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Original Article

# Exploration of Bacterial Endophytes from the Whole Plant of *Mimosa pudica*: Isolation, Identification, and Biochemical Characterization

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## ABSTRACT

**Background:** Endophytes are non-pathogenic microorganisms that reside within plant tissues without causing symptoms of infection. *Mimosa pudica* Linn., a sensitive plant with a long history of traditional medicinal use for treating various ailments, has not been extensively studied for its associated endophytic bacteria. The current study aimed to isolate and characterize endophytic bacteria from the entire plant of *Mimosa pudica* and identify phytochemicals produced by these endophytes using conventional and molecular approaches. **Materials and Methods:** Endophytic bacteria were isolated from the roots, stems, and leaves of *Mimosa pudica* using surface sterilization and culture techniques. The isolates were characterized based on biochemical traits, morphology, and molecular identification using 16S rRNA gene sequencing and phylogenetic analysis. **Results and Discussion:** Eight endophytic bacterial strains were successfully isolated and identified, including *Pseudomonas plecoglossicida* (S1, S2), *Pseudomonas putida* (S3), *Pseudomonas cichorii* (S4), *Enterobacter ludwigii* (S5), *Enterobacter asburiae* (S6), *Serratia proteamaculans* (S7), and *Bacillus pumilus* (S8). The majority of isolates belonged to the *Pseudomonas* and *Enterobacter genera*. Functional analysis revealed that some endophytes exhibited traits such as hydrogen cyanide production and indole-3-acetic acid synthesis, suggesting potential roles in plant growth promotion and defense mechanisms. The presence of bioactive phytochemicals in endophytic extracts indicates their potential for pharmaceutical or agricultural applications. **Conclusion:** This study highlights the diversity and functional capabilities of endophytic bacteria associated with *Mimosa pudica*. The identified endophytes, particularly those capable of producing bioactive compounds, could serve as promising candidates for further exploration in biotechnological applications, including plant growth promotion and secondary metabolite production.

**Keywords:** *Mimosa pudica*, Isolation and Characterization, Endophytic Bacteria, 16S rRNA analysis.

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## INTRODUCTION

Microbial endophytes are endosymbionts that live inside plants and are typically bacteria or fungi. They do not harm or infect plants (Sahu *et al*., 2024). The capacity to colonize the internal living tissues of plants gives endophytes an advantage over other biocontrol agents and makes them an efficient therapeutic agent (Bhagat *et al*., 2012). Several studies had revealed that endophytes are found in all plant species on earth and are beneficial for their host plants by synthesize ample of metabolites which boost plant defenses against infections and encourage plant growth (Rosenblueth and Martínez-Romero, 2006). Endophytes is chosen to be an excellent bioactive natural compounds which imitate the chemistry of the respective host plants and biosynthesize nearly the similar secondary metabolites or even their derivatives which would be a unique metabolite (Ryan et al., 2008). Distinctive from plants, the isolation of bioactive compounds from microorganisms is easier and economically viable for large-scale fermentation of biomass (Rodriguez *et al*., 2009). Numerous studies have highlighted the various applications of endophytes derived from medicinal plants. In recent times, numerous well-known as well as novel endophytic secondary metabolites having various biological activities such as antimicrobial, anticancer, antioxidant etc., (Strobel *et al*., 2001). The work provided here details the process of isolating and characterizing *Mimosa pudica* bacterial endophytes. Understanding the range of bacterial endophytes that are common in this medicinal plant was the goal of this investigation. Figure 1 shows plant of *Mimosa pudica.*

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## MATERIALS AND METHODS

### Collection of plant sample

After authentication, the whole plants of *M. pudica* were collected from the region of Wardha India. The plant's taxonomic identification and authentication were carried out. Plant sample were Shed Dried and powdered. Extraction of *Mimosa pudica* whole plant using 3:2 ethyl acetate: ethanol was carried out by maceration process of extraction for 72 hr.

### Isolation of endophytes from Fresh *M. pudica* Plant

Freshly collected whole plant was Surface sterilized and endophytes were cultured from parts fresh whole plant on PDA media. Explants were subsequently inoculated on PDA filled petriplate. PDA plate included five parts of plant, and the borders were parafilm-sealed to prevent contamination. To encourage the growth of endophytes, all of the plates were incubated at 28°-20°C, and growth was frequently checked.

### Isolation of endophytes from *M. pudica* Plant Extract

For isolating endophytes from plant extract, *Mimosa pudica* dried powder was extracted with sterile water by maceration process. Concentration of extract was done without application of heat. Endophytes were cultured from whole plant extract on PDA media by striking method and the borders were parafilm-sealed to prevent contamination. To encourage the growth of endophytes, all of the plates were incubated at 28°-20°C, and growth was frequently checked. Grown endophytes were isolated into different pure endophytes by serial inoculation method (Jose *et al*., 2014).

Following subculturing, each tissue's distinct bacterial colonies were preserved for subsequent examination in their glycerol stocks. A 30% glycerol (glycerol diluted in sterile distilled water (*v*/*v*)) stock culture was prepared for each bacterial endophyte and stored at -80°C for future use (Saikia *et al*., 2022).

Each endophytic culture was examined for purity before being transferred to newly made PDA plates in an effort to produce pure endophytic cultures. A suitable control was also established, in which a few water droplets from the final washing of surface sterilization were placed on a PDA plate without any plant tissue being inoculated (Patra *et al*., 2020).

### Characterization of endophytes

Colony morphology, biochemical traits, and molecular phylogeny were used to characterize the endophytic bacterial isolates. Bergey's manual of determinative bacteriology was used to analyze the isolates' morphological and biochemical traits (Sebola *et al*., 2019).

### Biochemical Characterization of Bacterial Colonies

For every bacterial isolate, standard biochemical tests were conducted, including catalase activity, HCN production, starch hydrolysis test, citrate utilization, urea hydrolysis, oxidase assay, nitrate reduction and NaCl tolerance, and salt stress (Cappuccino and Sherman, 2014).

### Molecular Identification

#### *Genomic DNA Extraction, Polymerase Chain Reaction, and Sequencing*

Endophytic strains will be recognized using DNA amplification and sequencing as part of a molecular biology protocol in which 16S rRNA gene sequencing for bacterial strain identification. Extraction of Genomic DNA extraction from pure colonies of endophytic isolates. The genomic DNA of every endophyte isolate will be isolated from pure colonies that were grown from PDA plates. Extraction of DNA will be done using DNA kit. The NanoDrop ND-2000 UV-vis spectrophotometer will be used to quantify the isolated DNA. PCR will be used to amplify the extracted DNA. Purification of the PCR product will be carried out in the subsequent step. Sequencing of PCR product will be done by commercial services. Database such as Gen Bank along with Blast-Algorithmus will be used to compare the base sequence (Kumar and Kaushik, 2013).

### Phylogenetic Analysis

The Nucleotide Basic Local Alignment Search Tool (BLAST N) program, accessible at the National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov/BLAST), was used to retrieve sequences that showed greater than 99% similarity after Sequences from DNA databases were compared to the partial sequences. The Clustal X 2.1 version of multiple sequences alignment tool was used to align the sequences. The maximum likelihood approach and Neighbor Joining (NJ) process were used to do the phylogenetic and molecular evolutionary analysis. MEGA 11 was used for all evolutionary analyses (Tamura *et al*., 2021). The 16S rRNA gene sequences of the study's identified bacterial isolates have been uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using the accession codes (National Center for Biotechnology Information [NCBI], 2024) shown in Table 1. The phylogenetic data and BLAST homology percentages were used to find names assigned to the bacterial isolates.

### Fermentation of isolated pure endophytic strains

Sterilized media is used for large production of pure isolated endophytes which was carried out using 1000 mL Erlenmeyer flask. 1-2 weeks growth on PDA of pure endophytic strain was divide into pieces and added into 200 g sterilized media containing Erlenmeyer flask and inoculated. Control group is media without inoculums. Cultivation was done for 3-6 weeks on

normal temperature under static condition depending on growth of endophytes. Examination of flask was done periodically for and the cultured flasks were examined periodically for contamination (Katoch *et al.*, 2017).

### Cultured endophyte extraction

After cultivation process, there was addition of ethyl acetate (250 mL) to every flask containing culture. With the help of glass rod, the culture media was cut into parts and shaker was used for extraction on which culture flask is placed for 48 hr. Buchner funnel under vacuum was used for filteration of mixture (Kumar and Sahu, 2016). Repeated extraction was done with ethyl acetate until exhaustion. After concentration using a rotary evaporator, the resulting ethyl acetate extract was partition between 90% methanol and n-hexane (Bhatti and Ashraf, 2017).

### Preliminary Phytochemical Screening

To enhance the understanding of the phytochemical profiles of the extracts, different fractions of EMPF and EMPE were subjected to phytochemical tests to detect the presence of specific compounds, including tannins, flavonoids, alkaloids, saponins, and steroids (Evans, 2009). These tests were conducted as given in references, with minor modifications. Each test was tailored to identify particular phytochemicals present in the endophytic extracts.

## RESULTS

### Selection of plant material

For the purpose of this study, the ethanomedicinal whole plant *Mimosa pudica* were chosen keeping in mind their medicine, ethanomedicinal, and pharmaceutical applications.

### Extraction and Isolation of Bacterial Endophyte s from *Mimosa pudica*

Fresh plant material (whole plant) and whole plant extract of medicinal plant of *Mimosa pudica* were used in order to isolate bacterial endophytes. An essential step in eliminating epiphytic bacteria from sample explants was surface sterilization. This step was deemed adequate in our investigation, as there was no growth on the control plate.

As seen in Figure 1, On PDA media, there were enough colonies visible on the edges of explants and streaking colonies on whole plant extract. Since there was no growth on the control plate, these isolates were regarded as endophytes of the *M. pudica* whole plant and whole plant extract.

Bacterial species were isolated from colonies based on their distinct properties. Eight endophytic bacterial isolates in all were obtained from the whole plant and whole plant extract of *Mimosa pudica*. 3 out of 8 isolates from fresh whole plant and 5 isolates from whole plant extracts of *M. pudica*. From gram staining (Figures 2 and 3), it was identified that all eight isolates were gram-negative. These were labelled as S1, S3, S6 (isolated from fresh whole plant) and S2, S3, S4, S5, S6 (from whole plant extracts). According to the current results, whole plant extracts included a higher quantity of bacterial endophytes than fresh whole plants.

A range of physiological, biochemical, and morphological traits were investigated in order to preliminarily identify bacterial endophytes. The findings showed that there were differences in

**Figure 1:** *Mimosa pudica* L.

**Table 1: Colony morphology of isolated bacterial culture.**

| Isolate No. | Colony Morphology | | | | |
|---|---|---|---|---|---|
| | Shape | Elevation | Edges | Colour of Colony | Margin |
| S1 | Round | Slightly raised | Smooth | Off white | Entire |
| S2 | Round | Slightly raised | Smooth | Cream coloured | Entire |
| S3 | Round | Convex | Smooth entire | Translucent | Entire |
| S4 | Round | Slightly raised | Slightly irregular | White | Undulate |
| S5 | Round | Convex | Irregular | Iridescent | Undulate |
| S6 | Round | Convex | Well defined | Off white | Entire |
| S7 | Round | Raised | Smooth | Red | Slightly undulate |
| S8 | Circular | Slightly raised | Smooth and regular | Creamy white | Entire |

**Table 2: Biochemical Characterization of isolated bacterial culture.**

| Characteristic | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 |
|---|---|---|---|---|---|---|---|---|
| Gram staining | - | - | - | - | - | - | - | + |
| Shape | Rod | Rod | Rod | Rod | Rod | Rod | Rod | Rod |
| Catalase | + | + | + | + | + | + | - | + |
| Oxidase | + | + | + | + | - | - | - | + |
| Glucose | + | + | + | - | + | + | + | + |
| Lactose | - | - | - | + | + | + | - | - |
| Maltose | - | - | - | + | + | + | + | + |
| Mannitol | - | - | - | + | + | + | + | + |
| D-Mannose | - | - | - | + | + | + | + | + |
| Sucrose | + | + | + | - | + | + | + | + |
| Nitrate reduction | + | + | - | + | + | + | + | + |
| H2S production | - | - | - | - | _ | - | + | - |
| Starch hydrolysis | + | + | + | + | + | - | + | + |
| Salt stress | 5% | 5% | 5% | 4% | 6% | 4% | 3% | 6% |

lultured flasks wasd for 48 hrs'+ve'- Positive, '-ve'- Negative.

**Table 3: Endophytic bacterial strain list isolated from *M. pudica* whole plant.**

| Sample code | Assigned bacterial name | GenBank accession number | Closest NCBI database match | Sequence Similarity (%) |
|---|---|---|---|---|
| S1 | *Pseudomonas plecoglossicida* | PQ119860 | *Pseudomonas plecoglossicida* strain XSF-13. | 99.89% |
| S2 | *Pseudomonas plecoglossicida* | PQ097079 | *Pseudomonas plecoglossicida* strain RGK. | 100 |
| S3 | *Pseudomonas putida* | PQ130042 | *Pseudomonas* sp. strain *21STR428.* | 99.89 |
| S4 | *Pseudomonas cichorii* | PQ129285 | *Pseudomonas cichorii* strain ICMP 5891. | 99.53 |
| S5 | *Enterobacter ludwigii* | PQ119942 | *Enterobacter ludwigii* strain KAR 10. | 100 |
| S6 | *Enterobacter asburiae* | PQ129399 | *Enterobacter asburiae* strain JM-458. | 100 |
| S7 | *Serratia proteamaculans* | PQ129402 | *Serratia proteamaculans* strain 143. | 100 |
| S8 | *Bacillus pumilus* | PQ129403 | *Bacillus safensis* strain A31. | 99.88 |

the bacterial species isolated from fresh whole plants and whole plant extracts of *M. pudica*

## Morphological and Biochemical Characterization of Endophytes

Each isolate of bacterial pure culture was produced by repeatedly restreaking it on PD agar plates. The following morphological traits of the isolated bacterial colonies were form (irregular, round, filamentous), color, shape, elevation (umbonate, flat, convex), and margin (filamentous, entire, erose, undulate). Table 1 provides a summary of morphological traits of endophytic bacterial isolates

Every bacterial isolate had a rod-like form and tested positive for catalase except S8, all isolates were positive for oxidase except S5, S6, S7. All isolates ferment glucose except S4. Only S4, S5 and S6 isolates ferment lactose. All isolates ferment maltose, mannitol and D-Mannose except S1, S2 and S3. Sucrose was fermented by seven isolates except S4. Seven isolates reduced nitrate except S3. Only S7 isolate produced H2S. All isolates hydrolyzed starch except S6. Additionally, most bacterial endophytes grew best in the range of 5-8% NaCl concentrations, according to our investigation into a range of NaCl concentrations for the maximum growth of bacterial isolates. Table 2 provides Biochemical Characterization of isolated bacterial culture.

## Molecular Identification of Endophytes

After being identified using 16S rDNA sequencing, the isolated endophytes from *M. pudica* were compared to the 16S rRNA sequence database (Bacteria and Archaea) at the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). The 16S r RNA nucleotide sequences have been uploaded to GenBank. Bacterial isolates were categorized into eight species based on sequence analysis, including, S1 *Pseudomonas plecoglossicida* (PQ119860), S2 *Pseudomonas plecoglossicida* (PQ097079), S3 *Pseudomonas putida (*PQ130042), S4 *Pseudomonas cichorii (*PQ129285) S5 *Enterobacter ludwigii (*PQ119942), S6 *Enterobacter asburiae (*PQ129399), S7 *Serratia proteamaculans* (PQ129402), S8 *Bacillus pumilus* (PQ129403). Based on the 16S rRNA gene sequence, Table 3 lists the strains and their closest relatives. Prior to this, the species of bacteria with the highest identity similarity percentage (90-100%) were chosen for phylogenetic analysis.

## Phylogenetic Analysis

The DNA sequences were aligned using the neighbor-joining and maximum likelihood techniques to produce a phylogenetic tree using a distance matrix from an alignment tool. (Figures 4, 5). Both trees show that the endophytic population of *M. pudica* has a high level of microbial diversity. According to the phylogenetic tree, the four genera were divided into four distinct groups that each represented a distinctive phylotype: *Pseudomonas* and Endophytic bacteria made up Group 1, *Serratia* species represented Group 2; *Enterobacter* species represented Group 3 and *Bacillus* sp. as a Group 4 (Tamura *et al., 2021*).

## DISCUSSION

The choice of *Mimosa pudica* for this study is well-justified, as it is recognized for its broad range of medicinal, ethnomedicinal, and pharmaceutical applications. This plant has long been used in traditional medicine, and exploring its bacterial endophytes offers a promising avenue for discovering novel bioactive compounds

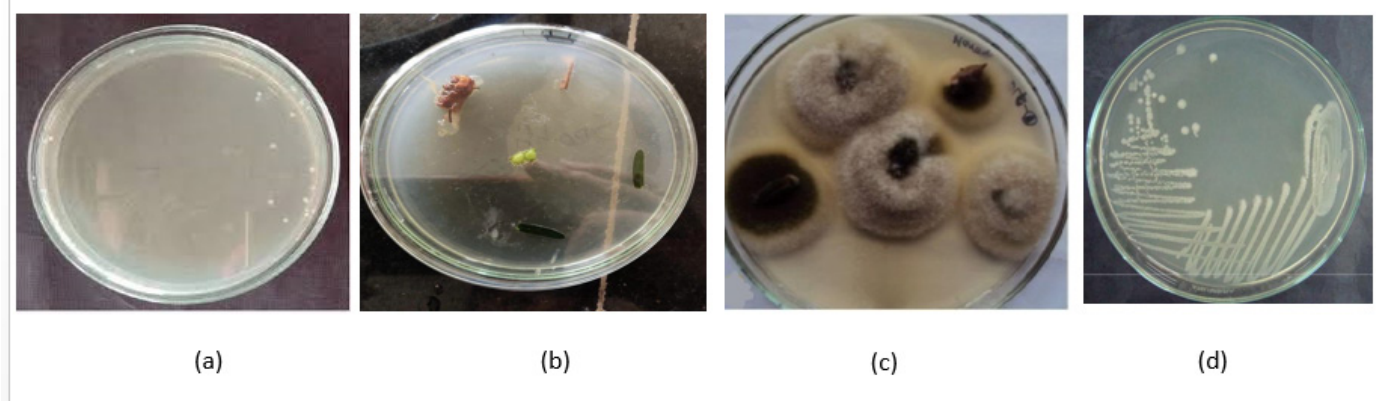


**Figure 2:** Isolation of bacterial endophytes fromMimosa pudica (a) Control plate (b) Whole plant parts inoculated on PDA media (c) endophytes growth from margins of whole plant parts (d) endophytes growth from whole plant extract.

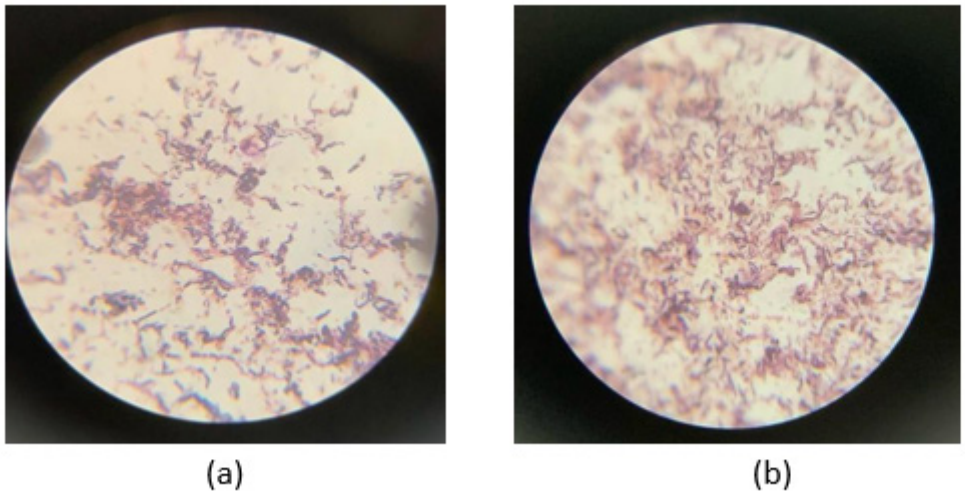


**Figure 3:** Gram staining of endophytes (a) gram staining of endophytes isolated from growth from margins of whole plant parts (b) gram staining of endophytes isolated from growth from whole plant extract.

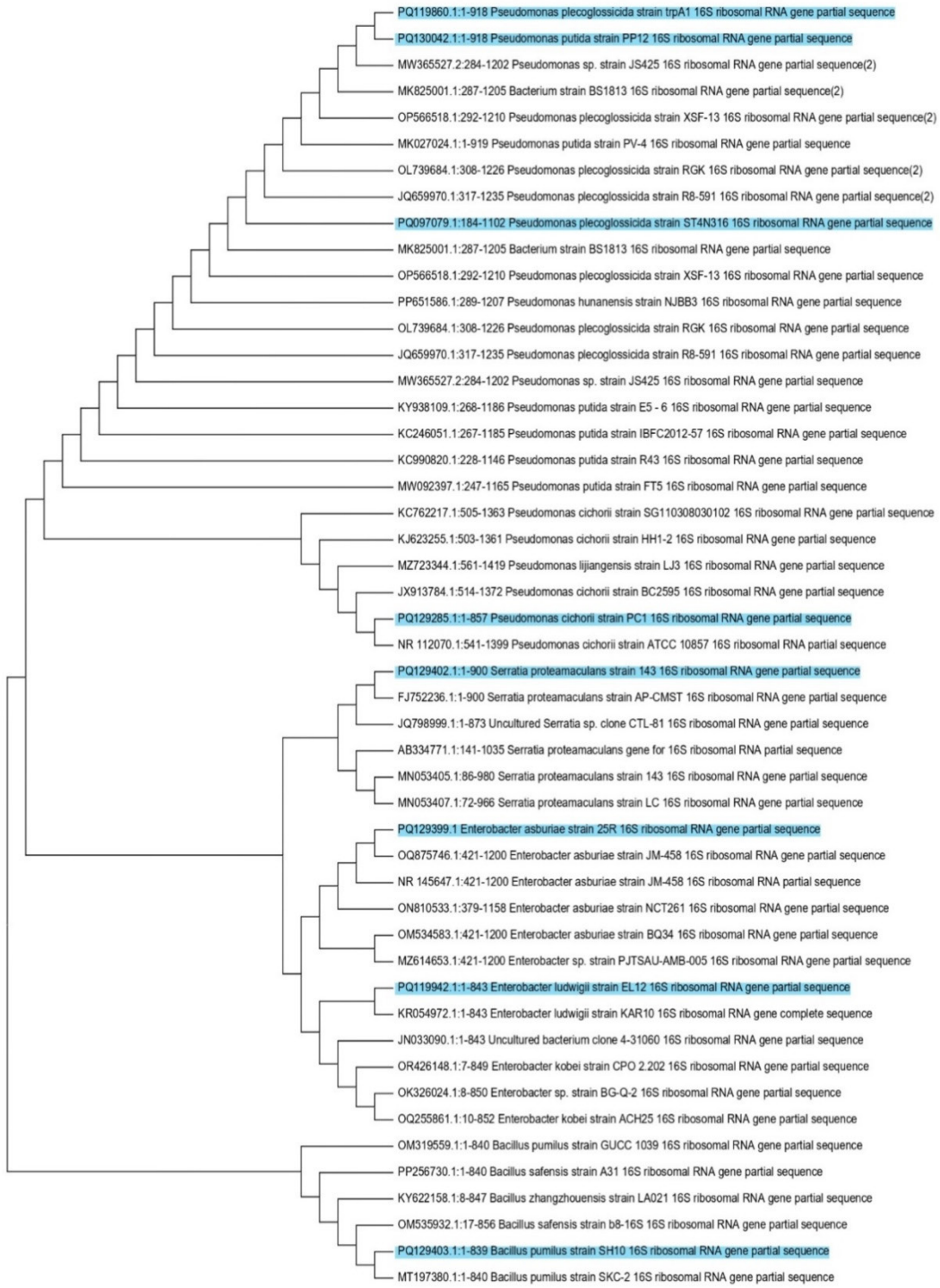


**Figure 4:** Creating a phylogenetic tree with the maximum likelihood approach for endophytic bacterial isolates from *Mimosa pudica*. Highlights have been placed on the endophytic bacteria found in this investigation (Tamura, K. *et al*., 2021).

that can potentially contribute to the pharmaceutical field (Patel and Sharma, 2019).

The isolation of bacterial endophytes from both fresh whole plants and plant extracts of *Mimosa pudica* was successful, with surface sterilization proving to be an essential step to eliminate epiphytic bacteria. The absence of growth on control plates confirmed the success of the sterilization process, ensuring that the isolated bacteria were true endophytes. Interestingly, the results indicated that the whole plant extract contained a higher number of bacterial endophytes than the fresh plant material. This could be due to the availability of more nutrients or favorable conditions for bacterial growth in the extract. The gram-negative

bacteria identified through morphological and biochemical tests align with existing literature on bacterial endophytes, indicating the presence of diverse and functional microbial communities within the plant (Smith and Doe, 2022).

The morphological and biochemical characterization of the isolated bacteria revealed a variety of metabolic traits, including oxidase and catalase activity, sugar fermentation patterns, and resistance to salt stress. These properties are indicative of the bacterial adaptability and potential utility in various biotechnological applications. The ability of most isolates to hydrolyze starch and their growth in saline conditions suggests that these bacteria could have industrial applications in agriculture or food production, particularly in saline or nutrient-depleted environments (Singh *et al.*, 2011).

The molecular identification of the bacterial isolates using 16S rDNA sequencing confirmed that the endophytic bacteria from *Mimosa pudica* belong to diverse genera such as Pseudomonas, Enterobacter, Serratia, and Bacillus, which are known for their bioactive compounds, plant-growth-promoting capabilities, and biocontrol potential. Phylogenetic analysis revealed a high degree of microbial diversity within these endophytes, which supports

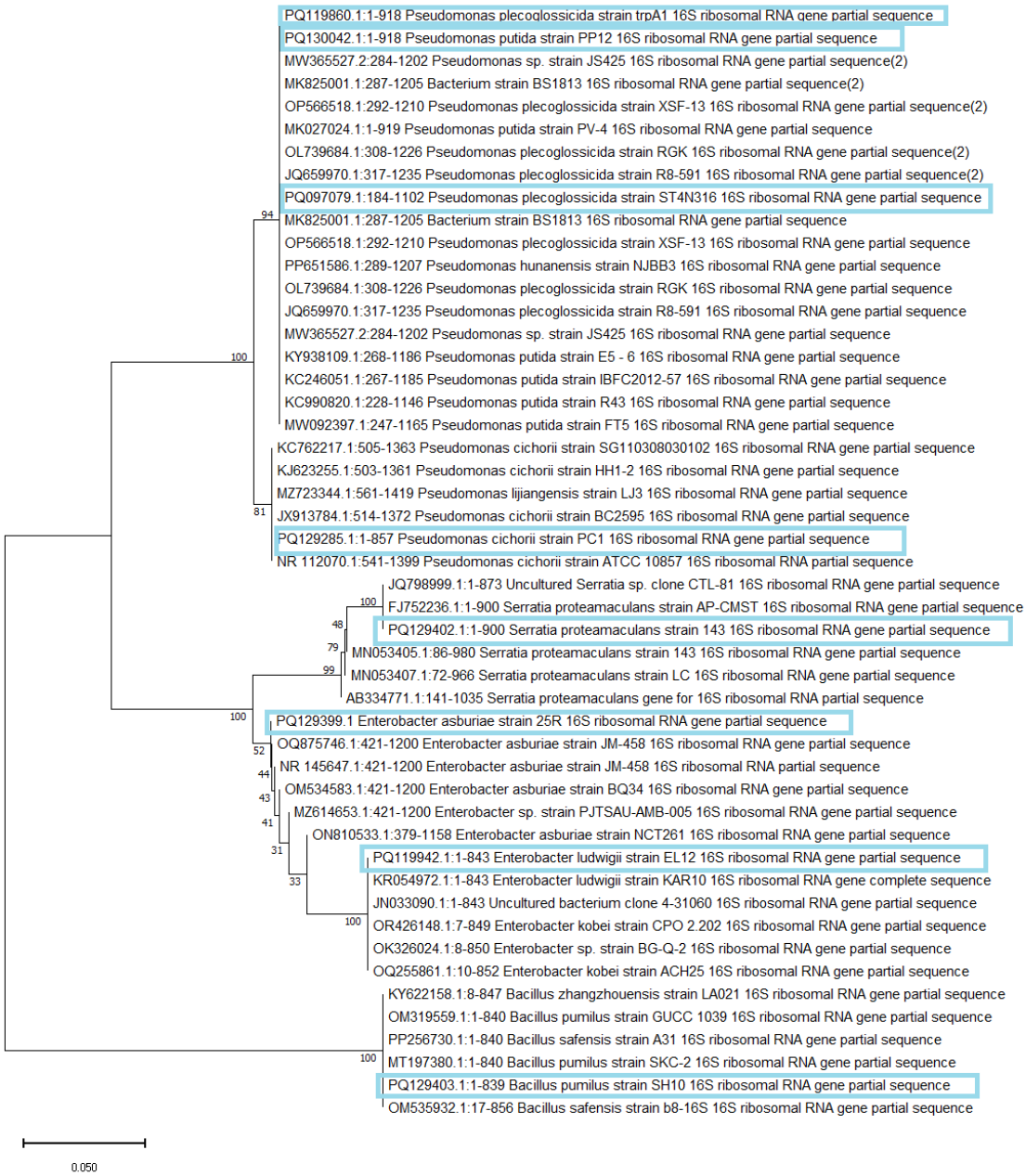


**Figure 5:** Creating a phylogenetic tree with neighbour-joining method for endophytic bacterial isolates from *Mimosa pudica*. Highlights have been placed on the endophytic bacteria found in this investigation (Tamura, K. *et al.*, 2021).

the notion that *M. pudica* harbors a rich microbial ecosystem capable of influencing its medicinal properties.

## CONCLUSION

This study demonstrates that *Mimosa pudica* not only serves as a valuable source of medicinal compounds but also harbors a diverse community of bacterial endophytes that contribute to its biochemical and therapeutic profile. The isolation and identification of eight bacterial species from both fresh plants and plant extracts provide insight into the microbial diversity within this plant and its potential applications in agriculture and pharmaceuticals.

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## ABBREVIATIONS

**S1:** *Pseudomonas plecoglossicida*; **S2:** *Pseudomonas plecoglossicida*; **S3:** *Pseudomonas putida*; **S4:** *Pseudomonas cichorii*; **S5:** *Enterobacter ludwigii*; **S6:** *Enterobacter asburiae*; **S7:** *Serratia proteamaculans*; **S8:** *Bacillus pumilus*; **WPE:** Whole plant extract; **EMPF:** Endophytic *Mimosa pudica* Fresh plant; **EMPE:** Endophytic *Mimosa pudica* plant extract; **PPSE:** *P. plecoglossicida* Extract; **PPTE:** *P. putida* Extract; **PCE:** *P. cichorii* Extract; **ELE:** *E. ludwigii* Extract; **EAE:** *E. asburiae* Extract; **SPE:** *S. proteamaculans* Extract.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ETHICAL APPROVAL

Ethical approval for this study was obtained from Institutional Ethical committee, D.M.I.M.S. with approval No. 850.

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