

Impact of Plant Growth Promoting Rhizobacteria in Sustainable Agriculture: An Important Natural Resource for Crop Improvement

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ABSTRACT

It is usually admitted that the chemical fertilizers and pesticides used in modern agriculture create a real environmental and public health problems. The increasing demand for production with a significant reduction of synthetic fertilizers and pesticides use is a big challenge nowadays. The use of plant growth promoting rhizobacteria or PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. They play an important role to increase in soil fertility, plant growth promotion and suppression of phytopathogens for development of ecofriendly sustainable agriculture. In view of the latest advances in PGPR biotechnology, this paper proposes to do the review on PGPR in rhizosphere and describes the different mechanisms used by PGPR to promote the plants growth and health. In prospect to a healthy and sustainable agriculture, the PGPR approach revealed as one of the best ecofriendly alternatives.

Key words: Crop yield, Plant growth promoting rhizobacteria (PGPR), Rhizosphere, Sustainable agriculture.

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INTRODUCTION

In modern cultivation process rapid use of fertilizers, has led to substantial pollution of soil, air and water. Excessive use of these chemicals exerts deleterious effects on soil microorganism, affects the fertility status and also pollutes environment (Yossef and Eissa, 2014). Over the last few decades, the agriculture policy has undergone a major change through diversification and emphasis on sustainable production system. Regarding that, soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. Plant bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. The term rhizosphere was introduced for the first time by Hiltner (1904). The major influences that the rhizosphere microorganisms have on plants today become important tool to guard the the health of plants in ecofriendly manner (Akhtar *et al.*, 2012). These microorganisms can effect plant growth often referred to as a plant growth promoting rhizobacteria (Kloepper *et al.*, 1980). The plant growth promoting bacteria or PGPR are capable of enhancing the growth of plants and protecting them from disease and abiotic stresses. Plant growth promoting rhizobacteria or PGPR, as on the majority of cases the effect is caused by the bacteria that exert highly beneficial effects on plant development by direct or indirect mechanisms. Some PGPR can be classified as biofertilisers and biocontrol agents or biopesticides (Glick, 2012). Biofertilisers are the rhizobacteria that under particular conditions mainly enhance plant growth via providing required nutrition. The can accelerate certain microbial processes in the soil that augment the availability of nutrients in a form easy to assimilate by plants. They can be grouped, based on their nature of function, as nitrogen fixing, phosphate mobilizing, or biofertilisers for micronutrients. Furthermore, plant growth promoting rhizobacteria can reduce chemical fertilizers application and economically, environmentally beneficial for lower production cost as well as recognize the soil and crop management practices to achieve more sustainable agriculture as well as fertility (Doornbos *et al.*, 2012; Maheswari *et al.*, 2012).

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Plant Growth Promoting Rhizobacterial Forms

Plant growth promoting rhizobacteria can be classified into extracellular plant growth rhizobacteria (ePGPR) and intracellular plant growth rhizobacteria (iPGPR) (Viveros *et al.*, 2010). The ePGPR may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex while iPGPR locates generally inside the specialized nodular structures of root cells. The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* belongs to ePGPR (Ahemad and Kibret, 2014). The iPGPR belongs to the family of Rhizobiaceae includes *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, endophytes and *Frankia* sps both of which can symbiotically fix atmospheric nitrogen with the higher plants (Ahemad and Kibret, 2014).

Plant growth promotion: Mechanism of action

Plant growth promotion by plant growth promoting rhizobacteria is a well-known phenomenon trait of rhizobacteria and this growth enhancement is due to certain traits of rhizobacteria. There are a number of mechanisms used by PGPR for enhancing plant growth and development in diverse environmental conditions (Fig. 1).

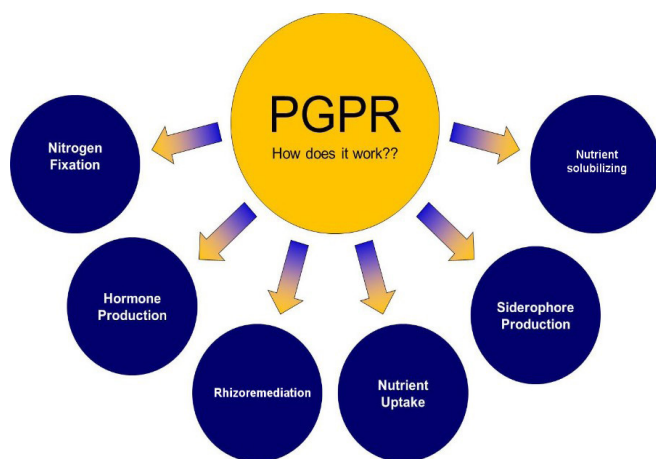


Fig. 1: Multidimensional action of PGPR.

Generally, plant growth promoting rhizobacteria promote plant growth directly (Fig. 1) by either often due to their ability for nutrient supply or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents, root colonizers, and environmental protectors (Bhattacharyya and Jha, 2012).

Direct mechanism

Plant growth promoting rhizobacteria having direct mechanisms that facilitate nutrient uptake or increase nutrient the availability by nitrogen fixation (Fig. 2), solubilization of mineral nutrients, mineralize organic compounds and production of phytohormones (Kloepper *et al.*, 1980).

Nitrogen fixation

Nitrogen is the most vital nutrient for plant growth and productivity. Although, there is about 78% nitrogen in the atmosphere, it is unavailable to the growing plants. The atmospheric nitrogen is converted into plant-utilizable forms by Biological nitrogen fixation which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase. Biological nitrogen fixation occurs, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature. Nitrogen-fixing bacteria fix atmospheric nitrogen by means of the enzyme nitrogenase, a two component metalloenzyme composed of (a) dinitrogenase reductase, a dimer of two identical subunits that contains the sites for Mg ATP Binding and hydrolysis and supplies the reducing power to the dinitrogenase, and (b) the dinitrogenase component that contains a metal co-factor. Nitrogen fixing organisms are generally categorized as (a)

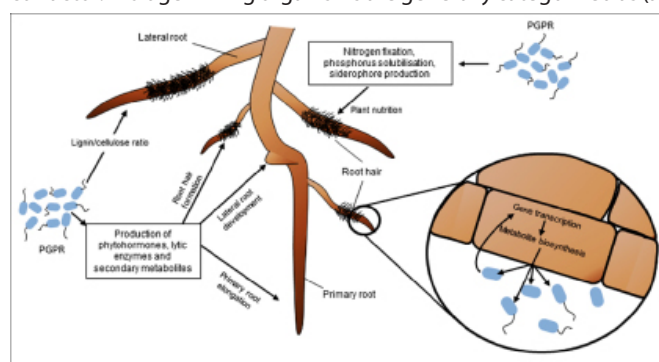


Fig. 2: Role of PGPR in nitrogen fixation.

symbiotic nitrogen fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. *Rhizobia*) (Zahran, 2001) and non-leguminous trees (e.g. *Frankia*) and (b) non-symbiotic nitrogen fixing forms such as cyanobacteria (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*, *Gluconoacetobacter diazotrophicus* and *Azocarus* etc. (Bhattacharyya and Jha, 2012). However, non-symbiotic nitrogen fixing bacteria provide only a small amount of the fixed nitrogen that the bacterially-associated host plants require (Glick, 2012). Symbiotic nitrogen-fixing rhizobia within the rhizobiaceae family infect and establish symbiotic relationship with the roots of leguminous plants. The establishment of the symbiosis involves a complex relationship between host and symbiont resulting in the formation of the nodules wherein the rhizobia colonize as intracellular-symbionts.

Phosphate solubilization

Since P 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. P in soil present in two main insoluble forms: mineral forms as well as organic forms. Solubilization and mineralization of P by Phosphate-Solubilizing Bacteria (PSB) is one of the most important bacterial physiological traits in soil biogeochemical cycles as well as in plant growth promotion by PGPR. Bacterial genera like *Azotobacter*, *Pseudomonas*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Rhizobium* and *Serratia* etc. are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012). Typically, the solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids which are synthesized by various soil bacteria (Zaidi *et al.*, 2009). Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters (Glick, 2012). Importantly, phosphate solubilization and mineralization can coexist in the same bacterial strain. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P by means of acid and alkali phosphatase enzymes. Because the pH of most soils ranges from acidic to neutral values acid phosphatases should play the major role in this process. The possibility of enhancing P uptake of crops by artificial inoculation with P-solubilizing strains of rhizobacteria presents an immense interest to agricultural microbiologists.

Siderophore production

Iron is an essential growth factor for living creatures. For the soil microorganisms, availability of solubilized ferric ion in soil is limited at neutral and alkaline pH. Siderophore producing plant growth promoting rhizobacteria can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the area around the root (Kuffner *et al.*, 2008). These siderophores binds with ferric ion and make siderophore-ferric complex which subsequently binds with iron limitation-dependent receptors at the bacterial cell surface. The ferric ion is subsequently released and active in the cytoplasm as ferrous ion. Various studies showed isolation of siderophore producing bacteria belonging to the *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Streptomyces* genera from the genera the rhizosphere (Fig. 3).

Synthesis of plant hormones

Phytohormones are signal molecules acting as chemical messengers and play a fundamental role as growth and development regulators in the plants. With the production of different phytohormones like indole-3-acetic acid, gibberelic acid and cytokinins PGPR can

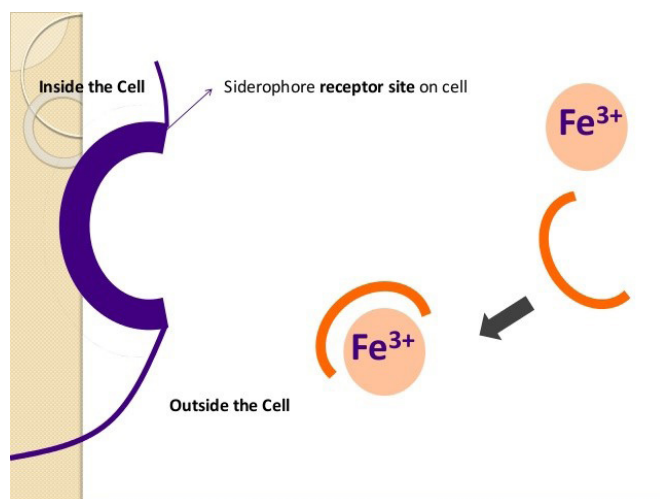


Fig. 3: Siderophore action.

increase root surface and promote in this way plant development (Kloepper *et al.*, 1980). IAA is the most important phytohormone produced by PGPR, and treatment with auxin-producing rhizobacteria increased the plant growth (Vessey, 2003). Among PGPR, a few PGPR strains were reported to produce cytokinins and gibberellins (Vessey, 2003). Bacteria like *Azospirillum* and *Pseudomonas* sp. produce cytokinins and gibberellins, in addition to indole-acetic acid. Many PGPR have the capability to produce ACC deaminase, an enzyme which cleaves ACC, the immediate precursor of ethylene in the biosynthetic pathway for ethylene in the biosynthetic pathway for ethylene in plants.

Indirect mechanism

Phytopathogenic microorganisms are a major and chronic threat to sustainable agriculture and ecosystem stability worldwide encompasses the soil ecology, disrupt environment, degrade soil fertility status and consequently show harmful effects on human health, along with contaminated ground water. PGPR is a promising sustainable and environmentally friendly approach to obtain sustainable fertility of the soil and plant growth indirectly. The indirect mechanism includes: the production of antibiotics, hydrolytic enzyme production, exopolysaccharides production or biofilm formation as well as induced systemic resistance or ISR.

Antibiotic production

Production of antibiotics is probably the best known and perhaps the most important mechanism used by PGPR to limit the pathogens invasion in the plant tissue. It consists to inhibit the development of plant pathogenic microorganisms through the production of secondary metabolites of low molecular weight, possessing antifungal or antibiotic properties. *Bacillus*, *Streptomyces*, and *Stenotrophomonas* strains produce a wide range of potent antifungal metabolites such as oligomycin-A, xanthobaccin (Compant *et al.*, 2005), kanosamine and lipopeptides of the surfactins and fengycin family. *Pseudomonas* strains are known for the production of amphisin, 2,4-diacetylphloroglucinol or DAPG, oomycin-A, tropolone, tensin and the cyclic lipopeptides. It was recently demonstrated the role of these lipoproteins in protective effect of a particular *B. subtilis* strain against *Pythium ultimum* pathogen of bean plants and against mould gray of apple after harvesting.

Lytic enzyme production

Some PGPR strains have the ability to degrade fungal cell walls through the production of hydrolytic enzymes such as

chitinases, dehydrogenases, β -glucanases, proteases, hydrolases, phosphatases, lipases, pectinolyases and cellulases (Joshi *et al.*, 2012). Various *Pseudomonas* strains showed *in vitro* antifungal activity against 3 zoospores fungi recently showed the synergistic effect of antagonistic fungi *Trichoderma* with combined application of *Pseudomonas* and rhizobial strains can protect chickpea from infection by the collar rot pathogen *Sclerotium rolfsii*. In this way through the production of various lytic enzymes PGPRs play a very vital role.

Induction of systemic resistance

PGPR can trigger the plants inducible defense mechanisms, phenotypically similar to normal defense reaction of plants, when attacked by a pathogen. This phenomenon called Induced systemic resistance (ISR) can make the plant much stronger and hardy against future aggression of pathogens. This phenomenon of systemic resistance induction by rhizobacteria is considered as a promising strategy for biological control of plant disease. The ISR can be induced by a wide range of microorganisms included Gram-positive bacteria such as *Bacillus pumilus*, or Gram-negative bacteria belonging to the genus *Pseudomonas* and *Enterobacteria* such as *Serratia* or *Pantoea agglomerans*. The IRS protects the plants against a large spectrum of pathogens not only fungal, bacterial and viral, but also against certain diseases caused by insects and nematodes. Several bacterial metabolites can induce an IRS. These metabolites include lipopolysaccharides, siderophores, cyclic lipopeptides, homoserine lactones and volatile compounds such as acetoin and 2,3-butanediol.

Exopolysaccharides production or biofilm formation

Certain bacteria synthesize a wide spectrum of multifunctional polysaccharides including intracellular polysaccharides, structural polysaccharides, and extracellular polysaccharides. Production of exopolysaccharides is generally important in biofilm formation as well as biofilm formation. Effective colonization of plant roots by EPS-producing microbes helps to hold the free phosphorus from the insoluble one in soils and circulating essential nutrient to the plant for proper growth and development and protecting it from the attack of foreign pathogens. Plant growth promoting rhizobacterial producing exopolysaccharides are highly important in promoting plant growth due to work as an active signal molecule during beneficial interactions, and provide defense response during infection process.

Commercialization of PGPR

The success and commercialization of plant growth promoting rhizobacterial strains depend on the linkages between the scientific organization and industries. PGPR-based commercialization is at a boom and several industries are commercializing bacterial and fungal strains as PGPR-based biofertilizers. Numerous work done showed different stages in the process of commercialization includes isolation of antagonistic strains, screening, fermentation methods, mass production, quality control and field efficacy etc. (Parada *et al.*, 2006). Moreover, the commercial success of PGPR strains requires economical and viable market demand, consistent and broad spectrum action, safety and stability, longer shelf life, low capital costs and easy availability of career of career materials.

Impact of PGPR on Sericulture

It is now proved that, the rhizosphere microflora plays a great role for growth promotion in mulberry (*Morus* sp.). In mulberry, production and quality of leaves are largely dependent on the

application of chemical fertilizers, specially nitrogen, phosphorus and potassium. Even these chemical fertilizers are not fully absorbed by the plants because of volatilization, leaching loss and quick fixation with soils, thus the farmers do not get the full benefit of the chemical fertilizers. Therefore, application of microbial agents in the form of biofertilizers is the key to sustain sericultural productivity. Efficient strains of some PGPR like *Azotobacter*, *Azospirillum*, *Bacillus megaterium* have been found to be highly beneficial for mulberry cultivation because of their ecofriendly utility and possibility of economizing mulberry cultivation. The beneficial roles of these organisms towards economization of nitrogenous and phosphatic fertilizers in mulberry cultivation has been well established. PGPR namely *Pseudomonas* sp. and *Bacillus* sp. have shown activity in suppressing the fungal infection. They activate systematically the plant's latent defense mechanisms against pathogens called induced systemic resistance. This mechanism operates through the activation of multiple defense compounds at the sites distant from the point of pathogen attack.

Recent Research and Development Strategies for sustainable technology with PGPR

The need to today's world is high output yield and enhanced production of the crop as well as fertility of soil in an eco-friendly manner. Hence the research has to be focused on the new concept of rhizoengineering based on favourably partitioning of the exotic bio-molecules which create a unique combination for the interaction between plant and microbes.

Future research in rhizosphere biology will rely on the development of molecular and biotechnological approaches to increase our knowledge of rhizosphere biology and to achieve an integrated management of soil microbial populations.

The application of multistrain bacterial consortium over single inoculation could be an effective approach for reducing the harmful impact of stress on plant growth. The addition of ice-nucleating plant growth promoting rhizobacteria could be an effective technology for plant growth at low temperature (Nadeem *et al.*, 2013).

Research on nitrogen fixation and phosphate solubilization by PGPR is progress on but little research can be done on potassium solubilization which is third essential macronutrient for plant growth. Future marketing of bioinoculant products and release of various transgenics into the environment as eco friendly alternations to agro chemicals will depend on the generation of biosafety data required for the registration of plant growth promoting rhizobacterial agent.

Apart from that future research in optimizing growth condition and increased self life of PGPR products, non phytotoxic to crop plants, tolerate adverse environmental condition, higher yield and cost-effective PGPR products for use of agricultural farmers will be also helpful.

CONCLUSION

This paper showed the beneficial effects of PGPR. PGPR improve soil fertility through increase plant nutrients available in soil. The phytohormones produced by PGPR are assimilated by plant for best growth. Also, PGPR inhibit plant pathogens growth through the production of antagonistic metabolites, induction of systemic resistance and nutrients. Additionally, PGPR polysaccharides alter soil porosity and consequently improve soil aeration. It is therefore clear that the objectives of chemical fertilizers and pesticides use can be reached with PGPR use. These rhizobacteria are the



Fig. 4: PGPR in sustainability.

best alternatives to use of chemical fertilizers and pesticides that generate many problems in our ecosystem. Thus, this technology based on the PGPR use, should be integrated into agricultural production strategies of all countries to a healthy and sustainable agriculture (Fig. 4).

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