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Plant Growth-Promoting Rhizobacteria: A Biological Approach towards the Production of Sustainable Agriculture

Rajesh Kumar Meena
Division of Agronomy, Indian Agricultural Research Institute, New Delhi -110012
Email: rajeshkumar2793@gmail.com

The presence of enormous numbers of microbial populations and species in the soil, especially in the rhizosphere and their intensive and extensive interactions with flora and fauna and plant roots, leads to plant growth promotion by rhizosphere phenomenon.

Introduction

Indiscriminate and unbalanced use of chemical fertilizers and pesticides in agriculture has created many environmental and health problems. Studies in Punjab, on the effects of synthetic nitrogen fertilizer on groundwater pollution in intensive agriculture areas shows that 20% of all sampled wells have nitrate levels above the WHO safety limit of 50 mg of nitrate per litre for drinking water (The Time of India; June 15, 2010). This nitrate pollution is due to higher usage of synthetic nitrogen fertilizers (urea) in the adjoining field. Nitrate pollution in drinking water is causing serious health impact on humans, especially for babies and children. Studies showed that long-term consumption of drinking water and food with nitrate concentrations even below 50 mg/ litre NO_3 has a potential role in development of cancers in digestive tract and is associated with other types of cancer (Forman *et al.* 1985). Ironically, intensive rice-wheat practice is also not living up to its promise of sustained increase in food production and now showing diminishing returns and declining factor productivity. Thus there is a need for sound and ecologically compatible strategies in agriculture. In this context, plant associated microorganisms fulfil important functions for plant productivity and the soil health as they participate actively in almost every chemical transformation taking place in soil. In particular, they play an active role in soil fertility, as a result of their involvement in the nutrient cycles of carbon and nitrogen, which are essential for plant growth. The presence of enormous numbers of microbial populations and species in the soil, especially in the rhizosphere and their intensive and extensive interactions with these flora and fauna and plant roots, leads to plant growth promotion by rhizosphere phenomenon. Even though, microorganisms fulfil important ecosystem functions for plants and

soil, the beneficial plant-microbe interactions have been ignored in the enhancing plant productivity.

Plant Growth Promoting Rhizobactria

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing better growth environment. These microbial floras cause a large number of biochemical changes in soil that largely determine the fertility of soil. PGPR enhances plant growth by a wide variety of mechanisms. PGPR are also termed as plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria. They also act as biofertiliser, bioprotectants and biostimulants. Such bacteria have been applied to a wide range of agricultural plant species for the purpose of growth enhancement, including increased seed emergence, plant weight, crop yields and disease control. They facilitate plant growth and development, both directly and indirectly. Direct facilitation may include supply of nitrogen to plants through nitrogen fixation, iron sequestered through bacterial siderophores, phosphate through solubilisation and phytohormones. Indirect stimulation of plant growth includes preventing phytopathogens (bio control) through production of antibiotics, siderophores and hydrogen cyanide and thus promotes plant growth and development (Glick *et al.* 2007). Such information reveals the need to evaluate the effect of inoculated microorganisms and plant responses, so as to build healthy interactions for enhanced crop yields. PGPR can be divided into two groups:

1. **Symbiotic nitrogen fixing**: These live inside the plant cells, produce nodules. Example: *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* and *Sinorhizobium sp.*
2. **Non-symbiotic:** These are free-living rhizobacteria and live outside the plant cells and do not produce nodules. *Azotobacter, Azospirillum, Acetobacter, Diazotrophs, Bacillus* and *Klebsiella sp*. belong to this group.

Function of PGPR

The means by which PGPR enhance the nutrient status of host plants can be categorized into following areas: (1) Biological N_2 fixation; (2) Increasing the availability of nutrients in the rhizosphere; (3) Increase root volume which related to more nutrient absorption; (4) To stimulate plant growth, e.g., through the production of plant hormones; (5) To control or inhibit the

activity of plant pathogens; (6) To improve soil structure; (7) Mineralization of organic pollutants, i.e. bioremediation of polluted soils.

Mechanism Shown by PGPR

1. Biological nitrogen fixation

Biological nitrogen fixation contributes 180 $\times 10^6$ metric tons/year globally, out of which symbiotic associations contribute 80% and the rest comes from free-living or associative systems (Graham, 1988). The use of biofertilizer and bio-enhancer such as N_2 fixing bacteria and beneficial micro-organism can reduce chemical fertilizer applications and consequently lower production cost. PGPR retain more soil organic N, and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhance release of the nutrients. These include symbiotic N_2-fixing forms, viz. *Rhizobium*, the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees, and non-symbiotic (free-living, associative or endophytic) *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas Acetobacter diazotrophicus*, *Azoarcus* etc., associated with the plant rhizosphere and fix atmospheric N_2 into form which are taken up by the plants. Important N inoculants are:

a. Rhizobium inoculants: *Rhizobium* inoculants help in establishing efficient symbiotic association with leguminous crops and thus can fix 50-100 kg N/ha. A 10-70% increase in yields of crop due to inoculation with *Rhizobium* inoculants over uninoculated has been reported. *Rhizobium* is specific to each legume crop and only recommended inoculant should be used for each leguminous crop such as peanuts, green gram, black gram, cow pea, pigeon pea, soybean, chick pea, peas, alfalfa, berseem, clover etc.

b. *Azotobacter* inoculants: *Azotobacter* is a free-living aerobic nitrogen-fixer which is recommended for non-leguminous crop like wheat, paddy, maize, barley, sugarcane, potato, tomato, cotton, mustard etc. *Azotobacter* fixes atmospheric nitrogen in soil and helps in saving chemical fertilizers by 15-20 kg N /ha. The family *Azotobacteriaceae* includes species *A. agilis, A. insignis, A. macrocytogenes A. chroococcum, A. vinelandii, A. beijerinckii, Azotobacter paspali*. In my M.sc study, i was found a positive influence of PGPR application on rice yield, soil microbial and plant defence enzyme in rice crop as shown in Table 1.

Table 1. Influence of PGPR application on soil microbial and plant defence enzyme in rice crop

Treatment	Rice grain yield (t ha^{-1})	Soil OC (%)	Soil chlorophyll (µg g^{-1})	Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})	ARA activity (n moles ethylene g^{-1} soil ha^{-1})	PPO activity (U min^{-1} g fresh wt^{-1})
N_0(control)	2.64	0.51(0)	0.324(0)	6.00(0)	0.755(0)	1.083(0)
N_{120}	4.79	0.54(5.8)	0.763(135)	11.81(96)	1.555(106)	1.700(5)7)
2/3 N+ BI	4.23	0.54(5.8)	0.607(87)	8.37(39)	1.308(73)	1.475(36)
2/3 N + BI + C	4.66	0.55(7.8)	0.699(115)	11.54(92)	1.517(101)	1.580(46)
2/3N + CI + C	4.35	0.55(7.8)	0.723(123)	10.83(80)	1.498(98)	1.603(48)
2/3N + CI + BI	4.58	0.55(7.8)	0.695(114)	10.10(68)	1.379(83)	1.507(39)
2/3N + BI + CI + C	5.02	0.56(9.8)	0.893(175)	14.78(146)	1.875(148)	1.753(62)
LSD (P=0.05)	**0.28**	**0.03**	**0.100**	**2.109**	**0.190**	**0.242**

*C= Compost @ 5.0 t ha^{-1} BI= Bacterial inoculation CI=Cyanobacterial inoculation
Data in parentheses show percent increase over control

Combined inoculation of bacterial and cyanobacterial inoculants contributed about 40 kg N ha^{-1} to rice crop and enhanced soil organic carbon by 5.8 to 9.8% besides significantly enhancing soil microbial properties. Plant defence enzymes like Peroxidase and poly phenol oxidase (PPO) also increased significantly due to PGPR inoculation in rice.

***c. Azospirillum inoculants*:** *Azospirillum* fix nitrogen under microaerophilic conditions and are frequently associated with root and rhizosphere of a large number of agriculturally important non-leguminous crops like sorghum, pearl millet, finger millet and other small millet. *Azospirillum* fixes atmospheric N in soil and helps to save chemical fertilizers by 15-20 kg N/ha and includes species like *lipoferum, brasilense, amazonense, halopraeferens, irakense, largimobile, doebereinerae, Oryzae, melini,* and *Canadensis.*

2. Phosphate solubilization

Phosphorus (P) is second most important plant nutrient but most of P remains fixed in soil which is not available to plants. Inoculation with an efficient P Solubilizing microorganism improve availability of P from insoluble form of P in soil and enhance use efficiency of phosphatic fertilizer such as super phosphate. There are number of inoculants which can even degrade

rockphosphate and soil fixed P. A number of metabolites are released by these strains which strongly affect the environment and increase nutrient availability for the plants viz. *B. subtilis, B. licheniformis, B. megaterium* var. *phosphaticum,* and *P. Lutea* (Chen *et al.* 2006).

3. Plant growth producers

Plant hormones are chemical messengers that affect a plants' ability to respond to its environment. Hormones are organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. They interact with specific target tissues to cause physiological responses, such as growth or fruit ripening. Botanists recognize five major groups of hormones: auxins, gibberellins, ethylene, cytokinins and abscisic acid (Ashrafuzzaman *et al.* 2009). Plant phytohormones produced by crop specific PGPR are given in Table 2.

4. Siderophore production

Siderophores (Greek: "iron carrier") are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria and fungi. Iron is an essential growth element for all living organisms. The scarcity of bio-available iron in soil habitats is common. Under iron-limiting conditions PGPR produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion. Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Soil bacteria isolates like *Azotobacter vinelandii, Bacillus megaterium* and *Bacillus cereus* produces siderophores and they can be used as efficient PGPR to increase the yield of the crop.

Table 2. Plant phytohormones produced by crop specific PGPR (Ashrafuzzaman *et al.* 2009)

Plant hormones	**PGPR**	**Host**
IAA	*Aeromonas veronii*	Rice
	Enterobacter cloacae	Rice
	Azospirillum brasilense	Wheat
	Enterobacter sp.	Sugarcane
Cytokinin	*Paenibacillus polymyxa*	Wheat
	Pseudomonas fluorescens	Soybean
Gibberellin	*Bacillus* sp.	Alder
ACC deaminase	*Bacillus pumilus*	Rape
	Pseudomonas cepacia	Soybean

5. Bio-control properties

PGPR are indigenous to soil and play a major role in the bio-control of plant pathogens. They can suppress a broad spectrum of bacterial, fungal, viral and nematode diseases. A major group of rhizobacteria with potential for biological control is the *Pseudomonas* sp. which is ubiquitous bacteria in agricultural soils. Among various bio-control agents,

Fluorescent pseudomonads, equipped with multiple mechanisms for bio-control of phytopathogens produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN catalase and can solubilize phosphorous. *Xanthomonas oryzae* pv. *oryzae* and *Rhizoctonia solani* – the bacterial leaf blight (BB) and sheath blight (ShB) pathogens of rice are suppressed by indigenous *Pseudomonas* strains isolated from rhizosphere of rice cultivated in the coastal agro-ecosystem under both natural and saline soil conditions.

6. Soil biological properties

Microbial biomass and soil enzymes are considered as potential indicator of soil quality. Application of PGPR increases the dehydrogenase activity, soil chlorophyll and acetylene reduction activity in rhizosphere Table 1 shows an example of PGPR effect on soil microbial properties in rhizosphere.

7. Effects on plant physiological attributes

In addition to the PR-proteins, the plants produce other enzymes of the defence, including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenoloxidase (PPO). Peroxidase and PPO are catalysts in the formation of lignin. PAL and other enzymes are involved in the formation of phytoalexins. These metabolic changes improve in the defence mechanism of plants so they are better performing under adverse condition. Table 1 evidenced an example of influence of PGPR on plant defence enzymes.

8. Effects of PGPR on plant growth and yield

a) Plant and root growth: PGPR can affect plant growth by production and release of secondary metabolites (plant growth regulators/phytohormones/biologically active substances), lessening or preventing deleterious effects of photopathogenic organisms in rhizosphere and facilitating the availability and uptake of certain nutrients from root environment.

PGPR like *Azospirillum brasilence* is helpful in proliferation of root hairs in maize, paddy, oat which could have dramatic effects on increasing root surface area. Most commonly, IAA-producting PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from soil.

b. Yield attributes and Yield: A range of PGPR participate in interaction with C_3 and C_4 plants (e.g., rice, wheat, maize, sugarcane and cotton), and significantly increase their vegetative growth and grain yield. Rice yield increased by 20-30% (Table 1) when inoculated with *Rhizobium leguminosarum*

bv. *trifolii* due to increase in number of panicles per plot and filled grains panicle^{-1} and also the total number of spikelet's plant^{-1} were increased as compared to uninoculated plants. Rhizobial inoculation increased sink size by either increase in panicle number or spikelet number.

c. **Nutrient uptake:** There are ample evidences that the mode of action of many PGPR is by increasing the availability of nutrients for the plant in the rhizosphere. The solubilization of P in the rhizosphere is the most common mode of action implemented in PGPR that increases nutrient availability to host plants. eg *Azotobacter chrooroccum*, *Bacillus circulans* and *Cladosporium herbarum* increase P availability in wheat. Inoculation of rice seedlings with *Rhizobium leguminosarum* bv. *trifolii* E11, *Rhizobium* sp. IRBG 74 and *Bradirhizobium* sp. increased N, P and K uptake by 10-28% as compared to uninoculated rice plant.

Methods of Application of PGPR Inoculants

A. **Seed treatment:** One packet of carrier based microbial inoculants (200 g) *viz*, *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB culture is enough for treating seeds sown in one acre. Dissolve 100-150 gm jaggery in water, boil the solution till a thick solution is obtained then allow the solution to cool to room temperature and add 100 g gum into the cooled solution and mix well then add one packet of microbial culture and mix thoroughly. Mix the required seed for one acre with the prepared culture thoroughly so that each seed is coated with a thin film of culture. Spread the treated seed on a non absorbent and clean surface under the shade for drying at room temperature and then seed should be sown on the same day.

B. **Seedling treatment:** This method is recommended for crops like paddy, tobacco, tomato, chilly, onion, cabbage, cauliflower etc. Prepare the suspension by mixing 1.0 kg of PGPR culture in 10-12 litres of water. Get seedlings required for one acre and make small bundles of seedlings. Dip the seedlings in the suspension for 15-20 minutes. Transplant treated seedling immediately.

Conclusion

PGPR is better option to enhance the crop productivity as well as quality. PGPR improves the chemical and microbial property of soil and enhances the amount of plant enzymes for better defence mechanism in plant. The use of PGPR is environmental friendly and thus plays role in sustainability of agriculture with high productivity.

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