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In vitro antifungal activity of *Mimosa pudica* rhizosphere bacteria against *Fusarium* spp.

Sriwulan Sriwulan[*1)], Dhina Mustikaningrum[2)], Nia Nurfitria[3)]
[*1]Biology Study Program, Faculty of Mathematic and Natural Sciences, Universitas PGRI Ronggolawe, Jl. Manunggal No. 61, Tuban, 62381, Indonesia
[2]Agroecotechnology Study Program, Faculty of Agriculture, Universitas Sunan Bonang, Jl. DR. Wahidin Sudirohusodo No.798, Tuban, 62315, Indonesia
[3]Mathematic Study Program, Faculty of Mathematic and Natural Sciences, Universitas PGRI Ronggolawe, Jl. Manunggal No. 61, Tuban, 62381, Indonesia

*Corresponding e-mail: biowulan08@gmail.com

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Abstract

This study aims to explore rhizosphere bacteria from *Mimosa pudica* L. as a source of antagonistic bacteria against *Fusarium* spp. Rhizosphere soil samples were collected, isolated, morphologically characterized, and tested for antifungal activity in vitro against *Fusarium* spp. The percentage of inhibition (PI) was calculated after 7 days. Six bacterial isolates were obtained and tentatively identified as *Bacillus*, *Cellulomonas*, *Arthrobacter*, and *Micrococcus* species. All isolates exhibited antagonistic activity against *Fusarium* spp., with inhibition percentages ranging from 61.99% to 81.83%. Isolate PM2 (tentatively identified as *Cellulomonas*) demonstrated the strongest inhibition at 81.83%, followed by isolate PM6 (tentatively identified as *Micrococcus*) at 79.25%. These results confirm the potential of *Mimosa pudica* L. as a source of biocontrol agents and warrant further investigation into their molecular identification, mechanisms of action, and in vivo efficacy.

Introduction

Fusarium spp. is a genus of fungal plant pathogens that is highly detrimental on a global scale (Ekwomadu & Mwanza, 2023; Nikitin et al., 2023; Shabeer et al., 2021). This fungus is responsible for various crucial diseases in agricultural crops, such as *Fusarium* wilt, root rot, and stem rot (Ekwomadu & Mwanza, 2023; Williamson-Benavides & Dhingra, 2021). The pathogenicity of *Fusarium* spp. is demonstrated not only through physical damage that impedes water and nutrient transport but also through the production of mycotoxins that are harmful to plants and human health (Awuchi et al., 2021; Perincherry et al., 2019). *Fusarium* spp. infections can lead to significant yield reduction, even up to total crop failure, thereby causing substantial economic losses for farmers (Petronaitis et al., 2021; Shukla et al., 2022).

For decades, control of *Fusarium* spp. has still heavily relied on the use of synthetic fungicides. Although effective in suppressing pathogen populations in the short term, over-reliance on these chemicals has led to various negative impacts. These include pathogen resistance and harmful chemical residues on agricultural products. In the environment, it caused soil and water pollution to disruption the balance of beneficial microorganisms in the soil ecosystem (Fenta & Mekonnen, 2024; Ishii, 2024). Therefore, the search for more environmentally friendly and sustainable control methods is become imperative.

One of the most promising alternative strategies is biological control using antagonistic agents such as rhizosphere bacteria (Azeem et al., 2020; Bonaterra et al., 2022; Sriwulan et al., 2019b). The rhizosphere, as the zone around plant roots rich in root exudates, serves as a habitat for highly diverse microbial communities, including bacteria with antifungal capabilities (Alawiye & Babalola, 2019; Vives-Peris et al., 2020). These bacteria can inhibit pathogen growth through various mechanisms, such as the production of antibiotics, siderophores, lytic enzymes (chitinases, cellulases), and competition for nutrients and space (Chowdhury et al., 2020; Riseh et al., 2024; H. Wang et al., 2021). Several studies have been conducted and shown that rhizosphere bacteria have the potential to be developed as biocontrol agents. Research by Flori et al. (2020) showed that *Bacillus* bacterial isolates from the rhizosphere of pepper plants could inhibit *Fusarium* sp. JDF in vitro with an inhibition zone diameter of 13.93 mm and were categorized as strong inhibitors. Research by Zunairoh et al. (2019) obtained 1 bacterial isolate from the strawberry rhizosphere that could inhibit the growth of *Fusarium* sp. Meanwhile, Nasution & Saryanah (2023) found that 2 bacterial isolates from the rhizosphere of *Celosia argantea* showed antifungal activity against *Fusarium* sp.

One plant that has the potential to be a source of antagonistic bacteria against pathogenic fungi is *Mimosa pudica*. This plant is known to have high adaptability to various types of soil (Febriyantiningrum et al., 2023). This adaptability is partly supported by the presence of microorganisms that form a symbiosis with this plant, both endophytic microorganisms and microorganisms in the rhizosphere(Kristianti et al., 2023). These microorganisms can produce bioactive compounds that play a role in protecting plants against biotic stress, one of which is pathogenic agents (Abdullahi et al., 2020; Sapiña-Solano et al., 2024). This is the basis for the assumption that *Mimosa pudica* rhizosphere bacteria also have potential as biocontrol agents against the pathogenic fungus *Fusarium*. Several studies have examined the potential of endophytic bacteria in the root nodules of *Mimosa pudica* as biocontrol agents (Méndez-Santiago et al., 2021; Sanchez-Cruz et al., 2019; Sharma & Goswami, 2020). However, exploration of *Mimosa pudica* rhizosphere bacteria as biocontrol agents against soil-borne pathogenic fungi has been limited.

This study investigated the antagonistic activity of rhizosphere bacteria from *Mimosa pudica* L. against *Fusarium* spp. The novelty of this study lies in the exploration of rhizosphere bacteria from *Mimosa pudica L.* as a source of new antagonistic agents against *Fusarium* spp., which has not been widely reported in the literature. Most previous studies have focused on cultivated plants, while wild plants like *Mimosa pudica L.* may harbor unexplored microbial diversity and superior potential. The findings provide a scientific basis for the development of new, effective, and environmentally friendly biological control agents as part of integrated plant disease management.

Materials and methods

This research was conducted from May to July 2025 at the Biology Laboratory, Universitas PGRI Ronggolawe.

Research Sampling

Rhizosphere soil samples were collected in an agricultural field with healthy, flowering *Mimosa pudica L.* in Leran Wetan Village, Palang District, Tuban Regency. Composite sampling technique was used to collect samples in five different points around the root zone at a depth of 10-20 cm (Sriwulan et al., 2022). Soil adhering to the roots was collected by gently brushing it off with a sterile brush and then mixed thoroughly (composited) to form one representative sample (Koyama et al., 2021). This composite sample was placed in a sterile plastic bag, labeled, and transported to the laboratory in a cool box for subsequent analysis.

Mimosa pudica L. Rhizospheric Bacteria Isolation and Characterization

Isolation and characterization of rhizospheric bacteria from the collected soil sample were carried out and began with a serial dilution. An aliquot of the composite soil sample was weighed 25 g and added to 225 mL of sterile 0.85% physiological NaCl solution in an Erlenmeyer flask (Jie et al., 2025). This suspension was shaken using an orbital shaker for 15 minutes at 120 rpm to obtain a 10^{-1} dilution. A serial dilution was then performed up to a 10^{-6} dilution.

Bacterial inoculation was carried out by taking 0.1 ml of suspension at 10^{-5} and 10^{-6} dilutions. Inoculation was carried out using the pour plate method on NA media. Incubation was carried out at room temperature (28 ± 2°C) for 2x24 hours. The growing bacterial colonies were observed, and colonies with different morphological characteristics were purified using the plate streak method on NA media. The isolates were then characterized based on colony morphology, gram staining, and biochemical characterization using the catalase test.

Propagation of *Fusarium* spp.

The propagation of *Fusarium* spp. isolates was carried out by inoculating pure cultures using the agar block transfer method (Ekwomadu & Mwanza, 2023). A 5 mm diameter agar block containing *Fusarium* spp. mycelium was taken aseptically using a sterile cork borer. The block was then placed in the center of fresh PDA media and incubated at room temperature (28 ± 2°C) for 5-7 days.

Antifungal Activity Test

The antifungal activity test of Mimosa pudica rhizosphere bacterial isolates against Fusarium spp. was carried out using the dual culture method on PDA media (Boulahouat et al., 2023). A 5mm agar block containing 7-day-old Fusarium spp. mycelium was placed on one side of a petri dish containing PDA media. On the opposite side, each purified isolate of Mimosa pudica rhizosphere bacteria was streaked, except for the control (no test bacterial isolate was streaked). Each bacterial isolate was repeated 3 times. The petri dish was then incubated at room temperature (28 ± 2°C) for 7 days.

The inhibition of fungal growth was observed. The radial growth diameter of Fusarium spp. towards the bacterial isolate was measured and compared to the growth diameter in the control plate (S. Wang et al., 2024). The percentage of inhibition (IP) was calculated using the following formula:

$$IP\ (\%) = [(dc - dt) / dc] \times 100\%$$

Where:
IP = Inhibition Percentage
dc = Radial growth diameter of the fungus in the control plate (mm)
dt = Radial growth diameter of the fungus towards the bacterial isolate in the treatment plate (mm)

Data Analysis

The inhibition percentage (IP) data obtained for each bacterial isolate were analyzed quantitative descriptive to identify which isolate exhibited the strongest antagonistic activity. Data was processed using SPSS 25.0 program in analysis of Variance (ANOVA) one-way test with confidence limit of 95% or p=0.05. If there is a noticeable difference effect between IP and isolate typed bacteria, further post-hoc Tukey's HSD test used to provided the highest mean inhibition percentage.

Results and discussion

1. Isolation and Characterization of *Mimosa pudica L.* Rhizosphere Bacteria

The bacterial isolates from the rhizosphere of *Mimosa pudica L.* were characterized based on colony morphology, microscopic characteristics, and biochemical tests. The characterization revealed six distinct isolates, and the characteristics were presented in Table 1. As shown in Table 1, the six isolates exhibited diverse characteristics. These observed characteristics were used as a basis for the preliminary genus-level identification of the isolates.

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

Isolate	Colony Morphology				Gram Type	Cell Shape	Catalase Test
	Form	Colour	Edge	Elevation			
PM1	Punctiform	White	Entire	Flat	Positive	Rod	Positive
PM2	Irregular	White	Undulate	Flat	Positive	Coccus	Positive
PM3	Irregular	White	Rhizoid	Flat	Positive	Rod	Positive
PM4	Circular	Yellow	Entire	Convex	Positive	Rod	Positive
PM5	Circular	Translucent White	Entire	Flat	Positive	Rod	Positive
PM6	Irregular	Yellowish-White	Rhizoid	Flat	Positive	Coccus	Positive

The characteristics of isolates PM1, PM3, and PM5 are indicative of the genus *Bacillus*. Bacteria from this genus initially often form punctiform colonies with entire edges (after short incubation periods). However, with longer incubation (typically 48 to 72 hours), these colonies can develop into larger, circular or irregular forms, sometimes appearing wrinkled due to endospore formation (Turenne et al., 2015). The punctiform colony with an entire edge observed for PM1 in this study is likely because characterization was performed after 24 hours of incubation. This morphological change was already observed in isolate PM3 and PM5, which can be attributed to differing growth rates among bacterial strains, even within the same genus. Bacillus isolates on Nutrient Agar (NA) generally appear translucent, white, cream, or brown. This group consists of Gram-positive, rod-shaped cells and tests positive for catalase (Bergey, 1994). Soil is a common habitat for this bacterial genus, which aligns with the origin of the sample in this research, rhizosphere soil of *Mimosa pudica L.*

The characterization results for isolate PM2, as shown in Table 1, characteristics pointing towards the genus Cellulomonas. This genus is characterized by short rod-shaped cells or cocci (in some species), which may align to form V-shaped structures. They are Gram-positive, catalase-positive, often motile with one or more flagella, do not form spores, are non-acid-fast, are facultative anaerobes, possess cellulolytic activity, and are commonly found in soil and other organic matter (Bergey, 1994; Wu et al., 2023).

The isolate PM4 exhibits characteristics indicative of bacteria belonging to the genus Arthrobacter. This genus is characterized by rod-shaped cells that undergo a rod-to-coccus cycle upon aging, a Gram-positive reaction, motility in some species, an inability to form endospores, an aerobic metabolism, a positive catalase test, and an optimal growth temperature of 25–30 °C. Arthrobacter species are widely distributed in nature, particularly in soil habitats (Busse et al., 2015; Khairani et al., 2019; Roy & Kumar, 2020).

Meanwhile, the characteristics of the PM6 isolate indicate a character that points to the genus Micrococcus. This genus is mixed with a group of bacteria that are gram-positive, have round cells that are paired, form tetrads, or grouped irregularly (Y. Wang et al., 2021). The results of the gram staining obtained by the PM6 isolate appear purple, indicating that it is a gram-positive bacteria and has coccus-shaped cells arranged in irregular groups. Micrococcus colonies on NA media appear yellow or red, where the PM6 isolate shows a yellow isolate color. Bacteria from the Micrococcus genus also show positive catalase results with one of their habitats in soil (Bergey, 1994).

2. Antifungal Activity of *Mimosa pudica L.* Rhizosphere Bacteria Agains *Fusarium* spp.

Table 2 below presents data on the percentage of inhibition of Mimosa pudica rhizosphere bacterial isolates against Fusarium spp. in vitro, which was observed on the 7th day of incubation.

Table 2. Inhibition Percentage (IP) of *Mimosa pudica* L. Rhizosphere Bacterial Isolates against *Fusarium* spp.

Isolate	Inhibition Percentage (%)
PM1	70.96^{ab}
PM2	81.83^{b}
PM3	70.14 ^{ab}

Isolate	Inhibition Percentage (%)
PM4	66.16 [a]
PM5	61.99 [a]
PM6	79.25[b]

Nb: Different letters indicate significant differences

The data in Table 2 shows that the six bacterial isolates obtained from the rhizosphere of *Mimosa pudica* in this study were able to inhibit *Fusarium* spp., with an inhibition percentage ranging from 61.99% to 81.83%. These results indicate that all isolates obtained in this study have antifungal activity against *Fusarium* spp.

The inhibition percentage data shown in Table 2 were analyzed using one-way ANOVA statistical analysis, which obtained a significant value of 0.01 < alpha 0.05. This indicates that differences in bacterial isolates affect their antifungal activity as indicated by their inhibition percentage. Meanwhile, further test results using the Tukey HSD test showed that Isolate PM2 provided the highest average inhibition percentage (81.83%), but was not significantly different from isolates PM6, PM1, and PM3.

These results indicate that the six isolates from the rhizosphere of *Mimosa pudica* plants have the potential to be developed as biocontrol agents, particularly against *Fusarium* spp. Rhizosphere bacteria capable of inhibiting the growth of pathogenic fungal isolates generally possess the ability to produce antibiotic compounds, siderophores, lytic enzymes, or a combination of these mechanisms (Sriwulan et al., 2019a).

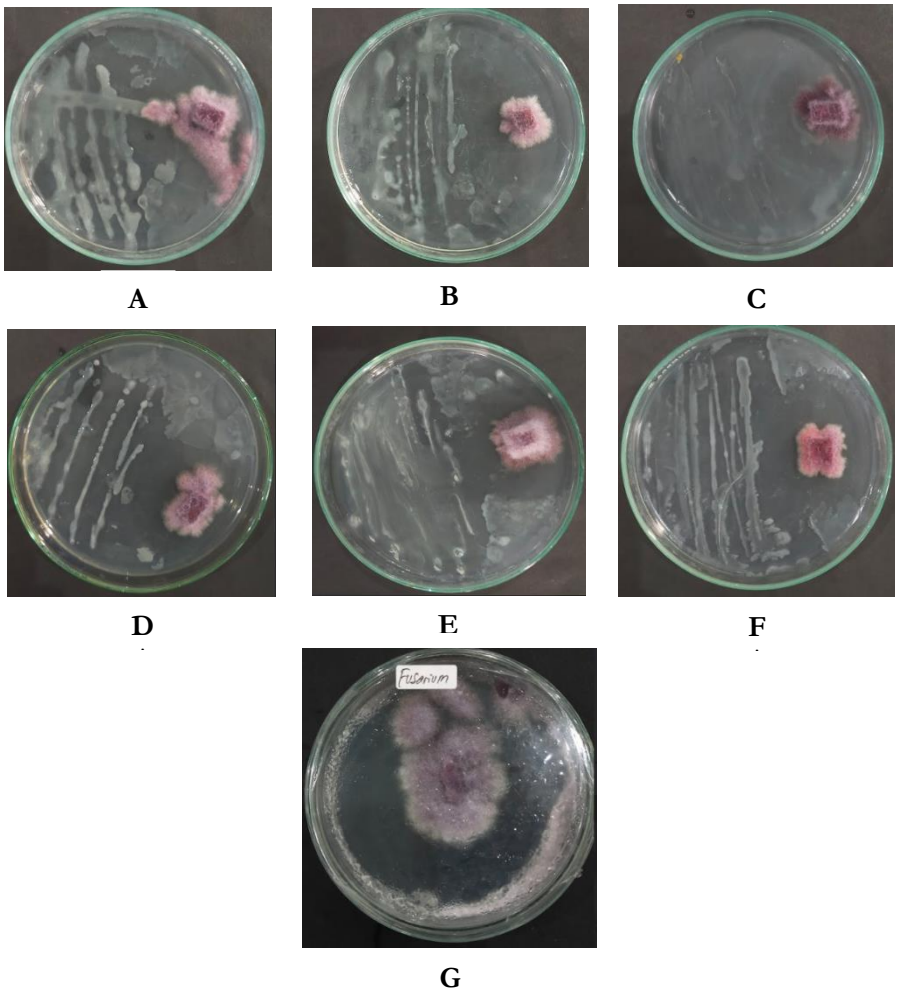

Figure 1. In Vitro Antifungal Activity Test of *Mimosa pudica* Rhizosphere Bacteria against *Fusarium* spp. (A. PM1 isolate agains *Fusarium* spp.; B. PM2 isolate agains *Fusarium* spp.; C. PM3 isolate agains

***Fusarium* spp.; D. PM4 isolate agains *Fusarium* spp.; E. PM5 isolate agains *Fusarium* spp.; F. PM6 isolate agains *Fusarium* spp.; G. *Fusarium* spp. only as control)**

Several bacterial species from the genus *Bacillus* are known to produce antibiotic compounds that can damage the cell membranes of pathogenic fungi, inhibit cell wall formation, and disrupt the metabolic processes of these pathogenic fungi (Puspitasari, 2023). Consistent with this finding, isolates PM1, PM3, and PM5 in this study, belonging to the genus *Bacillus*, were able to inhibit the growth of *Fusarium* spp. with inhibition rates of 70.96%, 70.14%, and 61.99%, respectively.

Meanwhile, isolate PM5 produced the lowest inhibition percentage, at 61.99%. However, this figure still demonstrates the isolate's promising potential for development as a biocontrol agent. Several species of the genus *Bacillus* are known to produce the enzymes chitinase and glucanase, which can damage fungal cell walls (Dawoud et al., 2020). Furthermore, *Bacillus amyloliquefaciens* is also known to stimulate host plants to increase their protective abilities against pathogen attacks (Luo et al., 2022; Ngalimat et al., 2021; Zalila-Kolsi et al., 2023).

The PM2 isolate, which demonstrated the highest antifungal activity in this in vitro test, is indicated as belonging to the genus *Celullomonas*. Bacteria from this genus exhibit antifungal activity through several mechanisms, including the ability to produce lytic enzymes that can damage fungal structures, such as chitinase and glucanase. These enzymes break down chitin and glucan, the main components and essential components of fungal cell walls. Furthermore, the metabolic activity of *Celullomonas* bacteria also alters microenvironmental conditions, particularly pH and oxygen levels. This will affect fungal survival. The ability of these bacteria to produce cellulase enzymes also allows them to release nutrients that can stimulate the growth of other antagonists (Ontañon et al., 2021; Wu et al., 2023).

Meanwhile, the antifungal mechanism demonstrated by the PM4 isolate may occur through its ability to produce siderophore compounds. This will make it difficult for *Fusarium* spp. to obtain iron ions and will disrupt metabolic processes. Furthermore, bacterial groups from the *Arthrobacter* genus are also known to have the ability to colonize rapidly, thereby outcompeting *Fusarium* spp. in the process of nutrient competition (Roy & Kumar, 2020). Several *Arthrobacter* species have been reported to be able to produce antibiotic compounds, volatile compounds, and lytic enzymes such as chitinase and glucanase that can inhibit fungal growth. This group of fungi can also induce systemic plant resistance . The same mechanism was also shown by the group of bacteria from the genus *Micrococcus* which in the study was represented by the isolate PM6 (Y. Wang et al., 2021).

Conclusion

Six isolates of Mimosa pudica rhizosphere bacteria that were successfully isolated and identified in this study have the potential to be used as biocontrol agents against *Fusarium* spp. The highest antifungal activity was shown by Isolates PM2 and PM6 with inhibition percentages of 81.83% and 79.25%, respectively. The results of this study require further research, especially in planta testing, to determine the effectiveness of these bacterial isolates in controlling Fusarium spp. on a field scale, so that they can be applied practically in integrated plant disease management..

Author Statements

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Generative AI: Not Applicable

Data availability: The raw data supporting the conclusion of this article, including serial dilatation, IP calculation will be made available to any qualified researcher.

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