

Review

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Review

# Microbial Biofertilisers in Plant Production and Resistance: A Review

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**Abstract:** To identify soil microbial communities, it is essential to understand their phenotypic and genetic characteristics. Plant growth-promoting rhizobacteria (PGPR) are bacteria capable of promoting plant growth. In addition, rhizobacteria also serve as biological control agents. In addition to fixing atmospheric nitrogen, solubilising insoluble phosphate and secreting hormones such as indoleacetic acid, kinetins and gibberellins, plant growth-promoting rhizobacteria help plants grow directly. Furthermore, they facilitate plant growth indirectly by inducing systemic resistance, siderophores, antibiotics, lytic enzymes, hydrogen cyanide production and stress regulation. This review examines in detail the direct and indirect mechanisms of PGPR action and their interaction in plant growth and resistance.

**Keywords:** microbial biofertilizers; microbial symbioses; plant interactions; crop resistance

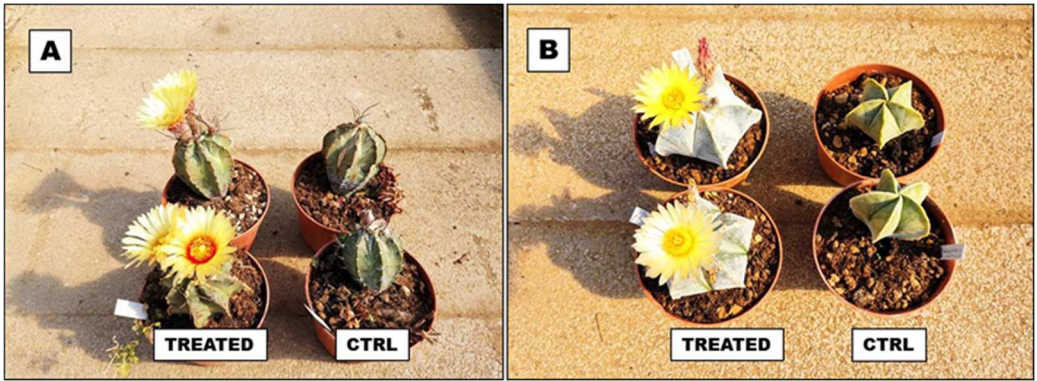
## 1. Introduction

Rhizobacteria that supports plant growth are known as Plant Growth-Promoting Rhizobacteria (PGPR) [1,2]. The diversity of phenotypic and genotypic characteristics of soil microbiomes makes them complex and difficult to characterise [3]. However, as the rhizosphere has become increasingly important to the bio-sphere in recent years, several PGPRs have been identified that significantly have a great impact in plant growth, primarily because they act as an ecological unit [4]. The PGPRs affect plant growth by solubilising insoluble phosphates, fixing atmospheric nitrogen, and secreting hormones that control plant growth [5]. In addition, through induced systemic resistance (ISR), nutrient competition, antibiotics, parasitism, and suppression of rhizobacterial growth, PGPRs indirectly benefit plant growth [6]. These communities are very diverse, and their actions can take many forms, including antagonistic action against pathogens in the soil and inducing systemic resistance against pathogens throughout the plant [7]. Plants can be indirectly aided in growing by antagonistic rhizobacteria because they produce various substances that can control pathogens [8]. If the inducing bacteria and the challenging pathogen remain spatially separated, inducing systemic resistance (ISR) can be compared to pathogen-induced acquired systemic resistance (SAR). Different plant species have induced resistance that makes uninfected parts of the plant more resistant to pathogen attack [9]. Induction of resistance occurs by rhizobacteria either through salicylic acid-dependent SAR pathways or through the bacteria's perception of jasmonic acid and ethylene. Among the many characteristics of rhizobacteria are their antagonistic effects and ability to trigger inflammatory responses. Many studies have examined the use of PGPRs as a substitute for crop protection agents (fertilisers and pesticides) for plant growth promotion in recent years [10,11]. The rhizobacteria can alter the soil structure, recycle essential elements, decompose organic matter, solubilise mineral nutrients, and serve as biocontrol agents for soil-borne and seed-borne pathogens [12–14]. A good understanding of rhizobacteria that promote plant growth and their interaction with

bi-otic and abiotic factors is crucial to bioremediation techniques. This is also relevant for energy generation processes, and biotechnology industries, such as pharmaceutical, chemical and food industries [15]; rhizobacteria are also useful for reducing the use of chemical fertilisers. The main advantage of this approach is the increasing the productivity and sustainability of agricultural systems and soil fertility [16]. As a result, production costs are reduced, and the best soil and crop management practices are identified [17]. The aim of this review was to show the possible benefits of the application of rhizobacteria in plants, the direct and indirect mechanisms they influence, the possible applications of PGPR-based formulations in agriculture and the prospects of the use of rhizobacteria on crops.

2. Plant and soil effects of PGPRs

Rhizobacteria that promote plant growth are well known and essential, and this growth enhancement is due to rhizobacteria's characteristics [18]. PGPRs can enhance plant growth and development through various mechanisms [19]. In particular, rhizobacteria produce a variety of substances that alter the entire microbial community in the rhizosphere; and they are capable of supplying nutrients (nitrogen, phosphorus, potassium and essential minerals) or producing plant hormones [20]. For example, the inoculation of rhizobacteria in *Astrophytum* spp. grown in biochar-enriched substrates improves vegetative and root growth and plant flowering (Figure 1) [21]. By acting as biocontrol agents, environmental protectors, and root colonisers, PGPRs can also indirectly promote plant growth by reducing the effects of pathogens [22,23]. Sustainable agriculture and ecosystem stability are severely threatened by phytopathogenic microorganisms, which degrade soil fertility, alter the environment, and, ultimately, harm human health and contaminate groundwater [24]. By fixing nitrogen, mineralising organic compounds, solubilising mineral nutrients, and producing phytohormones, PGPRs also facilitate plant uptake of nutrients and increased the and increase resistance to biotic and abiotic stresses. Many species are able to survive particular environmental conditions, such as high temperatures and drought (Table 1) [25]. As an indirect means of achieving soil fertility and plant growth, PGPRs are crucial to a sustainable and ecological approach. This can be achieved through various mechanisms, including antibiotics, HCNs, siderophores, and hydrolytic enzymes, and as outlined before, PGPRs can be exploited to decrease the need for agrochemicals such as fertilisers and pesticides and increase soil fertility [26].



**Figure 1.** Increased vegetative growth and flowering in *Astrophytum capricorne* (A) and *Astrophytum myriostigma* (B) in plants supplemented with rhizobacteria on biochar substrate [21].

**Table 1.** Bacterial genera and species that are drought resistant [25].

Bacteria	Crop	Action Mechanism
<i>Azospirillum</i> sp.	Wheat	Highest amounts of N and auxin

<i>Bacillus</i> sp.	Grass	Responses of antioxidant system and early proline accumulation
<i>Streptomyces</i> sp.	Tomato	Increase the content of different sugars
<i>Pseudomonas</i> sp.	<i>Arabidopsis</i>	Higher ACC deaminase activity gibberellic acid, abscisic acid, indole acetic acid, and exopolysaccharide
<i>Enterobacter</i> sp.	Bean	Enhance proline, malondialdehyde, and antioxidant enzymes
<i>Azospirillum brasilense</i>	Wheat	Less accumulation of H <sub>2</sub> O <sub>2</sub> with less enhanced production of proline and activities of catalase and peroxidase

### 3. Mechanisms activated directly by Plant Growth Promoting Rhizobacteria

In terms of plant growth, phytohormones play a critical role. These are plant hormones that affect the plant's response to its environment. These hormones are produced at one point in the plant and then transferred to another part of the plant, where they are used to promote the growth [1]. Roots and leaves grow due to the physical responses caused by these hormones [27]. Some essential plant hormones are auxins, gibberellins, ethylene, cytokinins, and abscisic acid [28]. Rhizobacteria produce these phytohormones. In addition to auxins and gibberellins, ethylene, cytokinins and abscisic acid are important phytohormones [29]. In plant roots and shoots, cytokinins (CKs) play a role in cell division [30]. Among their benefits, there is the growth of cells, differentiation of cells, apical dominance, axillary bud development, and leaf senescence [31,32]. Plants synthesise this hormone, but yeast strains and PGPR strains can also prepare it. As well as phytopathogens, some phytopathogens can synthesise cytokinins. It has been reported that *Azotobacter* species, *Pantoea agglomerans* strains, *Rhizobium* species, *Rhodospirillum rubrum* strains, *Bacillus subtilis* strains, *Pseudomonas fluorescens* strains, and *Paenibacillus polymyxa* species all produce the cytokinin hormone [33,34]. Some rhizobacteria are able by their action to mitigate the effect of different types of stress such as water, salt and heat stress (Table 2) [35]. A class of important plant hormones, gibberellins (GA) control various developmental processes in plants. Their functions include stem elongation, dormancy, germination, flowering, and flower development. Several cytokinin-producing polymeric protein receptors synthesise gibberellin, a phytohormone involved in breaking dormancy and other aspects of germination. Gibberellin is the most crucial phytohormone synthesised by some PGPRs. Production and regulation of gibberellin and cytokinin are extremely important [36]. PGPRs and plants produce a variety of phytohormones, including indoleacetic acid. In addition to cell division, other properties like gene expression, organogenesis, pigmentation, root development, seed germination, stress resistance, tropical responses, and photosynthesis, play an essential role in plant cellular responses [37]. Plants and bacteria influence the amount of IAA required to promote plant growth vigorously. The amount of IAA required to promote plant growth depends on the plant and



bacterial species. PGPRs produce indole-3-acetic acid, which is responsible for root elongation and the formation of roots. Nearly all plants produce ethylene as a growth hormone, which is key in many physiological changes [38]. Plants respond to biotic and abiotic stresses negatively, affecting root growth and plant growth [39]. The PGPR enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase can regulate ethylene production. Inoculation with PGPRs can maintain plant growth and development under stressful conditions, such as drought, salinity, cold, and soil pollution, plants synthesise abscisic acid [25]. This growth hormone activates stress-resistance genes. Abscissic acid producing strains, such as *Bacillus licheniformis* Rt4M10, *Azospirillum brasilense* sp. 245, and *Pseudomonas fluorescens* Rt6M10, increase the internal ABA content of plants. As a result, the plants become more resilient to drought. The unavailability of nitrogen can limit plant growth, but phosphorus is also essential for life [40]. There are large quantities of phosphate in soil, but they are in an insoluble form that plants cannot utilise for growth since they are insoluble. PGPRs can solubilise phosphate in soil via acidification, chelation or enzyme action [41]. For example, the PGPR *Gluconacetobacter diazotrophicus*, found in sugar cane, can solubilise phosphate by acidification [7].

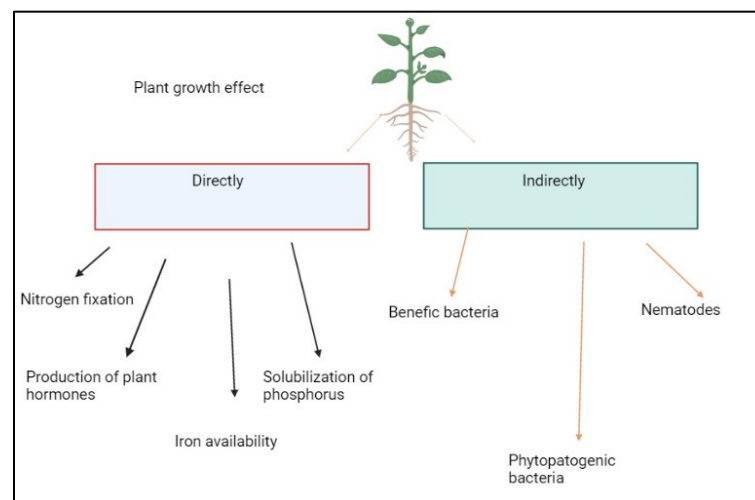
Table 2. Application of Rhizobacteria in mitigating heat stress in plants [35].

Microbes	Plant	Parameters	Stress
<i>Enterobacter</i> SA187	<i>Arabidopsis thaliana</i> , Wheat plant	Increased Biomass, height, seed weight	Long term
<i>Septoglomus</i> <i>deserticola</i>	<i>Solanum</i> <i>Lycopersicum</i>	Improved stomatal conductance, water content	Heat Drought
<i>Pseudomonas</i> <i>fluorescens</i> , <i>Pantoea</i> <i>agglomerans</i>	<i>Triticum</i> <i>aestivum</i>	Increased Antioxidant enzymes	High Temp.
<i>B.</i> <i>phytofirmans</i>	<i>Solanum</i> <i>tuberosum</i>	Increased Proline and glycine betaine	High Temp.
<i>B. cereus</i>	<i>Soybean</i>	Increased Chlorophyll and Carotenoid	High Temp.

4. Mechanisms indirectly activated by Plant Growth-Promoting Rhizobacteria

Microorganisms compete for nutrients and colonisation sites in their natural environment fiercely. Various mechanisms of PGPR species have evolved that allow them to reduce competition by releasing antibiotics, lytic enzymes, or weak organic acids into their environments (Figure 2) [21,42]. As a result, PGPRs are valuable tools that can be used against plant pathogens. However, there is a possibility of the development of resistant pathogens if antibiotic-producing bacteria are used more frequently. It was shown that PGPR enzymes secreted by these PGPRs could eliminate pathogens such as *Botrytis cinerea*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Phytophthora* spp, *Pythium ultimum* and *Rhizoctonia solani* [43,44]. They include cellulases, chitinases, lipases and proteases secreted by the plant. Plants respond to pathogens in two ways: acquired systemic resistance and induced systemic resistance. Induced systemic resistance is the same as Systemic Acquired Resistance (SAR) [45]. Acquired systemic resistance is triggered by the infection of a plant by a pathogen, whereas PGPR triggers acquired systemic resistance. The application of PGPR inoculum induces systemic resistance in the plant against many bacterial pathogens. Plants also need iron as a nutrient. Under aerobic conditions, iron is found as Fe3+, which is not soluble for use by microorganisms and

plants [46]. Therefore, microbes produce and secrete iron chelators, known as siderophores. The chelators are highly iron-adjacent. They function as iron solubilising agents under limiting conditions. In the cell,  $\text{Fe}^{3+}$  is transformed into  $\text{Fe}^{2+}$ , which is then released by the siderophores [47]. Rhizobacteria steal iron from other microorganisms by producing siderophores. In addition, PGPRs use siderophores to remove other heavy metals from soil and prevent the heavy metals from causing toxicity in plants. They can also be used for the bioremediation of heavy metal toxic soils [48]. As well as helping plants grow and ripen fruit, ethylene acts as a phytohormone in response to salt, drought or pathogenic bacteria. However, high amounts of ethylene can also cause plant harm. Many PGPR synthesises an ACC deaminase enzyme [50]. This enzyme destroys 1-aminocyclopropane-1-carboxylate, the precursor of ethylene. It relieves plant stress by reducing ethylene levels. Plant root surfaces can be colonised by harmful rhizobacteria that act as biocontrol agents for weeds. They produce toxic compounds known as cyanides, produced by many microorganisms, such as bacteria, algae, fungi and plants [51]. Biological weed control agents can be derived from host-specific rhizobacteria, which compete with their counterparts to survive. It has no negative impact on host plants when inoculated with cyanide-producing bacterial strains that produce cyanide [52]. In addition, weed biocontrol agents, such as hydro-gen cyanide, are produced, which inhibits the electron transport chain and energy supply to cells. Many harmful microbes compete with PGPRs for nutrients, but these nutrients are present only in trace amounts so that they can limit the disease's causative agent [53]. In fertile soils with abundant non-pathogenic microbes, they colonise plant surfaces quickly and utilise nutrients. These mechanisms can be challenging to study in the system because they inhibit pathogenic microbes from growing. One essential interaction that indirectly supports plant growth is the competition for nutrients between PGPR and pathogens [54].



**Figure 2.** PGPR promotes plant growth through the production of siderophores, increasing iron availability, and producing hormones such as auxins, gibberellins, and cytokinin that modulate the hormone balance of the host plant. The direct mechanism includes biological nitrogen fixation (BNF) by the activity of the nitrogenase enzyme complex; the solubilization of inorganic phosphate in the soil; and the production of siderophores. The indirect mechanisms are attributed to PGPR's occupation of niches and the production of substances that repel phytopathogens and nematodes [21].

## 5. Application of PGPR-based formulations

The support of crop management by beneficial microorganisms is an environmentally friendly alternative to the conventional techniques that are based on chemical inputs, with respect to the increasing consumer expectations of healthy products and current policies towards the implementation of environmentally friendly cropping systems [55]. Besides biotic stresses, useful microorganisms in agriculture have been shown to increase plant tolerance to abiotic stresses such as flooding, water shortages, and excess salinity [56]. Plant growth regulators of microbial origin are of great agrarian and ecological interest, since they offer significant opportunities for eco-friendly

agronomic applications. As a result of selected strains, these regulators can also be used in the open field today, thus overcoming certain limitations. It is difficult to colonize the rhizosphere of an adult plant that is already well colonized by resident microorganisms due to high competition [57]. Soil type, temperature, introduced strains, inoculant density, and plant species can all influence the immediate response to PGPR soil administration. After inoculation, the introduced population typically drops rapidly, and it is possible that the number of PGPR colonizing roots is insufficient to achieve the desired results [58]. Other times, the introduced microorganisms cannot find a free ecological niche in the soil. As well as maintaining the desired character characteristics, the strains used must be capable of surviving the stresses associated with concentration and stabilisation processes during production. Agricultural crops can be inoculated in a variety of ways:

- covering the seed at the time of sowing;
- using confected seed, i.e. covered with matrices that have included beneficial microorganisms;
- distributing the product directly in the furrows at the time of sowing;
- performing covering treatments during plant growth.

Using seed inoculation allows farmers to sow and inoculate at the same time, thus saving time and money. Another option is to encapsulate microbial cells in polymers, particularly alginate, which protect them from environmental stress and allow them to be released into the soil slowly and in large quantities [59]. For example, alginate preparations have been proposed for *Pseudomonas fluorescens* as a biocontrol and biostimulating agent, and for *Azospirillum brasilense* as a biofertilizing and biostimulating agent [60].

## 6. Perspectives on the use of Plant Growth Promoting Rhizobacteria in agriculture

An essential and safe method to increase yield is PGPR. In terms of increasing productivity, it is a promising solution [61,62]. In addition, it protects plants from chemicals used to control pests, which can also have a negative impact on the environment. Plant diseases and pests can also be controlled with PGPRs, improving yields. In laboratory and greenhouse experiments, PGPR strains are advantageous [63,64]. The field of genetic engineering is emerging as a means to improve PGPR strains and explore their potential applications. Besides all these advances, some environmental barriers and adverse conditions greatly influence the activity of PGPRs [65]. Mixing of strains, use of improved inoculation techniques and transfer of the active gene source of antagonists to the host plant can improve the variable efficacy of PGPRs [66]. Furthermore, biocontrol agents need a specific ecological environment to grow and survive, so different conditions may influence their efficacy and use [58,67]. The efficacy of biocontrol agents can be modified by using compatible mixed inocula in different ecological niches. Besides these advantages, PGPRs face several challenges [68]. Due to natural variations, it is difficult to predict the behaviour of bacteria in the laboratory and on the farm. These variations can have a significant effect on the entire experiment. Plant type and season can also influence the propagation of PGPRs to recover their viability and biological activity [69,70]. According to the notion that these bacteria can be applied as biofertilisers in agriculture and forestry [71,72], monitoring their activity under stress conditions such as salinity, soil pollution and other environmental conditions that alter crop productivity and yield is essential to understand their applications in different sectors of agriculture. Soil moisture, electrical conductivity and N, P and K concentrations must be monitored under different climatic conditions and bacterial concentrations. This is important in order to develop real, concrete microbial application protocols suitable for different geographical locations.

## 7. Conclusions

A significant improvement in plant growth, health and yield has been observed with the use of PGPRs. Plant growth, health and yield can be improved directly or indirectly by PGPRs through different mechanisms of action. Plants can also grow faster by reducing phytopathogens that reduce yield and growth with PGPRs. Several factors can influence the inoculation of PGPRs, including the age of the plants and the chemical, physical and biological properties of the soil. To overcome these

problems, field studies have to be carried out to identify the best application methods. This may one day ensure that PGPRs can replace chemical fertilisers in agriculture and ensure greater environmental sustainability.

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