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RESEARCH

In-Vitro Growth Pattern Estimation of Chickpea (*Cicer arietinum* L.) using Plant Growth Promoting Rhizobacteria (PGPR)

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Abstract

Nutrient deficiency in the agricultural field gives rise to a challenge for the production of grains at the global level. The utilization of chemical fertilizers to boost crop yield in the present causes detrimental effects on the health of biological communities, including the human population. Therefore, the present research has been designed to isolate two strains, i.e., *Pseudomonas* and *Azotobacter,* from the rhizospheric soil of *Cajanus cajan* (pigeon pea). Both strains of the PGPRs were characterized by performing morphological and biochemical analysis. The inoculation of isolated PGPR bacteria and their effect on *Cicer arietinum* (chickpea) were conducted under a plant growth chamber where *C. arietinum* plants were grown in three cups containing autoclaved soil, and each cup was marked for *Pseudomonas*, *Azotobacter,* and one for control. Separately, *Pseudomonas*, *Azotobacter* cultures were inoculated in two flasks containing 250 ml autoclaved distilled water, and one was used as a control. Incubate the inoculated flasks at 37℃ for three days. Each 50ml suspension was added to each marked cup after a three-day interval. Observed the efficacy of inoculation with PGPR strains separately. In the present study, the root and shoot length of the *C. arietinum* plant in the presence of bacterial strains were studied. The maximum growth occurs in the *Pseudomonas*-treated cup in comparison to the *Azotobacter*. The growth of the *C. arietinum* plant occurs due to the plant growth-promoting activity of these bacteria. Therefore, bacterial inoculation should be an effective biofertilizer for the growth of *C. arietinum*.

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Statement of Sustainability: In the present study, the use of plant growth-promoting rhizobacteria (PGPR) for crop cultivation aligns with sustainable agriculture goals by reducing the environmental impacts of chemical fertilizers that improve the soil health and productivity, along with plant growth.

1. Introduction

Biofertilizers are eco-friendly natural manures that contain living microorganisms. It provides primary nutrients to the plant and promotes its growth (Ammar et al., 2023). Living microorganisms naturally provide the nutrients to the plant by the processes of nitrogen fixation, phosphate solubilization, and stimulating plant growth through the synthesis of phytohormones (Timofeeva et al., 2023). With increasing use of microbes as biofertilizers reduces the need for chemical fertilizers that cause detrimental effects on the health of biological communities, including the human population. To overcome these problems by using biofertilizers, which are alternative and eco-friendly sources of nitrogen and other nutrients for plants. Biofertilizer has great importance in reducing the environmental pollution of chemical fertilizers (Ajmal et al., 2018). Cultivation with biofertilizers is one of the interesting methods for the development of sustainable agriculture by using beneficial bacteria that can enhance plant growth in of symbiotic association or as free-living cells in the soil. By using such biofertilizers, farmers can grow healthy plants and enhance

the fertility of the soil by producing organic nutrients or byproducts of microorganisms (Srivastava et al., 2013; Sonam et al., 2024). Because of their growth-promoting nature, researchers preferred a scientific term for such bacteria is "Plant-Growth Promoting Rhizobacteria (PGPR)".

Since biofertilizers do not contain any chemicals that spoil the texture of agricultural soil (Dar et al., 2021). The plant growth-promoting rhizobacteria (PGPR) were first described as soil bacteria that colonize the roots of plants following inoculation onto to seed, and they enhance plant growth (Kloepper and Schroth, 1978). There are several rhizobacteria, such as *Rhizobium*, *Azotobacter*, *Pseudomonas*, *Arthrobacter*, *Azospirillum, Bacillus,* etc., that are used as biofertilizers (Nosheen et al., 2021). Chickpea is one of the major pulse crops of the developing countries like India and other Asian countries (Merga and Haji, 2019). It is an important source of protein for millions of people. In spite of high protein contents, chickpea is rich in fiber, minerals, β-carotene, and a large amount of unsaturated fatty acids (Kumar et al., 2025). Besides playing an important role in the human diet, it also improves soil fertility by fixing the atmospheric nitrogen and is important as food, feed, and fodder (Suman et al., 2022; Verma et al., 2023; Heet et al., 2024). It has great economic importance as a source of protein for both human and animal nutrition. Due to its high content of protein source, the people of developing countries mostly depend on Chickpea.

Keeping in view the importance of Plant-Growth Promoting Rhizobacteria, the present study has been designed to isolate Rhizobial strains from rhizosphere soils of *Cajanus cajan* (pigeon pea) and to evaluate of growth pattern of *Cicer arietinum* (chickpea) by using isolated strains were conducted under in-vitro conditions.

2. Materials and Methods

2.1. Collection of Soil Sample

The rhizosphere soils were collected from cultivated *Cajanus cajan* (Pigeon pea) beside Amrit Sarovar, V.B.S. Purvanchal University, Jaunpur, India, during February 2025 and kept in a sterile bag. At each time of sampling, precautions were taken, and soil samples were kept in a sterile poly bag according to the standard procedure of transportation up to the laboratory.

2.2. Isolation of Bacterial Isolates

The soil samples were dried at room temperature. 1g of the soil sample was taken for serial dilution. 100µl 10^{-4}-10^{-6} dilutions were spread on King's B agar medium (King et al., 1954) containing a Petri plate for *Pseudomonas* and Jenson's agar medium (Norris and Chapman, 1968) for *Azotobacter*. Spread Petri plates were incubated at 37 °C for 24 hrs. The colonies were observed, and the isolated colonies were streaked on nutrient agar plates to obtain a pure culture.

2.3. Morphological and Biochemical Characteristics of PGPR Strains

The obtained pure cultures were identified by morphological and biochemical studies. The identification of isolates obtained in pure culture was based on Gram staining and biochemical characteristics (Cappuccino and Sherman, 1992; Aneja, 2003). The biochemical tests involved catalase, gelatine hydrolysis, Indole production, MR, VP, and Simmons' Citrate utilization.

2.4. PGPR Inoculation Plant Growth Measurement

This experiment was conducted under a plant growth chamber where chickpea plants were grown in three pots containing autoclaved soil, and each pot was marked as PGPR-1, PGPR-2, or control. Healthy seeds of chickpea were surface sterilized with 1% Mercuric chloride solution, then washed with sterile distilled water. For preparation of inoculants, take 250 ml autoclaved distilled water in three 500 ml capacity conical flasks. The bacterial isolates PGPR-1 and PGPR-2 cultures were inoculated into respective flasks along with the control. Incubate the inoculated flasks at 37 °C for three days.

A 50 ml suspension of each culture was added to the cultivated seed in pots after a three-day interval. Observed the efficacy of inoculation with PGPR strains separately after 15 days. The shoot and root length of Chickpea were measured in centimeters. Then the plants were dried in a hot air oven at 65 °C, and the weights were recorded in grams.

2.5. Statistical Analysis

The data was expressed as mean and standard deviation (Mean ± SD) and determined for all the parameters. Pearson correlation analysis was performed between the root and shoot length of treated and control plants, using Microsoft Office Excel (Version 2021).

3. Results and Discussion

3.1. Isolation and Characterization of PGPRs

Two bacterial isolates of plant growth-promoting Rhizobacteria were successfully isolated from the rhizosphere of cultivated *Cajanus cajan* (pigeon pea) beside Amrit Sarovar, V.B.S. Purvanchal University, Jaunpur. The results of isolated bacterial colonies from 10^{-6} dilution of soil samples were shown on King's B agar medium for *Pseudomonas* and Jenson's agar for *Azotobacter* (Figure 1a-c). The streaking of selected colonies on nutrient agar for obtaining a pure culture, which has been used for strain identification and evaluation of growth patterns of chickpea plants (Figure 1d-e). The strains were characterized by morphological and biochemical analysis.

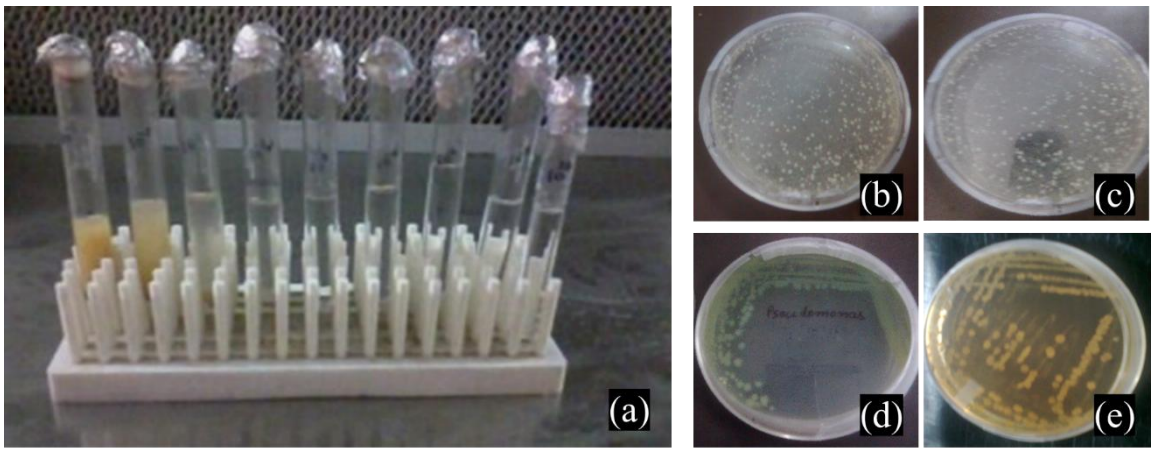

Figure 1. Photographs showing a) Sereal dilution of soil sample; b) PGPR-1 colony; c) PGPR-2 colony; d) PGPR-1 pure culture, and e) PGPR-2 pure culture.

3.2. Morphological Characterization

Based on morphological characters, PGPR-1 showed a circular, convex, opaque, and shiny appearance with greenish color colonies. PGPR-2 oval, flat, large, and can appear as milky white or creamy color colonies. The microscopic observation showed that both strains were of rod shape and Gram-negative.

3.3. Biochemical Characterization

The biochemical characterization of isolated bacterial strains, i.e., PGPR-1 and PGPR-2, was carried out based on different biochemical tests such as catalase, gelatin hydrolysis, IMViC (indole production, MRVP, Citrate). The catalase test was performed by the addition of 3% H_2O_2 bubbles had arisen from both strains. It was shown that both strains were catalase-positive. Gelatin hydrolysis was analyzed in gelatin medium containing tubes. The tubes were inoculated with both bacterial strains, and the results were shown as solid at 4 °C. This solidification of gelatin was shown to be a negative gelatin hydrolysis test. Indole production test was done by using Kovak's reagent, a cherry red color ring appeared in broth containing PGPR-2, hence indole +ve. No ring observation was shown in the broth containing PGPR-1. The presence of a ring was indicated positive test, while the absence of a ring indicated negative indole test. The MR test was performed by the addition of methyl red, at pH 4, the color of PGPR-2 broth was pink or red. The change in color from yellow to pink was indicated MR +ve test, while the PGPR-1 broth showed a yellow color, which was indicated as MR-ve. The VP test was analyzed after the addition of VP reagents; no color change in the VP broth of both strains inoculated tubes was shown VP-ve. The citrate test was performed in Simmons' citrate agar slant. In slants, bacterial growth was visible on the surface, and the medium color was changed from green to blue. These results showed positive tests for both strains. The obtained results were correlated with Bergey's Manual of Systematic Bacteriology to confirm the bacterial isolates as PGPR-1 as *Pseudomonas* and PGPR-2 as *Azotobacter*.

3.4. Estimation of Plant Growth Activities

Estimation of plant growth-promoting activities of PGPR was carried out under a plant growth chamber (Figure 2). Chickpea seeds were grown in a cup containing sterile soil in which an isolated bacterial culture was inoculated. The growth was observed that the PGPR isolates significantly affected the length of chickpea seedlings. Results reveal that the shoot and root length increased in PGPR-treated plants as compared to the control (Figures 3 and 4). It was observed that the highest shoot length (15.70±0.47cm) was recorded in the *Pseudomonas*-treated pot, followed by *Azotobacter* (12.17±0.42 cm) in comparison to the control (7.67±0.38 cm). The *Pseudomonas*-treated pots produced the highest root length (7.70±0.60 cm), followed by *Azotobacter* (6.73±0.11 cm) in comparison to the control (2.77±0.24 cm). The present study suggests that the PGPR isolates, *viz.,* PGPR-1 (*Pseudomonas*), are more effective than PGPR-2 (*Azotobacter*) as biofertilizer.

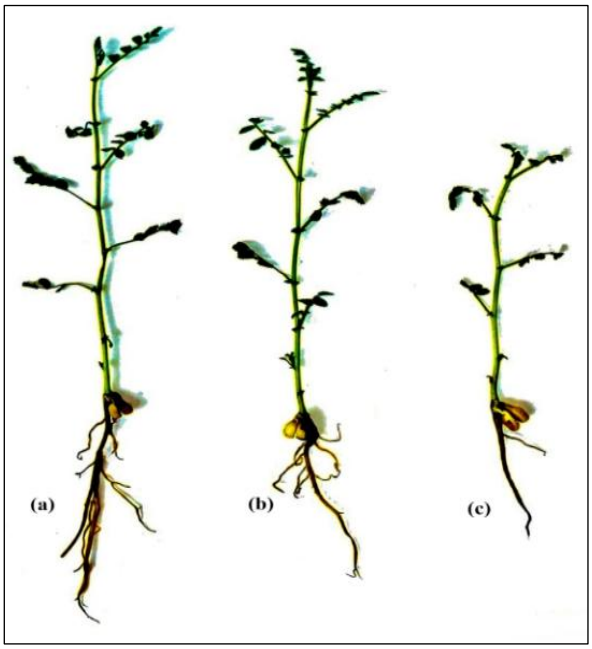

Figure 2. Growth pattern observation of *C. arietinum* plant: (a) PGPR-1; (b) PGPR-2, and (c) Control.

3.4. Pearson Correlation Analysis

A correlation analysis was conducted to examine the relationship between shoot length and root length under three treatment conditions: Control, *Pseudomonas*, and *Azotobacter*. The data consisted of shoot and root lengths (cm) measured across three replicates for each treatment. The Pearson correlation matrix revealed significant differences in the strength and direction of correlations across the treatments. Under the Control condition, a very strong positive correlation (r = 0.99) was observed between shoot and root length, indicating well-coordinated growth of aerial and below-ground parts in untreated plants. This suggests that in the absence of microbial inoculants, shoot and root development progress in a naturally proportional manner. The plants treated with *Pseudomonas* also exhibited a strong positive correlation (r = 0.93) between shoot and root length. Additionally, shoot and root lengths under this treatment were higher in absolute values compared to the control. The high correlation values not only within the treatment but also across treatments (e.g., shoot_*Pseudomonas* vs root_Control: r = 0.93) suggest that *Pseudomonas* may be enhancing overall plant Vigor by facilitating nutrient availability and growth hormone production. This indicates that *Pseudomonas* inoculation supports robust and balanced growth of both shoot and root systems. In contrast, the *Azotobacter*-treated plants showed a strong negative correlation (r = – 0.96) between shoot and root length. This negative association implies a growth imbalance where an increase in shoot length is accompanied by a reduction in root length, or vice versa. Although the average shoot and root values were higher than the control, their inverse relationship indicates a potential trade-off, possibly due to uneven resource allocation or physiological stress responses induced by the treatment (Vacheron et al., 2013). The correlation matrix heatmap (Figure 5) supports these interpretations, with warm color indicating strong positive relationships and cool color representing weak or negative correlations. The *Azotobacter* treatment is clearly distinguished by its inverse relationships compared to the highly correlated control and *Pseudomonas* treatments.

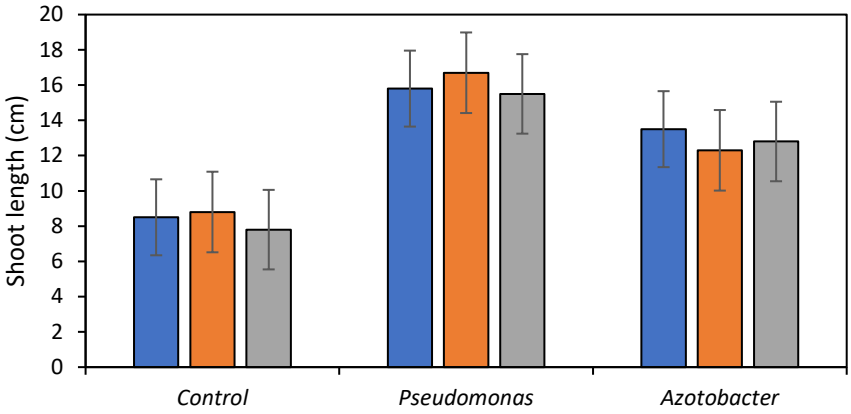

Figure 3. Variation in shoot length (cm) of *C. arietinum* on inoculation of *Pseudomonas* and *Azotobacter* culture along with the control.

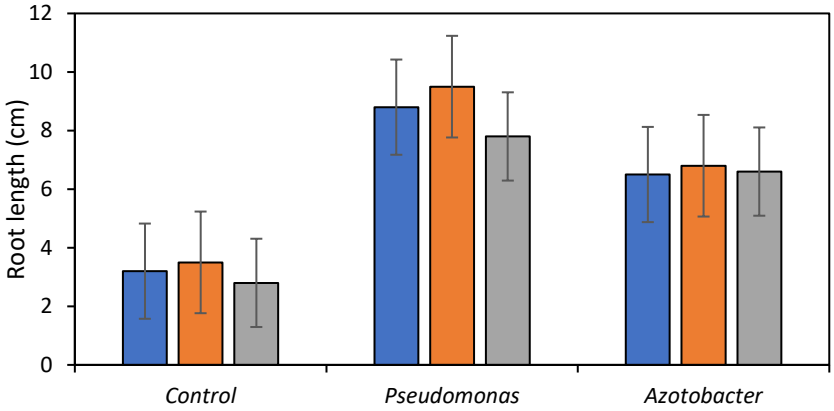

Figure 4. Variation in root length(cm) of *Cicer arietinum* on inoculation of *Pseudomonas* and *Azotobacter* culture along with control.

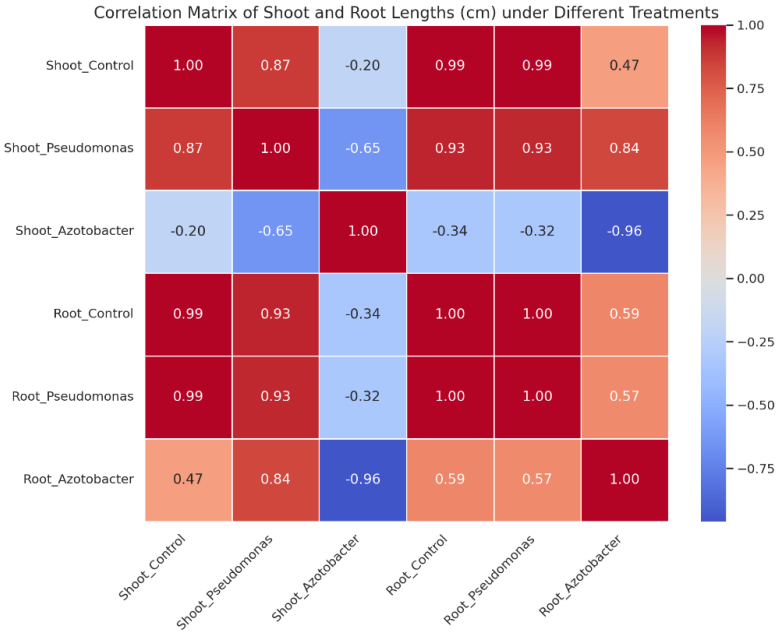

Figure 5. Pearson correlation analysis was performed between the root and shoot length of treated and control plants.

Overall, these findings suggest that while both microbial treatments enhance growth compared to the control, *Pseudomonas* promotes a more balanced development of shoot and root systems. In contrast, *Azotobacter* treatment, despite promoting individual growth parameters, may require optimization to achieve synchronized plant development.

This analysis underlines the importance of selecting appropriate microbial biofertilizers not just based on growth enhancement but also on their impact on the coordination between different plant parts.

4. Conclusion

Overall, in the present study, two bacterial strains were isolated from the soil sample collected from the rhizospheric soils of cultivated *Cajanus cajan* (Pigeon pea) beside Amrit Sarovar, V.B.S. Purvanchal University, Jaunpur. The bacterial strains were identified by performing morphological and biochemical tests. It was found that the isolated bacterial strains *Pseudomonas* (PGPR-1) and *Azotobacter* (PGPR-2) significantly enhanced the seed germination and seedling growth of Chickpea. Therefore, it is suggested that the use of PGPR isolates of *Pseudomonas* and *Azotobacter* as effective biofertilizers might be beneficial for *C. arietinum* cultivation. Such studies will also assist in identifying beneficial microorganisms from the agricultural soil that will help to develop eco-friendly microbial technologies for plant growth and yields.

Author Contributions: Awadhesh Kumar: Conceptualization, Investigation, Software, Visualization; Maruti Prasad Singh: Formal analysis, Resources; Dinesh Kumar: Conceptualization, Methodology, Data curation, Writing – original draft; Shree Prakash Tiwari: Supervision, Validation, Writing – review and editing; Rajesh Sharma: Writing – review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional/Ethical Approval: Not applicable.

Data Availability/Sharing: The datasets used and analyzed during the current study will be made available from the corresponding author upon a reasonable request.

Supplementary Information Availability: Not applicable.

References

Ajmal, M., Ali, H. I., Saeed, R., Akhtar, A., Tahir, M., Mehboob, M. Z., & Ayub, A. (2018). Biofertilizer as an alternative for chemical fertilizers. *Journal of Agriculture and Allied Sciences*, 7(1), 1-7.

Ammar, E. E., Rady, H. A., Khattab, A. M., Amer, M. H., Mohamed, S. A., Elodamy, N. I., & Aioub, A. A. (2023). A comprehensive overview of eco-friendly bio-fertilizers extracted from living organisms. *Environmental Science and Pollution Research*, 30(53), 113119-113137. https://doi.org/10.1007/s11356-023-30260-x

Aneja, K. R. (2003). Experiments in microbiology, Plant Pathology and Biotechnology, Fourth ed. New Age International Publishers, Daryaganj, New Delhi.

Cappuccino, J. C., & Sherman, N. (1992). In: Microbiology: A laboratory manual, Third ed. Benjamin /Cumming pub. Co. New York, pp. 125-179.

Dar, G. H., Bhat, R. A., Mehmood, M. A., & Hakeem, K. R. (2021). Microbiota and biofertilizers (Vol. 2). Springer Nature. https://doi.org/10.1007/978-3-030-61010-4

He, S., Li, L., Lv, M., Wang, R., Wang, L., Yu, S., & Li, X. (2024). PGPR: key to enhancing crop productivity and achieving sustainable agriculture. *Current Microbiology*, 81(11), 377. https://doi.org/10.1007/s00284-024-03893-5

Kloepper, J. W. & Schroth, M. N. (1978) Plant growth promoting rhizobacteria on radishes. In: *Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria* (ed. Station de Pathologie Végétale et Phytobactériologie, INRA, Angers), Volume 2, pp. 879–882.

Kumar, N., Hong, S., Zhu, Y., Garay, A., Yang, J., Henderson, D., & Li, Y. (2025). Comprehensive review of chickpea (Cicer arietinum): Nutritional significance, health benefits, techno-functionalities, and food applications. *Comprehensive Reviews in Food Science and Food Safety*, 24(2), e70152. https://doi.org/10.1111/1541-4337.70152

Merga, B., & Haji, J. (2019). Economic importance of chickpea: Production, value, and world trade. *Cogent Food & Agriculture*, 5(1), 1615718.

Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. *Sustainability*, 13(4), 1868.

Sonam, S., Srivastava, R., & Sharma, R. (2024). Assessment of Antifungal Potential of Rhizospheric Bacterial Isolates of *Daucus carota* against *Rhizoctonia solani*. *Journal of Pure & Applied Microbiology*, 18(3), 2036. https://doi.org/10.22207/JPAM.18.3.54

Srivastava, R., Tripathi, B. M., Singh, R. K., Srivastva, P., Kumari, P., Srivastav, M., & Tiwari, S. P. (2013). Profiling of plant growth promoting bacteria associated with Jaunpuri giant raddish rhizosphere. *International Journal of Agriculture, Environment and Biotechnology*, 6(2), 187-196. https://doi.org/10.3389/fsoil.2022.821589

Suman, J., Rakshit, A., Ogireddy, S. D., Singh, S., Gupta, C., & Chandrakala, J. (2022). Microbiome as a key player in sustainable agriculture and human health. *Frontiers in Soil Science*, 2, 821589.https://doi.org/10.3389/fsoil.2022.821589

Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2023). Plant growth-promoting soil bacteria: Nitrogen fixation, phosphate solubilization, siderophore production, and other biological activities. *Plants*, 12(24), 4074. https://doi.org/10.3390/plants12244074

Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënne-Loccoz, Y., Muller, D., & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*, 4, 356. https://doi.org/10.3389/fpls.2013.00356

Verma, M., Pandove, G., & Kaur, A. (2023). Role of PGPR as Microbial Inoculants in Improving Fodder Crops Productivity and Quality: A Review. *Forage Research*, 48(4), 420-429.