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Diversity of Beneficial Bacteria in Tamhini Ghat Region of Western Ghats, M.S., India

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ABSTRACT

The present study investigates that the isolation of beneficial bacteria from a soil sample collected from the Tamhini Ghat region of the Western Ghats. In October 2022, 15 soil samples were collected from Tamhini Ghat. Five different beneficial bacteria were isolated from these collected soil samples and characterized morphologically. The beneficial bacteria like *Azotobacter spp., Bacillus spp., Azospirillum spp., Pseudomonas spp*., and *Frateuria spp.* Isolation of these bacteria was carried out on a specific medium and observed after three days of the incubation period at 28±2 ^{0}C temperature. These isolated bacteria were purified on slants prepared by using a specific medium and stored at 4 ^{0}C for further study and observed under a microscope by performing a Gram staining test. These beneficial bacteria can be used for biofertilizer formulation in future studies. All over India, farmers are using chemical fertilizers, which directly impact beneficial soil microbe that leads to decreased soil health. For improving soil health, it is the need of the hour to integrate the use of chemical fertilizers along with biofertilizers as per the recommended dose of fertilizers (RDF) given by the agricultural universities for the specific crops.

Key words: *Beneficial bacteria, Forest soil, Bacillus spp., Microbial population, Western Ghats.*

Introduction

Forests cover more than 40 million square kilometers and account for 30% of the total global land area, making them one of the most important ecosystems on Earth. According to the Forest Survey Report (FSR 2022) by the Press Information Bureau, the total forest area recorded in India is about 71.37 million hectares. The soil microflora is a site where physical, chemical, and biological processes occur. Soil microorganisms enhance plant growth. A soil layer is made when rock weathers due to climate, vegetation, relief, and parent rock, and is the topmost section of the Earth's crust. The above-mentioned combination of factors has resulted in the making of Indian soil. Studies have revealed that soil microbial communities are altered by deforestation and soil cultivation, reducing biodiversity. Forest ecosystems depend on soils for nutrient recycling as well as the breakdown of organic matter. They are colonized by a variety of microbial groups responsible for the breakdown of organic matter, production of plant growth-promoting substances, accelerating the different nutrient uptake such as nitrogen fixation, phosphorus solubilization, IAA production, HCN production, and the production of siderophores.

The bacterial species isolated from the soils of

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Western Ghats are *Bacillus thioparus, Micrococus* spp., *Pontibacterniistensis, Paracoccus niistensis*, 300 new isolates of *Bacillus thuringiensis* (Bt), *Rhodobacterviridis.* In addition, 35 species belonging to *Acaulospora, Gigaspora, Glomus, Sclerocystis,* and *Scutellospora* were tested for fungal spores and endophytic fungi like *Aspergillus flavus, A. niger, A. ochraceus, Gliocladium viride, Penicillium* spp. and *Morpho* spp. were isolated from the soils of the Western Ghats. The Himalayan Forest soil has bacterial diversity of phosphate solubilizing *Pseudomonas spp., Bacillus spp., Enterobacter spp.,* and the fungi species like *Penicillium sp.,Aspergillus sp., and the Arbuscular Mycorrhizal (AMF).* Moreover, the microbial diversity of the hills of northeast India includes different species of *Bacillus* and *Pseudomonas* like *B. subtilis, B. clausii, P. aeruginosa, B. licheniformis, B. megaterium, B. thuringiensis* and *P. stutzeri* respectively. The bacteria isolated from the coastal sand dunes are from different families like Pseudomonadaceae, Enterobacteriaceae, Lavobacteriaceae, Burkholderiaceae, Xanthomonadaceae and Bacillaceae. There are a variety of PGPR strains isolated from the coastal area of the Andaman and Nicobar Islands, such as *Acinetobacter spp.,* fluorescent *Pseudomonas* spp., *Azotobacter* spp., *Bacillus* spp. and *Rhizobium* spp. Consequently, various species of plants were selected from the Tamhini Ghat forest areas for their rhizosphere soils to isolate native beneficial bacteria.The present study aims to isolate rhizobacteria from the plants grown in the forest area, morphological characterization, and identification of the isolated bacteria.

Materials and Methods

Soil Sampling

Tamhini Ghat soil was sampled from the rhizospheric soil of plants growing there *viz., Impatiens pulcherrima* L., *Boerhavia diffusa* L., *Chloris barbata* L., *Indigofera spicata* L., *Crotalaria retusa* L., of Western Ghat, Pune region, India. For the isolation of rhizobacteria of randomly selected plants, polythene zip bags were used to collect the soil sample.

Isolation of Bacteria

Isolation of rhizobacteria was achieved by adding 10 grams of soil sample to 100 ml of sterile distilled water, shaking for 30 minutes at 100 rpm on a rotary shaker.

The serial dilution was carried out upto 10^{-5}. The 1.0 ml of soil suspension from the 10^{-5} test tube was plated onto Jensen Medium for *Azotobacter spp.* (Vivek Kumar *et al.,* 1999), Malate Medium for *Azospirillum* spp. (Sadasivan *et al.*, 1985), Pikovskaya's Medium for *Bacillus spp.* (Maheshwar *et al.,* 2012), Alexandrov's Medium for *Frateuria spp.* (Anwar *et al.,* 2022), and King's B Medium for *Pseudomonas spp.* (Johnsen *et al.,* 1999). And incubated for 48 hrs at 30±2 ^{0}C. For further examination, the isolated bacterial colonies were purified and kept on the same medium agar slants at 4 ^{0}C.

Identification

Initially, bacteria were identified by studying the morphology of colonies. Isolates were identified by traditional methods based on morphological, and biochemical characteristics. All of the bacterial isolates were identified initially by the Gram staining method and also performed biochemical tests such as catalase activity, casein hydrolysis, gelatin hydrolysis, carbohydrate fermentation (Glucose and Sucrose), and starch hydrolysis capability.

Results and Discussion

The rhizospheric soil collected from *viz*., *Impatiens pulcherrima* L., *Boerhavia diffusa* L., *Chloris barbata* L., *Indigofera spicata* L., *Crotalaria retusa* L., is used for isolation of beneficial bacteria such as *Azotobacter spp., Azospirillum spp., Bacillus spp., Pseudomonas spp.,*

Table 1. Isolated Bacteria from Different Plant Species

Name of Isolated Bacteria	Plant Species
Azotobacter spp.	*Impatiens pulcherrima* L., *Crotalaria retusa* L.
Azospirillum spp.	*Chloris barbata* L.
Bacillus spp.	*Impatiens pulcherrima* L., *Boerhaavia diffusa* L., *Chloris barbata* L., *Indigofera spicata* L., *Crotalaria retusa* L.
Pseudomonas spp.	*Impatiens pulcherrima* L., *Boerhaavia diffusa* L., *Chloris barbata* L.
Frateuria spp.	*Indigofera spicata* L.

Table 2. Morphological Characteristics of Isolated Bacteria's

Name of Isolated Bacteria	Colony Morphology	Number of Isolates	Cell Shape	Gram Staining
Azotobacter spp.	Irregularly shaped, light brown colored pigmentation, large sized colony, Curled margin, rough texture, convex elevation, mucoid and opaque colonies.	2	Cocci	Negative
Azospirillum spp.	White-colored, round, convex, opaque, smooth, and shiny colonies	2	Vibrioid	Negative
Bacillus spp.	Rough, irregular, white to off-white	6	Bacilli	Positive
Pseudomonas spp.	Irregular-shaped, low convex, smooth, yellow pigmentation, translucent and opaque colonies	5	Rod	Negative
Frateuria spp.	Flat, irregular-shaped, waved edges, opaque and rough	1	Rod	Negative

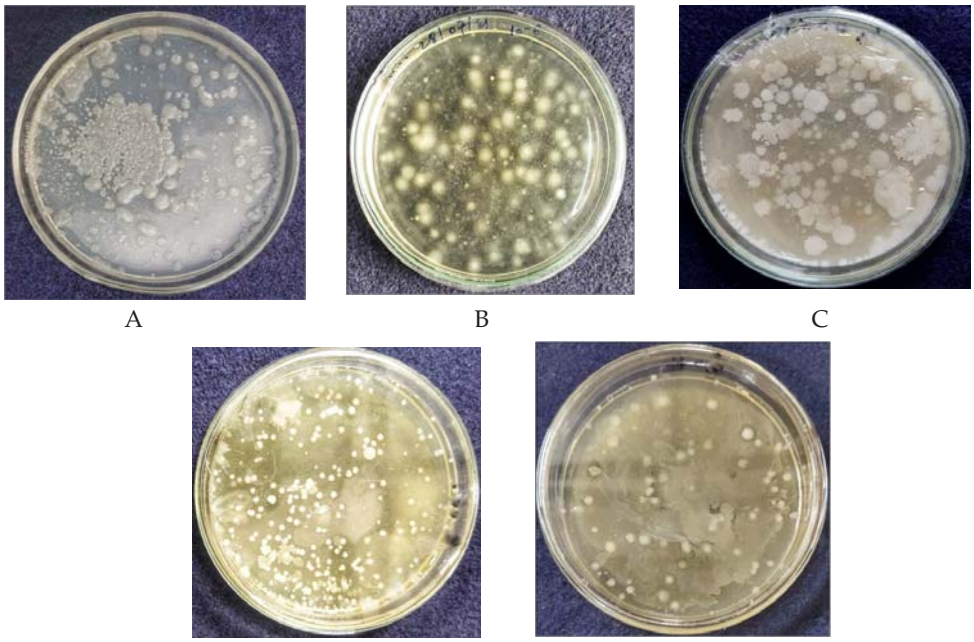

Fig. 1. Isolated bacterial species growing on Petri plates (A) *Azotobacter spp.* (B) *Azospirillum spp.* (C) *Bacillus spp*. (D) *Pseudomonas spp*. (E) *Frateuria spp.*

and *Frateuria spp.* respectively.Based on the colony's morphological characteristics, including size, shape, and colour, isolated bacteria were identified. Moreover, the identification was performed by Gram staining and biochemical activities.

These isolated bacterial species can be utilized in the production of biofertilizers to enhance crop productivity and soil health restoration.

Conclusion

The *Frateuria spp.* were isolated from *Indigofera spicata* L., and *Pseudomonas spp.* isolates were found in *Impatiens pulcherrima* L., *Boerhaaviadiffusa* L., *Chloris barbata* L. Moreover, the *Bacillus spp.* were isolated from four different plant species. *Azotobacter spp.* and *Azospirillum spp.* isolated from *Impatiens*

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