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Exploration and Characterization of Endophytic Bacteria from Antidiabetic Plants as Biocontrol Agents Fusarium Sp

Lita Nasution[1]*, Bahagia Sibotolungun Sipahutar[2], Ebin Hagata Pinem[3], Ersentabi Br Sembiring[4], Makhrani Sari Ginting[5]
[1]Dosen Program Studi Agroteknologi, Fakultas Sains dan Teknologi, Universitas Quality, Indonesia
[2]Dosen Program Studi Agribisnis, Fakultas Sains dan Teknologi, Universitas Quality, Indonesia
[3,4]Mahasiswa Program Studi Agroteknoogi, Fakultas Sains dan Teknologi, Universitas Quality, Indonesia
[5]Dosen Program Studi Proteksi Tanaman, Institut Teknologi Sawit Indonesia
Corresponding Author: Lita Nasution Litanasution@universitasquality.ac.id

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A B S T R A C T
This research aims to explore and characterize endophytic bacteria from the roots of antidiabetic plants and evaluate their potential as biocontrol agents against Fusarium sp. Endophytic bacteria were isolated from root tissues using surface sterilization methods, followed by colony morphology characterization, microscopic properties, and biochemical tests. Antagonistic activity was tested in vitro using the dual culture method. The research results showed that 12 endophytic bacterial isolates were obtained from root tissues, with three selected isolates (EAF-3, EAF-6, and EAF-7) exhibiting significant antagonistic activity against Fusarium sp. Isolate EAF-7 had the highest inhibitory power of 81.6%, followed by EAF-6 (74.2%) and EAF-3 (68.4%). Characterization shows that the promising isolates are closely related to the genera Bacillus and Pseudomonas, which are known as highly potential endophytic bacteria for controlling plant pathogens. These results indicate that endophytic bacteria from antidiabetic plants, particularly isolate EAF-7, have the potential to be developed as an environmentally friendly biocontrol agent for Fusarium wilt disease management in chili plants.

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INTRODUCTION

Chili pepper (Capsicum annuum L.) is an important horticultural commodity with high economic value, but its productivity is often reduced due to wilt disease caused by Fusarium sp. This soilborne pathogen is persistent and difficult to control using chemical fungicides on a sustainable basis. Therefore, an environmentally friendly and sustainable alternative approach is needed.

The use of endophytic bacteria as biocontrol agents and plant growth promoters has been widely reported to be effective in suppressing plant pathogens. Endophytic bacteria are known to produce antifungal metabolites, hydrolytic enzymes, and act as plant growth-promoting rhizobacteria (PGPR) (Sihotang et al., 2023a; Sartini et al., 2024; Sihotang et al., 2025a). Previous research has shown that endophytic bacteria are capable of inhibiting important plant pathogens thru antibiosis and nutrient competition.

Medicinal plants, including antidiabetic plants, are rich in secondary metabolites such as flavonoids, phenolics, and terpenoids, which support the selection of endophytic microbes with high metabolic capabilities. Exploratory studies on endophytic bacteria in various plants have been conducted and show great potential in controlling plant diseases (Noer et al., 2023; Azwana & Sihotang, 2023). In addition, endophytic bacteria also play a role in enhancing plant growth thru the production of growth hormones and increased nutrient uptake (Meyuliana et al., 2024; Sitepu & Sihotang, 2025).

Endophytic bacteria are microorganisms that live within plant tissues and will compete with pathogens, and their presence in plant tissues does not harm their host. Endophytic bacteria originate from the environment and enter plants thru stomata, lenticels, and wounds via lateral roots. The use of antagonistic endophytic bacteria has advantages over other antagonistic microbes, namely that endophytic bacteria are already formed within the plant, will remain throughout the plant's development, and provide protection for the plant. One type of endophytic bacteria that acts as an antagonistic agent against plant pathogens is antagonistic bacteria. Antagonistic bacteria can induce plant resistance to pathogens by activating signaling pathways and involving the plant hormones jasmonic acid and ethylene. Endophytic bacteria in red chili plants have been successfully isolated. Out of 109 isolates, 8 were able to suppress the development of the plant pathogen Colletotrichum sp. Based on characterization and identification, the isolates were identified as bacteria from the genera Pseudomonas and Bacillus. In the study, all the endophytic bacteria isolates successfully isolated from chili plants were able to suppress wilt disease caused by the pathogens Ralstonia and Fusarium. After identification, all isolates belong to the genus Bacillus. Bacillus is a widely used biological control agent for controlling soilborne pathogens.

Bacteria from the genus Pseudomonas are endophytic bacteria that act as biocontrol agents on plants, phytoremediation agents, and plant growth promoters. They mention that Pseudomonas sp. can produce antibiotic compounds, siderophores, and other secondary metabolites that can inhibit pathogen activity. Antidiabetic medicinal plants such as Andrographis paniculata (sambiloto), Momordica charantia (bitter melon), Moringa oleifera (kelor), and Syzygium cumini (duwet) are known to contain bioactive secondary metabolite compounds that are also thot to influence the diversity of their endophytic microbes. Exploring endophytic bacteria from these plants has the potential to discover isolates with natural antifungal activity against Fusarium sp.. Agriculture in Indonesia today is heavily reliant on the use of inorganic fertilizers and pesticides. The alternative used by farmers to control Fusarium wilt and anthracnose disease is the use of synthetic fungicides. Bacteria that can be PGPR are endophytic bacteria. Endophytic bacteria are bacteria found within the tissues of healthy plants that do not cause disease symptoms and are not harmful to the host plant. Endophytic bacteria can be isolated and extracted on bacterial growth media using surface sterilization techniques. The use of endophytic bacteria as a biological agent has advantages over other antagonistic microbes because endophytic microbes are already formed, live, and survive within the tissues during plant development and provide protection for the plant. The advantages of endophytic bacteria as biological agents compared to rhizosphere bacteria, beside their more protected existence and ability to colonize plant tissues, include better translocation of produced metabolic compounds into plant tissues (Hallmann et al. 1997 in Hanif and Susanti, 2017).

Therefore, this research is important to conduct in order to explore and characterize endophytic bacteria from antidiabetic plants as biocontrol agents against Fusarium sp. in chili plants. The research results are expected to serve as a basis for the development of environmentally friendly natural biofungicides that support sustainable agriculture. Based on the above description, the use of endophytic bacteria on plants is expected to increase plant growth and resistance. Research on the potential of endophytic bacteria from cucumber stems and chili seeds needs to be conducted to determine the effectiveness of these bacteria.

LITERATURE REVIEW

Endophytic bacteria are microorganisms that live inside plant tissues without causing harm to their host and are widely recognized for their role in plant disease suppression and growth promotion. These bacteria are able to colonize internal plant tissues and provide long-term protection against pathogens through mechanisms such as antibiosis, competition for nutrients and space, production of hydrolytic enzymes, and induction of systemic resistance. Compared to rhizosphere microorganisms, endophytic bacteria have advantages due to their protected niche within plant tissues and their ability to translocate bioactive compounds directly to target sites.

Several studies have reported the effectiveness of endophytic bacteria as biocontrol agents against soilborne pathogens, particularly *Fusarium* spp., which cause wilt diseases in various crops. Endophytic bacteria belonging to the genera *Bacillus* and *Pseudomonas* are among the most extensively studied due to their strong antagonistic activity and metabolic versatility. *Bacillus* spp. are known to produce lipopeptide antibiotics such as surfactin, iturin, and fengycin, which effectively inhibit fungal growth. Meanwhile, *Pseudomonas* spp. suppress pathogens through the production of siderophores, antibiotics, and volatile organic compounds, as well as by enhancing plant defense responses.

Medicinal plants, including antidiabetic plants, represent promising sources of endophytic bacteria with high biocontrol potential. These plants are rich in secondary metabolites such as flavonoids, phenolics, and terpenoids, which influence the diversity and functional capacity of their associated endophytes. Previous research has demonstrated that endophytic bacteria isolated from medicinal plants often exhibit strong antimicrobial activity and adaptability, making them suitable candidates for sustainable disease management strategies.

In addition to their antagonistic effects, endophytic bacteria function as plant growth-promoting rhizobacteria (PGPR) by producing phytohormones, enhancing nutrient uptake, and improving plant tolerance to biotic and abiotic stress. Their dual role as growth promoters and biocontrol agents makes endophytic bacteria an environmentally friendly alternative to synthetic pesticides, which are widely used but pose risks to human health and ecosystems.

Based on previous findings, exploration and characterization of endophytic bacteria from antidiabetic plant roots are essential to identify effective biological agents against *Fusarium* wilt disease in chili plants. Such studies contribute to the development of sustainable agriculture by reducing dependency on chemical fungicides and promoting eco-friendly plant disease management practices.

METHODOLOGY

Time and Place of Research This research was conducted in 2025 at the Microbiology Laboratory of the Faculty of Science and Technology, Quality University.

Plant Exploration and Sampling Antidiabetic plant samples were taken from naturally growing healthy plants. The plant part used is the root, as root tissue is the primary habitat for endophytic bacteria that interact directly with the soil and soilborne pathogens such as Fusarium sp. (Wijianto et al., 2023; Patel et al., 2018). Root samples were washed using running water to remove surface dirt.

Isolation of Endophytic Bacteria Endophytic bacteria were isolated using the surface sterilization method following standard procedures (Gouda et al., 2016; Nasution et al., 2025). The roots were cut to a length of ±1 cm, then sterilized using 70% ethanol for 1 minute, 2–3% sodium hypochlorite for 2 minutes, and rinsed three times with sterile distilled water. The success of sterilization was confirmed thru imprint tests and negative controls on Nutrient Agar (NA) medium. Tissue pieces were grown on NA medium and incubated at 28–30 °C for 48–72 hours. The growing colonies were purified until a pure isolate was obtained.

Morphological, Microscopic, and Biochemical Characterization Colony morphology was observed based on color, shape, elevation, and surface. Microscopic characterization was performed using Gram staining. Biochemical tests included catalase, oxidase, motility, and IMViC tests for initial isolate identification (Sihotang et al., 2023a).

In Vitro Antagonism Test against Fusarium sp. The antagonism test was conducted using the dual culture assay method on Potato Dextrose Agar (PDA) medium. A mycelial plug of Fusarium sp. was placed in the center of the dish, while the bacterial isolate was inoculated on the side of the dish. Incubation is carried out for 5-7 days. The percentage of pathogen growth inhibition was calculated by comparing the diameter of the pathogen mycelium in the treatment and control groups (Rahmadani & Aini, 2023).

Data Analysis Growth and disease intensity data were analyzed using ANOVA at a 95% confidence level. If significant differences were found, Tukey's or Duncan's post-hoc tests were performed to compare treatments. Antagonism test data is presented in the form of graphs and tables.

RESEARCH RESULT

Results of Endophytic Bacteria Exploration and Isolation from Roots The isolation of endophytic bacteria focused on the root tissues of antidiabetic plants. After surface sterilization and confirmation thru imprint tests/negative controls, endophytic isolates were obtained from the roots. Table 1. Number of Endophytic Bacterial Isolates from Roots Tissue Number of Isolates Percentage of Total Isolates (%) Root 12 44.4

Colony Morphology Characteristics of Root Isolate Observations of root isolate colony morphology were made after 48–72 hours of incubation (28–30°C). Colonies exhibit variations in color, shape, elevation, and surface. Table 2. Summary of Colony Morphology of Endophytic Isolates from Roots Parameter Dominant Category Description/Proportion Colony Color Milky white, cream, pale yellow milky white dominant Colony Shape Round / irregular round more common Elevation Convex / flat convex dominant Surface Smooth/glossy / rough/matte smooth dominant

Microscopic and Biochemical Characterization of Selected Root Isolates From the root isolates, the best isolates (EAF-3, EAF-6, EAF-7) were selected for further characterization.

Table 1. Gram Characterization and Biochemical Tests of Selected Root Isolates Isolate Gram Cell Shape Catalase Oxidase Motility Presumed Genus EAF-3 + Rod + + + Bacillus sp EAF-6 – Rod + + + Pseudomonas sp EAF-7 + Rod + + + Bacillus sp.

Antagonistic Activity of Root Isolates Against Fusarium sp. (In Vitro) Antagonism tests (dual culture) showed that the selected root isolates were able to significantly inhibit the growth of Fusarium sp.

Table 1. Percentage of Fusarium sp. Inhibition by Root Isolates

No	Root isolate	Percentage of Inhibition (%)
1	EAF-3	68,4
2	EAF-6	74,2
3	EAF-7	81,6

DISCUSSION

The exploration results indicate that the root network of antidiabetic plants is the primary source of endophytic bacteria, with the number of isolates reaching 12 isolates or 44.4% of the total isolates obtained. The dominance of isolates from root tissue aligns with the role of roots as the primary point of interaction between plants and soil microorganisms. Roots produce exudates containing amino acids, sugars, and phenolic compounds that support the colonization and persistence of endophytic bacteria. Additionally, roots are the primary entry point for endophytic microbes into plant tissues, resulting in higher endophytic diversity compared to other tissues.

Colony morphology observation showed a fairly wide variation in phenotypic characteristics, especially in color, shape, elevation, and colony surface. The dominance of milky white and cream-colored colonies with a round shape, convex elevation, and smooth surface indicates similarity to the bacterial colony characteristics of the genera Bacillus and Pseudomonas. This morphological character is often associated with endophytic bacteria that have high adaptability and strong metabolic activity, including the production of antibacterial and antifungal compounds.

Microscopic and biochemical characterization of the selected isolates (EAF-3, EAF-6, and EAF-7) showed that the isolates were dominated by rod-shaped bacteria with positive catalase and oxidase activity. Isolates EAF-3 and EAF-7 were Gram-positive and had characteristics similar to Bacillus velezensis and Bacillus subtilis, while EAF-6 was Gram-negative and had a typical IMViC pattern of Pseudomonas fluorescens. These two genera are widely recognized as superior endophytic bacteria that act as biocontrol agents and plant growth promoters. The positive catalase and oxidase activity indicates that the isolate is able to withstand oxidative stress, which is important for colonizing plant tissues and competing with pathogens.

In vitro antagonism tests showed that all selected isolates were able to inhibit the growth of Fusarium sp. with a high degree of inhibition, ranging from 68.4% to 81.6%. Isolate EAF-7 exhibited the highest inhibitory power at 81.6%, indicating very strong biocontrol potential. The high percentage of inhibition indicates that the isolates are capable of producing antifungal metabolites and/or hydrolytic enzymes that effectively damage the cell walls of pathogenic fungi. Differences in the level of inhibition between isolates are likely due to variations in secondary metabolite production capacity, growth rate, and colonization ability on the medium.

Overall, the results of this study indicate that endophytic bacteria from antidiabetic plant roots, particularly isolate EAF-7, have great potential as biocontrol agents for Fusarium sp. These findings support the concept of utilizing endophytic bacteria as an environmentally friendly and sustainable alternative for plant disease control.

CONCLUSIONS AND RECOMMENDATIONS

This research indicates that the root network of antidiabetic plants is a potential source of endophytic bacteria with biocontrol activity against Fusarium sp. A total of 12 endophytic bacterial isolates were successfully isolated from the roots, with three selected isolates (EAF-3, EAF-6, and EAF-7) showing significant in vitro antagonistic ability. Isolate EAF-7 exhibited the highest inhibitory effect on the growth of Fusarium sp. Morphological, microscopic, and biochemical characterization indicated that the superior isolates belonged to the genera Bacillus and Pseudomonas.

ADVANCED RESEARCH

The genera Bacillus and Pseudomonas, which are known to be effective as environmentally friendly biocontrol agents for plant disease management.

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