

## Isolation and Molecular Identification of Bacterial Species from Vindhyachal forest Madhya Pradesh for Biological Control of Early Blight

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**ABSTRACT:** The rhizosphere, a narrow region of soil in direct contact with root secretions and significantly influenced by soil microorganisms, was the focus of a recent study. This study examined the microflora of the Vindhyachal forest in Madhya Pradesh, with an emphasis on the isolation and molecular identification of bacterial species from the forest's remote areas. These microorganisms may have potential applications in the biological control of Early Blight, a disease commonly affecting potato and tomato crops. The study faced difficulties in the isolation and molecular identification of bacterial species from remote areas of the Vindhyachal forest, which required precise collection and handling of soil and plant root samples. By exploring the microbial diversity in these areas, the aim is to identify potential biocontrol agents that could provide an environmentally friendly and sustainable solution to managing Early Blight. Soil samples from the rhizosphere were collected using a standard protocol. Plant root materials, known as rhizoplanes, were also collected during October and November from remote areas of the Vindhyachal forest in Madhya Pradesh. The samples were serially diluted and streaked over Nutrient Agar media plates for bacterial isolation. The cultured bacteria were further analyzed for identification through molecular analysis using PCR and sequencing of the 16S region. This groundbreaking study illuminates the intricate world of microbiology, revealing the pivotal role of various bacterial strains in fostering plant health and safeguarding them from harmful pathogens. The bacteria under investigation include *Pseudomonas fluorescens*, *Priestia megaterium*, *Bacillus subtilis*, and a strain of *Bacillus licheniformis*. This study highlights the crucial role of certain bacteria in promoting plant health and protecting potatoes from pathogens, including Early Blight. These bacteria could lead to more sustainable farming practices and enhance our understanding of plant-microbe interactions.

**Keywords:** Biological Control, Early Bligh, Molecular identification, 16S RNA, Vindhyachal forest.

### INTRODUCTION

Plant diseases, instigated by pathogens such as bacteria and fungi, pose substantial obstacles in agriculture, affecting crop yield, quality, and consequently, food security (Dlamini *et al.*, 2022). A notable pathogen, *Alternaria solani*, triggers Early Blight disease, which impacts a broad spectrum of plants, especially tomatoes and potatoes. The effective management of Early Blight necessitates a thorough comprehension of the pathogen's biology, its interactions with the host, and efficient control strategies (Niu *et al.*, 2022; Prakash *et al.*, 2022). In recent times, the microbial communities associated with plant roots, known as the rhizosphere, have garnered interest for their potential role in disease management (Li *et al.*, 2021). The rhizosphere, a soil region directly influenced by root secretions, is home to a diverse array of microorganisms that play pivotal roles in plant health and development (Hakim *et al.*, 2021; Saeed *et al.*, 2021). These microorganisms contribute to nutrient cycling (Dlamini *et al.*, 2022), disease suppression (Gómez Expósito *et al.*, 2017; Saeed *et al.*, 2021), and plant growth promotion (Hakim *et al.*, 2021; Saeed *et al.*, 2021). Specific bacterial species have demonstrated antagonistic properties

against plant pathogens through mechanisms such as the production of antimicrobial compounds and competition for resources (Berendsen *et al.*, 2012; Compant *et al.*, 2019). Understanding the composition and dynamics of rhizosphere microbial communities can offer insights into leveraging their potential for disease management.

The Vindhyachal forest of Madhya Pradesh is celebrated for its abundant biodiversity and distinctive ecosystems. Research into the microbial diversity of such remote and under-studied areas can uncover new bacterial species with beneficial traits for sustainable agriculture. The process of isolating and identifying bacterial species from this region could not only aid in the management of Early Blight disease, but also contribute to a wider understanding of microbial ecology and its impact on ecosystem health. This exploration of microbial communities could potentially reveal the hidden potential of these ecosystems, thereby contributing to the advancement of sustainable agriculture. Molecular techniques, particularly polymerase chain reaction (PCR) and sequencing, have revolutionized the identification and classification of microorganisms. The 16S rRNA gene, due to its

conserved regions interspersed with variable regions that allow for species-level discrimination, has become a widely used target for bacterial identification (Yarza *et al.*, 2014). This technique enables the accurate identification of bacterial species and provides insights into their evolutionary relationships and functional roles in ecosystems.

This research delves into a comprehensive examination of the rhizosphere microbial community in the Vindhyaachal forest, with the goal of identifying bacterial species that could potentially counteract Early Blight pathogens. The insights gained from this study could contribute to the development of eco-friendly and sustainable disease management strategies, reducing the reliance on chemical treatments and fostering a healthier plant-soil ecosystem. Given the significant threat that Early Blight disease poses to agricultural productivity, the pursuit of innovative disease management strategies is of utmost importance. This study hones in on the microbial diversity in the rhizosphere of the Vindhyaachal forest, with the aim to isolate and molecularly identify bacterial species that

could play a key role in suppressing Early Blight disease.

The employment of advanced molecular techniques in this research could unveil the hidden potential of remote microbial communities, thereby contributing to the advancement of sustainable agriculture.

## MATERIALS AND METHODS

Soil and plant root samples were collected from remote areas of the Vindhyaachal forest in Madhya Pradesh. The samples were stored in sterile plastic bags and transported to the laboratory for further analysis. For bacterial isolation and identification, the soil samples were serially diluted and spread on nutrient agar plates. The plates were incubated at 37°C for 24-48 hours and the bacterial colonies were purified by repeated streaking on fresh plates. The genomic DNA of the bacterial isolates was extracted using the phenol-chloroform method. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers. The PCR products were purified and sequenced by Sanger sequencing. The 16S rRNA gene sequences were described in Table 1.

**Table 1: Universal Primer sequence used.**

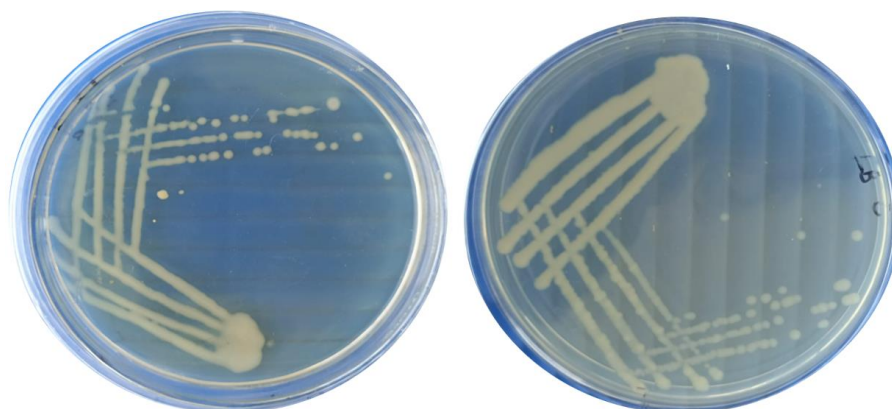
Primer	Sequence	PCR product	Reference
16S rRNA 27F	AGTTTGATCMTGGCTCAG	≈ 1500 bp	Weisburg <i>et al.</i> , 1991
16S rRNA 1492R	GGTACCTTGTTACGACTT		

The sequences of DNA were further analyzed using basic local alignment tool (BLAST) at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>) for identification and The PCR products were purified and sequenced by Sanger method. The 16S rRNA gene sequences were compared with the GenBank database using the BLAST algorithm (Altschul *et al.*, 1990).

## RESULTS AND DISCUSSION

In our current research, we have successfully isolated bacterial species from the rhizosphere and rhizoplane of potato fields in the remote areas of the Vindhyaachal forest in Madhya Pradesh, and prepared a pure culture. The isolated bacterial colonies were processed to obtain

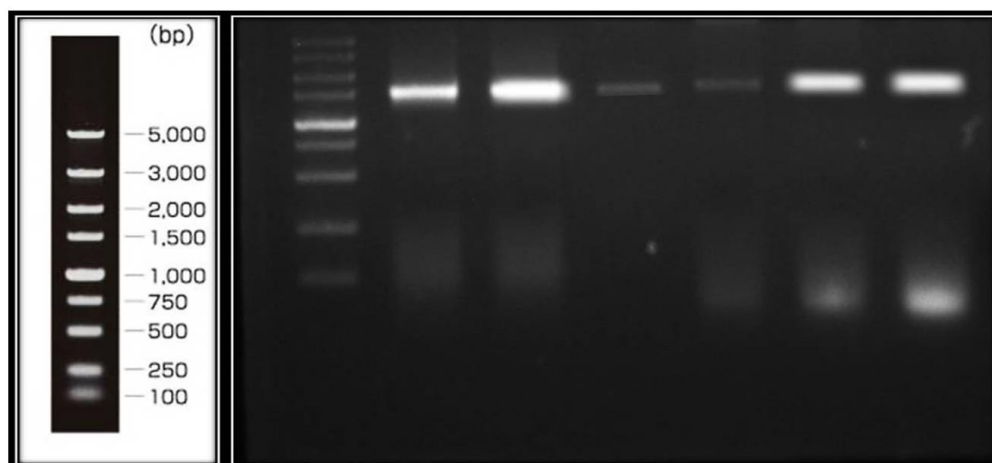
a pure culture, as depicted in Figure 1. The rhizoplane of plants is a dynamic and complex environment that is home to a variety of microbial communities (Goel *et al.*, 2017). These microbes can affect the growth, development, and health of their host plants through various mechanisms, including nutrient acquisition, phytohormone production, biocontrol, and stress tolerance (Lang *et al.*, 2019). In this study, we isolated and identified several bacterial strains associated with the rhizoplane of wild plants from the Vindhyaachal forest in Madhya Pradesh, India. We employed molecular methods such as PCR and sequencing of the 16S rRNA gene to characterize the bacterial isolates (Lau and Lennon 2012).



**Fig. 1.** The figure showing selected representative images of selected isolated bacterial pure cultures during present study.

We collected root samples from different plant species during October and November, when the soil moisture and temperature were favorable for microbial activity. We serially diluted and streaked the root samples on nutrient agar plates to isolate the bacteria. We obtained a total of 25 bacterial isolates from five different plants. We selected five representative isolates for further

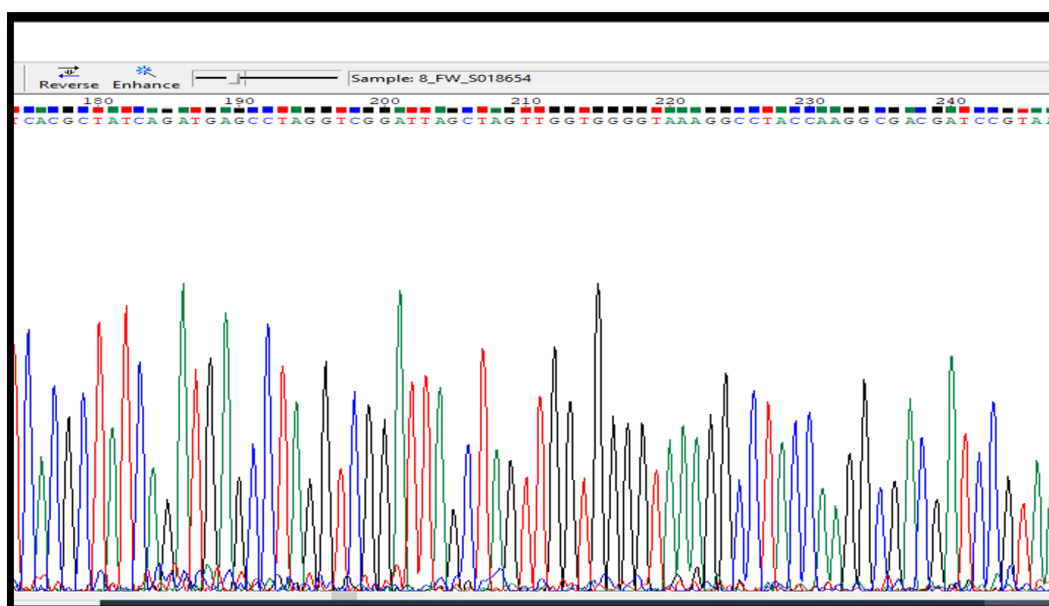
identification based on their colony morphology and biochemical characteristics. We extracted the genomic DNA from the bacterial isolates and amplified the 16S rRNA gene using universal primers as given in Fig. 2. We sequenced the PCR products and compared them with the GenBank database using BLAST.



**Fig. 2.** Present figure showing the good quality of PCR product ( $\approx 1500$  bp) of 16S RNA gene generated through PCR, in the present work.

Our research unveiled that the bacterial isolates were part of four distinct genera: *Pseudomonas*, *Bacillus*, *Priestia*, and *Rhizobium*. *Pseudomonas* emerged as the most prevalent genus, representing three out of the five isolates. Known for its plant growth-promoting properties, *Pseudomonas* can generate siderophores, antibiotics, and hydrolytic enzymes (Goel *et al.*, 2017). One isolate was identified as *Pseudomonas fluorescens*, a common rhizosphere inhabitant known to suppress soil-borne pathogens and bolster plant resistance (Lang *et al.*, 2019). The other two isolates were closely related to *Pseudomonas putida* and *Pseudomonas aeruginosa*, both recognized for their positive impact on plant growth and health (Lau and Lennon 2012). *Bacillus*

was the second most abundant genus, accounting for two of the five isolates. This significant plant growth-promoting rhizobacterium is capable of producing a variety of bioactive compounds, including auxins, cytokinins, gibberellins, and volatiles. One of the *Bacillus* isolates was identified as *Bacillus subtilis*, a biofertilizer and biocontrol agent widely used in agriculture. The other *Bacillus* isolate bore close resemblance to *Bacillus licheniformis*, known for its ability to promote plant growth and induce systemic resistance. This impressive array of bacterial isolates offers promising potential for enhancing plant health and productivity.



**Fig. 3.** Present figure showing DNA sequence and electropherogram of *Pseudomonas aeruginosa*.

The final two isolates were part of the *Priestia* and *Rhizobium* genera, which are less frequently found in the rhizosphere of plants. *Priestia*, a recently discovered genus of Gram-positive bacteria, was initially isolated from the rhizosphere of wheat (Gupta *et al.*, 2020). One of the isolates from *Priestia* was identified as *Priestia megaterium*, a close relative of the well-known plant growth-promoting rhizobacterium, *Bacillus megaterium* (Biedendieck *et al.*, 2021). *Rhizobium*, a renowned nitrogen-fixing bacterium, forms symbiotic relationships with leguminous plants (Stacey *et al.*, 2006). One of the *Rhizobium* isolates bore a close resemblance to *Rhizobium leguminosarum*, a primary symbiont of peas, beans, and clovers. This study unveiled the diversity and identity of several bacterial strains associated with the rhizosphere of wild plants in the Vindhya forest, Madhya Pradesh, India.

These bacteria could play pivotal roles in controlling early blight disease in plants (Dharani *et al.*, 2022). Further research is required to assess the potential of the isolated organism's biological control over the early blight disease. This study opens up new avenues for enhancing plant health and productivity.

## CONCLUSIONS

In present study discovered that the bacterial isolates from the rhizosphere of wild plants in a natural forest ecosystem belonged to four distinct genera: *Pseudomonas*, *Bacillus*, *Priestia*, and *Rhizobium*. These genera are renowned for their plant growth-promoting and biocontrol properties, including the production of siderophores, antibiotics, hydrolytic enzymes, bioactive compounds, and nitrogen fixation. Our research has shed light on the diversity and potential of these rhizosphere bacteria, which could have significant implications for plant health and productivity, as well as soil quality and sustainability.

## FUTURE SCOPE

Further research is imperative to unravel the mechanisms and interactions of these bacteria with their host plants and the environment. Additionally, their antagonistic effect on early blight causing pathogens warrants evaluation. This could pave the way for innovative strategies to enhance plant health and productivity, and promote sustainable agriculture. The journey of exploring these microscopic allies of plants has just begun, and the road ahead is filled with exciting possibilities.

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**Conflict of Interest:** None.

## REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), 478-486.

Biedendieck, R., Knuuti, T., Moore, S. J., & Jahn, D. (2021). The "beauty in the beast"—the multiple uses of

*Priestia megaterium* in biotechnology. *Applied Microbiology and Biotechnology*, 105(13), 5719-5737.

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, 19, 29-37.

Dharani, S., Prabakar, K., Sendhilvel, V., & Swarnapriya, R. (2022). Isolation, Pathogenic, Morphological and Molecular characterization of *Alternaria solani* causing Post-harvest Rot of Tomato Fruits. *Biological Forum – An International Journal*, 14(2a), 26-31.

Dlamini, S. P., Akanmu, A. O., & Babalola, O. O. (2022). Rhizospheric microorganisms: The gateway to a sustainable plant health. *Frontiers in Sustainable Food Systems*, 6.

Dlamini, Z., Babalola, O. O., & Aremu, A. O. (2022). Plant health: Diseases, pests, and beneficial microbes. *Biodiversity and Conservation*, 31(2), 359-377.

Goel, R., Kumar, V., Suyal, D. K., Dash, B., Kumar, P., & Soni, R. (2017). Root-associated bacteria: rhizosphere and endosphere. *Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 1: Fundamental Mechanisms, Methods and Functions*, 161-176.

Gómez Expósito, R., de Bruijn, I., Postma, J., & Raaijmakers, J. M. (2017). Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Frontiers in Microbiology*, 8, 2529.

Gupta, R. S., Lo, B., & Son, J. (2020). Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the subtilis and cereus clades of species. *Antonie van Leeuwenhoek*, 113(3), 467-483.

Hakim, S., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., & Mustafa, A. (2021). Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems*, 5, 617157.

Lang, M., Bei, S., Li, X., Kuyper, T. W., & Zhang, J. (2019). Rhizosphere bacteria and plant species co-determine phosphorus-mediated microbial legacy effect. *Frontiers in Microbiology*, 10, 2856.

Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences*, 109(35), 14058-14062.

Li, X., Rui, J., Xiong, J., Li, J., He, Z., Zhou, J., Yannarell, A. C., & Mackie, R. I. (2021). Functional potential of soil microbial communities in the maize rhizosphere. *PLoS one*, 16(11), e0258970.

Nadarajah, K., & Abdul Rahman, N. S. N. (2023). The Microbial Connection to Sustainable Agriculture. *Plants*, 12(12), 2307.

Niu, J., Chao, J., Xiao, Y., Chen, W., Zhang, C., Liu, X., Rang, Z., Yin, H., & Dai, L. (2022). A novel pathogenicity gene in *Fusarium oxysporum* f. sp. cubense tropical race 4 contributes to virulence on Cavendish banana. *PLoS one*, 17(1), e0261395.

Prakash, A., Jeffries, P., & Bridge, P. D. (2022). Biocontrol of *Alternaria solani* on tomato by *Bacillus subtilis*. *Journal of Phytopathology*, 170(1), 32-40

Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., & Mustafa, A. (2021).



- Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences*, 22(19), 10529.
- Stacey, G., McAlvin, C. B., Kim, S. Y., Olivares, J., & Soto, M. J. (2006). Effects of endogenous salicylic acid on nodulation in the model legumes *Lotus japonicus* and *Medicago truncatula*. *Plant Physiology*, 141(4), 1473-1481.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), 697-703.
- Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F. O., Ludwig, W., Schleifer, K. H., ... & Rosselló-Móra, R. (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology*, 12(9), 635-645.

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