Isolation of Plant Growth-Promoting Rhizobacteria and Its Correlation with Soil Physio-Chemical Properties

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Abstract

PGPR belongs to the rhizospheric bacterial species that promote plant growth through various mechanisms, e.g., phosphate solubilizing, nitrogen fixation, siderophore synthesis for effective iron uptake, and many similar responsibilities that encourage plant growth. The average bacterial density ranged from 10^5 to 10^7 CFU/gram soil from different sampling sites. Our findings revealed that the Bulk Density, pH, CEC, EC, OC, and Soil Moisture Content were found more in the organic agriculture field. The physicochemical properties of soil revealed that the Bulk Density, pH, CEC, EC, OC, and Soil Moisture Content were found more in the organic agriculture field. In contrast, Nitrogen, P_2O_5, and K_2O were measured at higher concentrations in the chemical agriculture field. PGPR also play a crucial role in enhancing soil's physicochemical properties, vital for maintaining soil health, fertility, and sustainable agricultural productivity. The PGPR bacteria are integral to sustainable agriculture, as they reduce the reliance on chemical fertilizers and pesticides, stimulate plant growth, and contribute to long-term soil fertility and productivity.

Keywords: *PGPR, rhizospheric bacteria, sustainable agricultural, soil health, physicochemical properties*

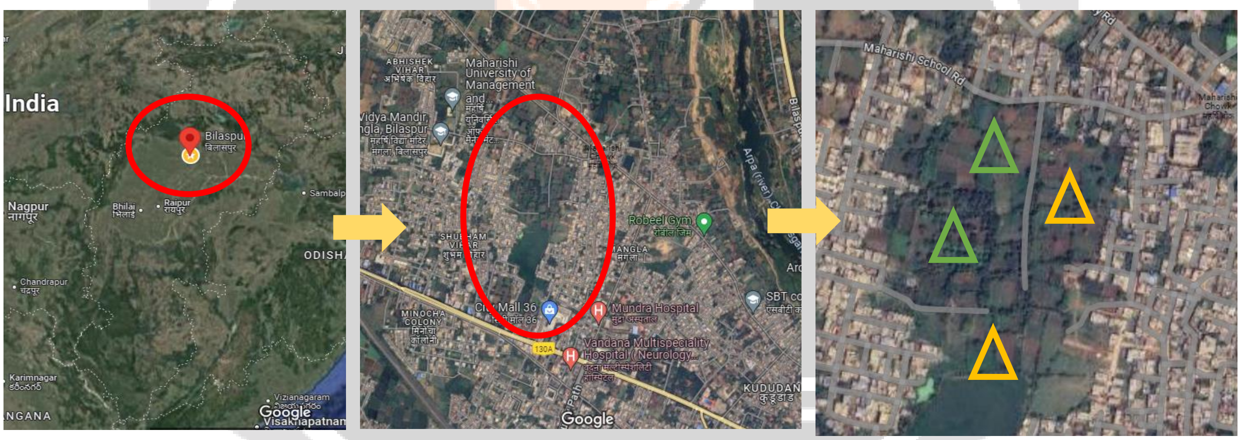
Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) positively influence plant growth and development by colonizing symbiotically in the plant root system (Kloepper & Beauchamp, 1992). PGPR can enhance crop growth, improve nutrient availability, help to tolerate abiotic stresses (e.g., drought and salinity), and suppress plant pathogens. PGPR can synthesize and secrete plant hormones such as auxins, gibberellins, and cytokinins, which are crucial for plant growth, development, and stress response. For example, auxins promote root development and cell division, whereas gibberellins stimulate stem elongation and germination (Ahmad et al., 2017). Phosphate-soluble Bacteria (PSB) transform insoluble phosphate (Pi) to soluble phosphate (Ps) and regulate the biogeochemical cycling of phosphate in the soil system (Sperber, 1957). PSB enhances soils by facilitating nutrient availability. PSB, e.g., *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., and *Escherichia* sp., serve as the largest microbial communities capable of solubilization of phosphate in soil (Tian et al., 2021). PGPR conquers plant pathogens through various mechanisms, including competition for nutrients, production of antimicrobial compounds, and induced systemic resistance (ISR) in the plant. ISR involves activating plant defence mechanisms in response to PGPR colonization (Van Loon et al., 1998). Effective PGPR-based biofertilizers and biocontrol agents are vital for sustainable agriculture.

Soil physiochemical properties, such as pH, organic matter content, nutrient availability, and soil texture, significantly influence the efficiency of PGPR. Evaluating the correlation between PGPR and soil properties is essential for optimizing agricultural practices and improving crop yields. Soil pH usually influences the solubility of nutrients, microbial activity, and the overall structure of soil microbial communities. Research has shown that PGPR bacteria, Bacillus and *Pseudomonas* sp., thrive in neutral to slightly alkaline soils (Naseem et al., 2018). Extreme pH levels often reduce plant growth by inhibiting PGPR activity. High organic matter content enhances the richness and diversity of PGPR (Egamberdieva et al., 2015). Besides, the organic matter creates a favorable environment for PGPR by improving soil structure, enhancing water retention, facilitating cation exchange capacity, and ensuring nutrient availability. PGPR, e.g., *Azospirillum* and *Rhizobium*, are well-studied in fixing atmospheric nitrogen for plants in nitrogen-deficient soils (Bhattacharyya & Jha, 2012). Soil texture influences the distribution and activity of PGPR. Loamy soils have a higher density and diversity of PGPR because they have a favorable balance of aeration, water retention, and nutrient availability (Marschner et al., 2011). The clayey soil has low humus and an anaerobic environment) while sandy soils have high water retention. Therefore, the present research focused on isolating Plant Growth-Promoting Rhizobacteria and assessing their correlation with the physiochemical properties of Soil towards sustainable agriculture.

Materials and Methods

The research was done to isolate the PGPR from the rhizospheric soil and assess their correlation with the soil's physiochemical properties in the interest of sustainable agriculture. The soil samples were collected from the Agriculture field of the Mangla area (Geographical coordinate: 22.101206482827983, 82.12481191569194) located near Bilaspur City.



Bilaspur, Chhattisgarh **Mangla Area, Bilaspur City** **Sampling Site**

Fig. 1. Details of Sampling Sites of Mangla Area, Bilaspur Chhattisgarh, India

△ **Organic agriculture Field** △ **Chemical agriculture Fields**

Isolation of bacteria

The bacterial strains were isolated from the collected soil samples, and serial dilution was followed. They were further used to isolate bacterial colonies. The Nutrient agar media (NAM) was prepared and autoclaved at 121°C for 15 minutes for sterilization (Atlas, 2010), and aseptically, the NAM plates were prepared. A 0.5 ml of serially diluted 10^{-5} dilution) the sample was then spread over NAM plates and incubated at 37ºC for 48 h. After incubation, the inoculated NAM plates were observed for bacterial colonies. Later, the pure culture of each bacterial colony was prepared by the streak plate method (Cappuccino & Sherman, 2005) and incubated at 37ºC for 48 h.

Colony characterization

The incubated pure cultures were further examined for colony morphology, including color, shape, surface texture, and opacity (Cappuccino & Sherman, 2005).

Microscopic characterization

The gram staining and motility test were performed, as documented in Aneja (2003).

Biochemical Characterization

The Catalase, Oxidase, and Indole Production test was done per the protocol mentioned in Aneja (2003). The PSB bacteria were screened using a Pikovskayas agar medium (Karpagam & Nagalakshmi, 2014). The colonies with clear zones around bacterial colonies are considered positive for PSB.

Physicochemical analysis of Soil

The pH, Electrical Conductivity (EC), Organic Carbon (OC), Nitrogen (N), Phosphorus (P_2O_5), Potassium (K_2O), Soil Texture, Bulk Density, Cation Exchange Capacity (CEC), Soil Moisture Content was measured as mentioned in the manual of Department of Agriculture & Cooperation, Ministry of Agriculture, India (2011).

All the experiments were carried out in triplicates, and the standard deviation was calculated using the M.S. Office 2021.

Results and Discussion

A PGPR bacteria was isolated from the soil samples, and the soil's physiochemical properties were assessed to correlate soil properties and the diversity of PGPR in the agricultural fields. The bacterial density was recorded at an average of 10^5 to 10^7 CFU/gram soil from different sampling sites. The Colony and Biochemical Characteristics exhibited *Bacillus* sp., *Pseudomonas* sp., *Azospirillum* sp., *Rhizobium* sp., and *Enterobacter* sp. in the samples (Table 1). Further correlation with the standard bacterial identification key revealed that the aforementioned bacterial genera were close to *Bacillus subtilis*, *Pseudomonas fluorescens*, *Azospirillum brasilense*, *Rhizobium leguminosarum*, and *Enterobacter cloacae*, respectively. In the present work, *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp. were observed as dominant PGPR bacteria genera in the Mangla area of Bilaspur, Chhattisgarh. Karpagam and Nagalakshmi (2014) reported that *Pseudomonas*, *Rhizobium*, and *Bacillus* genera are in rhizospheric soil. The literature divulged that *Bacillus* sp. and *Pseudomonas* sp. participated in phosphate solubilization (Qingwei et al., 2023; Janati et al., 2022), while recently, Deb & Tatung (2024), the *Enterobacter* sp. and *Pseudomonas* sp. have been stated as the most dominant siderophore-producing genera. Further, *Rhizobium* sp. and *Azospirillum* sp. have been documented as the dominant nitrogen-fixing bacterial genus (Widawati & Suliasih, 2001). In the present investigation, the density of PGPR-producing bacteria was higher in organic agriculture fields (10^7 CFU/gram soil) than in chemical agriculture fields (10^5 CFU/gram soil). In comparison, Chandna et al. (2013) revealed 10^5 to 10^9 CFU/ gram soil in composting agricultural byproducts.

The physicochemical properties of soil of agriculture fields were examined. Our findings revealed that the Bulk Density, pH, CEC, EC, OC, and Soil Moisture Content were found more in the organic agriculture field (Table 2; Fig.1; Fig. 2) because a higher density of PGPR increases soil texture and organic carbon nutrient bioavailability, supplies hormones, and synthesized growth factors for crops. In contrast, Nitrogen, P_2O_5, and K_2O were measured at higher concentrations in the chemical agriculture field (Table 2; Fig. 2) in the present study because chemical fertilizers often serve Nitrogen, P_2O_5, and K_2O. PGPR plays a complex role in enhancing the physicochemical profile of soil by improving nutrient availability, enriching soil structure, regulating pH, increasing organic carbon content, and improving overall soil health.

Table 1: Colony, Microscopic and Biochemical Characteristics of isolated bacterial strains having potential as PGPR

S. No.	Colony, Microscopic and Biochemical Characteristics	*Bacillus sp.*	*Pseudomonas sp.*	*Azospirillum sp.*	*Rhizobium sp.*	*Enterobacter sp.*
1.	Colony Morphology	Creamy, irregular, dry, wrinkled	Greenish, circular, smooth, shiny	Pinkish, irregular, mucilaginous	White, circular, translucent	Creamy, circular, smooth, shiny
2.	Gram Staining	+	-	-	-	-
3.	Catalase Test	+	+	+	-	+
4.	Oxidase Test	-	+	+	+	+
5.	Motility	Motile	Motile	Motile	Motile	Motile
6.	Indole Production	-	-	-	-	+
7.	Phosphate Solubilization	+	+	-	-	-
8.	Siderophore Production	+	+	-	+	+

Table 2: Physicochemical Properties of Organic Manure and Chemically Fertilized Soils

Parameters	**Organic Manure Fertilized Soil (Calculated value** ±SD)	**Chemically Fertilized Soil (Calculated value** ±SD)
pH	6.9 ±0.17	6.3 ±0.12
EC (dS/m)	0.52 ±0.02	0.67 ±0.04
OC (%)	0.94 ±0.05	0.56 ±0.03
Nitrogen (kg/ha)	353 ±4.7	441 ±5.2
P_2O_5 (kg/ha)	58 ±2.1	68 ±2.5
K_2O (kg/ha)	325 ±3.8	363 ±4.4
Bulk Density (g/cm^3)	1.27 ±0.21	1.42 ±0.32
CEC (cmol/kg)	26.8 ±1.5	19.6 ±1.1
Soil Moisture Content (%)	21.2 ±1.2	17.4 ± 0.9

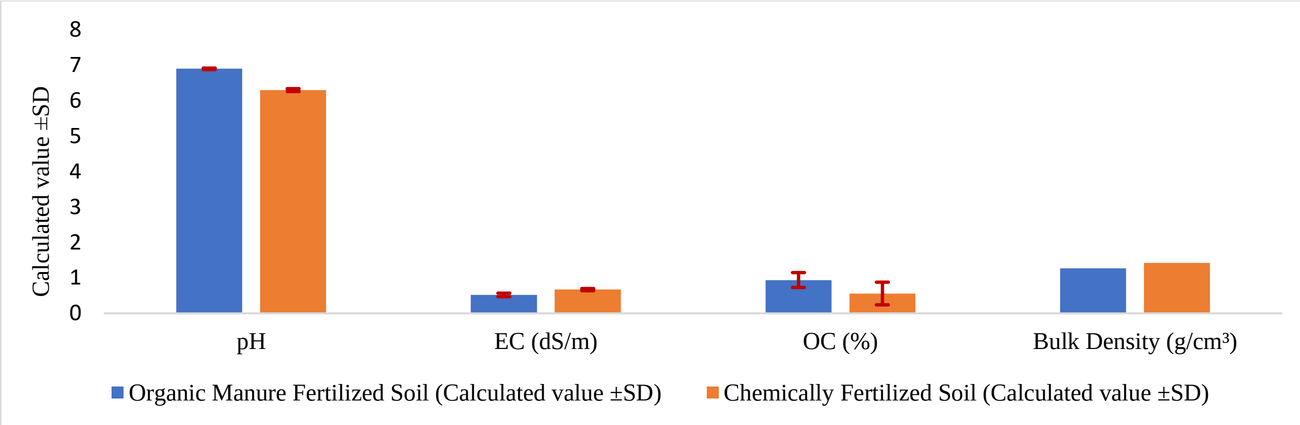


Fig. 1: A comparative account of pH, EC, OC, and Bulk Density of organic manure and chemically fertilized soil

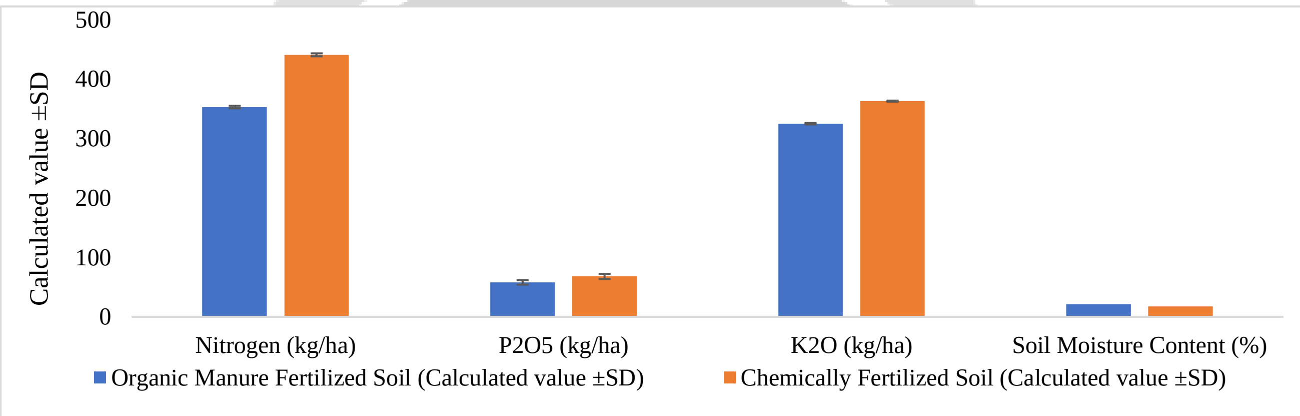


Fig. 2: A comparative account of Nitrogen, P_2O_5, K_2O, and Soil Moisture Content of organic manure and chemically fertilized soil

Conclusion

PGPR is pivotal in sustainable agriculture by enhancing crop productivity and soil health. These beneficial bacteria subsidize nutrient cycling, enhancing soil structure, pH regulation, and accumulating organic carbon. PGPR diminishes the dependency on chemical fertilizers and promotes eco-friendly farming practices. Moreover, PGPR contributes to soil structure by producing exopolysaccharides and decomposing organic matter, which enhances soil aggregation, water retention, and aeration. PGPR also can suppress soil-borne pathogens. In addition, PGPR also maintains the diversity of beneficial bacteria in soil and stimulates the overall physiological properties of the soil. The effective integration of PGPR into agricultural practices offers a promising pathway towards achieving higher crop yields, improved soil fertility, and reduced environmental impact, ultimately towards the sustainability and resilience of agricultural ecosystems.

References

Ahmad, I., Zahir, A., & Ashraf, M. (2017). Role of plant growth-promoting rhizobacteria (PGPR) in improving plant tolerance to abiotic stress. *Environmental Science and Pollution Research*, 24(14), 13887-13909.

Aneja, K.R. (2003). Experiments in Microbiology, Pant Pathology and Biotechnology. 4th Ed. *New Age International Limited Publisher, New Delhi*, India.

Atlas, R. M. (2010). Handbook of Microbiological Media. 4th ed. *CRC Press*.

Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*(4), 1327-1350.

Cappuccino, J. G., & Sherman, N. (2005). Microbiology: A Laboratory Manual. 7th ed. *Pearson/Benjamin Cummings*.

Chandna, P., Nain, L., Singh, S., & Kuhad, R.C. (2013). Assessment of bacterial diversity during composting of agricultural byproducts. *BMC Microbiology*, 13 (1). https://doi.org/10.1186/1471-2180-13-99

Deb, C. R., & Tatung, M. (2024). Siderophore producing bacteria as a biocontrol agent against phytopathogens for a better environment: A review. *South African Journal of Botany*, 165, 153–162. https://doi.org/10.1016/j.sajb.2023.12.031

Department of Agriculture & Cooperation, Ministry of Agriculture, India (2011). Soil testing manual. Accession Dated - 2-10-2023. (https://agriculture.uk.gov.in/files/Soil_Testing_Method_by_Govt_of_India.pdf)

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., & Hashem, A. (2015). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 6, 521.

Janati W, Mikou K, El Ghadraoui L, & Errachidi F. (2022). Isolation and characterization of phosphate solubilizing bacteria naturally colonizing legumes rhizosphere in Morocco. *Front Microbiol*., 26(13), 958300. https://doi.org/10.3389/fmicb.2022.958300

Karpagam T. and Nagalakshmi, P.K. (2014). Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil. *Int. J. Curr. Microbiol. App. Sci.*, 3(3), 601-614. https://www.ijcmas.com/vol-3-3/T.Karpagam%20and%20P.%20K.%20Nagalakshmi.pdf

Kloepper, J.W. & Beauchamp, C.P. (1992). Plant growth-promoting rhizobacteria: A review. *Advances in Agronomy*, 48, 133-161.

Marschner, P., Crowley, D., & Yang, C. H. (2011). Development of specific rhizosphere bacterial communities concerning plant species, nutrition and soil type. *Plant and Soil*, 340(1-2), 1-16.

Naseem, H., Ahsan, M., & Mohsin, M. (2018). Role of plant growth-promoting rhizobacteria in agriculture. *Microbiome in Plant Health and Disease*, 1-22.

Qingwei, Z., Lushi, T., Yu, Z., Yu, S., Wanting, W., Jiangchuan, W., Xiaolei, D., Xuejiao, H., & Bilal, M. (2023). Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere of poplar on road verge and their antagonistic potential against various phytopathogens. In BMC Microbiology (Vol. 23, Issue 1). https://doi.org/10.1186/s12866-023-02953-3

Rousk, J., Brookes, P.C., & Bååth, E. (2010). The microbial PLFA composition as affected by pH in an arable soil. *Soil Biology and Biochemistry*, 42(3), 516-520.

Sperber, J.I. (1957). Solution of mineral phosphate by soil bacteria. *Nature*, 180, 994-995. https://doi.org/10.1038/180994a0

Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X. (2021). Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology (Basel)*, 10(2), 158. https://doi.org/10.3390/biology10020158

Van Loon, L.C., Bakker, P.A.H.M., & Pieterse, C.M.J. (1998). Systemic induced resistance by rhizosphere microorganisms. *Trends in Plant Science*, 3(12), 443-448.

Widawati, S. & Suliasih (2001). Biodiversity of Mount Halimun National Park. *Biology News*, 5 (6).