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Induction of Endophytic Bacteria to Enhance Intracellular Immunity of Arabica Coffee (*Coffea arabica* L.) Infected with *Fusarium* sp. in vitro

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ABSTRACT

This study aimed to isolate and characterize endophytic bacteria from the roots of Arabica coffee (*Coffea arabica* L.), investigate their growth patterns, and evaluate their antagonistic activity against the pathogenic fungus *Fusarium* sp. Through surface sterilization and subsequent cultivation on Nutrient Agar, six distinct endophytic bacterial isolates were obtained, exhibiting diverse colony morphologies ranging from circular to irregular, and colors from white to cream or yellowish. Antagonism assays indicated varying degrees of inhibitory effects against Fusarium sp.: Sp2At produced the largest inhibition zone with an average of 17.9 mm, followed by Sp3At at 16.86 mm, while Sp4At and Sp6At showed smaller zones of 6.16 mm and 6.9 mm, respectively. Furthermore, the highest indole-3-acetic acid (IAA) production was detected at pH 7.5 with 291.64 ppm, whereas pH 6.5 resulted in the lowest IAA production at 281.04 ppm after 72 hours of incubation. These results suggest the potential of these isolates to promote plant growth through phytohormonal pathways.

Keywords: Antagonist, *Coffea arabica* L, Colony morphology, Endophytic bacteria, *Fusarium* sp.

INTRODUCTION

Coffee (*Coffea* sp.) is one of the plantation commodities that significantly contributes to the Indonesian economy. However, according to data from the Badan Pusat Statistik, (2024) national coffee production has shown a declining trend from 2021 to 2023. In 2022, coffee production decreased by 1.43 percent, falling from 786.19 thousand tons to 774.96 thousand tons. This downward trend continued in 2023, with production declining by 16.24 thousand tons, or 2.10 percent, compared to the previous year. This decline in production is largely due to various diseases affecting coffee plants, one of the main ones being coffee berry rot. According to Leonardo & Milantara, (2023) this disease is caused by the pathogenic fungus *Fusarium* sp., which acts as a parasite that can infect various parts of the plant, particularly the coffee fruit and beans. *Fusarium* sp. infection typically causes symptoms such as wilting, early tissue damage (damping off), and rotting of the coffee fruit and beans.

Efforts to minimize losses caused by plant disease infections can be implemented through targeted and appropriate control methods. The excessive and uncontrolled use of pesticides may have detrimental effects on the environment as well as on the quality of the coffee fruit itself (Hartatie & Donianto, 2021). Therefore, an environmentally friendly approach to disease management is necessary to preserve ecosystem balance. One promising strategy involves the use of ecological biological control agents, such as antagonistic bacteria, particularly endophytic bacteria (Maya Listya et al., 2017).

Endophytic bacteria have gained widespread application as biological control agents due to their capacity to produce antimicrobial compounds and growth-regulating hormones, as well as their roles in nitrogen fixation and phosphate mobilization, which collectively enhance plant growth and resilience (Etminani & Harighi, 2018). Research on indigenous endophytic bacteria from Tapanuli Selatan is still very limited. Further studies are needed to examine the diversity of isolates and their potential to inhibit *Fusarium* sp. infestation in coffee plants. This study aims to identify endophytic bacterial isolates from Tapanuli Selatan Regency and their potential as biological control agents for the pathogen *Fusarium* sp.

MATERIALS & METHODS

This research was conducted at the Laboratory of Pests and Diseases, Faculty of Science and Technology, University of Pembangunan Panca Budi, Medan, North Sumatra, from December 2024 to May 2025. The equipment used in this study included petri dishes, test tubes, tube racks, measuring cups, beakers, Erlenmeyer flasks, autoclave, oven, spatulas, inoculation needles, centrifuge, incubator, hot plate, stirring rods, analytical balance, sprayers, laminar airflow cabinet, glass bottles, aluminum foil, cotton, cutters, Bunsen burners, microscopes, and orbital shakers. The materials utilized comprised coffee root samples, *Fusarium* sp. fungal isolates, Nutrient Agar (NA) media, Potato Dextrose Agar (PDA) media, Nutrient Broth (NB) media, Salkowski solution, distilled water, 70% alcohol, and 5.25% sodium hypochlorite solution.

Isolate Endophytic Bacteria

Isolation of endophytic bacteria was performed using roots from healthy Arabica coffee plants (*Coffea arabica* L) free from pathogen infection. Root samples were collected from coffee plants cultivated in Aek Sabaon Village, Marancar District, South Tapanuli Regency. The isolation procedure followed the methods developed by (Singh et al., 2022) with certain modifications made to accommodate the specific research conditions.

The isolation process began with washing the roots under running water to remove soil and surface microorganisms. The roots were then cut into small segments. Surface sterilization was performed by first immersing the root segments in 70% ethanol for 2 minutes, followed by immersion in a 5.25% sodium hypochlorite solution for an additional 2 minutes. Subsequently, the root segments were rinsed several times with sterile distilled water to eliminate any residual sterilizing agents. The sterilized samples were then dried using sterile wipes under aseptic conditions.

Following the sterilization process, the root segments were aseptically sectioned and inoculated onto sterile Nutrient Agar (NA) plates. The plates were then incubated at room temperature for three days to facilitate the growth of endophytic bacteria.

Antagonists Test

The antagonistic activity of endophytic bacteria against *Fusarium* sp. growth was assessed using the dual culture method on Potato Dextrose Agar (PDA) media in petri dishes. Endophytic bacteria were inoculated at the center of the dish, while *Fusarium* sp. was placed in one of the quadrants. The experiment was conducted in triplicate to ensure result consistency. Fungal growth was monitored periodically from the second to the fourteenth day after inoculation.

The percentage of radial growth inhibition (PIRG) of the fungus was calculated to evaluate the effectiveness of inhibition exerted by the endophytic bacteria. PIRG was calculated using the formula developed by (Skidmore & Dickinson, 1976)

$$PIRG = \frac{R_1 - R_2}{R_1} \times 100\%$$

Where R_1 represents the diameter of fungal growth on the control medium without antagonistic bacteria, and R_2 represents the

diameter of fungal growth on the medium inoculated with endophytic bacteria.

RESULT

Isolation and Characterization of Endophytic Bacteria

According to (Zit et al., 2023), pure endophytic bacterial isolates were identified morphologically based on colony color, edge shape, elevation, and growth characteristics. Of the endophytic bacterial isolates obtained from coffee plant roots (*Coffea arabica* L.), six isolates showed diverse colony morphological characteristics. Each isolate had different colony shape, edge shape, elevation, and color. These results are consistent with research (Purba et al., 2023) on bacterial isolation and endophytic characteristics in coffee plants, resulting in four isolates with different characteristics. The morphological characteristics of endophytic bacterial colonies from coffee plant roots are presented in Table 1.

Isolate	Colony Morphology			
	Shape	Margin	Elevation	Color
sp1At	Punctiform	Filamentous	Flat	Yellowish
Sp2At	Circular	Circular	Flat	Cream
Sp3At	Circular	Filamentous	Flat	Yellowish
Sp4At	Irregularize	Undulate	Flat	White
Sp5At	Filamentous	Filamentous	Flat	White
Sp6At	Irregularize	Irregulars	Flat	White

Table 1. Morphological Characteristics of Endophytic Bacterial Colonies of Coffee Plants.

Uji IAA

Indole-3-acetic acid (IAA) production by endophytic bacterial isolates Sp3At was evaluated by incubating cultures in media adjusted to pH values of 6.5, 7.0, and 7.5 for 72 hours. IAA concentrations were measured spectrophotometrically at a wavelength of 530 nm following reaction with Salkowski's reagent. This procedure aligns with the method used by (Khianngam et al., 2023), who also measured IAA in endophytic bacterial isolates using NB media with varying pH and performed spectrophotometric measurements after reaction with Salkowski's reagent. This demonstrates that the approach used in this study is consistent with protocols validated in previous studies.

The results of absorbance measurements and IAA concentration calculations in endophytic bacterial cultures, conducted under varying pH conditions of the culture medium, demonstrated an increase in IAA production corresponding to the rise in medium pH, as follows:

Treatment (pH)	Concentration (ppm)
6,5	281,04
7	283,64
7,5	291,64

Table 2. IAA production by endophytic bacteria

These data indicate that IAA production by endophytic bacteria increases as the pH of the medium rises from 6.5 to 7.5, with pH 7.5 yielding the highest IAA concentration after 72 hours of incubation.

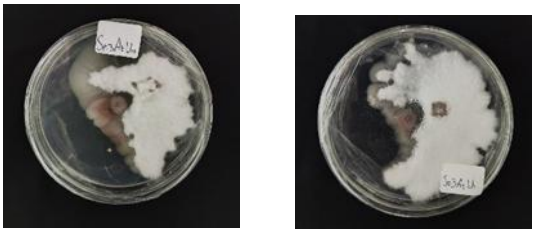
Antagonist Test Observation Results

The results of the antagonistic activity test of endophytic bacteria isolated from coffee roots against *Fusarium* sp. demonstrated the presence of an inhibition zone, indicating the ability of the endophytic bacteria to suppress the growth of *Fusarium* sp. Quantitative observations are presented in Table 3.

Species	Inhibition zone (mm)
Sp1At	8,6
Sp2At	17,93
Sp3At	16,86
Sp4At	6,16
Sp5At	8,3
Sp6At	6,9

Table 3. Diameter of inhibition zones produced by endophytic bacterial antagonists against *Fusarium* sp. fungus.

Figure 1. Growth antagonist test against *Fusarium* sp



DISCUSSION

Characterization of Endophytic Bacteria from Coffee Plant Roots

Bacteria were characterized based on colony morphology, including colony shape, elevation, margin, and color. Similar studies by Warsito et al., (2024) reported that endophytic bacterial isolates from coffee plants exhibited considerable diversity in colony morphology, ranging from circular and irregular to filamentous shapes, along with color variations spanning white, cream, and yellowish hues. This morphological diversity serves as a critical criterion for the selection of isolates for further applications, such as biological control agents or plant growth promoters.

IAA Test for Endophytic Bacteria

The significant increase in IAA production at pH 7.5 compared to pH 6.5 and 7.0 underscores the importance of optimizing culture conditions to enhance the potential of endophytic bacteria as plant growth biostimulants.

Test Antagonists

The results of the antagonistic activity test of endophytic bacteria against fungi showed significant variations in inhibitory efficacy between bacterial isolates. The Sp2At isolate produced the largest inhibition zone, with an average diameter of 17.9 mm, followed by the Sp3At isolate which showed an inhibition zone of 16.86 mm. In contrast, the Sp4At and Sp6At isolates showed smaller average inhibition zones, namely between 6.16 and 6.9 mm. This difference reflects the variability in the inhibitory efficacy of each isolate against the growth of pathogenic fungi in vitro.

The observed inhibition zones provide evidence of the antagonistic activity of endophytic bacteria, which can arise through various mechanisms, including the production of antifungal metabolites, competition for space and nutrients, and the secretion of enzymes that impede fungal growth (Galih Alifianto et al., 2025). This finding aligns with research Warsito et al., (2024) which showed that endophytic bacteria isolated from the stems and roots of Arabica coffee (*Coffea arabica* L.) were able to suppress the growth of Upas fungus (*Corticium salmonicolor* B. Et. Br.) by showing an inhibition zone measuring 6.10 mm to 13.20 mm.

The antagonistic potential of these isolates is believed to be associated with the production of secondary metabolites such as iturin, phenazine, and siderophores, which contribute to damaging fungal cell membranes or disrupting the metabolism of pathogenic fungi (Oktavianti et al., 2024). Therefore, isolates exhibiting the largest inhibition zones have the potential to serve as effective biological control agents for managing fungal diseases in coffee plants.

CONCLUSION

Endophytic bacteria isolated from Arabica coffee roots exhibited distinct variations in colony morphology, including shape, margins, elevation, and color. Indole-3-acetic acid (IAA) testing at pH 7.5 demonstrated the highest concentration after 72 hours of incubation. Furthermore, antagonistic testing revealed that several isolates, particularly Sp2At and Sp3At, exhibited significant inhibitory effects against the pathogenic fungus *Fusarium* sp.. These findings suggest that certain endophytic bacteria from coffee roots possess strong potential as natural biological control agents and plant growth promoters through mechanisms involving the production of antifungal metabolites and the synthesis of IAA.

Declaration by Authors
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