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PGPR Isolated from Rhizosphere can be an Eco-Friendly Fertilizer for Promotion Plant Growth and Antagonistic Activity against Phytopathogen

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are advantageous bacteria that dwell around plant roots and make better plant growth by a commodious variety of direct and indirect mechanisms such as producing IAA, siderophore, and phytase in addition to producing lytic enzymes, solubilized various sources of organic and inorganic phosphates and zinc. PGPR also shows a strong antagonistic activity against the growth of several phytopathogens. So PGPR can be utilized as a potential biofertilizer and plant growth-yield promoter in the replacement of injurious chemical fertilizer.

KEYWORDS: Antagonistic activity, Biofertilizer, PGPR, Phosphate solubilization, and Siderophore production

INTRODUCTION

Soil bacteria having beneficial effect on plant health are commonly referred to Plant growth promoting rhizobacteria.(PGPR) are such a group of bacteria that actively colonize plant roots and exert positive effect on growth and productivity. There are many reports which reveal that PGPR not only increases the plant growth but also increases in seed germination rate, root growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight, and delayed leaf senescence [1]. The PGPR can promote the plant growth by either direct or indirect mechanism. The direct mechanisms are as Nitrogen fixation, Solubilisation of mineral phosphates and other nutrients, Production of phytohormones like IAA, GA, cytokinins. The indirect mechanisms include Antibiotic production, Siderophore production, Synthesis of anti-fungal compound and fungal cell wall lysing enzymes such as cellulase, protease, amylase, chitinase, and cyanide and Competition for sites on roots and induced systemic resistance [2,3]. Now days the utilization of PGPR is steadily increasing in agriculture the strains such *as Bacillus, Pseudomonas, Paenibacillus, BradyrhizobiumKlebsiella, Streptomyces, Pantoea, Rhizobium* etc.have strong potential to be successful eco-friendly biofertilizers and bioenhacers and offers an attractive way to replace chemical fertilizers and pesticides.

LITERATURE REVIEW

The use of PGPR as bio fertilizer is steadily increasing in agriculture recently not only to improve plant growth and to manage plant disease but also offers an attractive way to replace chemical fertilizers, pesticides, and supplements causing environment pollution. In world India is one of the major crop producing country and thus is also susceptible to attack by insect and fungus. The soil borne plant pathogenic fungi are very much threatening to crop grown in India. In certain yield region and season fungi cause yield reducers [4]. Hence to avoid crop losses, extensive use of insecticides, chemical fertilizers and fungicides have been used. Consequently these cropping practices have brought about significant undesirable changes in soil microbial activity due to release of byproduct of pesticides into the soil. As a result land gradually losses its fertility. This approach is costly and imposes threat to the environment and human health [3].For the last few years scientists are searching an alternative and sustainable way to overcome this problem. Application of plant growth promoting bacteria may be the way [5].Concept of rhizosphere was first given by Hiltner to depicture the zone of soil surrounding the roots where microbial populations are accelerated by the activity of root and PGPR term was coined for the first time by Kloepper and Schroth to narrate this microbial population in the rhizosphere which is beneficial, colonize the roots of plants and shows plant growth promotion activity . The term “Plant Growth Promoting Rhizobacteria” is refer to bacteria colonizing in the root cause increase in growth and yield and to differentiate them from other microorganisms placed in rhizosphere that do not colonized in root and enhance plant growth. This is well established that only 1 to 2% of rhizosphearic bacteria promote plant growth [6]. Recent study revealing that in the rhizosphere 2-5% of bacterial population is PGPR.There are many reports presented the soil attached to the root system as a hot spot of microbial abundance and activity due to the presence of root exudates and rhizodiposits. These compounds secreted by roots into the soils are generally called as root exudates. Root exudates can serve as a food source and chemoattractant for microbes which then attach to the root surface and form microcolonies. Common sites for bacterial attachment and colonization are at epidermal cell junctions, root hairs, axial groves, cap cells, and sites of emerging lateral roots [7].The PGPR can promote the plant growth by either direct or indirect mechanism but the proper mechanisms by which they can act beneficially on plant growth have not been fully explained. PGPR may increase plant growth by several mechanisms, for example, by solubilizing nutrients such as P , Releasing phytohormone and decreasing heavy metal toxicity [8].The use of PGPR, including phosphate potassium and zinc solubilizing bacteria, as bio-fertilizers was suggested as a sustainable solution to improve plant nutrient and production [9]. Among these the direct plant growth activities are as 1.Nitrogen fixation 2. Solubilisation of mineral phosphates and other nutrients 3.Production of

Phytohormones like auxin, gibberellins, cytokinins. The indirect mechanisms of plant growth promotion by PGPR include 1. Antibiotic production; 2.Siderophore production; 3.Synthesis of anti fungal compound and fungal cell wall lysing enzymes such as cellulase, protease, chitinase, and cyanide. 4. Competition for sites on roots and induced systemic resistance [2,3].

DIRECT MECHANISMS

Nitrogen Fixation

Nitrogen(N) is one of the chief plant nutrients, and has become one of the yield-limiting factors in plant growth due to rainfall and mineral leaching into the ground water. There are number of Plant Growth Promoting Bacteria (PGPB), which are able to fix atmospheric nitrogen (N_2) and make it much more available to plants. They are able to greatly increase the intake of nitrogen by the plants, due to their effect on shoot elongation and stimulation of nitrate (NO^{3-}) transport systems, despite not fixing enough nitrogen on its own for sustenance (Mantelin et al. 2003). There are two types of biological fixation: Symbiotic and Non-symbiotic. The first is the most important mechanism by which most atmospheric N is fixed, but it is only found to legume plant species and various shrubs and trees that form actinorrhizal roots with Frankia. This process is carried out in well-defined nodule structures. Many reports suggest that symbiotic bacteria are *Rhizobium*, *Bradyrhizobium, Sinorhizobium* and *Mesorhizobium*[10]. Although the beneficial effects of the symbiotic association of rhizobia with legume plants is known, these bacteria are not considered as PGPR, except when associated with non-legume plants [1]. On the other hand, non-symbiotic biological N fixation is carried out by free living diazotrophics, and this can stimulate non-legume plants growth . Nitrogenase (nif) genes required for nitrogen fixation include stuctural genes, genes involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme. In diazotrophic (nitrogen fixing) bacteria, nif genes are typically found in a cluster of around 20-24Kb with seven operons encoding 20 different proteins. Because of the complexity of this system, genetic strategies to improve nitrogen fixation have been elusive. Since the process of nitrogen fixation requires a large amount of energy in the form of ATP, it would be advantageous if bacterial carbon resources were directed toward oxidative phosphorylation, which results in the storage of energy in the form of glycogen. In one experiment, a strain of *Rhizobium tropici* was constructed with a deletion in the gene for glycogen synthase. Recently, Unkovich and Baldock (2008) pointed out that the contribution of N by free living soil bacteria for crop growth in Australia in probably <10kg ha-1 yr-1[11]. Peoples *et al*.(2002) present a N fixation value of 0 to 15kg ha-1 yr-1 [12]and Bottomley&Myrold (2007) suggest annual values between <1 and 10kg ha-

1[13]. For this reason, the ability of PGPR to fix N is no longer an important criterion for classification of a bacterium as a biofertilizer.

Phosphate Solubilization

After nitrogen, phosphorus is an essential macronutrient for plant growth and has only limited bioavailability, it is considered to be one of the elements that limit plant growth [14]. The amount of phosphorus (P) usually in soil is between 400 and 1,200 mg kg-1 of soil, the concentration of soluble P in soil is typically ~1mg kg-1 or less . Two main insoluble form of phosphorus present in soil are mineral and organic forms. Mineral forms viz. Apatite, hydroxyapatite, oxyapatite and organic forms including inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters and phosphotriesters[15]. According to Gyaneshwar *et al.*(1999) and Mullen (2005), the rhizosphere bacteria has the ability to solubilize insoluble P minerals which have been attributed to their capacity to reduce pH by the excretion of organic acids(e.g. gluconate,citrate, lactate and succinate) and protons (during the assimilation of NH_4^+)[16, 17] . From different rhizospheric soils, the phosphate solubilizing bacteria have been characterize including *Enterobactor, Bacillus, Burkholderia, Klebsiella, Streptomyces, Pantoea and Pseudomonas* genera[18]. The phytase enzymes are produced by most of the bacteria for the minerilization of phytates .Based on the synthesis of low molecular weight organic acids viz. gluconic and citric acid, inorganic phosphates are solubilized by the use of phosphate solubilizing bacteria (PSB)[19] . These organic acids bind phosphate with their hydoxyl and carboxyl groups there the contribution of N by free living soil bacteria for crop growth in Australia in probably <10kg ha-1 yr-1. Peoples *et al.(*2002) limb chelating cautions and also inducing soil acidification, both resulting in the release of soluble phosphate [12] .Other mechanisms that have been implicated in solubilzation of inorganic phosphate are the release of pH [20], the production of chelatin substances and inorganic acids [21] . In addition, exopolysaccharide synthesized by PSB participate indirectly in the solubilization of tricalcium phosphates by binding free P in the medium, affecting the homeostasis of Psolubilization[22]. Thus solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait in PGPB.

Sequestering Iron By Siderophores

Iron is known as the most abundant element on earth [23]. On the other hand, in aerobic soils, iron is frequently precipitated as hydroxides, oxyhydroxides and oxides so that iron is not readily assimilated by either bacteria or plants because ferric ion or Fe^{3+}, which is the predominant form of nature, is only sparingly soluble so that the amount of iron available for assimilation by living organisms is extremely low corresponding from 10^{-7} to 10^{-23} M at pH 3.5 and 8.5 [23]. According to Loper& Buyer

(1991) andGuerinot& Ying (1994), both microorganisms and plants need a high level of iron, and getting sufficient iron is even more problematic in the rhizosphere where plant, bacteria and fungi compete for iron. To survive with such a limited supply of iron, bacteria synthesize low-molecular weight compounds, siderophores (~400-1500 Da) as iron (Fe) chelating agents. These molecules have high affinity for Fe^{3+} (ranging from 10^{23} to 10^{52}) as well as membrane receptors which is able to forming the Fe-siderophore complex, thereby facillating iron uptake by microorganisms [24]. Siderophore producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+}, around the root area. Many reports have isolated siderophore producing bacteria including to the *Pseudomonas*,*Rhizobium*, *Bradyrhizobium*, *Serratia* genera from the rhizosphere[25,26]. At the current time, there are over 500 known siderophoresthe chemical structures of 270 of these compounds have been determined. The direct benefits of Siderophore production by bacteria on the growth of plants have been confirmed in several different types of experiments. For example, (i) several studies using radiolabeled ferric-siderophores as a sole source of iron showed that plants are able to take up the labeled iron, (ii) mung bean plants, inoculated with the siderophore producing *Pseudomonas* strain GRP3 and grown under iron limiting conditions, showed decreased chlorotic symptoms and an superior chlorophyll level compared to uninoculated plants [27] (iii) the Fe-pyoverdine complex synthesized by *Pseudomonas* fluorescence C7 was taken up by *Arabidopsis thaliana* plants, leading to an boost up of iron inside plant tissue and to improve plant growth.

Production of Phytohormones

The synthesis of Phytohormones by PGPR is now considered to be one of the most significant mechanisms by which many rhizobacteria promote plant growth [28]. These phytohormones are generally signal molecules acting as chemical messengers and play a vital role as growth and development regulators in the plants. In extremely low concentrations, phytohormones stimulate biochemical, physiological and morphological processes in plants and synthesis of phytohormones are highly regulated [29]. There are several bacterial and fungal species that can produce phytohormones. Studies have confirmed that the PGPR can stimulate plant growth through the production of high levels of endogenous ethylene in the plant , cytokinins, gibberellines and auxins (indol acetic acid) [30]. Subsequently many plant growth promoting bacteria has the ability to control the hormonal balance in plants.

Indole Acetic Acid (IAA) Producing Rhizobacteria

Indole 3 acetic acid (IAA) is the principal native auxin of higher plants. Many important plant microbial interactions center on the production of auxins, IAA being the main plant auxin. The IAA is responsible for the division, expansion and differentiation of

plant cells and tissues, beside this, auxin stimulate root elongation, seed and tuber germination; increase the rate of xylem, control processes of vegetative growth, initiate lateral and adventitious roots; trophic responses, flowering and frutification of plants; and also affect photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions [31]. The capability to make IAA has been discovered in many rhizobacteria as well as in pathogenic, symbiotic and free living bacterial species[31]. By different pathways these type of rhizobacteria are synthesized from tryptophan, although it can synthesized by tryptophan independent pathway [28]. In contrast, the acid indole pyruvic pathway appears to be the main pathway present in plant growth promoting beneficial bacteria. In bacteria, auxin biosynthesis is affected by several types of factors including environmental stress, pH, osmotic and matrix stress, carbon starvation, and the composition of the root exudates. One of the key effects of bacterial IAA is the prolongation of lateral and adventitious root that leading to improve mineral and nutrient uptake and root exudation that in turn stimulates bacterial proliferation on the roots [32].

INDIRECT MECHANISMS

The major indirect mechanism of plant growth promotion of rhizobacteria is to take action as bio-control agents . PGPR showed several attributes to be the potent strains can be used as bio-fertilizer as well as bio-control agents**.** Biological control is the reduction of disease caused by pathogen by the help of other organism. PGPR are biological agent with the potential to interfere in the life process of plant pathogen such as fungi, bacteria etc. The PGPR shows its effects via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant. Beattie (2006) reported that bacteria that reduce the occurrence or severity of plant diseases are often referred to as bio-control agents whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists[33]. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirect promotion of growth in many plants. The following rhizospheric environment and bacterial antagonistic activities can be highlighted: (1) production of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, that can cause lysis pathogenic fungal cells , (2) competition for nutrients for utilizing of same niches at the root surface , (3) regulation of plant ethylene levels through the ACC-deaminase enzyme, which is able to modulate the level of ethylene in a plant in response to stress imposed by the infection , and (4) By produce siderophores and antibiotics[34,35].

Production Of Lytic Enzymes

Lytic enzymes produced by bacteria are glucanases, proteases , cellulases, and chitinases. Bacteria could parasitize disease-causing fungal pathogen by the

production of these enzymes. Some enzyme producing bacteria are able to demolish oospores of phytopathogenic fungi and affect the spore germination and germ-tube elongation of phytopathogenic fungi [36]. A positive relationship was observed between chitinase production and the antifungal activity of chitinolytic *P. fluorescens* isolates. Production of extracellular cell wall degrading enzymes has been associated with bio-control abilities of the producing bacteria [36]. Tn5 mutants of one of the Enterobacter which were deficient in chitinolytic activity were unable to protect plants against the disease. In addition, enzyme producing bacteria were successfully used in combination with other bio-control agents, leading to a synergistic inhibitory effect against pathogen [37].

Among all the plant growth promoting bacteria the use of *Bacillus* is advantageous as because Bacillus sp. are able to form endospores that allow them to survive for extended periods under unfavorable environmental conditions. This trait is relevant in their relative durable viability when stored for a relatively long period (shelf-life) in the opposite to *Pseudomonas* and other nonspore-forming bacteria. Studies also reveal that *Bacillus* species are among the most noticeable bacteria found to colonize plant root and soil populations [38]. *Bacillus* species are generally present in the immediately vicinity of plant root.

Application of *Bacillus* and or *Paenibacillus* species to seeds or roots has been shown to cause alterationin the composition of rhizosphere leading to increase in growth and yield of different crops. Disease suppression by *Bacillus cereus* enhanced nodulation and seedling emergence in common bean, soybean, cowpea, and pea and also been demonstrated as beneficial effects on plants. Bacilli are also very attractive as potential inoculants in agriculture, as they produce very hardy spores that can survive for prolonged periods in soil and in storage containers.

Different species of *Paenibacillus* can induce plant growth by fixing atmospheric nitrogen, and producing auxins and cytokinin. Though there is very little evidence of production of gibberellins by the plant growth promotory rhizobacteria. Yet, it has been reported to be produced by some soil rhizospheric bacteria's like *Bacillus licheniformis* and *Bacillus pumilus*. Several species *Bacillus* have been reported as plant promoting bacteria in a wide range of plants[39]. Different *Bacillus* species were reported to be effective biocontrol agents in greenhouse or field trials [39]. *Bacillus* spp. members were reported as generator of antibiotics suppressing various phytopathogens including *F. oxysporum* f. sp. ciceri and *Rhizoctonia solani*. Bacillus strains were also reported as capable inducers of systemic resistance (ISR). Jetiyanon et al. (2003) observed that one PGPR mixture, *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b, defended plants by inducing systemic resistance (ISR) against southern blight of tomato, a disease caused by *Sclerotium rolfsii*[40].

CONCLUSION

In developing countries like India, the necessity of chemical fertilizers for crop production has increased tremendously due to the invention of various high yielding and nutrient requiring varieties of crop plants. But the use of chemical fertilizers has resulted not only in the deterioration of soil health but also has led to some major environmental problems, such as soil and water pollution and other health related problems. In order to effectively reduce the excessive use of chemicals in agriculture, currently, much emphasis is being laid on use of eco friendly biological materials for use in sustainable agriculture. Thus the use of rhizospheric bacteria having plant growth promoting activity for the betterment of the health of plant is one of the most promising avenues of research in modern science. Further investigation and proper identification of different types of PGPR may lead to establishment of new plant growth promoting and bio controlling agents which can be alternative of deleterious chemical fertilizer and can control phytopathogen also in eco-friendly way.

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