P demands of growing plants. However, a large portion of soluble inorganic P applied to the soil as fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Moreover, the concentration of soluble P in soil solution is very low ($400\text{--}1,200\text{ mg kg}^{-1}$ of soil) (Ehrlich, 1990; Rodriguez and Fraga, 1999). Attempts to overcome the P deficiency by applying phosphatic fertilizers is expensive and ecologically unsafe practice because the efficiency of the added P fertilizer is as low. This has led to search environment-friendly and economically feasible alternative strategies for improving crop production in low P soils. In this study, organisms endowed with phosphate solubilizing activity, often termed Phosphate Solubilizing Microorganisms (PSM), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers (Khan *et al.*, 2006). Of the various PSM (s) inhabiting rhizosphere, Phosphate-Solubilizing Bacteria (PSB) are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available by various mechanisms (Fig. 5) (Khan *et al.*, 2006). Though, PSB are commonly found in most soils (Wani *et al.*, 2007c; Ahemad and Khan, 2010h); their establishment and performances are severely affected by environmental factors especially under stress conditions (Wani *et al.*, 2007c; Ahemad and Khan, 2010g). However,

the beneficial effects of the inoculation with PSB used alone (Poonguzhali *et al.*, 2008; Chen *et al.*, 2008; Ahemad and Khan, 2010b, f) or in combination with other rhizospheric microbes have been reported (Zaidi and Khan, 2005; Zaidi and Khan, 2006; Vikram and Hamzehzarghani, 2008).

Besides providing P to the plants, the PS bacteria also augment the growth of plants by stimulating the efficiency of BNF, enhancing the availability of other trace elements (such as iron, zinc) and by synthesizing important plant growth promoting substances (Ponmurugan and Gopi, 2006; Mittal *et al.*, 2008) (Table 3) including siderophores (Vassilev *et al.*, 2006; Wani *et al.*, 2008), antibiotics (Fernando *et al.*, 2006) and providing protection to plants against soil borne pathogens (biocontrol). Accordingly, these bacterial communities when used either singly (Chen *et al.*, 2008) or as consortia, in combination with other rhizosphere microbes (Mishra *et al.*, 2009) have shown a substantial improvement in crop productivity in different agro-ecosystems.

Siderophores: Iron-chelating molecules termed siderophores are generally less than 1000 molecular weight and are produced by many microorganisms. More than 500 different siderophores, mostly produced by Gram-positive and Gram-negative bacteria (Wani *et al.*, 2007a; Ahemad and Khan, 2010a, b), have been described. Despite this great variety, most have a peptide backbone with several non-protein amino acid analogs including

Fig. 5: Mechanisms of P solubilization by phosphate solubilizing bacteria

Table 3: Growth promoting substances released by phosphate solubilizing bacteria

Phosphate solubilizing bacteria	Plant growth promoting traits	References
<i>Pseudomonas aeruginosa</i> PS1	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2010b), Ahemad and Khan (2010f)
<i>Klebsiella</i> sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2010g)
<i>Enterobacter asburiae</i>	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan (2010h), Ahemad and Khan (2010f)
<i>Pseudomonas</i> sp.	ACC deaminase, IAA, siderophore	Poonguzhali <i>et al.</i> (2008)
<i>Bacillus subtilis</i>	IAA, siderophore, antifungal activity	Singh <i>et al.</i> (2008)
<i>Serratia marcescens</i>	IAA, siderophore, HCN	Selvakumar <i>et al.</i> (2008)
<i>Pseudomonas fluorescens</i>	ACC deaminase	Shaharoon <i>et al.</i> (2008)
<i>Acinetobacter</i> sp., <i>Pseudomonas</i> sp.	ACC deaminase, IAA, antifungal activity, N ₂ fixation	Indiragandhi <i>et al.</i> (2008)
<i>Enterobacter</i> sp.	ACC deaminase, IAA, siderophore	Kumar <i>et al.</i> (2008)
<i>Burkholderia</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization	Jiang <i>et al.</i> (2008)
<i>Pseudomonas jessenii</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization	Rajkumar and Freitas (2008)
<i>Pseudomonas aeruginosa</i>	ACC deaminase, IAA, siderophore	Ganesan (2008)
<i>Pseudomonas</i> sp.	ACC deaminase, IAA, siderophore, heavy metal solubilization	Rajkumar and Freitas (2008)
<i>Azotobacter</i> sp., <i>Mesorhizobium</i> sp., <i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	IAA, siderophore, antifungal activity, ammonia production, HCN	Ahmad <i>et al.</i> (2008)
<i>Bacillus</i> spp.	IAA, siderophores, ammonia production, HCN, chromium reduction, metal solubilization	Wani <i>et al.</i> (2007a, b, c)
<i>Bacillus subtilis</i>	IAA	Zaidi <i>et al.</i> (2006)
<i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	IAA, siderophore	Rajkumar <i>et al.</i> (2006)
<i>Pseudomonas putida</i>	Antifungal activity, siderophore, HCN	Pandey <i>et al.</i> (2006)
<i>Pseudomonas fluorescens</i> PRS ₉	IAA, siderophores	Gupta <i>et al.</i> (2005)
<i>Pseudomonas fluorescens</i> GRS ₁		

both modified and D-amino acids. In general, rhizosphere bacteria differs with respect to siderophore cross-utilizing abilities; some are proficient at using siderophores of the same genus (homologous siderophores) while others could utilize siderophores produced by other rhizospheric bacteria of different genera (heterologous siderophores) (Khan *et al.*, 2009).

Siderophore production and utilization by rhizobacteria is of particular interest due to the dominant role of iron in the nitrogen fixation and assimilation process. The iron enzymes involved include nitrogenase, leghemoglobin, ferredoxin and hydrogenase with nitrogenase and leghemoglobin constituting up to 12 and 30% of total protein in the bacterial and infected plant cells, respectively (Verma and Long, 1983).

Siderophores chelate iron with high affinity (the calculated affinity constant being above 10^{30} M^{-1}). Siderophores are highly electronegative and bind Fe (III), preferentially forming a hexacoordinated complex. The iron ligation groups have been tentatively classified into three main chemical types: hydroxamate (e.g., aerobactin and ferrichrome), catechol rings (e.g., enterobactin) and hydroxyacid (e.g., pyochelin). Some siderophores contain more than one of these three iron-chelating groups (Stintzi *et al.*, 2000).

In Gram-negative bacteria, the ferric-siderophore complex must cross the outer membrane and the cytoplasmic membrane before delivering iron within the cytoplasm. The ferric complexes are too large for passive diffusion or nonspecific transport across these membranes (Stintzi *et al.*, 2000); ferric-siderophore uptake is both receptor and energy dependent. Translocation of

Fig. 6: The usual siderophore iron delivery mechanism. Adopted from Stintzi *et al.* (2000)

iron through the bacterial outer membrane as the ferric-siderophore requires the formation of an energy transducing complex with the proteins TonB, ExbB and ExbD which couple the electrochemical gradient across the cytoplasmic membrane to a highly specific receptor and so promote transport of the iron complex across the outer membrane (Stintzi *et al.*, 2000). Once in the periplasmic space, the ferric-siderophore binds to its cognate periplasmic binding protein and is then actively transported across the cytoplasmic membrane by an ATP-transporter system. Two mechanisms have been proposed (i) the usual siderophore iron delivery mechanism (Fig. 6): (a) ferric siderophore is bound to the protein receptor,

Fig.7: The siderophore shuttle iron delivery mechanism. Adopted from Stintzi *et al.* (2002)

Fig. 8: Impact of microbially secreted siderophores on plant growth

causing a conformational change in the protein (b) the ferric siderophore is pumped through the receptor into the periplasmic space and (c) on release of the ferric siderophore, the receptor protein returns to its original conformation and (ii) the siderophore shuttle iron delivery mechanism (Fig. 7): (a) with the iron-free siderophore initially bound to the receptor protein, a second, iron-loaded siderophore binds to the receptor (b) iron exchange between the two siderophores occurs (c) this iron exchange causes a conformational change and the iron complex of the originally iron-free siderophore enters the cytoplasm (d) on release of the ferric siderophore, the receptor protein returns to its original conformation, now with the originally iron-loaded siderophore bound to the receptor protein (Stintzi *et al.*, 2000).

One of the suggested modes of growth promotion of nodulated legumes under field conditions is by microbial production of siderophores which facilitate the uptake of iron from the environment (Ahemad and Khan, 2010c, d). The effects of microbial siderophores on growth and development of plants are presented in Fig. 8. A

nodulated legume has been found to have an increased demand for iron compared to that of a non-nodulated plant (Derylo and Skorupska, 1993). For example, *Pseudomonas* sp. strain 267 enhanced symbiotic N₂ fixation in clover under gnotobiotic conditions, produced fluorescent siderophores under low-iron conditions and secreted B group vitamins (Kozaczuk and Skorupska, 2001).

However, Tn5 insertion mutants of strain 267 defective in siderophore production did not differ from the wild-type in promoting the growth of clover (*Medicago sativa*) suggesting that the siderophore production had no effect on stimulating nodulation. In contrast, Gill *et al.* (1991) demonstrated that mutants of *Rhizobium melioli* that were unable to produce siderophores were able to nodulate the plants but the efficiency of N₂ fixation was less compared to the wild-type, indicating the importance of iron in N₂ fixation. In a similar study, *Kluyvera ascorbata*, a siderophore-producing PGPR was able to protect plants from heavy metal toxicity (Burd *et al.*, 1998).

CONCLUSION

The discussion clearly demonstrated that PGPR adopt different mechanisms to promote the plant growth and these rhizobacteria due to versatile plant-beneficial traits are promising the environment-friendly tools for sustainable agriculture. Due to a great variation in soil ecology of different regions, no single PGPR can be used universally as a bio-inoculant. Hence, the next step should be to explore the rhizobacteria (with multiple plant growth promoting properties) which might flourish well in most of the geographical niches. In this context, the optimization of PGPR inoculums must be rigorously tested in the presence of diverse biotic and/or abiotic factors. In addition, to maintain the maximum viability and activities of PGPR, an appropriate carrier should be developed.

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