

Mechanism of Plant Growth Promoting Rhizobacteria (PGPR) in enhancing plant growth – A Review

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ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) are the soil bacteria inhabiting the root surface as a population that competitively colonizes plant root and increases their growth and also reduces plant diseases. Few properties strictly associated through PGPR, are their properties of insistent migration and plant growth stimulation and their biocontrol ability. The use of PGPRs has been demonstrated to be an environmentally sound method of growing crop yields by facilitating plant growth all the way through either a direct or indirect mechanism. The mechanisms of PGPR include amendable hormonal and nutritional equilibrium, inducing conflict against plant pathogens, and solubilizing nutrients for simple uptake by plants. In accumulation, PGPR illustrate synergistic and antagonistic connections with microorganisms inside the rhizosphere and outside in bulk soil, which ultimately boosts plant growth rate. This review accentuates that PGPRs are not only coupled with the root to wield valuable effects on plant development although also have affirmative effects on calculating phytopathogenic microorganisms. Further, overt outlooks on the different mechanisms of rhizobacteria mediated plant growth promotion in detail with the current development and research to provide it as one of the dynamic ingredients in biofertilizer formulation.

Keywords: Biofertilizer, Mechanism, PGPR, Rhizobacteria, Rhizosphere

1. INTRODUCTION

Plants are associated with complex microbiomes, which have an ability to promote plant growth and stress tolerance, support plant nutrition, and antagonize plant pathogens. Depending on the behavior, the microorganisms can be beneficial or pathogenic, or even neutral. The integration of beneficial plant-

microbe and microbiome interactions may represent a promising sustainable solution to improve agricultural and forest production [1]. Several exciting new studies are unveiling the way in which the plant microbiome performs its duties. They indicate that, like any other species, microorganisms operate in interlocked networks [2] possessing microbial hubs. Within the networks reside certain keystone species that are critical for the plant-microbe interactions. The concentration of bacteria found around the roots of plants is generally much greater than in the bulk soil. Therefore different soil microbes that have been reported as Plant Growth Promoting Rhizobacteria (PGPR), belong to the genera which exert a beneficial effect to the plant growth includes *Pseudomonas*, *Bacillus*, *Azospirillum*, *Agrobacterium*, *Azotobacter*, *Arthrobacter*, *Alcaligenes*, *Serratia*, *Rhizobium*, *Enterobacter*, *Burkholderia*, *Beijerinckia*, *Klebsiella*, *Clostridium*, *Vario-vovax*, *Xanthomonas* and *Phyllobacterium* [3]. Plant growth promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species and confer beneficial effects, such as increased plant growth and reduced susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses and nematodes [4]. Based on the interactions with plants, PGPR can be separated into symbiotic bacteria, whereby they live inside plants and exchange metabolites with them directly, and free-living rhizobacteria, which live outside plant cells [5]. The enhancement of plant growth by the application of these microbial populations is well known and proven [6] [7]. Over the years, it has been seen that, the PGPR's increase crop yield by 20- 30%, replace chemical nitrogen & phosphorus by 25%, stimulate plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne diseases and these PGPR's have gained worldwide importance and acceptance for agricultural crops, which lead to enhanced growth and biomass content [8]. Therefore, PGPR serve as one of the active ingredients in biofertilizer formulation. The present review is an effort to elucidate the concept of the beneficial microbes especially plant growth promoting rhizobacteria in the current scenario and their core mechanisms of plant growth promotion with current updates.

2. RHIZOSPHERE

Rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro and micronutrients are extracted. The microbial population present in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth [9]. It has also been defined as the zone that includes the soil influenced by the root along with the root tissues, supporting large active groups of microorganisms [10]. The rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil which may be due to the increased availability of soluble organic compounds (carbohydrate monomers, sugars and amino acids) secreted from plant root exudation. The rhizosphere is broadly divided into the three zones, named as endorhizosphere, rhizoplane and

ectorrhizosphere [11]. Besides these three fundamental zones, some other layers are also found which include the mycorrhizosphere, rhizosheath, and bulk soil. Rhizosphere being an active region of microbial diversity is considered as the hot spot of soil microbes [12]. The intense and complex communication among microorganisms plays a fundamental role in recruiting and shaping the microbial community in the rhizosphere [13]. The rhizo-microbiome is important for plant health and is able to influence the structure of the microbial community. The rhizosphere is also called as human gut microbiome for plants and is considered as the lavish fountain in the arid region [14]. Rhizosphere has the greater combined genome than that of plant and thus it is called as plant's second genome. Several reports have shown that the difference in microbial communities between the bulk and rhizosphere soil of land plants [15]. Recently [16] investigated the microbial communities inhabiting the rice field ecosystem and concluded that the microbes within the rice root interior, the rhizoplane and the rhizosphere had considerable variation. In addition, the microbial communities in various zones, such as rhizosphere, anoxic bulk soil, and oxic surface soil have been reported [17].

3. MECHANISM OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

According to [18], PGPR-mediated plant growth promotion occurs by the alteration of the whole microbial community in the rhizosphere niche through the production of various substances [18]. Generally, PGPR promote plant growth directly by either facilitating resource acquisition like nitrogen, phosphorus, and essential minerals via biological nitrogen fixation, phosphate solubilization, and iron sequestration by siderophore, respectively, or modulating plant hormone levels such as auxins, gibberellins (GAs), cytokinins (CK), and nitric oxide (NO), or indirectly with rhizosphere competition, induced systemic resistance (ISR), and biosynthesis of stress-related phytohormones like jasmonic acid (JA), cadaverine (Cad), or the ethylene catabolism-related enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Fig 1).

3.1. DIRECT MECHANISMS

Direct PGPR enhance plant growth in the absence of pathogens. In accordance with [19], soil bacterial species in the plant rhizosphere which grow in, on, or around plant tissues stimulate plant growth by a plethora of mechanisms. In addition to providing the mechanical support and facilitating water and nutrient uptake, microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants.

3.1.1. Nitrogen fixation

Nitrogen (N) is the most vital nutrient for plant growth and productivity. Although, there is about 78% N₂ in the atmosphere, it is unavailable to the growing plants. The atmospheric N₂ is converted into plant-utilizable forms by biological N₂ fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase [20]. Biological nitrogen fixation occurs, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature [21]. Furthermore, BNF represents an economically beneficial and environmentally sound alternative to chemical fertilizers. Nitrogen fixing organisms are generally categorized as (a) symbiotic N₂ fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. *rhizobia*) and non-leguminous trees (e.g. *Frankia*) and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena*, *Nostoc*, *Azospirillum*, *Azotobacter*, *Gluconoacetobacter diazotrophicus* and *Azocarus* etc. [7]. However, non-symbiotic nitrogen fixing bacteria provide only a small amount of the fixed nitrogen that the bacterially-associated host plant requires [22]. Symbiotic nitrogen fixing rhizobia within the rhizobiaceae family (α-proteobacteria) infect and establish symbiotic relationship with the roots of leguminous plants. The establishment of the symbiosis involves a complex interplay between host and symbiont [23] resulting in the formation of the nodules wherein the rhizobia colonize as intracellular symbionts (Fig 2). Plant growth-promoting rhizobacteria that fix N₂ in non-leguminous plants are also called as diazotrophs capable of forming a nonobligate interaction with the host plants [24]. The process of N₂ fixation is carried out by a complex enzyme, the nitrogenase complex. However, it is generally believed that free-living bacteria provide only a small amount of the fixed nitrogen that the bacterially associated host plant requires. Nitrogenase (nif) genes required for nitrogen fixation include structural 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 [25]. In diazotrophic (nitrogen fixing) bacteria, nif genes are typically found in a cluster of around 20–24 kb with seven operons encoding 20 different proteins [22].

3.1.2. Phosphate Solubilization

Phosphorus (P), the second most important plant growth limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms [26]. This low availability of phosphorous to plants is because the majority of soil P is found in insoluble forms, whereas plants absorb it only in two soluble forms, the monobasic (H₂PO₄) and the dibasic (HPO₄²⁻) ions [7]. To overcome P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb low amounts of

applied phosphatic fertilizers, and the rest are rapidly converted into insoluble complexes in the soil [25]. But regular application of phosphate fertilizers is not only costly but is also environmentally undesirable. This has led to a search for an ecologically safe and economically reasonable option for improving crop production in low P soils. In this context, organisms coupled with phosphate-solubilizing activity, often termed phosphate-solubilizing microorganisms (PSM), may provide the available fertilizers [27]. Of the various PSM(s) inhabiting the rhizosphere, phosphate-solubilizing bacteria (PSB) are considered as promising biofertilizers because they can supply plants with P from sources otherwise poorly available by various mechanisms [28]. Bacterial genera like *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate-solubilizing bacteria [29]. Rhizobacteria can solubilise inorganic P sources and enhance growth and yield of crop plants. Examples of some widely reported P solubilising microbial species intimately associated with a large number of agricultural crops like potato, tomato, wheat, and radish are *Azotobacter chroococcum*, *Bacillus circulans* and *Cladosporium herbarum*, *Bradyrhizobium japonicum*, *Enterobacter agglomerans*, *Pseudomonas chlororaphis* and *P. putida*, and *Rhizobium leguminosarum* [30]. The ability of PGPRs to solubilize mineral phosphate, therefore, has been of immense interest to agricultural microbiologists since it can enhance the availability of phosphorus for effective plant growth. PGPRs have been recorded to solubilize precipitated phosphates to plants, representing a possible mechanism of plant growth promotion under field conditions [31]. Synthesis of organic acids by rhizosphere microorganisms could be the possible reason for solubilization of inorganic P sources. Unfortunately, because of variable results, the commercial application of phosphate-solubilizing bacteria has been quite limited. In fact, the most consistent positive effects of applying phosphate-solubilizing bacteria are seen when these bacteria are co-inoculated with bacteria with other physiological capabilities such as fixation, or with mycorrhizal or non-mycorrhizal fungi [32].

3.1.3. Siderophore production

Iron is among the bulk minerals present on the surface of the earth, yet it is unavailable in the soil for plants. Iron is commonly present in nature in the form of Fe^{3+} , which is highly insoluble; to solve this problem, PGPR secrete siderophores. Siderophores are low molecular weight iron binding protein compounds involved in the process of chelating ferric iron (Fe^{3+}) from the environment. When Fe is limited, microbial siderophores provide plants with Fe, enhancing their growth. These molecules act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Siderophores, generally form 1:1 complexes with Fe^{3+} , which are then taken up by the cell membrane of bacteria, where the Fe^{3+} is reduced to Fe^{2+} and released from the siderophore into the cell.

PGPR have been demonstrated as enhancing the plant-growth producing very efficient extracellular siderophores which allow control of several plant diseases by depriving the pathogen of iron nutrition,

thus resulting in increased crop yield. In addition to iron, siderophores can also form stable complexes with other metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn [33] have shown that the presence of heavy metals induces bacterial siderophore production. Paradoxically, plants grown in metal-contaminated soils are often iron deficient and the bacteria may help plants to obtain sufficient iron. Microbial siderophores are used as metal chelating agents that regulate the availability of iron in plant rhizosphere. This in turn helps plants to alleviate the toxicity of metals as reported for arsenic uptake by several plants.

3.1.4. Phytohormone production

Microbial synthesis of the phytohormone auxin (indole-3-acetic acid/indole acetic acid/IAA) has been known for a long time. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites [34]. Generally, IAA secreted by rhizobacteria interferes with the many plant developmental processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria. Evidently, IAA also acts as a reciprocal signaling molecule affecting gene expression in several microorganisms. Consequently, IAA plays a very important role in rhizobacteria-plant interactions [35]. Generally, IAA affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. IAA produced by rhizobacteria likely, interfere the above physiological processes of plants by changing the plant auxin pool. Moreover, bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria [22]. Thus, rhizobacterial IAA is identified as an effector molecule in plant–microbe interactions, both in pathogenesis and phytostimulation. An important molecule that alters the level of IAA synthesis is the amino acid tryptophan, identified as the main precursor for IAA and thus plays a role in modulating the level of IAA biosynthesis [36]. Most *Rhizobium* species have been shown to produce IAA. Since, IAA is involved in multiple processes including cell division, differentiation and vascular bundle formation, these three processes are also essential for nodule formation. Hence, it seems likely that auxin levels in the host legume plants are necessary for nodule formation. It is also reported that the inoculation with *Rhizobium leguminosarum* by *viciae* wherein the IAA biosynthetic pathway had been introduced, produced potential nitrogen fixing root nodules containing up to 60-fold more IAA than nodules formed by the wild-type counterpart in *Vicia*

hirsute [37]. Environmental stress factors which modulate the IAA biosynthesis in different bacteria include acidic pH, osmotic and matrix stress, and carbon limitation. Among genetic factors, both the location of auxin biosynthesis genes in the bacterial genome (either plasmid or chromosomal) and the mode of expression (constitutive vs. induced) have been shown to affect the level of IAA production. The location of auxin biosynthesis genes can affect the IAA level, as plasmids are mostly present in multiple copies. This can be illustrated by the difference in the IAA level between the rhizobacterial strains, *Pseudomonas savastanoi* pv. *savastanoi* and *P. syringae*. In the former strain, the genes for auxin biosynthesis genes are present on a plasmid, while in the latter one the corresponding genes are located on the chromosomal DNA, resulting in a lower IAA production. The IAA production in *P. syringae* could be increased many fold by introducing a low-copy plasmid, carrying the IAA biosynthetic operon [38].

3.1.5. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase

Generally, ethylene is an essential metabolite for the normal growth and development of plants [39]. This plant growth hormone is produced endogenously by approximately all plants and is also produced by different biotic and abiotic processes in soils and is important in inducing multifarious physiological changes in plants. Apart from being a plant growth regulator, ethylene has also been established as a stress hormone [40]. Under stress conditions like those generated by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects the overall plant growth. For instance, the high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance [41]. Plant growth promoting rhizobacteria which possess the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, inducing salt tolerance and reducing drought stress in plants [42]. Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* etc. [43]. Such rhizobacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3 . Several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic microorganisms (viruses, bacteria, and fungi etc.), and resistance to stress from polycyclic aromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, draft, extremes of temperature, high light intensity, and flooding [44]. As a result, the major noticeable effects of seed/ root inoculation with ACC deaminase- producing rhizobacteria are the plant root elongation, promotion of shoot growth, and enhancement in rhizobial nodulation and N, P and K uptake as well as mycorrhizal colonization in various crops [45].

INDIRECT MECHANISM

The application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach [44]. The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents [22]. In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR. Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin [7]. Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses. This phenomenon is called induced systemic resistance (ISR). Moreover, ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses against a variety of plant pathogens [22]. Many individual bacterial components induce ISR, such as lipopolysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2,4-diacetylphloroglucinol, homoserine lactones, and volatiles like, acetoin and 2,3-butanediol [44].

4. FIGURES

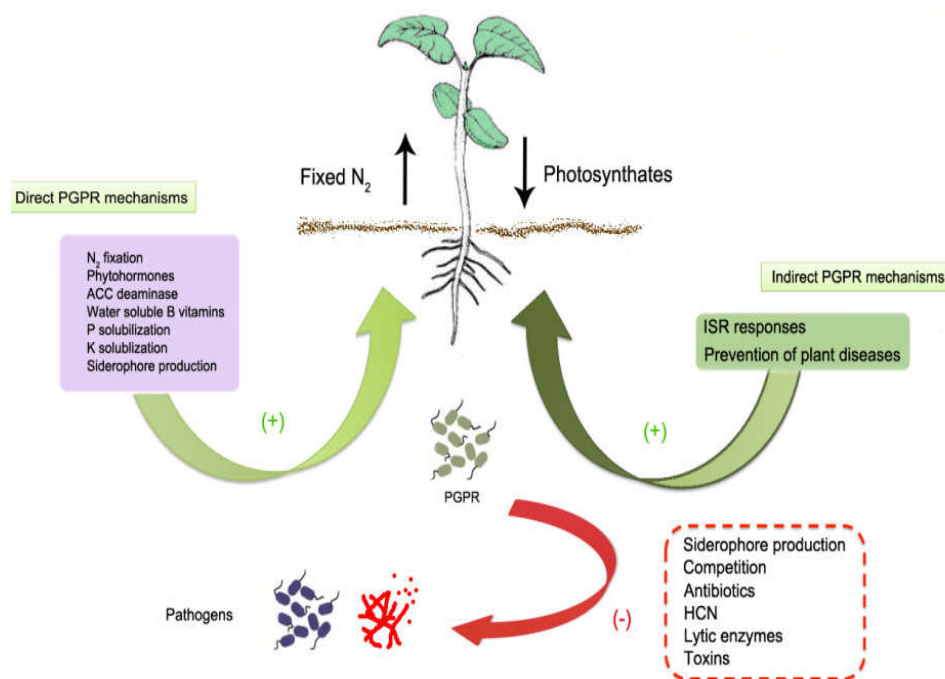


fig 1: mechanism of plant growth promoting rhizobacteria (pgpr)

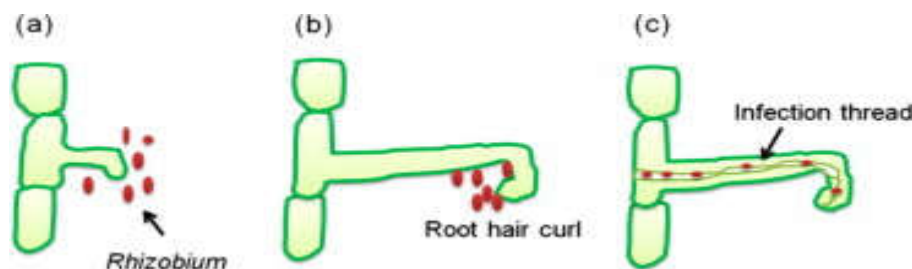


fig 2: process of nodulation (a) interaction of rhizobial rhicadhesin with host lectins and rhizobial attachment with root cells. (b) excretion of nod factors by rhizobia causes root hair curling. (c) rhizobia penetrate root hair and form an infection thread through which they penetrate the cortical cells and form bacteroid state thereby nodules are formed.

5. CONCLUSION

Plant growth promoting rhizobacteria, having multiple activities directed towards plant growth promotion in relation to exhibiting bioremediating potentials by detoxifying various pollutants and controlling a range of phytopathogens, have shown spectacular results in different crop studies. The productive efficiency of a specific PGPR may be further enhanced with the optimization and acclimatization according to the prevailing soil conditions. In future, they are expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side-effects to sustainable agriculture. Further research and understanding of mechanisms of PGPR mediated-phytostimulation would overlay the way to find out more proficient rhizobacterial strains which may work under varied agro-ecological conditions.

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