

## A Brief Review on Plant Growth Promoting Rhizobacteria

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### Abstract:

Plant growth-promoting rhizobacteria (PGPR) are helpful bacteria residing in the rhizosphere (root zone) of plants. The PGPR also refers to free-living soil bacteria that are favourable to plant development and can colonize plant roots. This book chapter explores the prospects of PGPR in promoting plant growth and development. It highlights how PGPR employ several mechanisms, for example, N<sub>2</sub>-fixation, nutrient solubilization, phytohormone production, and induced systemic resistance, to enhance plant health and yield. The increasing demand for eco-friendly agricultural practices positions PGPR as an auspicious substitute for chemical fertilizers and pesticides. Finally, current challenges and future directions in exploring the full potential of PGPR for sustainable agriculture are discussed, emphasizing the need for further research and technological innovations to optimize their efficacy and application strategies.

### Introduction:

The ever-growing demand for food security necessitates a sustainable approach to agriculture (Shah et al. 2021). Chemical fertilizers and pesticides, while historically effective in boosting yields, have raised concerns about environmental pollution and potential harm to human health (Dhakal et al. 2026; Banerjee et al. 2021). In this context, PGPR has appeared as a capable alternative (Shah et al. 2021). PGPR is nitrogen-fixing bacteria or diazotrophs that support plant growth and/or prevent and reduce disease. These are members of a class of bacteria present at root surfaces, close to roots, and in the rhizosphere (Tang et al. 2021). Main crops like rice, wheat, maize, and sugarcane are associated with PGPR, which promotes plant growth by various means.

As stated by Davison (Davison 1988), Brierley (Brierley 1985), and Ehrlich (Ehrlich 1990), these bacteria's primary roles include: (1) providing nutrients to crops; (2) inducing plant hormones to promote plant development; (3) regulating or suppressing the action of plant pathogens; (4) enhancing soil structure; and (5) bioaccumulation or microbial leakage of chemicals. Recently, bioremediation of contaminated soils has also employed microorganisms in the soil to mineralize organic contaminants. Based on their level of

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adherence to the plant, two categories of microorganisms are distinguished: endophytic and rhizospheric colonizers.

PGPR can enhance plant growth directly through boosting nutrient cycling processes including biological nitrogen fixation, phosphorus solubilization, and phytohormone synthesis, or indirectly through manufacturing biocontrol chemicals that impede phytopathogens (Tang et al. 2021). Plant-microbe relations in the rhizosphere are crucial for sustainable crop production because they influence nutrient mobilization, transformation, and solubilization from a limited pool. This allows plants to absorb essential nutrients and reach their full genetic potential. In a cohesive nutrient management in plants, the employment of biological techniques is currently gaining popularity as a complement to chemical fertilizers for increasing crop production. Several researchers reported the utilization of PGPR has found a possible part in emerging sustainable crop production. Numerous non-symbiotic (*Azospirillum*, *Azotobacter*, *Bacillus*, and *Klebsiella sp.*) and symbiotic (*Rhizobium sp.*) bacteria are currently being employed globally to increase plant productivity (Hayat et al. 2010).

Burr and Caesar referred PGPR as nodule-promoting rhizobacteria (NPR) or plant-health promoting rhizobacteria (PHPR) (Burr and Caesar 1984). These bacteria are linked to the rhizosphere, a crucial soil biological habitat for relations between microbes and plants. Symbiotic bacteria and free-living rhizobacteria are the two categories into which PGPR can be separated based on their interactions with the plants.

Several bacteria, such as species of *Azospirillum*, *Pseudomonas*, *Azotobacter*, *Azoarcus*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Klebsiella*, *Herbaspirillum*, and *Serratia*, have exhibited plant development features (Kour et al. 2019). Numerous researchers have confirmed the ability of PGPR in plant growth development (Hayat et al. 2010). According to studies, PGPR bacterial isolates can fix N<sub>2</sub> and generate species that endorse plant development.

It has been established that the interior of sugarcane is home to several types of diazotrophic bacteria (Taulé et al. 2012; Sharma et al., 2024). The roots and stems of sugarcane comprise high concentrations of *Acetobacter diazotrophicus*, a recently identified diazotrophic bacteria that can reach 106 per gram fresh weight (Sinha et al. 2024). After infecting sugarcane tissues, research using electron microscopy of *A. diazotrophicus* gave precise information regarding where the bacteria were located in the sugarcane tissues of micropropagated plantlets. The  $\alpha$ -*Proteobacterium Herbaspirillum seropedicae* was found in large quantities in the sugarcane leaves, stems, and roots. *Herbaspirillum rubrisubalbicans*, which was previously known as *Pseudomonas rubrisubalbicans*, is also a close relative of *H. seropedicae*, and it causes mottled stripe disease in sugarcane (Hayat et al. 2010). *H. rubrisubalbicans* is also capable of fixing nitrogen. Sugarcane tissue frequently contained a second novel type of diazotrophic bacteria, previously known as isolate E-bacteria but now

provisionally called *Burkholderia brasilensis*. Also found in maize plants were *Herbaspirillum seropedicae* and *Burkholderia sp.*

This book chapter aims to provide an outline of how PGPR promotes plant growth and elucidate their potential applications in modern agriculture. By delving into the latest research findings and highlighting current challenges and opportunities, this review seeks to underscore the significance of PGPR as an encouraging avenue for sustainable crop production.

### Diversity of rhizospheric bacteria and crop improvement

The diversity of rhizospheric bacteria plays a critical role in crop improvement and agricultural sustainability (Mukhtar et al. 2019). The rhizosphere, the soil region directly encouraged by plant roots, harbors a complex microbial community that interacts with plants in several ways, influencing their development, health, and productivity. The rhizosphere is a hotspot for microbial activity due to the constant supply of nutrients from root exudates. Certain rhizosphere bacteria, called PGPR, directly contribute to development of plant and health by several methods. The use of diazotrophs as biofertilizers for rice production has been the subject of widespread study for several years (Jha et al. 2020). The "hot-spot," or aggregates with accrued organic compound and the rice rhizosphere, is the only area with significant microbial activity. Rice agro-ecosystem soils of India comprise a rich diversity of PGPR enhanced development and yield of cultivated rice (Kumar et al. 2020). Various scientists and researchers all over the world have isolated and utilised these bacteria for crop improvement in different crops (Kumar et al. 2020). They isolated bacteria from rhizospheric soil or non-rhizospheric soil. Plant growth supporting bacteria were selected for use in crop improvement. The diazotrophs are isolated much from rhizospheric soil compared to non-rhizospheric soil because plant exudes and rotting plant materials provide a source of organic substance to help the growth of these diazotrophs. The presence of microorganisms in the root tissue or rhizoplane area of a plant is termed root colonisation, while the existence of microbes in the adjacent soil of the root is termed rhizosphere colonisation (Compant et al. 2010). *Citrobacter*, *Enterobacter*, *Erwinia*, and *Klebsiella* are among the facultative anaerobic bacteria whose associations and capacities for nitrogen fixation. Important agricultural plants like rice, maize, wheat, and some grasses have been found to harbor a variety of bacterial species, including *Gluconacetobacter diazotrophicus*, *Herbaspirillum*, *Azoarcus*, *Azospirillum*, *Serratia*, *Burkholderia*, *Enterobacter*, *Rhizobium*, and *Klebsiella* (Kour et al. 2019). *Alcaligenes faecalis*, *Enterobacter cloacae*, *Klebsiella planticola*, *Azospirillum brasilense*, *Klebsiella oxytoca*, and *Azospirillum lipoferum* are the commonly associated N<sub>2</sub>-fixing bacteria in the rice rhizosphere (Afzal et al. 2019). Certain strains of *Azospirillum brasilense* and *Alcaligenes faecalis* have been identified. The only strains that have been shown to be safe and effective for use as biofertilizer are *Enterobacter cloacae*, *Alcaligenes faecalis*, *Azospirillum*, and *Klebsiella pneumoniae*. Associative N<sub>2</sub>-fixing

bacteria can have up to  $10^3$ – $10^7$  cells/g of soil, which is a higher density in paddy than in dry land (Burns and Hardy 2012). In the rhizosphere, associative  $N_2$ -fixing bacteria are primarily found. Microaerophilic bacteria, including *Azospirillum spp.*, *Burkholderia spp.*, and *Herbaspirillum spp.* were found colonizing the shoots, roots, and leaves of maize, rice, and wheat (Alves et al. 2015). Research on nitrogen-fixing bacteria linked to cereals has grown significantly. Numerous non-diazotrophic bacterial species and an extensive variety of plant species have been found to exhibit bacterial colonization of the internal tissues of healthy plants.

From the rhizosphere of wetland rice, diazotrophic bacteria have been recovered, including *Klebsiella oxytoca*, *Enterobacter cloacae*, *Alcaligenes*, and *Azospirillum*. Nitrogen-fixing bacteria that can invade the inner workings of plant tissues have been discovered. *Herbaspirillum seropedicae* was isolated from rice, sorghum, and maize plants, and *Gluconacetobacter diazotrophicus* (synon. *Acetobacter diazotrophicus*) was isolated from sugarcane plants. *Herbaspirillum* is an endophyte that colonizes a variety of cereals, including rice, sugarcane, maize, and sorghum. *Herbaspirillum* was able to fix  $N_2$  under aseptic conditions at an estimated  $33$ – $58$  mg tube<sup>-1</sup>. *Herbaspirillum seropedicae* colonizes the inside of wheat roots between cells, acting as an endophytic diazotroph of wheat. *Herbaspirillum rubrisubalbicans*, as an obligatory endophyte of stems, roots, and leaves, *Herbaspirillum seropedicae* is similarly present in the stems and roots of sugarcane (Monteiro et al. 2012).

Reis et al., reported that *B. tropicalis* is limited to the stems and roots of sugarcane, but *Burkholderia brasilensis* is an endophyte of the plant's stems, roots, and leaves (Reis et al. 2000). Additionally, these organisms can create compounds that are hostile to nematodes. The  $N_2$ -fixing bacteria that were recovered from the paddy field ranged from  $1.41 \times 10^6$  cfu to  $1.24 \times 10^8$  cfu/g weight of the soil.

The  $N_2$ -fixing capacity of strains of *Stenotrophomonas maltophilia*, *Pseudomonas fluorescens*, and *Bacillus fusiformis* were reported (Park et al. 2005). These strains have been found from the rhizosphere soils of wheat, maize, and rice crops rich in inorganic fertilizers. A nitrogen-fixing bacterium called *Sphingomonas azotifigens sp. nov.* was identified from the roots of *Oryza sativa*, the cultivable endophytic bacterial community—which included *A. junii*, *Acinetobacter calcoaceticus*, *Burkholderia sp.*, *B. gladioli*, *Enterobacter sakazaki*, *Klebsiella pneumoniae*, *Pseudomonas oryzihabitans*, *P. straminea*, *Ralstonia pickettii*, and *Sphingomonas sp.*—was extracted from soybean stems, roots, and leaves (Xie & Yokota 2006).

*Derxia* has been found in the rhizosphere of various rice cultivators. Diazotrophs are found in many species of the Enterobacteriaceae family, especially those found in the rice rhizosphere. These genera include several unnamed species along with *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Pseudomonas*, which are examples of diazotrophs having PGP activity. These plant-associated bacteria include *Pseudomonas putida* or *Pseudomonas*

*fluorescens*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*. Several N<sub>2</sub>-fixing bacteria were reported in graminaceous plants. These included rhizospheric bacteria (*Azotobacter paspali* and *Beijerinckia fluminensis*), associative bacteria (*A. amazonense*, *Azospirillumlipoferum*, *A. brasilense*), and endophytic bacteria (*H. rubrisubalbicans*, *Herbaspirillum seropedicae*, *Burkholderia brasiliensis*, *Gluconacetobacter diazotrophicus*, and *B. tropica*) (Andrews et al. 2003).

Zhang et al. detected soil bacteria that belonged to genera like *Alcaligenes*, *Azotobacter*, *Beijerinckia*, *Azospirillum*, *Bacillus*, *Derxia*, *Campylobacter*, numerous members of Enterobacteriaceae (*Pantoea*, *Klebsiella*), and *Pseudomonas stutzeri* by using traditional microbiological techniques that involved culturing bacteria (Zhang et al. 2008). Some isolates, like those of *Gluconacetobacter* and *Herbaspirillum*, *Azoarcus*, *Burkholderia*, or *K. pneumoniae* strain 342, happened to be endophytes. In cultivated rice (*Oryza sativa* L. cultivar KDML-105), the population of feasible endophytic diazotrophic bacteria was examined in various soil settings (Prakamhang et al. 2009). The nitrogen fixing capacity of particular consortia and single isolates was evaluated. It was demonstrated that a single isolate from every diazotrophic consortia could both promote and inhibit N<sub>2</sub>-fixation. It was discovered that a few isolates shared close ties with *Pseudomonas spp.*, *Pantoea agglomerans*, *Brevundimonas aurantiaca*, *Enterobacter dissolvens*, and Enterobacteriaceae.

*Stenotrophomonas maltophilia*, which was formerly known as *Pseudomonas maltophilia* and *Xanthomonas maltophilia*, is commonly found in close proximity to plants. It has been identified and isolated from the rhizosphere of various plants, including grass, oat, wheat, cucumber, oilseed, grape, maize, potato, and lettuce. Understanding and employing rhizosphere bacterial diversity is a propitious method for sustainable agriculture.

### Effect of rhizosphere on the diazotrophs of rice plants

The rhizosphere, the soil area impacted by plant roots, plays a significant role in shaping the population and activity of diazotrophic bacteria connected with rice (Dobbelaere et al. 2003). Diazotrophs are microorganisms capable of atmospheric N<sub>2</sub>-fixing into a form that plants can use, thus contributing to the N<sub>2</sub> nutrition of rice, that is essential for their growth and development. Numerous chemical, physical, and biological aspects of the soil affect the development and colonization of bacteria on plant roots. Rice liberates several organic molecules, for example, amino acids, sugars, organic acids, and phenolic compounds, into the rhizosphere through root exudation. These biomolecules assist as an energy and carbon source for diazotrophic bacteria, promoting their growth and activity. The prevalence and dispersal of diazotrophic bacteria in maize plants can be exaggerated by biological aspects, for example, cultivar category and stage of plant development. Research on the microbial colonization of maize roots confirmed that plant development significantly impacts on fluorescent pseudomonas populations that reside in maize roots.

Muthukumarasamy et al., working with 16 kinds of sugarcane, found that N<sub>2</sub>-fertilizer was the restraining aspect for the separation of *Gluconacetobacter diazotrophicus*, but that N<sub>2</sub>-fertilized samples had no effect on the isolation of *Herbaspirillum spp* (Muthukumarasamy et al. 1999). It was discovered that high N<sub>2</sub>-fertilization levels had a detrimental impact on *G. diazotrophicus* capacity to form colonies (CFU). However, since the nitrogen fertilizer changes the physiological condition of the plant, which in turn disturbs the plant's relationship with the endophyte, this detrimental effect on the bacteria does not appear to be a straight result of the nitrogen fertiliser.

The availability of nitrogen is a significant issue that influences the action of diazotrophic bacteria. The accessibility of C, N, or P can restrict the growth of bacteria in aquatic environments. Microbial development was facilitated by carbon and energy supplies found in organic matter derived from organic rich strata. The ideal conditions for microbial development and nutrient availability are often found in soil solutions with a neutral pH. The interrelationship between bacteria and plants can also be impacted by the type of soil, as it might have different chemical and biological qualities. It was found that ammonium in the soil can prevent diazotrophic bacteria from growing. This detrimental effect is also seen in the diazotrophic population living in plants with high N<sub>2</sub>-fertilization levels.

Biotic and environmental factors can impact the number of diazotrophic bacteria that are certainly linked with the maize rhizosphere. The study assessed the impact of two distinct maize genotypes, both with and without N<sub>2</sub>-fertilizer, on the distribution and population dynamics of diazotrophic bacteria linked to maize across various stages of plant ontogenesis. Utilizing maize cultivars (Santa Helena 8447 and Santa Rosa 3063) that had been earlier chosen from 32 cultivars against the highest and lowest retort to N<sub>2</sub>-fertilizer (Roesch et al. 2006). The analysis was conducted in an area with and without N<sub>2</sub>-fertilizer. They concluded that because roots were the favoured place of colonization regardless of cultivar category or development phase, the ontogenic phase of the maize plants had an impact on the dynamics of the diazotrophic bacterial population but not the cultivar type. Additionally, they noticed that the diazotrophic bacterial population was adversely disturbed by adding N<sub>2</sub>-fertilizer during the initial phases of maize growth.

### Plant-bacteria interaction

Plant-bacteria interactions are fundamental to the health and development of plants, in addition to the functioning of ecosystems (Singh et al. 2017). These interactions may be helpful, neutral, or detrimental, reliant on various influences, for example, the identity of the plant and bacteria species involved, environmental conditions, and the specific mechanisms of interaction. Some bacteria form symbiotic relationships with plants, where both partners benefit. Examples include N<sub>2</sub>-fixing bacteria, for example, *Rhizobium spp.* in legumes and *Frankia spp.* in actinorhizal plants, which provide plants with fixed N<sub>2</sub> in exchange for organic compounds. PGPR are free-living soil bacteria which are good for plant development. They can colonize roots and endorse plant development. PGPR are related to the rhizosphere,

a crucial soil ecological habitat for plant-microbe interactions. PGPR can be separated in two categories depending on their interactions with plants: free-living rhizobacteria and symbiotic bacteria.

A wide variety of bacteria, such as species of *Azospirillum*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Bacillus*, *Azotobacter*, *Arthobacter*, *Burkholderia*, *Alcaligenes*, and *Serratia*, have been isolated from the rhizosphere of diverse agricultural plants (Di Benedetto et al. 2017). The majority of these bacteria have several features that endorse plant development, such as the fixation of atmospheric N<sub>2</sub>, the solubilization of minerals, the synthesis of phytohormones (gibberellic acid and IAA), siderophores, and some antagonistic compounds that are useful in the administration of plant diseases. The potential of the advantageous PGPR as biofertilizers has led to a sharp rise in attention in them in recent years. The environment and the genotype of the organisms are both crucial for a productive plant-bacterium relationship. Engelhard et al. documented the impact of plant genotype in wild rice types and traditional rice that assist a better population of *Azoarcus sp.* compared to current variations (Engelhard et al. 2000). It was also found that *Asaia bogorensis*, a species of *Acetobacterium*, has been shown to increase the development of pineapple plants, most likely by creating phytohormones or by engaging in N<sub>2</sub>-fixation activities. The availability of nitrogen to the roots plays a significant role in the interface between plants and microorganisms. Even while just a few non-legumes have been identified to contain biologically fixed nitrogen, this process has significantly affected the ecology of both wild and developed ecosystems. According to Pereg Gerk et al., the discovery of spontaneous *A. brasilense* mutants with increased acetylene reduction ability with wheat suggested that populations in nature are highly flexible and that few genotypes that fix a lot of nitrogen might be preferred under specific circumstances (Pereg Gerk et al. 2000). Sugar cane is an excellent crop that help N<sub>2</sub>-fixation since some cultivars may obtain more than 150 kg N/ha/year from BNF. Thus, the capability of related bacteria for N<sub>2</sub>-fixation benefits certain crops inherently. Okon and Labandera-Gonzales found that *Azospirillum* inoculation may significantly boost yields, from 5 to 30%, especially when chemical N<sub>2</sub>-fertilizer use was minimal (Okon and Labandera-Gonzales 1994). This was based on their analysis of 20 years of field inoculation globally. Nevertheless, they thought that the growth endorsing impact was most likely related to phytohormone synthesis.

Following the inoculation of rice with certain strains of *A. amazonense*, considerable nitrogen fixation was found, as revealed by the <sup>15</sup>N isotope dilution process. Several researchers also provided evidence of promoting plant development resulting from endophyte nitrogen fixation. This supports the idea that studying a relationship between the rhizosphere and agriculture could be very beneficial. It was observed that fusion transcripts of *nifH* were found in rice inoculated with *Azoarcus sp.* BH72 when altered with a negligible organic source. However, the *nifH* reporter was relatively weak and delayed. Hurek et al. discovered that significant transcripts of *nifH* were observed in Kallar grass strain BH72, both in plants

grown in greenhouses and in noninoculated plants growing in the wild, and that this strain bestowed to N<sub>2</sub> uptake (Hurek et al. 2002).

Comprehending the interactions between plants and bacteria is crucial for various applications in agriculture, including the development of sustainable crop management practices, biofertilizers, biocontrol agents, and the exploitation of beneficial microbial communities to enhance crop productivity while minimizing environmental impacts.

### Inoculation of diazotrophs for crop improvement

Inoculating rice with bacteria is one of the sustainable and viable methods. Inoculation of diazotrophic bacteria for crop improvement involves the deliberate introduction of N<sub>2</sub>-fixing bacteria in the rhizosphere or plant tissues to enhance nitrogen availability and promote plant growth (Doty 2011). This practice is particularly beneficial in agricultural systems with high nitrogen demand, such as those involving N<sub>2</sub>-fixing crops like legumes or in areas where synthetic nitrogen fertilizers are expensive or environmentally unsustainable. As a result of their capability to fix atmospheric N<sub>2</sub>, certain soil bacteria, including *Clostridium*, *Azospirillum*, *Herbaspirillum*, and *Burkholderia*, may provide rice with additional N<sub>2</sub>. Some of these bacteria can also create phytohormones that promote plant development and/or antagonists that prevent soil-borne plant diseases and fix nitrogen dioxide. It has been demonstrated that diazotrophs, which effectively colonize the root endosphere, fix nitrogen. Diazotrophic bacteria can fix N<sub>2</sub> in a form that plants can utilize (ammonium). Inoculating crops with these bacteria can supplement or even replace the need for synthetic nitrogen fertilizers, reducing production costs and minimizing environmental pollution from nitrogen runoff.

Microscopic inspection revealed that gus-tagged *Achromobacter xyloxidans* had colonized the vascular zones and intercellular spaces of roots. The researchers observed a noteworthy rise in the length of shoots and roots, weight, and content of chlorophyll-a of rice plants upon inoculation with gus-tagged WM234C-3. The cross-infection ability and characteristics that promote plant growth indicated that, following a thorough and critical pathogenicity test, endophytic bacteria can be used as agricultural agents for several crops.

The impact of PGPR on field grownup maize yield, seed germination, and seedling growth was investigated by Gholami et al. *Pseudomonas putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* strain DSM-50090, *P. putida* strain DSM291, *A. lipoferum* DSM1691, and *A. brasilense* DSM 1690 were the bacterial strains utilized in the experiment (Gholami et al. 2009). According to the findings, maize seed inoculation greatly improved seed germination and seedling vigor.

In their study, Mirza et al. (2006) explored the consequences of inoculating two rice types, Super Basmati and Basmati 385, with the K1 strain of the genus *Pseudomonas* (Mirza et al. 2006). They then compared the results to those of three non-*Pseudomonas* N<sub>2</sub>-fixing PGPR, namely *Azospirillum lipoferum* strain N4, *Azospirillum brasilense* strain Wb3, and *Zoogloea* strain Ky1. They stated that in every infected treatment, there was a greater shoot biomass



and/or grain production for both types of rice compared to the noninoculated control plants. For both rice types, the impact of *Pseudomonas* strain K1 on grain yield was similar to that of *Zoogloea* sp. Ky1 and *A. brasilense* Wb3. These findings demonstrate the value of investigating N<sub>2</sub>-fixing pseudomonads as possible PGPR inoculants for rice. Feng et al. also examined how the rice endophyte *Pantoea agglomerans* YS19 affected photosynthetic allocation and host plant development (Feng et al. 2006). They found that endophyte YS19 stimulates photosynthetic allocation and influences host plant growth. Inoculating endophytic diazotroph *Pantoea agglomerans* YS19 increased the biomass of the host rice seedlings grown for 12 days, by 18.7% on N<sub>2</sub>-supplemented medium or by 63.4% on N<sub>2</sub>-free media. YS19 cell culture sprayed on rice during the premilk phase greatly improved the product of photosynthetic absorption. Applying YS19 cell culture during the late milk stage prevented the formation of the plant sink.

Tran Vân et al. found that the inoculation of rice with *Burkholderia vietnamiensis* caused in a considerable upsurge in grain yields, reaching up to 8 t ha<sup>-1</sup> (Tran Vân et al. 2000). It was discovered that this strain may save 25–30 kgN ha<sup>-1</sup>. In greenhouse research by Mirza et al., inoculating rice with *Herbaspirillum* resulted in a considerable increase in production, reaching 7.5 g plant<sup>-1</sup> (Mirza et al. 2000). They researchers measured BNF in super basmati and basmati rice by means of various *Herbaspirillum* strains. For basmati and super basmati, the %N values were 19.5–38.7 and 38.1–58.2, respectively.

Sharma et al. (2010) investigated a study in the summers of 2008 and 2009 to examine the effects of four NPK fertiliser levels combined with varying concentrations of biofertilizers (*Azotobacter* and PSB) on the development and production of the hybrid cauliflower variety known as "Swati" (Sharma and Sharma 2010). Both alone and in combination, the application of biofertilizers improved height of plant, number of leaves/plants, gross weight/plant, curd depth, curd diameter, curd yield, and profit cost ratio. When 100% NPK + seedlings dipping of both inoculants were added to the plots, the highest commercial output (247 q/ha) was detected. Additionally, the yield (238.66 q/ha) attained by applying 75% NPK + *Azotobacter* + PSB was found to be equivalent to that of 100% prescribed NPK, ensuing in a savings of 25% NPK fertilisers with the highest profit cost ratio.

It was observed that the performance of phosphate solubilising bacteria in increasing the development and production of sunflowers (*Helianthus annuus* L.) in the presence of phosphorous fertiliser (Zehra 2010). The intention of this analysis was to assess the result of the PSB, *Bacillus* M-13, with and without variable quantities of phosphorous fertiliser on the development and production of sunflowers in field circumstances. The PSB mobilised phosphorous proficiently in the sunflower and enhanced quality of seed and oil production. Furthermore, when PSB was employed in combination with phosphorous fertilisers, a superior result was observed.

Inoculation of diazotrophic bacteria offers a promising strategy for improving crop productivity, reducing environmental impacts, and promoting sustainable agricultural

development. Moreover, the success of inoculation depends on aspects, for example, the selection of effective bacterial strains, proper application methods, and environmental conditions conducive to bacterial colonization and nitrogen fixation.

### Molecular characterization of diazotrophs

Molecular characterization of diazotrophs involves the use of numerous molecular biology methods to recognize, classify, and analysis the genetic diversity, phylogeny, and functional genes related with N<sub>2</sub>-fixing bacteria (Liu et al. 2017). These techniques provide insights into the taxonomic composition, ecological roles, and N<sub>2</sub>-fixation potential of diazotrophic communities in different environments. The discovery of diazotrophs in rice paddy field soil has, up to this point, primarily been accomplished through cultivation-based study, which is followed by morphological and/or genetic recognition of specific isolates. Molecular biological approaches have provided considerable support for identifying and classifying of bacterial isolates in recent times. After the invention of *nif* gene cloning and DNA sequencing amplification by polymerase chain reaction (PCR), an alternate method of identifying nitrogen fixers gained popularity. As a result, *nif* DNA was found in hypothetical nitrogen-fixing isolates and was established by PCR amplification and nucleotide sequencing of the amplified sequence.

Zehr and McReynolds (1989) created a set of universal *nifH* primers, among other oligonucleotides, to amplify *nifH* remains from ecological materials (Zehr and McReynolds 1989). This is significant for ecological investigations of N<sub>2</sub>-fixers in rhizosphere soil. Another benefit is that it allows one to evaluate the variety of bacteria while accounting for the non-culturable population, all without isolating strains. Utilizing conserved 16S rRNA gene sequences, strain-specific probes were also created to track populations of specific nitrogen-fixing species in environmental samples.

Noteworthy progresses have been attained in the targeted identification of bacteria using rRNA-targeted oligonucleotide probes. These probes can target regions of the 23S or 16S rDNA with highly variable sequences. Numerous phylogenetic oligonucleotide probes were created for *Burkholderia sp.*, *Acetobacter diazotrophicus*, *Azoarcus*, *Herbaspirillum spp.*, and *Azospirillum spp.* One of the earliest genes still in existence and active in the annals of gene evolution is the nitrogenase iron protein gene (*nifH*). The contours of the *nifH* tree are said to be substantially in line with the 16S rRNA phylogeny.

N<sub>2</sub>-fixing heterotrophic bacteria are linked to fixation of N<sub>2</sub> in the rice root region. However, because it is generally accepted that only a tiny proportion of naturally occurring prokaryotes may indeed be culturable, investigations on the rhizospheric nitrogen fixing microflora have, up till now, suffered from the usage of specific media for counts and isolation.

It is now feasible to depict both non-culturable and culturable bacteria thanks to the progress of novel molecular methods and tactics like rRNA sequencing, FISH (fluorescence in situ hybridization), random amplified polymorphic DNA (RAPD), DGGE (denaturing

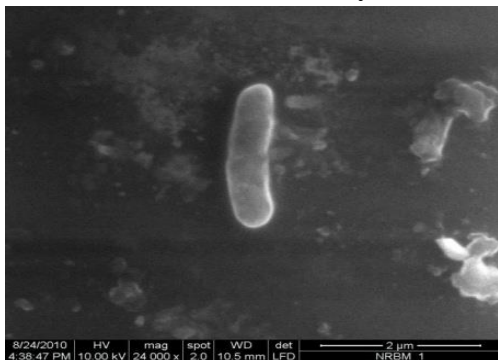
gradient gel electrophoresis), TGGE (temperature gradient gel electrophoresis), and SSCP (single strand conformation polymorphism) (Dubey et al. 2006). But little is known about the intricate web of interactions that makes up life in the rhizosphere of crops, and its revelation is anticipated to unveil many previously unknown truths.

Six *Burkholderia* isolates were isolated by Roy et al. (2013) from rice rhizosphere soils in South Assam, India's tropical lowlands. SDSA-I10/1, one of the identified *Burkholderia* isolates, was chosen for 16S rDNA sequencing due to its increased nitrogen fixing capacity (Roy et al. 2013). *Burkholderia caribensis* strain SDSA-I10/1 (GU372342) was isolated from the isolate SDSA-I10/1, which exhibited the strongest similarity to *Burkholderia caribensis* MWAP84 (Y17011). This strain can be employed as an indigenous microbial inoculant for exhaustive rice harvesting in tropical lowlands since it considerably increases the development and production characteristics of rice when injected than uninoculated control plants.

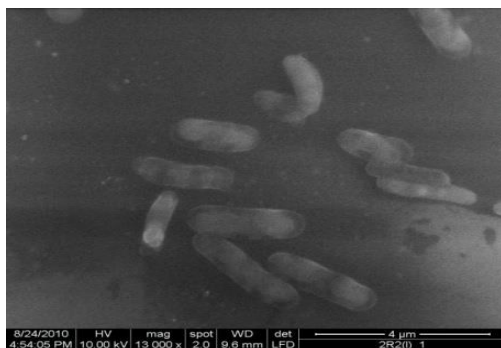
Shu et al. employed DGGE) and qPCR techniques to evaluate the molecular diversity and extent of *nifH* gene sequences in bulk paddy soil and rhizosphere, respectively, in orthodox administration and varying durations of organic compounds (2, 3, 5, 9 years) (Shu et al. 2012). Based on the *nifH* gene sequence, the phylogenetic distribution of clones revealed that the taxonomic groups comprised Betaproteobacteria (24.1%), Alphaproteobacteria (27.6%), and Gammaproteobacteria (48.3%). The majority of the diazotrophs were members of the orders Rhizobiales and Pseudomonadales. Using qPCR, they quantified the amount of *nifH* gene sequences in the soil;  $2.27 \times 10^5$  to  $1.14 \times 10^6$  copies/g were found. They discovered that *nifH* gene numbers in organic soil, both rhizosphere and bulk, were substantially greater compared to CM soil, except two years of organically managed soil. Furthermore, compared to bulk soil, the organic rhizosphere soil (3, 5, and 9 years) had noticeably greater *nifH* gene copy numbers. Shahi et al. (2011) used conventional microbiological and biochemical approaches to isolate and screen 114 diazotrophic bacteria from the rice rhizosphere of 5 districts in Eastern Uttar Pradesh, India, for PGP actions (Shahi et al. 2011). Amplified ribosomal DNA restriction analysis (ARDRA) and DGGE were used to analyze the molecular diversity of the isolates, and the results showed clear variations between each isolate. Twenty-one (21) isolates out of the 114 displayed numerous features that promoted plant development and were very active in PGP. Based on 16S rDNA sequencing, these isolates were identified, and they belonged to the following genera: *g*-proteobacterium strain VA3S1, *Rhizobium*, *Agrobacterium*, *Pantoea*, *Bacillus*, *Microbacterium*, *Sphingomonas*, *Pseudomonas*, *Enterobacter*, *Advenella*, *Ancylobacter*, and *Microbacterium*.

Roesch et al. examined the effects of two genotypes of maize, both with and without N<sub>2</sub>-fertilizer, as well as the size of the population of diazotrophic bacteria that are certainly linked with the maize rhizosphere (Roesch et al. 2006). Using two cultivars of maize (Santa Helena 8447 and Santa Rosa 3063), the population dynamics and distribution of diazotrophic bacteria related with maize over several plant ontogenic phases were also investigated. They

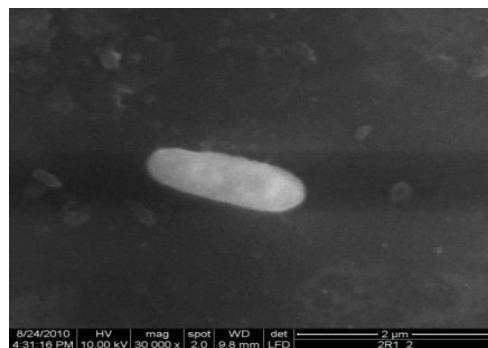
employed molecular and microbiological methods to describe the population structure of diazotrophic bacteria. The most likely number of bacteria was determined by means of semi-solid N<sub>2</sub>-free media. Additionally, DNA was extracted from soil and plant tissue and amplified using nested PCR for *nifH* gene fragments, and restriction endonucleases TaqI and HaeIII were used for RFLP analysis.



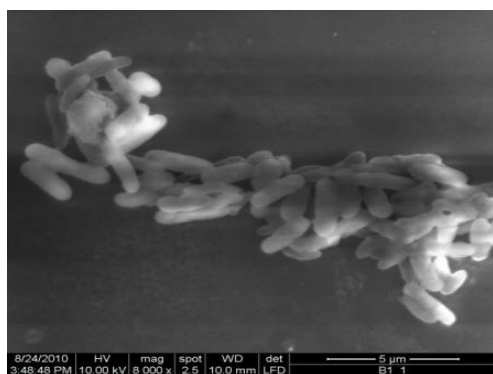
*Sphingomonas azotifigens.*



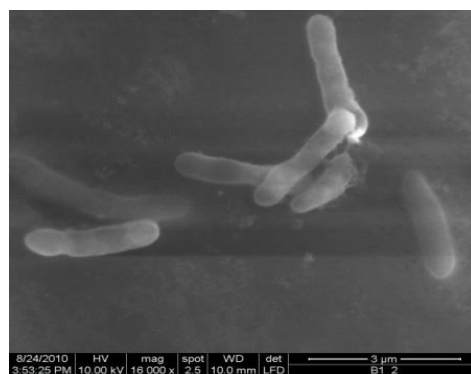
*Stenotrophomonas maltophila.*



*Pseudomonas putida.*



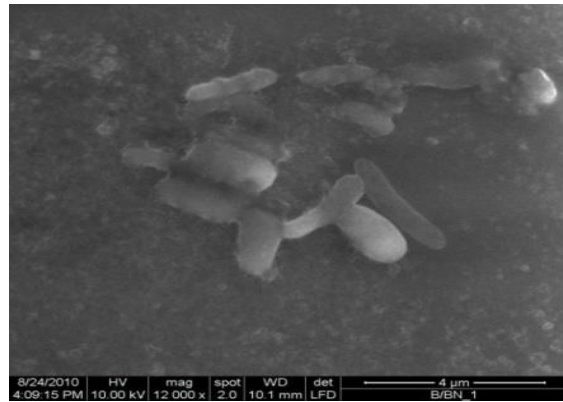
*Herbispirillum sp.*



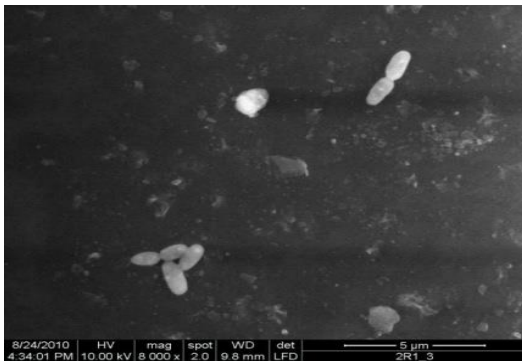
*Herbispirillum rubrisubalbicans.*



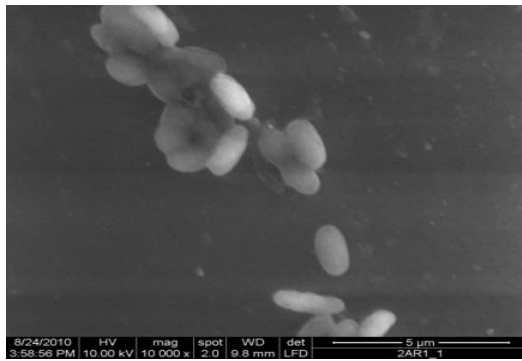
*Acinetobacter radioresistans.*



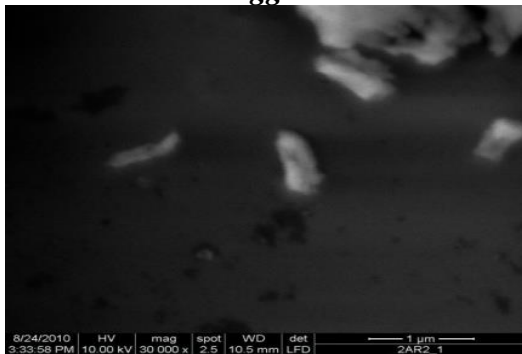
*Enterobacter cloacae subsp. dissolvens.*



*Pantoea agglomerans.*



*Achromobacter xyloxidans.*



*Alcaligenes faecalis*



*Klebsiella pneumoniae*

**Figure 1. Electron micrographs diazotrophs**

By employing molecular techniques, researchers can gain a deeper comprehending of the diversity, ecology, and functional potential of diazotrophic bacteria in various ecosystems, paving the way for the progress of novel approaches for crop improvement, sustainable agriculture, and environmental management.

### **Challenges and Future Perspective:**

Despite the immense potential of PGPR, there are still challenges, such as regulatory hurdles, market acceptance, and widespread adoption. Addressing these challenges will be decisive for understanding the full prospective of PGPR in sustainable agriculture. Different PGPR strains have varying effects on different plant species. Identifying and selecting the most effective strains for specific crops and soil conditions remains a challenge. Furthermore, developing cost-effective methods for large-scale production and formulating PGPR into user-friendly biofertilizers is crucial for practical application. Indeed, environmental factors can impact the efficiency of PGPR in the field. A deeper consideration of the complex interactions between PGPR, plants, and the surrounding microbiome is necessary to optimize their beneficial effects.

Studying the composition and interactions of the entire rhizosphere microbial community will provide insights for exploring the synergistic effects of PGPR with other beneficial microbes. Screening for PGPR with unique and potent plant growth-promoting traits from diverse environments remains a promising avenue. Genetic modification could potentially improve specific functionalities of PGPR strains, such as nitrogen fixation or stress tolerance. Advances in biotechnology offer opportunities to enhance the effectiveness of PGPR. This includes genetic engineering to improve traits such as stress tolerance, nutrient utilization, and colonization ability. Moreover, developing efficient methods for applying PGPR to crops and integrating them into existing agricultural practices is essential for wider adoption. By addressing these challenges and exploring future directions, researchers can continue to advance our understanding of PGPR and unlock their full potential for sustainable agriculture.

### **Conclusion:**

In conclusion, the study of PGPR offers promising intuitions into sustainable agricultural development. The book chapter shows that PGPR play fundamental roles in increasing plant growth, enhancing nutrient uptake, and conferring resistance against pathogens. Their multifaceted mechanisms, for example, N<sub>2</sub>-fixation, phosphate solubilization, and generation of phytohormones, underscore their significance in agricultural ecosystems. Furthermore, the symbiotic connection between plants and PGPR highlights the potential for these bacteria to be biofertilizers and biocontrol agents, reducing reliance on chemical inputs and mitigating environmental degradation. However, despite considerable advancements in understanding PGPR functions, challenges remain in optimizing their application, ensuring efficacy across different environmental conditions, and scaling up their use in commercial agriculture. Moving forward, interdisciplinary research efforts encompassing microbiology, plant biology, and agronomy will be critical for unlocking the potential of PGPR in sustainable agriculture, ultimately contributing to global food security and environmental sustainability. Further exploring PGPR diversity and formulation strategies will be crucial for maximizing their beneficial effects and fostering a more sustainable and productive agricultural landscape.

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