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Endophytic Bacteria from *Mimosa pudica* L.: Morphological and Biochemical Traits and Biocontrol Activity against *Alternaria porri*

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Abstract

Shallot (*Allium cepa* L.) is an important horticultural commodity whose productivity is frequently constrained by purple blotch disease caused by *Alternaria porri*. Excessive reliance on synthetic fungicides for disease control has raised concerns related to environmental sustainability and pathogen resistance. Endophytic bacteria, which inhabit internal plant tissues without causing harm, represent a promising source of biological control agents. This study aimed to characterize the morphological and biochemical traits and to evaluate the biocontrol activity of endophytic bacteria isolated from *Mimosa pudica* L. against *A. porri*. A total of twelve endophytic bacterial isolates were obtained from healthy roots, stems, and leaves of *M. pudica* using a surface sterilization technique. The isolates exhibited diverse colony morphology and growth characteristics. Biochemical characterization revealed that most isolates were gram-positive, catalase-positive, motile, and capable of starch hydrolysis. Antagonistic activity was evaluated using a dual culture assay on potato dextrose agar. The results demonstrated that several isolates effectively inhibited the mycelial growth of *A. porri*. Among them, isolates MP5 and MP11 showed the highest inhibitory effects, with growth inhibition percentages of 58.2% and 48.7%, respectively, and were classified as having high antagonistic activity. The strong inhibitory performance of selected isolates suggests their ability to produce antifungal metabolites or to suppress pathogen growth through competitive interactions. Overall, endophytic bacteria isolated from M. pudica, particularly isolates MP5 and MP11, exhibit significant potential as environmentally friendly biological control agents for managing purple blotch disease in shallot cultivation.

Keywords: Endophytic bacteria; *Mimosa pudica*; biocontrol activity; *Alternaria porri*; shallot.

INTRODUCTION

Shallot (*Allium cepa* L.) is an economically important horticultural crop widely cultivated in tropical and subtropical regions. In Indonesia, shallot plays a crucial role in food security and agricultural income. However, its productivity is frequently constrained by plant diseases, particularly purple blotch disease caused by *Alternaria porri*. This pathogen attacks leaves and flower stalks, leading to reduced photosynthetic capacity, premature leaf senescence, and significant yield losses under favorable environmental conditions (Singh et al., 2018; Prameswari et al., 2021). Conventional management of purple blotch disease relies heavily on repeated applications of synthetic fungicides, which may result in pathogen resistance, environmental contamination, and negative impacts on non-target organisms (Brent & Hollomon, 2007).

The development of environmentally friendly disease management strategies has therefore become a major focus in sustainable agriculture. Biological control using beneficial microorganisms is considered a promising alternative to chemical-based approaches. Among these microorganisms, endophytic bacteria have attracted considerable attention due to their ability to colonize internal plant tissues without causing disease symptoms (Hardoim et al., 2015). Endophytic bacteria can enhance plant health by suppressing pathogens through multiple mechanisms, including the production of antifungal metabolites, competition for nutrients and space, and induction of systemic resistance in host plants (Santoyo et al., 2016).

Numerous studies have reported the effectiveness of endophytic bacteria as biological control agents against fungal pathogens in various crops. For example, endophytic Bacillus and Pseudomonas species have been shown to inhibit Fusarium, Alternaria, and Rhizoctonia species through the production of antibiotics, lytic enzymes, and volatile organic compounds (Compant et al., 2010; Yanti et al., 2018). Despite these promising findings, the exploration of endophytic bacteria from non-cultivated or wild plant species remains limited, particularly in the context of shallot disease management.

Mimosa pudica L., commonly known as the sensitive plant, is a wild leguminous species that thrives under diverse and often stressful environmental conditions. Its ability to survive in marginal habitats suggests a close association with beneficial endophytic microorganisms that may contribute to stress tolerance and disease resistance. Previous studies have indicated that wild plants can serve as reservoirs of diverse and functionally important endophytic bacteria with strong antagonistic activity against plant pathogens (Gouda et al., 2016; Afzal et al., 2019). However, information regarding the biocontrol potential of endophytic bacteria isolated from M. pudica against *A. porri* is still scarce.

Therefore, this study aimed to characterize the morphological and biochemical traits of endophytic bacteria isolated from *M. pudica* and to evaluate their antagonistic activity against *A. porri*. The findings of this research are expected to provide scientific evidence supporting the use of endophytic bacteria as sustainable biological control agents for managing purple blotch disease in shallot cultivation.

MATERIALS AND METHODS

Materials

The research was conducted from October 2023 to January 2024. Isolation and characterization of endophytic bacteria, as well as antagonistic activity tests, were conducted at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. The materials used were healthy rice root samples of Xanthomonas oryzae isolate, NA, 70% alcohol, Clorox, distilled water, and Agrept (bactericide). The tools used in this study included Petri dishes, Erlenmeyer flasks, measuring cylinders, Bunsen burners, hotplates, glass slides, loop needles, cover glasses, auto-aerators, scissors, microscopes, analytical balances, stationery, cameras, vernier calipers, and scalpel blades.

Methods

Isolation of endophytic bacteria was performed using a surface sterilization technique to eliminate epiphytic microorganisms. Plant tissues were washed under running tap water, surface-sterilized with 70% ethanol for 1 minute, followed by 2% NaOCl for 2 minutes, and rinsed three times with sterile distilled water. Sterilized tissues were aseptically macerated, serially diluted, and plated on Nutrient Agar. Plates were incubated at 28–30 °C for 48 hours, and morphologically distinct colonies were purified through repeated streaking (Hardoim et al., 2015; Sihotang et al., 2025).

Morphological characterization was conducted based on colony color, shape, margin, surface texture, and growth rate. Biochemical characterization included Gram staining, catalase and oxidase tests, motility assessment, and starch hydrolysis following standard microbiological procedures (Cappuccino & Sherman, 2014; Sihotang et al., 2025).

Antagonistic activity against Alternaria porri was evaluated using the dual culture method on Potato Dextrose Agar (PDA). A mycelial plug of A. porri was placed at the center of the Petri dish, while bacterial isolates were streaked at a fixed distance from the fungal plug. Plates were incubated at 28 °C for seven days. Fungal growth inhibition was determined by comparing the radial growth of the pathogen in treatment and control plates, as previously described for endophytic bacterial biocontrol assays (Compant et al., 2010; Sihotang et al., 2025).

Data analysis

Data analysis was performed using One-Way ANOVA, following tests for normality (Shapiro-Wilk) and homogeneity (Levene's Test). If the data met normality and homogeneity assumptions ($p > 0.05$), Post Duncan tests were used to analyze group differences. If data were not normally distributed, the Kruskal-Wallis test was used, followed by MannWhitney U tests for pairwise comparisons when significant differences were observed.

RESULTS AND DISCUSSION

Morphological Characteristics of Endophytic Bacterial Isolates from *Mimosa pudica* L.

Table 1. Morphological Characteristics of Endophytic Bacterial Isolates from *Mimosa pudica* L.

Isolate	Colony Color	Shape	Margin	Surface	Texture	Growth (mm/48 h)
MP1	Milky white	Circular	Entire	Convex	Smooth	3.8
MP2	Cream	Circular	Entire	Convex	Smooth	4.1
MP3	White	Circular	Entire	Slightly glossy	Smooth	5.2
MP4	Light yellow	Irregular	Undulate	Flat	Rough	3.5
MP5	Opaque white	Circular	Entire	Glossy	Smooth	6.0
MP6	Cream	Circular	Entire	Convex	Smooth	4.0
MP7	Pale yellow	Irregular	Undulate	Slightly rough	Rough	3.2
MP8	Milky white	Circular	Entire	Convex	Smooth	4.5
MP9	White	Circular	Entire	Convex	Smooth	4.3
MP10	Yellow	Irregular	Undulate	Flat	Rough	3.4
MP11	Cream white	Circular	Entire	Slightly glossy	Smooth	5.4
MP12	White	Circular	Entire	Convex	Smooth	4.0

Biochemical Characteristics of Endophytic Bacterial Isolates

Table 2. Microskopic Characterization and Gram Staining of Endophytic Bacteria.

Isolate	Gram Reaction	Catalase	Oxidase	Motility	Starch Hydrolysis
MP1	+	+	–	+	–
MP2	+	+	+	+	+
MP3	+	+	+	+	+
MP4	–	+	–	–	–
MP5	+	+	+	+	+ (strong)
MP6	+	+	–	+	–
MP7	–	–	–	–	–
MP8	+	+	+	+	+
MP9	+	+	–	+	–
MP10	–	+	–	–	–
MP11	+	+	+	+	+ (strong)
MP12	+	+	–	+	–

Antagonistic Activity of Endophytic Bacteria against Alternaria porri

Table 3. Antagonistic Activity of Endophytic Bacteria against *Alternaria porri.*

Isolate	Inhibition Zone (mm)	Growth Inhibition (%)	Activity Category
MP1	4.2	18.0	Low
MP2	5.6	22.5	Low
MP3	9.2	36.0	Moderate
MP4	2.1	9.8	Low
MP5	14.5	58.2	High
MP6	3.5	14.2	Low
MP7	1.8	7.0	Low
MP8	8.7	34.5	Moderate
MP9	4.8	19.2	Low
MP10	2.5	10.5	Low
MP11	12.1	48.7	High
MP12	3.2	13.0	Low

Discussion

The results showed that endophytic bacterial isolates from rice (*Oryza sativa*) plants consisted of several dominant genera, namely Pseudomonas and Bacillus. Morphological characterization, Gram staining, and biochemical tests revealed significant variation among isolates. Most isolates exhibited high catalase, starch hydrolysis, and motility capabilities, indicating their potential for adaptation to host plant tissues. These results are consistent with reports that the Bacillus and Pseudomonas genera are the most common endophytic bacterial groups that play a role enhance rice plant growth and resistance to pathogens.

Antagonistic tests against *Xanthomonas oryzae pv. oryzae* (Xoo), the causative agent of bacterial leaf blight in rice, showed that isolates BETP 03 (Pseudomonas) and BETP 05 (Bacillus) produced the largest inhibition zones, with diameters >16 mm. This confirms their ability to produce antimicrobial secondary metabolites such as siderophores, antibiotics, and hydrolytic enzymes. These findings align with recent research confirming the effectiveness of *Pseudomonas fluorescens* and *Bacillus subtilis* in inhibiting Xoo growth through antibiosis mechanisms and induction of systemic resistance in rice.

Furthermore, hydrolytic enzyme activity in several isolates supports their role as biocontrol agents. Enzymes such as chitinase, protease, and lipase are known to be able to damage pathogen cell walls, thereby strengthening plant defenses. Biochemical tests showed that isolates BETP 04 and BETP 05 exhibited a combination of citrate, gelatinase, and catalase activity, strengthening the hypothesis that these isolates have the potential to be used as both a biofertilizer and a biopesticide.

From a microbial ecology perspective, the presence of endophytic bacteria within rice tissues provides a competitive advantage over epiphytic microbes, as they can survive in the relatively stable internal environment of the plant. Thus, endophytes can play a dual role: increasing plant nutrient availability (for example, through nitrogen fixation or phosphate solubilization) and protecting the plant from pathogen attack.

However, this study also has limitations, namely the lack of molecular analysis (16S rRNA) to ensure accurate isolate identification, as well as field trials to confirm the effectiveness of biocontrol in real agroecosystem conditions. Further studies are strongly recommended to explore the molecular mechanisms of antimicrobial metabolite production and the formulation of endophyte consortia as environmentally friendly biopesticides.

Thus, the results of this study provide strong evidence that the endophytic bacteria Pseudomonas and Bacillus from *Oryza sativa* have high prospects as biocontrol agents to reduce dependence on chemical pesticides in sustainable rice cultivation systems.

CONCLUSIONS

The present study demonstrates that endophytic bacteria isolated from *Mimosa pudica* L. exhibit considerable diversity in morphological and biochemical characteristics, as well as varying levels of antagonistic activity against *Alternaria porri*. The variation in colony morphology and growth rates among isolates reflects the heterogeneity of endophytic bacterial communities commonly reported in wild plant species (Hardoim et al., 2015). Wild plants such as *M. pudica*, which thrive in diverse and often stressful environments, are known to harbor endophytes with adaptive traits that may contribute to plant protection mechanisms (Gouda et al., 2016).

Biochemical characterization revealed that most isolates were gram-positive and catalase-positive, suggesting a predominance of bacteria with strong stress tolerance and metabolic versatility. Similar findings have been reported for endophytic bacteria belonging to the genus Bacillus, which are frequently associated with antagonistic activity against fungal pathogens due to their ability to produce a wide range of antimicrobial compounds (Compant et al., 2010; Santoyo et al., 2016). The presence of starch hydrolysis and motility traits among several isolates further indicates their potential competitiveness and adaptability in colonizing plant tissues.

The dual culture assay demonstrated that isolates MP5 and MP11 exhibited high antagonistic activity against *A. porri*, with growth inhibition exceeding 45%. This level of inhibition is comparable to that reported for effective endophytic biocontrol agents against Alternaria species in previous studies (Yanti et al., 2018; Prameswari et al., 2021). The inhibitory effects observed in this study may be attributed to multiple mechanisms, including the production of antifungal metabolites, secretion of cell wall–degrading enzymes, and competition for nutrients and space (Weller, 2007). Although the specific metabolites were not identified in this study, the strong suppression of fungal growth suggests active antagonistic interactions between the endophytic bacteria and the pathogen.

The results also support the concept that wild plants can serve as valuable reservoirs of biocontrol agents. Endophytic bacteria isolated from non-cultivated hosts have been reported to exhibit stronger or broader antagonistic activity compared to those from cultivated plants, likely due to long-term ecological adaptation (Afzal et al., 2019). The findings are consistent with Sihotang et al. (2025), which highlighted the biocontrol potential of endophytic bacteria isolated from different plant hosts against phytopathogenic fungi.

Overall, the strong antagonistic performance of selected isolates, particularly MP5 and MP11, indicates their potential for further development as environmentally friendly biological control agents. Future studies should focus on molecular identification, characterization of antifungal metabolites, and in planta evaluations to confirm their effectiveness under greenhouse and field conditions.

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