

## Review

# Plant growth promoting rhizobacteria and sustainable agriculture: A review

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Plant growth promoting rhizobacteria have gained extensive importance worldwide. These are naturally occurring bacteria that actively colonize the plant roots and improve plant growth. Plants inoculated with some potential PGPR strains exhibit improved biomass production. It has been observed that PGPR exert their growth promoting effects on plants by utilizing a number of mechanisms. Taken as an example, PGPR improve legumes growth due to their potential involvement in nitrogen fixation. Supply of nutrients like phosphorous, copper, iron, sulphur etc. is enhanced in the plants having rhizosphere enriched with plant growth promoting rhizobacteria. PGPR also act as bio-control agents by imposing their negative effects on the growth of disease causing bacteria, fungi and help in controlling insect pests. The research on PGPR has been on rise for the past few decades and several products containing PGPR have been commercialized for their use in agriculture. The more emphasis of scientists on these PGPR is due to the fact that these could only be the best alternative for the chemical products being utilized on a wide scale for getting improved yield. These chemical products are known to add to the pollutants prevailing in our environment. Therefore, the focus of this review is on the potential characteristics of PGPR that make them suitable alternative for chemical products being extensively exploited in agricultural practices.

**Key words:** PGPR, Nitrogen fixation, Bio-control agents.

## INTRODUCTION

There is a variety of biotic and abiotic factors considered to influence the growth of plants. A thin layer of soil that immediately surrounds the plant roots is an extremely important area for root metabolism. This active zone of soil surrounding the plant roots is known as rhizosphere. Hiltner (1904) was the first person who introduced the

term rhizosphere of rhizosphere as the area of soil surrounding the roots where microbes flourish due to active metabolic activities of roots in this zone. This concept has been further extended to include the soil whose physical, chemical and biological activities are expected to change due to root growth (McCully, 2005). Plant roots offer a niche for the proliferation of soil bacteria that thrive on root lysates and root exudates. Population densities of bacteria is 100-fold higher in rhizosphere than in bulk soil and up to 15% of plant roots are covered by micro-colonies of a variety of bacterial strains

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(Van Loon, 2007). Plants release certain chemical products as root exudates which define in part of the microbial community that is expected to improve the plant growth and help protect the plant from disease causing organisms (Marilley and Aragno, 1999).

Bacterial species belonging to genera *Serratia*, *Rhizobium*, *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Erwinia*, *Burkholderia*, *Bacillus*, *Acinetobacter*, *Arthrobacter*, *Alcaligenes*, and *Azospirillum* are well known for their growth promotion effects on plants (Tilak et al., 2005; Egamberdiyeva, 2007). The chemical constituents secreted by plant roots play a very crucial part in the selection and enrichment of particular type of bacteria in rhizosphere. Thus, the bacterial community developed in the rhizosphere is the result of certain types of organic constituents in root exudates and the relevant dependence of bacteria on these root exudates for energy source (Curl and Truelove, 1986).

PGPR promote plant growth by exploiting either of direct or indirect mechanism. The direct mechanism of plant growth promotion by PGPR include production of metabolites, that is, phytohormones or enhanced availability of nutrients. In contrast, induced systemic resistance, antibiotic protection against pathogens, reduction of iron availability by sequestration with siderophores, synthesis of antifungal enzymes or lytic enzymes are included in indirect mechanisms of growth promotion by PGPR (Burdman et al., 2000; Dobbelaere and Okon, 2007; Lucy et al., 2004).

## MECHANISMS OF GROWTH PROMOTION BY PGPR

### Production of phytohormones

It has been widely known that plants synthesize a variety of chemical compounds, called phytohormones, that are actively involved in the regulation of plant growth and development (Santner et al., 2009). Mechanisms of growth improvement may involve modulation of plant regulatory mechanisms through the production of hormones or other compounds which influence plant growth (Van Loon, 2007). Several bacterial strains possess the ability to produce auxins and/or ethylene. In addition, production of cytokinins and gibberellins has also been reported in the literature (Van Loon, 2007). Pieterse and Van Loon (1999) reported 30% growth improvement due to inoculation with *Pseudomonas fluorescens* in arabidopsis accession Co1-0 grown in autoclaved soil. Likewise, when arabidopsis seedlings were treated with WCS417, a significant growth promotion was recorded. This growth promotion could be attributed to the potential of WCS417 strain to produce auxins known to promote lateral root formation (Tanimoto 2005). Improved lateral root formation results in better nutrient uptake. In another study, growth promotion in wheat and pearl millet treated with *Azospirillum brasilense* was due to the ability of this

strain to produce auxins (Barbieri and Galli, 1993).

However, bacteria lacking ACC deaminase have also been shown to increase plant growth and such observations cannot be explained by known mechanisms. It is presumed that under such conditions bacterial cells possess certain surface components or secrete compounds that act as 'elicitors' of plant growth. Plant roots must be able to perceive and recognize such elicitors in ways similar to the recognition of elicitors from plant pathogens. In fact, plant pathogens might interfere with the action of PGPR by being perceived by similar receptors.

Cytokinins and gibberellins have also been reported to stimulate shoot development. The effects of these hormones on the root growth have not been well documented. Ethylene is known as inhibitor of plant growth but ethylene at low concentrations may promote growth in several plant species including Arabidopsis (Pierik et al., 2006). The moderate levels of ethylene inhibit root and shoot elongation and elevated levels may induce senescence and organ abscission (Abeles et al., 1992). ACC (1-aminocyclopropane-1-carboxylic acid) is precursor of ethylene biosynthesis. ACC is secreted in root exudates by plants. Rhizobacteria that possess the enzyme ACC deaminase can utilize ACC as carbon source by degrading it. As a consequence, the levels of ACC in plants fall because of limited re-uptake of ACC by roots. This leads to lowered production of ethylene that relieves inhibition of root growth. Rhizobacteria with ACC deaminase enzyme are known to improve root growth by lowering the levels of ACC (Glick, 2005; Van Loon, 2007). Bacteria lacking ACC deaminase could also promote plant growth by employing such mechanisms which are not well known. However, it is presumed that bacterial cells secrete certain compounds that act as elicitors of plant growth. Plant roots perceive and recognize such elicitors in the ways similar to the recognition of elicitors from plant pathogens. In this way, plant pathogens interfere with the action of PGPR due to being recognized by the same receptors (Van Loon, 2007).

Production of plant growth regulators is considered one of the modes of action by which PGPR stimulates plant growth (Barea et al., 1976). For example, about 80% of the naturally occurring bacteria in soil are capable of producing auxin (Khalid et al., 2004; Patten and Glick, 1996). These bacteria utilize tryptophan secreted by plants as root exudates to synthesize auxin since tryptophan is considered as the precursor for synthesis of this hormone. Plant growth promotion has been reported in the plants inoculated with the bacterial strains capable of producing these chemical compounds (Patten and Glick, 1996). These soil borne bacteria are known to produce variable amounts of plant hormones. Mansour et al. (1994) evaluated 24 strains belonging to genus *Streptomyces* for their ability to produce plant hormones, all the strains exhibited the synthesis of auxin, gibberellins and cytokinins in liquid medium. The production of plant

growth promoting substances by PGPR has also been confirmed by García de Salamone et al. (2001).

### Biological nitrogen fixation

Biological nitrogen fixation is considered as one of the major mechanisms by which plants get benefited from PGPR. According to an estimate, global contribution of biological nitrogen fixation is  $180 \times 10^9$  metric tons per year. Of this contribution, 83% comes from symbiotic associations, while the rest part of it is provided by free living or associative systems (Graham, 1988). Archaea and bacteria are the only living forms that are capable of fixing the atmospheric nitrogen and enrich the soil with this form of nitrogen (Young, 1992). These include symbiotic nitrogen fixers (*Rhizobium* in legumes, *Frankia* in non-leguminous trees) and non-symbiotic nitrogen fixers such as *Azoarcus*, *Acetobacter diazotrophicus*, *Azotobacter*, *Azospirillum*, cyanobacteria etc.

Diazotrophic microorganisms are known to provide fixed nitrogen in exchange of fixed carbon secreted by plant as root exudates (Glick, 1995). The beneficial effects of symbiotic association of *rhizobia* with legumes have been well documented. In addition, several free living bacteria and rhizobial strains being capable of fixing atmospheric nitrogen are known to promote the growth of cereal plants by providing fixed nitrogen (Malik et al., 1997; Antoun et al., 1998; Biswas et al., 2000a). For example, it has been reported that the growth of rice could be stimulated by nitrogen fixing PGPR (Ladha et al., 1998). Likewise, the growth promotion effects of diazotrophic PGPR strains on rice have also been reported by Biswas et al. (2000a). In this way, PGPR provide an attractive alternative for chemical source of nitrogen fertilization.

Rhizobia are the most studied PGPR for their potential to fix  $N_2$  in the legumesous plants. *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* are among the most exploited PGPR (Hansen, 1994; Gualtieri and Bisseling, 2000; Schultze and Kondorosi, 1998; Sessitsch et al., 2002). It is interesting to note that a large number of PGPR strains have the ability to fix  $N_2$ . However, stimulation of plant growth through this type of mode of action is rarely credited to biological nitrogen fixation. PGPR strains that have the ability to fix  $N_2$  but there is little evidence to support the fact that improvement in growth of host plant is due to nitrogenase activity of bacterial strains. Such PGPR include *Azoarcus* sp. (Hurek et al., 1994), *Beijerinckia* sp. (Baldani et al., 1997), *Klebsiella pneumoniae* (Riggs et al., 2001), *Pantoea agglomerans* (Riggs et al., 2001), and *Rhizobium* sp. (Antoun et al., 1998; Yanni et al., 2001).

There has been extensive research on associative  $N_2$  fixation in non-legume crops in 1980s (Wani, 1986). But

still there is little evidence that supports the fact that improvement in growth of non-legume crops is due to the ability of strains to fix nitrogen. For example, it was believed that the beneficial effects of *A. brasilense* on non-legume crops were due to BNF. However, it is now well established that the growth primitive effects of *A. brasilense* were due to other mechanisms including production of phytohormones, effects on root morphology etc.

### Enhanced availability of nutrients

Plants require an adequate supply of nutrients for their proper growth and development. Plants growing on the soils enriched with nutrients may still exhibit nutrient deficiencies due to unavailability of these mineral nutrients. However, plant growth promoting rhizobacteria are actively involved in the solubilization of important minerals such as phosphorous, 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; Chabot et al., 1996; Biswas et al., 2000b; Dazzo et al., 2000). For example, Dazzo et al. (2000) reported that certain strains of *rhizobia* are capable of phosphorous solubilization. A prominent increase (13 to 23%) in phosphorous uptake has been reported in rice inoculated with rhizobial strains by (Biswas et al., 2000a). Likewise, when a combined effect of phosphorous solubilizing bacteria and arbuscular mycorrhizal fungi was evaluated on *Allium cepa* growing on soil with low in P contents, a significantly higher endogenous levels of nitrogen and phosphorous were found in plant tissues (Toro et al., 1997). It could be inferred from this study that interaction between fungi and bacteria help plant in getting sustainable supply of nutrients.

These PGPR can also alter the solubilization of mineral nutrients by releasing certain organic acids. In this context, Pietr et al. (1990) isolated 748 bacterial strains from the rhizosphere of different field crops and reported that 26% bacterial strains were able to solubilize calcium phosphate. These researchers further suggested that secretion of organic acid is the major mechanism for converting the insoluble phosphorous compounds to soluble forms. A number of other scientists also stated that PGPR improve mineral nutrient solubilization by creating an acidic environment (Webley and Duff, 1962; Moghimi et al., 1978; Alexander, 1977).

Some studies on PGPR also indicate their ability to produce high affinity siderophores considered to be involved in increasing the mobility and availability of micronutrients. Siderophores are the low molecular weight compounds that combine with  $Fe^{2+}$  and make it available to microorganisms (Leong, 1986). Plants are capable of

using this siderophore-Fe complex of microorganisms as a source of obtaining iron (Wang et al., 1993). For example, Hughes et al. (1992) reported siderophore production as the major contributor to improved iron uptake in oat. However, extensive research is needed in this area to establish quality, quantity and optimum conditions for siderophore production and their ability to influence plant growth and development.

## BIOLOGICAL CONTROL OF PLANT DISEASES BY PGPR

Soil-borne pathogens have been responsible for their devastating effects on plant growth and yield. For better crop yields, it is imperative to search for the cheap and effective ways to cope with the damaging effects of different pests or diseases. Plant growth promoting rhizobacteria are now recognized as the potential inducers of systemic resistance in crops against a number of pathogens (Vidhayasekaran and Muthamilan, 1999; Viswanathan and Samiyappan, 1999). PGPR strains have been reported to induce resistance in plants against bacterial, viral, fungal pathogens (Liu et al., 1995; Maurhofer et al., 1998), nematodes (Sikora, 1988) and insects (Zehnder et al., 1997).

Plant growth promoting rhizobacteria are known to decrease the population of pathogenic organisms by producing toxic metabolites (Glick, 1995; Kloepper, 1996). For example, production of siderophores and subsequent binding with Fe negatively affect the availability iron to other pathogenic microorganisms (Berthelin et al., 1991; Subba Rao, 1993). For example, Berthelin et al. (1991) reported that siderophore chelating of iron made iron unavailable for harmful organisms in sterilized medium. Production of antibiotics by PGPR is another important aspect of biological control. However, in some cases it is very difficult to distinguish between competition and antibiosis. There are a number of reports stating the suppression of pathogenic organisms by antibiotic production (phycocyanin, 2,4-diacetyl phloroglucinol, pyrrol-nitrin etc.) of microbial inocula (Pierson and Thomashow, 1992; Kloepper, 1993; Subba Rao, 1993; Glick, 1995; Thomashow and Weller, 1995). The other factors contributing in biological control of pathogens by PGPR include competition for nutrients and infectious sites, degradation of fungal cell wall by the action of lytic enzymes like chitinase and  $\beta$ -1, 3- glucanase (Potgieter and Alexander, 1996; Glick, 1995; Velazhahan et al., 1999).

## APPLICATION OF PGPR IN AGRICULTURE

The use of PGPR as biofertilizers has gained importance worldwide. PGPR is also considered as the potential alternative for chemical fertilizers. For example, the growth primitive effects of PGPR have been reported in

the literature on a number of crops. For example, Javed and Arshad (1997) isolated 38 cultures of rhizobacteria from soil and screened them on the basis of their ability to produce IAA (indoleacetic acid). Then the selected strains were used as inoculants for wheat plants of two cultivars (InqLab and LU-26S) grown under field conditions with minimal fertilization. A significant improvement in grain yield was observed in both cultivars inoculated with PGPR. Similar effects of PGPR have also been reported in rice. For example, Sakthivel et al. (1986) isolated different strains of PGPR and used them as inoculum for rice grown in pots. They observed a significant increase in plant height of inoculated plant over non-inoculated control plants. Likewise, Van et al. (2000) determined the beneficial effects of PGPR inoculation on rice grown either in pots or fields. They used *Burkholderia vietnamiensis* as inoculum and observed a marked positive effect on plant biomass and number of tillers than that of non-inoculated control. When maize plants were inoculated with PGPR, a significant increase in yield was reported (Javed et al., 1998). Of the 11 isolates used in this study, 5 isolates identified as *Pseudomonas* were more consistent in improving growth and yield of maize plant. A marked improvement in growth of maize seedlings in response to inoculation with phosphorous solubilizing PGPR strains has also been reported (Berthelin et al., 1991). The beneficial effects of PGPR have also been well documented on crops other than cereals, for example, potato (Zahir and Arshad, 1996), tomato (Gagne et al., 1993), rapeseed (Mei, 1989) and canola (Tang et al., 1995; Shah et al., 1998; Glick et al., 1995).

## CONCLUSION

Keeping in view the above-given discussion on the different characteristics of PGPR, it is amply clear that the chemical fertilization can be minimized to a great extent using PGPR. The use of these PGPR is an attractive as well as economic approach for sustainable agriculture. The commercialization of PGPR as biofertilizers should be emphasized. There is need to create awareness among the farmers about the potential benefits that could be obtained using these microorganisms rather than focusing on cost ineffective approaches based on the use of chemical fertilizers.

Moreover, the use of PGPR is environmental friendly approach. Agriculturists from all over the world should focus on the research centered on unrevealing the hidden potential of these microorganisms.

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