



**Review Article** 

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## Plant Growth Promoting Rhizobacteria as Growth Promoters for Wheat: A Review



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#### Abstract

The microorganisms with the aim of improving nutrients availability for plants are an important practice and necessary for agriculture. During the past couple of decades, plant growth-promoting rhizobacteria (PGPR) will begin to replace the use of chemicals in agriculture, horticulture, silviculture, and environmental cleanup strategies. Scientific researches involve multidisciplinary approaches to understand adaptation of PGPR, effects on plant physiology and growth, induced systemic resistance, biocontrol of plant pathogens and biofertilization. This is due to the emerging demand for dependence diminishing of synthetic chemical products, to the growing necessity of sustainable agriculture within a holistic vision of development and to focalize environmental protection. The PGPR are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on roots and shoots growth. In this review, we have discussed about rhizobacteria which acts as plant growth promoter and the other desirable properties exhibited by them.

Keywords: Wheat; PGPR; Rhizosphere; Rhizobacteria; PGP attributes

### Introduction

## Wheat

Wheat is the major grain that sustains humanity. Wheat grown in temperate climate and it is staple food for 35% of world's population. On other hand, it provides more calories and protein in the diet than any other crop. Wheat (Triticum spp., most commonly T. aestivum) is a cereal grain (botanically, a type of fruit called a caryopsis) originally from the Levant region but now cultivated worldwide. In 2016, world production of wheat was 749 million tonnes, making, it the second most-produced cereal after maize (1.03 billion tonnes), with more than rice (499 million tonnes) (United Nations, Food and Agriculture Organization. 2016). Since 1960, world production of wheat and other grain crops has tripled and is expected to grow further through the middle of the 21st Century. World trade in wheat is greater than for all other crops combined. Globally, wheat is the leading source of vegetal protein in human food, having a protein content of about 12% [1,2], which is relatively high as compared to other major cereals and staple foods.

When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber, and is associated with lower risk of several diseases, including coronary heart disease, stroke, cancer and diabetes. PGPR are beneficial for plant growth and also referred as Increasing Bacteria (YIB). They can affect plant growth and yield in a number of ways and enhancement reproductive growth is documented in a range of crops like cereals, pulses, ornamentals, vegetables, plantation crops and some trees. Treatments with PGPR increase germination percentage, seedling vigour, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, grains, fodder and fruit yields etc [3,4].

**Nutrition:** In 100 grams, wheat provides 327 calories and is a rich source (20% or more of the Daily Value, DV) of multiple essential nutrients, such as protein, dietary fibers, manganese, phosphorus and niacin. Several B vitamins and other dietary minerals are in significant content. Wheat seeds contain 13% water, 71% carbohydrates, and 1.5% fat. Its 13% protein content

is comprised mostly of gluten as 75-80% of total wheat protein which upon digestion contributes amino acids for human nutrition.

**Production and consumption:** In 2016, global wheat production was 749 million tonnes. Wheat is the primary food staple in North Africa and the Middle East, and is growing in uses in Asia. Unlike rice, wheat production is more widespread globally, though 47% of the world total in 2014 was produced by just four countries - China, India, Russia and the United States. During the past century, industrialization of agriculture has provoked a significant and essential productivity increase, which has led to a greater amount of food available to the general population.

Along with this abundance the appearance of serious environmental and social problems came with the package: problems that must be faced and solved in the not too distant future. Nowadays, it is urgent to maintain that high productivity, but it is becoming urgent to alter as little as possible the environment. Clearly we must then head for a more environmentally sustainable agriculture while maintaining ecosystems and biodiversity. One potential way to decrease negative environmental impact resulting from continued use of chemical fertilizers, herbicides and pesticides is the use of plant growth-promoting rhizobacteria (PGPR). This term was first defined to describe soil bacteria that colonize the rhizosphere of plants, growing in, on or around plant tissues that stimulate plant growth by several mechanisms.

#### Rhizosphere

The term rhizosphere is coined more than hundred years ago by Hiltner [5]. It is a nutrient-rich habitat for microorganisms, where severe, intense interaction stake place between the plant, soil, and micro fauna [6]. They may have positive, negative or no visible effect on plant growth. Plant growth and productivity is highly affected by these interactions. Different types of microorganisms such as bacteria, fungi, protozoa, algae coexist among them. Bacteria contribute most to plant health by releasing many organic exudates, thus creating a selectively sensitive environment where diversity is less [7]. Out of them, plant growth promoting bacteria (PGPR) are most abundant among all others in the rhizosphere.

Role of PGPR: PGPR are free living bacteria that resides in soil. They either directly or indirectly assist rooting [8]. They play different roles in the soil which proves beneficial for plant health and productivity. They colonize the rhizosphere and protect plant from its pathogens, produce secondary metabolites such a santibiotics that suppress harmful rhizobacteria, produce siderophores, and phytohormones, can fix atmospheric nitrogen, and help in providing nutrition uptake by solubilizing phosphate and produce biologically active substances which influence the plant growth and development.

**Occurrence and forms of PGPR:** The mechanism by which PGPR exerts their beneficial effect on plants can be very diverse. They can establish themselves on root surface or inside the roots. PGPR can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) that may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex. The bacterial general such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Flavo bacterium,* Pseudomonas and Serratia belong to ePGPR. The other category is intracellular plant growth promoting rhizobacteria (iPGPR) that locates generally inside the specialized nodular structures of root cells [9]. It belongs to the family of *Rhizobiaceae* includes *Allorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium,* endophytes and Frankia species both of which can symbiotically fix atmospheric nitrogen with the higher plants.

Role of plant growth promoting rhizobacteria for plant growth enhancement: PGPR plays an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PGPR that promotes plant growth includes (i) abiotic stress tolerance in plants;(ii) nutrient fixation for easy uptake by plant (iii) plant growth regulators; (iv) the production of siderophores; (v) the production of volatile organic compounds; and (vi) the production of protectio lyticenzyme such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases [10,11]. Nutrient availability for plant uptake PGPR has the ability to increase the availability of nutrient concentration in the rhizosphere [10] by fixing nutrients, thus preventing them from leaching out. As an example, nitrogen, which is needed for the synthesis of amino acids and proteins, is the most limiting nutrient for plants. The mechanisms by which atmospheric nitrogen is added into organic forms that can be assimilated by plants are exclusive to prokaryotes [12,13]. A rare example of a free-living nitrogen-fixing organism is Azospirillum, often associated with cereals in temperate zones and also reported to be able to improve rice crop yields [14].

Plant growth regulators: Plant growth regulators, also termed plant exogenous hormones, are synthetic substances that are similar to natural plant hormones. They are used to regulate the growth of plants and are important measures for boosting agricultural production. One of the terms for the prominent modes of action for growth promotion by PGPR is phyto stimulator, or plant growth regulator. This is defined as microorganisms that have the ability to produce or alter the concentration of growth regulators such as IAA, GA, cytokinins, and ethylene. The mechanism that is being projected is the production of phytohormones (plant hormones) such as auxins, cytokinins, and GA [15,16].

## Auxin

Auxin is one of the crucial molecules, regulating most plant processes directly or indirectly as was further proven when [17] reported that auxins-producing Bacillus spp. inflicts a positive

effect on Solanun tube rosum's growth. The most active and famous auxins in plants is indole-3-acetic acid (IAA) area and length. The processes of seed germination and emergence, floral induction, flower and fruit development, and stem and leaf growth include the involvement of gibberellin (GA), 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 [18,20].

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 [21]. Moreover, down-regulation of IAA as signaling is associated with the plant defense mechanisms against a number of phyto-pathogenic bacteria as evidenced in enhanced susceptibility of plants to the bacterial pathogens by exogenous application of IAA or IAA produced by the pathogen [21]. 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 (Glick, 2012). Thus, rhizobacterial IAA is identified as an effect or molecule in plant-microbe interactions, both in pathogenesis and phyto stimulation [21].

## Gibberellin (GA) and Cytokinins

This is one of the phytohormones (Bottini.2004). However, the most dominant physiological effect of GA is shoot elongation [21,22] showed that tomato plants inoculated with the gibberellin-producing Sphingomonassp.LK11 strain have a significant increase in various growth characteristics. Cytokinins stimulate plant's cell division, vascular cambium sensitivity, and vascular differentiation and induce the proliferation of root hairs, but inhibit lateral root formation. Ortíz-Castro et al. [23] reported the identification of a Bacillus megaterium strain that promoted the growth of A. thaliana and P. vulgaris seedlings through cytokinins production. Other different bacterial genera Proteus, Klebsiella, Escherichia and Pseudomonas have also been reported to possess the ability to produce cytokinins [24]. Over the course of time, detection and quantification of gibberellins is possible using spectroscopy, high-performance thin layer chromatography (HPLC) etc. The extraction and detection procedures for quantifying gibberellins from microbes are described by Patel et al. [25]. Effect of PGPR producing GAs on plant is not exactly known but such bacteria are used in the seed germination. Whereas, several reports suggests that GAs producing fungi are considered as phytopatogens [26].

#### Production of siderophores

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 Fe3+, 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 (iii)) 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 Fe3+, which are then taken up by the cell membrane of bacteria, where the Fe3+is reduced to Fe2+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 [27] 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.

## Phosphate Solubilizing Bacteria (PSB)

The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, Phosphate solubilization is equally important. Phosphorus (P) is major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilising the insoluble inorganic P of soil and make it available to he plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields [28,29]. The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture [30]. The use of phosphate solubilising bacteria as inoculants increases the P uptake by plants [29,31]. Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solubilising Microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. These organisms in addition to providing P to plants also facilitate plant growth by other mechanisms. Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems [32]. PSM include largely bacteria and fungi. Bacterial strains Azotobacter vinelandii and Bacillus cereus

when tested in vitro are found to solubilise Phosphate and thus help in the growth of plants [33].

## **HCN** production

One group of microorganisms which acts as biocontrol agents of weeds include the *Deleterious Rhizobacteria* (DRB) that cancolonize plant root surfaces and able to suppress plant growth [34]. Many Deleterious Rhizobacteria (DRB) are plant specific [35]. Cyanide is a dreaded chemical produced by them as it has toxic properties. Although cyanide acts as a general metabolic inhibitor, it is synthesized, excreted and metabolized by hundreds of organisms, including bacteria, algae, fungi, plants, and insects, as a mean to avoid predation or competition. The host plants are generally not negatively affected by inoculation with cyanide-producing bacterial strains and host-specific rhizobacteria can act as biological weed-control agents [36].

A secondary metabolite produced commonly by rhizosphere pseudomonads is Hydrogen Cyanide (HCN), a gas known to negatively affect root metabolism and root growth [37] and is a potential and environmentally compatible mechanism for biological control of pathogens [38]. The HCN production is found to be a common trait of Pseudomonas (88.89%) and Bacillus (50%) is the rhizospheric soil and plant root nodules and is a serious environmental pollutant and a biocontrol metabolite in Pseudomonas species [27]. The Pseudomonas fragiCS11RH1 (MTCC 8984), a psychrotolerant bacterium produces hydrogen cyanide (HCN) and the seed bacterization with the isolate significantly increases the percent germination, rate of germination, plant biomass and nutrient uptake of wheat seedlings [39].

## Lytic enzymes

Growth enhancement through enzymatic activity is another mechanism used by plant growth promoting rhizobacteria. Plant growth promoting rhizobacterial strains can produce certain enzymes such as chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases etc. [40,41] exhibit hyper parasitic activity, attacking pathogens by excreting cell wall hydrolases. Through the activity of these enzymes, plant growth promoting rhizobacteria play a very significant role in plant growth promotion particularly to protect them from biotic and abiotic stresses by suppression of pathogenic fungi including Botrytis cinerea, Sclerotium rolfsii, Fusarium oxysporum, Phytophthorasp., Rhizoctonia solani, and Pythium ultimum [42,43]. Cell wall-degrading enzymes such as β-1,3-glucanase, chitinase, cellulase, and protease secreted by biocontrol strains of PGPR exert a direct inhibitory effect on the hyphal growth of fungal pathogens by degrading their cell wall. Chitinase degrades chitin, an insoluble linear polymer of β-1, 4-N-acetylglucoseamine, which is the major component of the fungal cell wall.

The  $\beta$ -1,3-glucanase synthesized by strains of Paeni bacillus and Streptomyces spp. can easily degrade fungal cell walls of

pathogenic F. oxysporum, is reported [44]. In a similar manner, Bacillus cepacia synthesizes  $\beta\text{-1,3-glucanase,}$  which destroys the cell walls of the soil borne pathogens R. solani, P. ultimum, and Sclerotium rolfsii [45]. Potential biocontrol agents with chitinolytic activities include B. licheniformis,B. cereus, B. circulans, B. subtilis, and B. thuringiensis [46].

Among the Gram-negative bacteria, Serratia marcescens, *Enterobacte ragglomerans, Pseudomonas aeruginosa*, and P. fluorescens have been found to possess chitinolytic activities [47] studied the chitinolytic and antifungal activities of a potent biocontrol strain of SerratiamarcescensB2 against the soil borne pathogens Rhizoctonia solani and Fusarium oxysporum. The mycelia of the fungal pathogens co-inoculated with this strain showed various abnormalities such as partial swelling in the hyphae and at the tip, hyphal curling, or bursting of the hyphal tip.

## **Applications of PGPR**

As it is been mentioned on maize and rice, the use of PGPR for improving crop production, thus reducing the need for chemical fertilizers, is becoming a frequent strategy for sustain able agriculture. Inoculation of the wheat seed with ACC-deaminase producer P. fluorescents strains allowed the diminishing of N, P and K fertilizer rates and, in general, crops presented higher grain yields, harvest index and protein content with lower fertilizer doses, along with PGPR, than those conventionally applied. Significantly enhanced yields of wheat have been obtained when consortia of PGPR and AMF were applied, particularly if they exhibit different and complementary abilities.

Studied the effect of inoculation with the AMF Glomus sp. 88 and two phosphate (PO43) solubilizing microorganisms (PSM), Bacillus circulars and Cladosporium herbarum, in the presence or absence of rock phosphate in a natural P-deficient sandy soil on wheat crops. The significant increase in grain and straw yields due to inoculation with the consortia could be attributed to a high absorption of nutrients. The effects of the application of the consortia AMF and PGPR on wheat crops were investigated in a two-year experiments in different agro-climate zones of India at seven locations extending from the Himalayan foothills to the Indo-Gangetic and it was seen that dual inoculation of this cereal increased crop yield, grain and soil quality and the nutrient uptake of wheat.

# Commercialization of PGPR as biofertilizers and biocontrol agents

Several PGPR bacterial strains are commercially available in the form of formulated products which is used as biofertilizers and biocontrol agents [48]. Bacterial biofertilizers are formulated in variety of ways and available in the market. Formulation of the sporulating, Grampositive bacteria are resistant to desiccation. Gram-positive micro-organisms possess heat-resistantspores that are exploited to formulate stable and dry powder products [49]. Alternative to solid-powdered formulation is the suspension

of organisms in oil, wherethe purpose is to exclude oxygen which prevents respiration [50].

The problems faced by biocontrol developers is that crops are grown under a multiplicity of climatic and environmental conditions which include temperature, rainfall, soil type, crop variety which change from farm to farm or even within one field, and such variations causes disparity in the potentiality of PGPR based biofertilizers. However, over the period of time, researchers have been able to develop better biofertilizers with improved shelf life and possessing better and efficient strains. From present scenario for the use of PGPR in sustainable agriculture, there is still a huge scope of enhancing agricultural productivity using this technique [51].

The success and commercialization of plant growth promoting rhizobacterial strains depend on the linkages between the scientific organizations and industries. Numerous work done showed different stages in the process of commercialization include isolation of antagonist strains, screening, fermentation methods, mass production, formulation viability, toxicology, industrial linkages, quality control and field efficacy [52]. Moreover, 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 materials.

# Future Research and Development Strategies for Sustainable Technology

The need of today's world is high output yield and enhanced production of the crop as well as fertility of soil to get in an ecofriendly manner. Hence, the research has to be focused on the new concept of rhizo engineering based on favorably partitioning of the exotic biomolecules, which create a unique setting for the interaction between plant and microbes [53]. 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. Fresh alternatives should be explored for the use of bio inoculants for other high value crops such as vegetables, fruits, and flowers. The application of multi strain bacterial consortium over single inoculation could bean 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 enhancing plant growth at low temperature [42]. Research on nitrogen fixation and phosphate solubilization by plant growth promoting rhizobacteria is progress on but little research can be done on potassium solubilization which is third major essential macronutrient for plant growth. This will not only increase the field of the inoculants but also create confidence among the farmers for their use. In addition, future marketing of bioinoculant products and release of these trans genics into the

environment as eco-friendly alternations to agrochemicals will depend on the generation of bio safety data required for the registration of plant growth promoting rhizobacterial agents. A part from that future research in optimizing growth condition and increased shelf life of PGPR products, not phytotoxic to crop plants, tolerate adverse environmental condition, higher yield and cost effective PGPR products for use of agricultural farmer will be also helpful.

## **Conclusions and Perspective**

This review has focused on a heterogeneous group of microorganisms that can be found in the rhizosphere. They live in association with roots and stimulate the plant growth and/or reduce the incidence of plant disease. Among the numerous PGP bacteria and fungi described up to now, the bacteria Azospirillum, Bacillus, and Pseudomonas. The plant growth promoting phenomenon can be attributed to the ability of the isolate to produce IAA, as IAA positively influences root growth and development, thereby enhancing nutrient uptake [54]. It is a well-established fact that improved phosphorous nutrition influences overall plant growth and root development [55].

Siderophore production by the isolate assumes significance for iron nutrition of plants grown under iron deficient conditions [56] worldwide there is a profound need to explore varied agroecological niches for the presence of indigenous beneficial microorganisms. With increasing awareness about the problems of chemical fertilizers based agricultural practices [57-60], it is important to search for region-specific microbial strains which can be used as a potential plant growth promoter to achieve desired production of strains. It would be very useful to match correctly the appropriate PGPR with the right plant and environmental condition to achieve the best results on plant growth. In this sense [61], more effort should be done on the development of good inoculant delivery systems that facilitate the environmental persistence of the PGPR and this fact would allow diminishing the amount of chemical fertilizers and pesticides used for enhance soil fertility and crop productivity [62,63].

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