Review Paper:

Plant Growth promoting Rhizobacteria (PGPR) for enhancing Sustainable Agriculture and Revolutionized Tools for Farmers

Kaur Manjit[1]* and Mathur Abhishek[2]
1. Department of Zoology, RIMT University, Mandi Gobindgarh, Punjab, INDIA
2. NCS Group, Nagpur, Maharashtra, INDIA
*manjitkaur380@gmail.com

Abstract

The aim of the present review is to study the effect of plant growth promoting rhizobacteria (PGPR) on crops. These PGPRs are meant to be the natural and alternate substitute of chemicals in agriculture. The plant growth promoting rhizobacteria are the naturally occurring soil bacteria which enhance the plant growth, also protect plant in stress condition without causing any adverse effect on the environment. The use of PGPRs for sustainable and safe agriculture has increased globally in last couple of decades. PGPRs are meant to reduce the soil toxicity which occurs due to surplus use of chemicals and pesticides.

Scientific researches involve multidisciplinary approaches to understand the working of PGPR, effects on plant growth and physiology, biocontrol of plant pathogens, induced systematic resistance and phyto-stimulation. PGPR had shown both antagonistic and synergistic interaction with their surrounding microorganism to boost the plant favorably. The present review has tried to describe all the important aspects of PGPRs and thus can be described as revolutionized tools for farmers for sustainable agriculture.

Keywords: Plant growth promoting rhizobacteria (PGPR), rhizosphere, agricultural productivity, biocontrol agents, soil toxicity, sustainable agriculture.

Introduction

Agriculture plays an important role in economic stability of India as well as it also provides the food security and employment to the people of our country. But in today's scenario, where there is a rapid increase in population, the demand of food is increasing day by day whereas the land for agriculture is decreasing day by day mean while the excessive use of synthetic chemical and fertilizers causes future environment damage with the potential risk to human health.

Thus, the sustainable agriculture is important in today's world because it gives the potential to achieve our future agriculture needs, something that traditional agriculture will not be able to do[1,2]. By the excessive use of chemical nitrogen fertilizer, nitrous oxide (N_2O) present abundantly in the atmosphere is the main cause of global warming.

As per the study, 74% of the total U.S. N_2O emissions in 2013 accounted by agricultural soil management. Farmers apply high amount of nitrogen fertilizers (ammonium nitrate) to fertilize their soil to grow crops, now due to the influx of ammonium, plants no longer need the symbiotic microbes to provide ammonium leading to the degree of symbiosis being diminished, as a result they (nitrogen fertilizers) also reduce biological nitrogen fixation in the soil. This high amount of nitrate is utilized by denitrifying bacteria to produce N_2O and extra nitrate leaches into the groundwater.

Thus, increased microbial processes of denitrification and nitrification increase the natural production of N_2O. Denitrification is the step where nitrogen oxides are reduced by soil bacteria to gaseous product and released into the environment. Nitrification is a process of ammonium (NH_4) being converted into nitrate (NO_3^-) by soil bacteria. In reference towards the sustainable agriculture vision, crops produced should be disease resistance, drought tolerant, heavy metal stress tolerant, salt tolerant and better nutritional value with high amount to yield to fulfill today's world needs.

So, to fulfill all the above desired crop characters, one possibility is use of soil microorganisms (algae, bacteria, fungi etc.) which increase the nutrient uptake capacity of plant and also water use efficiency. Among all soil microorganisms, bacteria known as plant growth promoting rhizobacteria (PGPR) are the most potential and promising. From the above it may sense that PGPR may be used to enhance the plant growth as well as plant growth without hampering the environment leaving no to toxic residues within the soil. These PGPRs themselves are beneficial microbes and produce every substance (metabolites, derivatives, enzymes, phyto-harmones etc.) which is biodegradable, biocompatible and environment friendly[3].

What is PGPR?: The term 'rhizobacteria' was introduced by eminent researchers[4]. This term was given to those soil bacteria that competitively colonized plant roots and enhanced the growth rate of plant and reduced the incidence of plant disease. PGPR can be defined as the indispensable part of rhizosphere biota that when grown in association with the host plant can stimulate the growth of the host. Due to their adaptability, faster growth rate and presence of versatile biochemical metabolize a wide range of natural and xenobiotic compounds. PGPRs are successfully getting established in soil ecosystem[5]. PGPR are the significant component in the management of agriculture due to their innate genetic ability[6].

Types of PGPR: According to the degree of association with the plant root cells, PGPRs are broadly divided into two categories i.e. ePGPR and iPGPR.

ePGPR: These are extracellular plant growth rhizobacteria which may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex. Example:- *Bacillus, Azotobacter, Arthrobacter, Caulobacter, Chromobacterium, Agrobacterium, Pseudomonas, Azospirillum, Burkholdaria, Enterobacter, Flavobacterium, Rhizobium, Serratia, Erwinia, Serratia, Micrococcus* etc.[7]

iPGPR: As name indicates, it locates generally inside the specialized nodular structures of root cells. Example: Endophyte and Frankia species both sp. symbiotically fix atmospheric nitrogen with the higher plants[8].

Interaction of PGPR with crops: Basically there are three types of interaction between PGPR and growing crops[9].

Neutral Interaction: Rhizobacteria associated with plants are commensals in which bacteria establish an innocuous interaction with the host plant, thus exhibit no visible effect on the growth and overall physiology of the plant[10].

Negative Interaction: In this interaction, the phyto-pathogenic rhizobacteria produce phytotoxic substances such as ethylene, hydrogen cyanide which negatively influence the growth and physiology of the plant[11].

Positive Interaction: This interaction is the counter to deleterious bacteria, there are some PGPRs which can exert a positive plant growth by direct mechanism (solubilization of nutrients ,production of growth regulators, nitrogen fixation) and indirect mechanism (stimulation of mycorrhizae development removal of phytotoxic substances etc.)[12, 13] (Image 1).

Role of PGPR in Agriculture: Plant growth promoting Rhizobacteria are free living bacteria residing in soil, they assist roots directly or indirectly[14]. PGPRs play unique roles in the soil that enhance the plant productivity as well as plant health. These PGPRs colonize in the rhizosphere, help in producing secondary metabolites, protect plant from pathogens, produce siderophores, can fix atmospheric nitrogen, produce phyto-hormones and help in providing nutrition uptake by solubilizing phosphate and also produce biologically active substances which influence the plant development and growth. Thus, PGPRs are playing very important roles in the life of plant.

PGPR as Bio fertilizer: Bio fertilizers are the substances, prepared from living microorganisms that, when applied to the plant surface or seeds colonize rhizosphere or inside the plant body thus promote root growth by providing fundamental nutrients to crop plant. Bio fertilizer is the very crucial aspect of organic farming and also plays important role in economy on global level. Several studies show that biofertilizer prepared by compost and PGPR both could trigger the growth of plant also showing bio-control of plants[15].

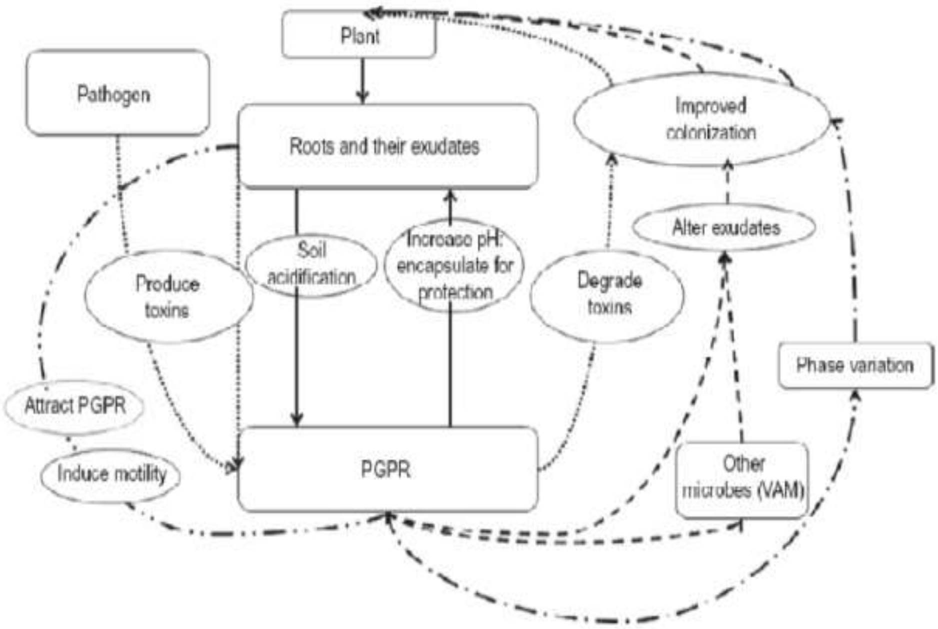

Image 1: Interaction of plant root exudates, pathogens, PGPR and other beneficial microbes in the rhizosphere

PGPR can only come into the category of biofertilizers when they act as a plant nourishment and enrichment source that could reconstruct the nutrient cycle between the plant soil and microorganism present within soil. Biofertilizer products are usually based on the plant growth promoting microorganisms (PGPM)[16]. They are divided into three categories-arbuscular mycorrhizal fungi (AMF)[17], plant growth promoting rhizobacteria (PGPR)[18] and nitrogen fixing rhizobia[19]. Among these three, it has been reported that the most effective and worldwide used biofertilizer is PGPR which contributes to increased crop yields and soil fertility. Therefore, with the potential of PGPRs, this leads to sustained agriculture and forestry.

PGPR act as Nitrogen Fixer: Nitrogen is an essential for all living organisms, it is also the vital nutrient for plant growth and productivity. Nitrogen is present almost 78% of the atmosphere but it remains unavailable to the plant. Plants cannot utilize the atmospheric nitrogen directly. Thus, the atmospheric nitrogen is converted into plant utilizable by microorganism by using complex enzyme system known as nitrogenase. They convert nitrogen to ammonia, which can be utilized by plants[20]. Plant growth promoting Rhizobacteria have the ability to convert atmospheric nitrogen and provide it to plants. This can be done by two mechanisms:

Symbiotic Nitrogen Fixation: In this type of fixation, microbes and plant have a mutual relationship. The microbe first enters into the root and then moves to the nodules where nitrogen fixation occurs. Rhizobia have the ability to create symbiotic interactions by colonization and formation of root nodules with leguminous plants. Nitrogen is converted to ammonia and makes available to plants[21]. For example, *Bradyrhizobium*, *Sinorhizobium* and *Mesorhizobium aremutual* with leguminous plants and Frankia with non-leguminous trees[22].

Non-symbiotic Nitrogen Fixation: This can be seen in non-legume plants such as radish and rice. Non-symbiotic nitrogen fixation is carried out by free living diazotrophs. Examples are *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Gluconacetobacte*r, *Nostoc*, *Anabena* etc[23,24]. *Nif* are the nitrogen fixation genes in both symbiotic and free living systems[25]. Nitrogenase (*nif)* genes are involved in activation of the fe protein, cofactor synthesis, iron molybdenum, electron donation, structural genes. Inoculation of this gene on crop provides multidisplinary approach for promoting growth, disease management and maintaining the nitrogen level in agricultural soil[26].

PGPR as the source for siderophores: Iron is vital element for the growth and development of a plant. It is present in bulk minerals on the surface of the earth; still it is not available to plant. Iron is commonly present in nature in the form of Fe^{3+} which is insoluble.

In order to solve this problem, PGPR secrets siderophores which are basically protein with low molecular weight, have the ability to bind iron, thus involved in the process of chelating ferric iron from the environment. When iron is limited, microbial siderophores provide plant with iron, thus enhancing their growth[27]. The assumed flow of this mode of action is shown in given image 2.

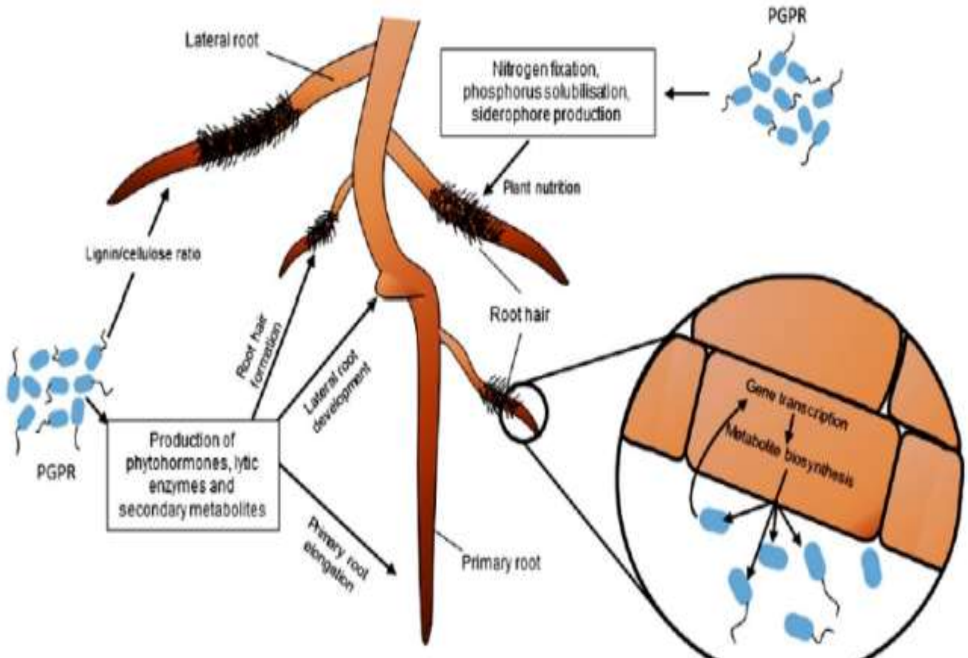

Image 2: The possible mode of action used by plant growth promoting rhizobacteria in plants. The flow and location of nitrogen fixation, phosphorus solubilization and siderophore production are shown.

PGPR as phyto-stimulator: Several investigations on PGPR proved that it can promote plant growth mainly by following means;
(1) Production of ACC deaminase to reduce the level of ethylene in the roots of developing plants.[28]
(2) Production of plant growth regulators like indole acetic acid (IAA)[29], gibberellic acid[30], cytokinins[31] and ethylene[32].
(3) PGPR assist in diminishing the accumulation of ethylene level and re-establish a healthy root system required to cope with environmental stress. Most of the studies explain the concept rhizosphere bacteria such as *Azospirillum, Bacillus, Pseudomonas, Enterobacter* and *Rhizobium* with ACC deaminase activity.[33-35]

Rhizobia are the first group of bacteria, which release IAA that can help to promote the growth and pathogenesis in plants[36]. IAA (indole-3-acetic acid) is the member of the group of phytohormones and is considered the most important auxin[37]. The functions of auxin act as signal molecule, which help in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division and differentiation and gene regulation[38]. Various bacterial species possess the ability to produce the auxin phytohormone IAA by different biosynthesis pathways and redundancy for IAA biosynthesis is widespread among plant-associated bacteria. Interactions between IAA-producing bacteria and plants results in diverse outcomes on the plant side and vary from pathogenesis to phytostimulation.

Also, on the other hand bacteria also use this phytohormone to interact with plants as part of their colonization strategy including phytostimulation and circumvention of basal plant defense mechanisms. Moreover, various recent reports indicate that IAA can also be a signaling molecule in bacteria also and thus can have a direct effect on bacterial physiology[39]. Altogether evidences indicate that PGPR influence the plant growth and development by the production of phytohormones such as auxins, gibberellins and cytokinins. The effects of auxins on plant seedlings are concentration dependent i.e. low concentration may stimulate growth while high concentrations may be inhibitory[40]. Different plant seedlings respond differently to variable auxin concentrations[41].

PGPR as Phosphate Solubilizers: Phosphate solubilizing bacteria (PSB) could play an efficient role in supplying phosphate to plants in a more environment friendly manner. These naturally abundant PSB solubilize calcium-bound phosphate compounds in an alkaline soil environment and convert the insoluble compounds into soluble forms and make them available to plants. PSB are widely used in agronomic practices in order to increase the productivity of crops and also maintaining the health of soils[42]. The most powerful phosphate solubilizing bacteria are belonging to the genera *Bacillus, Rhizobium* and *Pseudomonas*. There are also reports of phosphate solubilization by Azotobacter (non-symbiotic nitrogen fixer)[43].

It is reported that phosphorus-solubilizing bacteria convert phosphorus from organic and inorganic phosphorus and release into pools through mineralization and solubilization. Lowering of soil pH by microbial production of organic acids such as acid phosphatases, lactate, citrate and succinate gluconic and keto gluconic acids etc.[44,45] and proton extrusion is the main principal mechanism of mineralization of organic form of phosphorus.

Release of phosphorus from insoluble and adsorbed forms is also an important part/step of phosphorus solubilizing bacteria regarding phosphorus availability in soils. Phosphorus solubilizing bacteria transform soil phosphorus to forms that can easily be taken up by crops. Bacteria assimilate soluble phosphorus and make it available by preventing it from adsorption[46]. Bacteria also enhance phosphorus availability to crops by solubilizing precipitated forms of phosphorus[47].

PGPR as Biocontrol agents: PGPR play a major role in the biocontrol of plant pathogens. They have the ability to suppress a broad spectrum of bacterial, fungal and nematode diseases. PGPR also help in providing the protection against viral diseases. Recent studies demonstrate their diversity, colonizing ability and mechanism of action, should facilitate their development as reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. The application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization[48].

The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and as biocontrol agents as they show various plant growth promoting activities[49]. A major group of rhizobacteria with potential for biological control is the *Pseudomonades*[50]. Researches revealed that *Pseudomonads* possess many traits that make them well suited as biocontrol agent[51] and these include the ability to-

(i) grow rapidly *in vitro* and to be mass produced.
(ii) rapidly utilize seed and root exudates.
(iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant.
(iv) produce a wide spectrum of bioactive metabolites (i.e. antibiotics, siderophores, volatiles and growth-promoting substances).
(v) compete aggressively with other microorganisms.
(vi) adapt to environmental stresses.

These are the characteristics that make *Pseudomonads* as a strong biocontrol agent but these do not have the ability to produce resting spores (as do many bacillus spp.) which complicate formulation of the bacteria for commercial use[52-55].

PGPR in stressed conditions: Agricultural crops are exposed to many stresses that are induced by both biotic and abiotic factors. These stresses decrease the crops production. Abiotic stress factors are temperature variation, salinity, drought, flooding, U.V. rays, air pollution (ozone) and heavy metals. These stress condition decrease the crop yield from 50% to 82%, depending on the crop. In high salinity conditions, plants show reduced leaf growth rate due to decreased water uptake, which restricts photosynthetic capacity. Plant involves a number of metabolic and physiological changes in response to salt stress and water deficiency (drought)[56]. The inoculation of salt-stressed plants with PGPR strains alleviates the salinity stress in plants. Heavy metal condition is one of the most severe factors for plant growth and development.

Researches reviewed recent advances in effect and significance of Rhizobacteria in phytoremediation of heavy metal contaminated soils. Cd in soil leads in plant-stress ethylene biosynthesis[57] and probably also accumulation of ACC in roots, the PGPR protects the plants against the inhibitory effects of cadmium[58,59]. A plant growth-promoting bacterium, *Kluyvera ascorbata* SUCD165 containing high level of heavy metals is resistant to the toxic effects of Ni^{2+}, Pb^{2+} and Zn^{2+}. This bacterium decreases nickel toxicity in the seedlings and thus enhances soil fertility[60]. PGPR can have positive effects on vigor and productivity, especially under stress conditions[61].

Defense mechanism shown by PGPR

Induced systemic resistance (ISR): Biopriming plants treated with plant growth promoting rhizobacteria can also provide systemic resistance against a wide range of plant pathogens. Diseases origin from fungus, bacteria and virus and in some cases, even damage caused by insects and nematodes can be reduced after application of plant growth promoting rhizobacteria[62]. The induced systemic resistance (ISR) mechanism involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses against a variety of plant pathogens[63].

Many individual bacterial components induce induced systemic resistance such as lipo-polysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2, 4-diacetyl phloroglucinol, homoserine lactones and volatiles like acetoin and 2, 3-butanediol[64].

Beneficial and Harmful Aspects of Plant Growth Promoting Rhizobacteria: It is undisputed that rhizobacteria play an important role in maintaining soil fertility and enhancing plant growth and development. This growth betterment takes place with the help of several mechanisms as mentioned in this review paper, although the reverse is true in some other research[65]. As an example, some species of *Pseudomonas* show the characteristic of production of cyanide. Here, cyanide production by the bacteria is considered as a growth promotion as well as a growth inhibition character. Cyanide acts as a biocontrol agent against certain plant pathogens; on the other hand, it can also cause adverse effects on plant growth[66]. Auxin production by PGPR can also cause positive as well as negative effects on plant growth. It is important to note that the functioning of auxin relies upon its concentration. For instance, at low concentrations, it enhances growth of plant, whereas at a high level it inhibits growth of root[67].

In addition, *Bradyrhizobium elkanii* produce rhizobitoxine which has dual effects. Since it is an inhibitor of ethylene synthesis, it can alleviate the negative effect of stress-induced ethylene production on nodulation[68]. On the other hand, rhizobitoxine is also considered a plant toxin due to ability to cause foliar chlorosis in soybeans[69, 70].

Conclusion

It has been concluded that, PGPRs are beneficial in promoting plant growth of the crops by enhancing agricultural productivity via different activities such as nitrogen fixation, potash mobilization, phosphate solubilization etc. These are found antagonistic against soil borne pathogens. These are also utilized as insecticidal and pesticidal agents. The complete awareness and utilization of such beneficial strains of PGPRs can be boon to farmers for enhancing agricultural productivity and maintaining sustainable agriculture.

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