



REVIEW ARTICLE

APPLICATION AND PERSPECTIVE OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN
DEVELOPMENT OF SUSTAINABLE AGRICULTURE

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ABSTRACT

At a molecular level, plants are rather leaky. They are perpetually sloughing off cells, leaking moisture, nutrients, carbohydrates and other compounds. Because the plant roots provide food, shelter and energy, areas of very high biological diversity are found directly on roots and the areas next to them. This area is collectively called the rhizosphere. The role of rhizobia, mycorrhizae, biological control organisms, and the whole of the soil food web in maintaining soil quality for crop production have been reported by various researchers. However, rhizobia and mycorrhizae are just a small portion of the total biodiversity in the soil. Although our knowledge has increased, we remain woefully ignorant about soil biology. There is a group of soil bacteria known as plant growth promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. Inoculations of crop plants with certain strains of PGPR at an early stage of development improve biomass production through direct effects on root and shoot growth. Inoculation of agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, plant vigor, plant height, shoot weight, and nutrient content of shoot tissues. PGPR are reported to influence the growth, yield and nutrient uptake by an array of mechanisms. There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for various crops. Several reviews have discussed specific aspects of growth promotion by PGPR. In this review, we have discussed various bacteria which act as PGPR, mechanisms and the desirable properties exhibited by them.

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INTRODUCTION

Soil plant microbe is highly complex and dynamic ecosystem. Among these, soil ecosystem represents a heterogeneous environment, since it comprises of several habitats each with own trophic characteristics, thus contributing towards specific microbial population structure. In soil, a compartment of major interest is rhizosphere defined as a part of soil under the direct influence of plant roots. Indigenous microbial population, specifically rhizobacteria exhibit positive interaction with plant roots (Plant growth promoting rhizobacteria, PGPR) and plays a key role in establishing microbial communities with beneficial properties (Lynch, 1996). Rhizosphere is a rich reservoir of microbial gene pool on account of available nutrient resources derived from secretions, sloughed-root cells, mucilage and dead biomass. Both are important habitats wherein competitive interactions of the highest order prevail

and niche diversification is a way of life. Many bioactive molecule secreting forms have been recovered from these hot spots of diversity including bacteria and fungi. Among the heterotrophic bacterial forms, bacilli and pseudomonads have attracted special attention since they are not only dominant in these ecosystems but are capable of helping plants withstand abiotic and biotic stress through direct and indirect mechanisms that lead to improved plant fitness and better soil health (Lucy *et al.*, 2004). While the direct mechanisms revolve around release of growth promotory substances and action of ACC deaminase, indirect promotion is known to occur through release of siderophores that chelate iron (deprive phytopathogens), antifungal that help suppress the colonization capacity of pathogenic root/ shoot and soil borne fungi, and building up plant defense machinery through the induction of systemic resistance (Jacobsen *et al.*, 2004).

What are plant growth promoting rhizobacteria?

Bacteria that colonize roots effectively are termed "Rhizobacteria". Root colonization is the process where

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bacteria survive on seeds, multiply in spermosphere in response to seed exudates which is rich in carbohydrates and amino acids (Kloepper *et al.*, 1989) attach on to the root surfaces and colonize the developing root system. Thus, colonization of roots is an active process and not a transitory relation between bacteria and roots in the soil.

About 2 to 5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed plant growth promoting rhizobacteria (Arshad *et al.*, 2003). PGPR are free-living bacteria and some of them invade the tissues of living plants and cause unapparent and asymptomatic infections. These rhizobacteria are referred to as endophytes, and in order to invade roots they must first be rhizosphere competent. It is important to note that the term endorhizosphere, previously used in studies of the root zone microflora, is semantically incorrect and should not be used (Kloepper *et al.*, 1992). The original definition of rhizobacteria was restricted to free-living bacteria to differentiate them from nitrogen-fixing rhizobia and *Frankia*. Overtime, some authors have used a less restrictive definition of rhizobacteria as any root-colonizing bacteria. With the original definition, rhizobia and *Frankia* would not be considered as PGPR, while they would be PGPR with broader definition of rhizobacteria. It is generally accepted now that growth stimulation resulting from the biological dinitrogen fixation by rhizobia in legume nodules or by *Frankia* in nodules of *Alnus* spp., is not considered as a PGPR mechanism of action, but rather as the result of the establishment of these well-known symbioses producing nodules (Bashan *et al.*, 2004).

PGPR may induce plant growth promotion by direct or indirect modes of action. Direct mechanisms include the production of stimulatory bacterial volatiles and phytohormones, lowering of the ethylene level in plant, improvement of the plant nutrient status (liberation of phosphates and micronutrients from insoluble sources; non-symbiotic nitrogen fixation) and stimulation of disease-resistance mechanisms (induced systemic resistance). Indirect effects originate for example when PGPR act like biocontrol agents reducing diseases, when they stimulate other beneficial symbioses, or when they protect the plant by degrading xenobiotics in inhibitory contaminated soils (Zaki *et al.*, 2006). PGPR are classified as biofertilizers (increasing the availability of nutrients to plant), phytostimulators (plant growth promoting, usually by the production of phytohormones), rhizoremediators (degrading organic pollutants) and biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites) (Somer *et al.*, 2004).

Diversity of Plant Growth supporting Microorganisms

Association of microorganisms and plant system is considered not only intimate but one where positive and negative influences can be found within a group with ease and exploitative potential considerable. Besides a dominant constituent of the bulk soil, microbial populations resident in rhizosphere are known to be not only large but also much diverse on account of resource availability and competition. However, from the point of view of beneficial influence on

plant health and fitness, functionality of the effective microbial populations is essential. Since Indian soils are deficient in nitrogen and phosphorous, considerable research effort has been directed towards assessment of diversity that could lead to recovery of potentially exploitable forms. In doing so both, non-symbiotic and symbiotic nitrogen fixers have been surveyed across the country and evaluated for field performance utilizing the extension machinery of the agricultural system. While a great deal has been achieved here, variability, stability and effective root colonization has been difficult to maintain. This scenario has slowly changed with emphasis on selection protocols applied on large indigenous pools of bacterial diversity, multistep screening procedures, coupled to community dynamics data that permits closer monitoring of perturbations as a consequence. Whereas symbiotic rhizobial diversity has been a focus of attention on account of legume productivity, associative forms such as *Azospirillum* have been investigated for cereals. However, for quite some time now, free-living, heterotrophic growth promoting rhizobacteria have received considerable emphasis on account of their role in soil health and plant growth. Pseudomonad diversity has been a focus of attention since populations that are neutral, deleterious, and promotory can be found within a single gene pool with soil and plant influencing their composition.

Such physico-chemical and biological-influences can also delineate populations that are functionally relevant, viz, antibiotic-producers, siderophore producers, P-solubilisers, degraders of hemicellular and those inducing systemic resistance through cross-signally with plants. Endophytic bacterial communities of especially agricultural crops are now a focus of attention by virtue of their adaptive behaviour, functionality and phylogenies. Species richness within a single plant system can be large to suggest that such discreet populations are important in sustenance and natural protection of plants. Since crop management based on reduced or low input is considered ideal as a long-term strategy to achieved sustainability, bacterial population and community dynamics of rhizosphere ecosystem are highlighted especially for wheat which is under considerable discussion on account of productivity decline and soil salinization.

In agro-ecosystem, sustainability is dependent on the biological balance in the soils that is governed by the activity of microbial communities. Soil microbial populations are involved in various interactions known to affect plant fitness and soil quality, thereby the stability and productivity of both the agro-ecosystem and natural ecosystem (Azcón *et al.*, 2005). The global necessity to increase agricultural productivity from steadily decreasing land resources base has placed significant strain on the fragile agro-ecosystems. Therefore, it has become necessary to adopt strategies to maintain and improve agricultural productivity through the employment of high input practices. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, and relies on soil biological processes and soil biodiversity. Hence it is necessary to understand perspectives of microbial diversity in the agricultural context in order to arrive at measures, which can act as indicators of soil quality and plant

productivity (Johri *et al.*, 2005). Phytopathogens are major and chronic threats to food production and ecosystem stability worldwide. As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. Despite inconsistency in field performance, biological control is considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Gerhadson *et al.*, 2002).

Genera of PGPR

The number of bacterial species identified as PGPR increased recently as a result of the numerous studies covering a wider range of plant species (wild, economically important and tree) and because of the advances made in bacterial taxonomy and the progress in our understanding of the different mechanisms of action of PGPR. Presently, PGPR include representatives from very diverse bacterial taxa a few examples to illustrate the biodiversity of these beneficial bacteria are (Vessey *et al.*, 2003, Glick *et al.*, 2004).

Diazotrophic PGPR

Azospirillum known for many years as PGPR was isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates (Steenhoudt *et al.*, 2002). This bacterium was originally selected for its ability to fix atmospheric nitrogen (N₂), and since the mid-1970s, it has consistently proven to be a very promising PGPR, and recently the physiological, molecular, agricultural and environmental advances made with this bacterium were thoroughly reviewed (Bashan *et al.*, 2004). Presently PGPR for which evidence exists that their plant stimulation effect is related to their ability to fix N₂ include the endophytes *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotrophicus* and *Herbaspirillum* sp. and, the rhizospheric bacteria *Azotobacter* sp. and *Paenibacillus polymyxa* (Vessey *et al.*, 2003).

Bacilli

Bacillus spp. is able to form endospores that allow them to survive for extended periods under adverse environmental conditions. Some members of the group are diazotrophs and *B. subtilis* was isolated from the rhizosphere of a range of plant species at concentration as high as 10⁷ per gram of rhizosphere soil. *Bacillus* species have been reported to promote the growth of a wide range of plants however; they are very effective in the biological control of many plant microbial diseases (Agrawal *et al.*, 2011; Kokalis-Burelle *et al.*, 2002). Under field conditions in Thailand, Various scientist observed that a PGPR mixture containing *B.amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b, induced systemic resistance against southern blight of tomato (*Lycopersicon esculentum*) caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper (*Capsicum annum* var. *acuminatum*) caused by *Colletotrichum gloeosporioides*, and mosaic disease of cucumber (*Cucumis sativus*) caused by cucumber mosaic virus

(CMV) (Fowler *et al.*, 2003). *Bacillus megaterium* KL39, a biocontrol agent of red-pepper Phytophthora blight disease, produces an antifungal antibiotic active against a broad range of plant pathogenic fungi (Jung *et al.*, 2003). *B.subtilis* also synthesizes an antifungal antibiotic inhibiting *Fusarium oxysporum* sp. *ciceris*, the agent of fusarial wilt in chickpea and strain RB14 produces the cyclic lipopeptides antibiotics iturin A and surfactin active against several phytopathogens. This strain has a very good potential to be used for the biological control of damping-off of tomato caused by *Rhizoctonia solani* (Asaka *et al.*, 1996).

Pseudomonads

Early observations on the beneficial effect of seeds or seed pieces bacterization were first made with *Pseudomonas* spp. isolates, on root crops. By treating potato (*Solanum tuberosum* L.) seed pieces with suspensions of strains of *Pseudomonas fluorescens* and *P. putida* obtained statistically significant increases in yield ranging from 14 to 33% in five of nine field plots established in California and Idaho. Substantial increase in the fresh matter yield of radish (*Raphanus sativus* L.) was obtained by seed inoculation with fluorescent pseudomonads (Burr *et al.*, 1978, Kloepper *et al.*, 1978). Several *Pseudomonas* isolates are able to solubilize sparingly soluble inorganic and organic phosphates (Lee *et al.*, 2009). The beneficial effects of these bacteria have been attributed to their ability to promote plant growth and to protect the plant against pathogenic microorganisms. Production of indole acetic acid (IAA) by *Pseudomonas putida* GR12-2 plays a major role in the root development of canola (*Brassica rapa*) root system as evidenced by the production of roots 35 to 50% shorter by an IAA-deficient mutant (Glick *et al.*, 2002). IAA may promote directly root growth by stimulating plant cell elongation or cell division or indirectly by influencing bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. ACC is the direct precursor of ethylene an inhibitor of root growth, and strain GR12-2 like several other bacteria produces ACC-deaminase which degrades ACC, thus preventing plant production of inhibitory levels of ethylene (Glick *et al.*, 1994).

Rhizobia

Among the groups that inhabit the rhizosphere are rhizobia. Strains from this genus may behave as PGPR when they colonize roots from nonlegume plant species in a nonspecific relationship. It is well known that a number of individual species may release plant growth regulators, siderophores and hydrogen cyanide or may increase phosphate availability, thereby improving plant nutrition (Vessey *et al.*, 2003). An increase in rhizosphere populations has been reported after crop rotation with nonlegumes which resulted in the abundance of benefiting subsequent crops (Bruijn *et al.*, 1997; Biederbeck *et al.*, 2004).

Plant Growth Promotion

Rhizobacterial strains were found to increase plant growth after inoculation in seeds and therefore called "Plant growth promoting rhizobacteria". The mechanisms of growth promotion by these PGPR are complex and appear to comprise

both changes in the microbial balance in the rhizosphere and alterations in host plant physiology. Plant growth promoting rhizobacteria, including fluorescent *Pseudomonads* are capable of surviving and colonizing the rhizosphere of all field crops. They promote plant growth by secreting auxins, gibberellins and cytokinins (Kamilova *et al.*, 2009). PGPR has a significant impact on plant growth and development in both indirect and direct ways. Indirect promotion of plant growth occurs when bacteria or prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms. On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Vessey *et al.*, 2003). Plant growth benefits due to the addition of PGPR include increase in germination rates, root growth, yield including grain, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senescence (Glick *et al.*, 2004). Seed treatment with PGPR resulted in increased yield and growth in potato under field conditions (Kloepper *et al.*, 1980). The increased root and shoot fresh weight of tomato, cucumber, lettuce and potato has been reported as a result of bacterization with *Pseudomonas* strains (Peer *et al.*, 1988).

Siderophore Production

Siderophores are low molecular weight, extracellular compounds with a high affinity for ferric iron, that are secreted by microorganisms to take up iron from the environment and their mode of action in suppression of disease were thought to be solely based on competition for iron with the pathogen (Duijff *et al.*, 1997). Fluorescent *Pseudomonas* is characterized by the production of yellow-green pigments termed pyoverdines which fluoresce under UV light and function as siderophores. The role of siderophores produced by fluorescent *Pseudomonads* in plant growth promotion was first reported (Peer *et al.*, 1988). The siderophores of fluorescent *Pseudomonads* were later reported to be implicated in the suppression of plant pathogens, competition for iron between pathogens and siderophores of fluorescent *Pseudomonads* has been implicated in the biocontrol of wilt diseases caused by *Fusarium oxysporum* damping off cotton caused by *Pythium ultimum* and pythium root rot of wheat (Bakker *et al.*, 1982, Loper *et al.*, 1988, Janzen *et al.*, 1989). Pyoverdines chelate iron in the rhizosphere and deprive pathogens of iron which is required for their growth and pathogenesis (Leong *et al.*, 1986). Rhizobacteria produce various types of siderophores (Pseudobactin and ferrioxamine B) that chelate the scarcely available iron and there by prevent pathogens from acquiring iron (Buyer *et al.*, 1991). The fluorescent *Pseudomonads* had the property to form ferric siderophores complex which prevent the availability of iron to the microorganisms (Leong *et al.*, 1986). Ultimately this led to iron starvation and prevented the survival of the microorganisms including nematodes. *Pseudomonas aeruginosa* strain IE-6 and its streptomycin resistant strain IE-6+ markedly suppressed nematode population densities in root and subsequent rootknot development.

Phosphate solubilization

The improvement of soil fertility is one of the most common strategies to increase agricultural production. Phosphate solubilization is very important in enhancing the soil fertility. Phosphorus (P) is major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields. The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture (Chaiharan *et al.*, 2008). The use of phosphate solubilizing bacteria as inoculants increases the Phosphorus uptake by plants (Arun *et al.*, 2006). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solubilizing Microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. These organisms in addition to providing Phosphorus to plants also facilitate plant growth by other mechanisms. Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems. PSM include largely bacteria and fungi. The most efficient PSM belong to genera *Bacillus*, *Rhizobium* and *Pseudomonas* amongst bacteria, and *Aspergillus* and *Penicillium* amongst fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Mateos *et al.*, 2006).

Hydrogen Cyanide (HCN) Production

The cyanide ion is exhaled as HCN and metabolized to a lesser degree in to other compounds. HCN first inhibits the electron transport, and the energy supply to the cell is disrupted leading to the death of the organisms. It inhibits proper functioning of enzymes, natural receptors mechanism of inhibition and is also known to inhibit the action of cytochrome oxidase (Brown *et al.*, 2008). HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens (Dangar *et al.*, 2013). Production of HCN by certain strains of fluorescent pseudomonads has been involved in the suppression of soil borne pathogens. Suppression of black root rot of tobacco and take-all of wheat by *P. fluorescens* strain CHAO was attributed to the production of HCN (Defago *et al.*, 1986, Defago *et al.*, 1990). *Pseudomonas fluorescens* HCN inhibited the mycelial growth of *Pythium in vitro*. The cyanide producing strain CHAO stimulated root hair formation, indicating that the strain induced and altered plant physiological activities (Somers *et al.*, 2004). Hydrogen cyanide is a broad spectrum antimicrobial compound involved in biological control of root disease by many plant associated fluorescent pseudomonads (Defago *et al.*, 2003). Further, they noted that the enzyme HCN synthase is encoded by three biosynthetic genes (*henA*, *henB* and *henC*).

Indole-3-Acetic Acid (IAA) Production

IAA hormone is very commonly produced by PGPR. The production of this hormone has been reviewed and implicated it in the growth promotion by PGPR (Vessey *et al.*, 2003). 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. Bacterial IAA from *P. putida* played a major role in the development of host plant root system (Glick *et al.*, 2002).

Rhizobacteria in the Management of Plant Diseases

PGPR are having the ability to protect above ground plant parts against fungal, bacterial and viral diseases by induced systemic resistance (ISR). Among the PGPR, fluorescent pseudomonads are the most exploited bacteria for biological control of soil borne and foliar plant pathogens. Among the various isolates tested, *P. fluorescens* isolate Pfl effectively inhibited mycelial growth of the pathogen *in vitro* conditions and decreased the fruit rot incidence under greenhouse conditions (Raguchander *et al.*, 2001, Agrawal *et al.*, 2011). The application of biocontrol PGPR strains has given promising results in cereals, vegetables, fruit and ornamental plant production under glass house and field conditions (Kloepper *et al.*, 2000). In greenhouse and field experiments, PGPR strain *B. Pumilus* INR-7 effectively protected pearl millet against downy mildew (Amruthesh *et al.*, 2003). PGPR mediated resistance in mango trees infected with *Colletotrichum gloeosporioides* significantly reduced the anthracnose infection besides enhancing fruit yield under field conditions (Ramanathan *et al.*, 2004). These studies clearly indicate the PGPR have diverse mechanism to operate and to combat the pests and pathogens and work efficiently in both greenhouse and field conditions.

Influence of PGPR on Nutrient Uptake

The combined application of *Azospirillum*, phosphobacteria and VAM with 75 % of recommended NPK (90:90 kg/ha) recorded higher yield of potato (14.96 t/ha) which was 21 per cent higher than uninoculated control (11.93 t/ha) (Nanjan *et al.*, 1998). The inoculation of *Rhizobium*, *P. striata* or *B. polymyxa* significantly increased nitrogen and phosphorus uptake by chickpea over control. The uptake was further enhanced with the application of 10 kg N and 60 kg P per ha. The highest N and P uptake was recorded in *Rhizobium* + 20 kg N and 60 kg P per ha (Alagawadi *et al.*, 1988). Two equal splits of 100 per cent NPK with biofertilizers such as *Azospirillum* and Phosphobacterium significantly influenced the uptake of NPK by different plant parts of potato over treatment receiving recommended dose of fertilizers alone (Mahendran *et al.*, 1996). Seed bacterization with *Bacillus polymyxa* and *Pseudomonas striata* when used as single and mixed inoculants in potato crop increases the yield and Phosphorus uptake. When the phosphobacteria were inoculated together, the increase was 35.20 % followed by *Pseudomonas striata* 30.8 % and *B. polymyxa* 22.90 % (Gaur *et al.*, 1989). Use of PSB to increase phosphorus availability from rock phosphate in groundnut was also studied (Hebbara *et al.*, 1990). One hundred isolates from different rhizobial

genus on the basis of microscopic and biochemical tests were selected. All rhizobial isolates evaluated on their potential production of auxin hormone (IAA and its homologues) on LB solid medium which was improved with TRP. Application effect of IAA+ super strains inoculants on wheat growth indexes were evaluated. The results were indicated that indigenous rhizobia have the potential of Indole Acetic Acid production (IAA). Depending on ANOVA results, significant difference ($P < 0.001$) has been observed in rhizobial groups and also in the strains of each rhizobial group from the point of IAA production capability. Greenhouse test results on wheat showed that bacterial treatment and also the Ag and Trp treatments had significant effect on measured parameters. Comparison of means shows that there is a significant difference between the rhizobial treatment effects on the measured parameters too. So, the most important promotion mechanism by rhizobial strains, is production of Indole phytohormones (IAA) which results in the better root growth, which increases water and micronutrient (N, P and K) uptake by the plant and which resulted in the increase of the plant growth (Alikhani *et al.*, 2009). The impact of inoculating *Ocimum basilicum* roots with plant growth-promoting rhizobacteria (PGPR) on plant growth indices such as shoot wet weight, shoot dry weight, root fresh weight, shoot height, and N, P, K content were estimated. The control treatment was not inoculated; *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5, and *Azospirillum lipoferum* were used to inoculate PGPR treatment. In PGPR treatments all factors were increased as compared to control treatment. The maximum Root fresh weight (3.96 g/plant), and N content (4.72%) were observed in the *Pseudomonas* + *Azotobacter* + *Azospirillum* treatment which was significantly different when compared to other treatments. All factors were higher in the *Pseudomonas* + *Azotobacter* + *Azospirillum* and *Azotobacter* + *Azospirillum* treatments, which stated positive synergistic interactions between them when treatment on *Ocimum basilicum* growth compared to the other PGPR treatments (Ordookhani *et al.*, 2011).

A study on tomato, the most popular garden vegetable in the world was conducted. Vitamin A and C are present in high amount in Tomatoes. An increase in absorption of water and nutrients from soil has been observed when plant-growth promoting rhizobacteria (PGPR) has been inoculated. To evaluate the effects of some PGPR on growth and nutrients uptake of tomato (*Lycopersicon esculentum*) plants a green house experiment was conducted. The control was compared with the seven treatments used for bacteria (*Pseudomonas*, *Azotobacter*, *Azospirillum*, *Pseudomonas* + *Azotobacter*, *Pseudomonas* + *Azospirillum*, *Azotobacter* + *Azospirillum* and *Pseudomonas* + *Azotobacter* + *Azospirillum*). At Prebloom stage the plants were cut. In *Azotobacter* + *Azospirillum*, *Pseudomonas* + *Azotobacter* + *Azospirillum* and *Azospirillum* treatments maximum shoot fresh weight was shown which differs significantly from other treatments. *Pseudomonas* + *Azotobacter* + *Azospirillum* treatment has given highest amount of N, P and K and *Pseudomonas* + *Azotobacter* treatment has given the lowest amount (Sharafzadeh *et al.*, 2012).

Application of PGPR as Bioinoculant

The use of rhizosphere-associated microorganisms as biofertilizers is now being considered as having potential for improving plant productivity. Bio-fertilizers are defined as substances that contain living microorganisms that when applied to seed, plant surfaces, or soil, colonize the plant and promote its growth by increasing the nutrient availability (Vessey *et al.*, 2003). Rhizosphere associated nitrogen fixing and phosphate-solubilizing bacteria have been used as inoculum for non-legume crop species such as corn, rice, wheat, and sugarcane (Dobereiner *et al.*, 1997). Many of the bacteria that increase plant growth were shown to possess the ability to solubilize phosphate, increase the efficiency of biological nitrogen fixation, improve the availability of Fe and Zn, and alter the growth of roots or shoots by production of plant hormones (Janzen *et al.*, 1989). However, the actual mechanisms have rarely been clearly identified except for some bacteria that act as biological control agents. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were particularly effective in increasing root and shoot elongation in canola, lettuce, and tomato and yield of potato, radish, rice, sugar beet, tomato, lettuce, apple, citrus, bean, ornamental plants, and wheat (Fraga *et al.*, 1999).

Conclusion

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Rhizobium* and *Serratia* have reported to enhance plant growth. The direct promotion by PGPR entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR prevent deleterious effects of one or more Phytopathogenic microorganisms.

The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene (ii) asymbiotic N₂ fixation, (iii) antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide (Johri *et al.*, 2003, Flaishman *et al.*, 1996, Arshad *et al.*, 2003). (iv) solubilization of mineral phosphates and other nutrients (Goel *et al.*, 2004). In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil (Mittal *et al.*, 2007). Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained *in vitro* cannot always be dependably reproduced under field conditions (Chanway *et al.*, 1993). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on

plant. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other microorganisms. Therefore, it is necessary to develop efficient strains in field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of PGP activities and well adapted to particular soil environment. As our understanding of the complex environment of the rhizosphere, of the mechanisms of action of PGPR, and of the practical aspects of inoculants formulation and delivery increases, we can expect to see new PGPR products becoming available. The success of these products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculant formulation and delivery. Genetic enhancement of PGPR strains to enhance colonization and effectiveness may involve addition of one or more traits associated with plant growth promotion (Bloemberg *et al.*, 2001). Genetic manipulation of host crops for root-associated traits to enhance establishment and proliferation of beneficial microorganisms is being pursued. The use of multi-strain inoculate of PGPR with known functions is of interest as these formulations may increase consistency in the field. They offer the potential to address multiple modes of action, multiple pathogens, and temporal or spatial variability. PGPR offer an environmentally sustainable approach to increase crop production and health. The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with improved effectiveness (Jetiyanon *et al.*, 2002).

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