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Isolation and Screening of Endophytic Bacteria Against *Colletotrichum* sp. Causing Antracnose in Edamame

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Abstract: Edamame (*Glycine max* L. Merr.) is a horticultural commodity with high potential as a protein-rich functional food, in line with the increasing demand from both domestic and export markets. However, edamame productivity in Indonesia continues to face serious constraints due to anthracnose disease caused by *Colletotrichum* sp. This pathogen can infect nearly all parts of the plant and significantly reduce both yield quality and quantity. Conventional control strategies relying on synthetic fungicides have caused various negative impacts; therefore, alternative, environmentally friendly, and sustainable approaches are urgently required. This study aimed to isolate endophytic bacteria from edamame plant tissues and to screen their antagonistic activity against *Colletotrichum* sp. Endophytic bacteria were isolated from the roots, stems, and leaves of edamame, and their antagonistic activity was assessed using the dual culture method. The results revealed several endophytic bacterial isolates with varying abilities to inhibit the growth of *Colletotrichum* sp. Selected isolates exhibited high inhibition percentages and did not induce hypersensitive reactions in the tobacco assay, indicating their potential to be further developed as biological control agents in edamame disease management systems.

Keywords: anthracnose, biological control, *Colletotrichum* sp, edamame, endophytic bacteria

1. Introduction

The demand for edamame continues to rise in line with the global trend toward healthy lifestyles, which has positioned edamame as a protein-rich functional food. However, edamame productivity in Indonesia still faces significant challenges, particularly anthracnose disease caused by *Colletotrichum* sp., which can markedly reduce both the quality and quantity of yield. *Colletotrichum* sp. is capable of infecting seeds up to the post-harvest stage and can attack all parts of the plant, including leaves, stems, pods, and soybean cotyledons. Symptoms typically begin with the appearance of sunken, irregularly shaped black lesions on stems, petioles, leaves, and pods. Lesions on leaves generally cause curling and the formation of dark brown necrotic areas.

Efforts to control anthracnose disease have so far been dominated by the use of synthetic fungicides. Although effective, intensive fungicide application has negative consequences, such as chemical residues in agricultural products, pathogen resistance, environmental degradation, and potential risks to human health. Therefore, alternative control strategies that are environmentally friendly, sustainable, and supportive of export-oriented agricultural systems are urgently required. One promising approach that has gained increasing attention is the use of endophytic bacteria as biological control agents.

Endophytic bacteria are microorganisms that live symbiotically within host plant tissues and possess the ability to produce bioactive compounds similar to those of their host plants, including antibacterial agents.

Endophytic bacteria can play a crucial role in inducing the host plant to produce phytoalexins, enhancing tolerance to stress conditions, and acting directly as biological control agents (Sulistiyani, 2020). Indigenous endophytic bacteria are considered to possess greater adaptability to local environments and exhibit higher competitiveness compared to non-indigenous strains (Yanti et al., 2019). The utilization of antagonistic endophytic bacteria isolated from healthy plants to control diseases in infected crops has been relatively limited. Therefore, it is necessary to research the isolation and screening of beneficial antagonistic bacteria as potential biological control agents.

2. Materials and Methods

2.1. Time and Place of Execution

Final Project research was implemented from July-August 2025 at the Laboratory of Plant Protection of the Department of Agricultural Production, Politeknik Negeri Jember.

2.2. Research Implementation

1. The Isolation of Endophytic Bacteria

The isolation of endophytic bacteria was carried out using a modified method (Munif, 2015). Root and leaf samples of cabbage plants were washed under running tap water for 2 minutes to remove adhering surface debris. The fresh weight of each root and leaf sample was then recorded. Surface sterilization was performed by immersing the root and leaf samples in 70% ethanol for 1 minute, followed by 3 minutes in sodium hypochlorite (NaOCl), and finally rinsing three times with sterile distilled water for 1 minute each. The sterilization process using these solutions aimed to eliminate residual debris and minimize unwanted contamination. To confirm sterilization effectiveness, 0.1 ml of the final rinse water was plated onto the Nutrient Agar (NA) medium to check for microbial growth. Subsequently, the root and leaf tissues were aseptically homogenized using a sterile mortar and pestle until a fine consistency was obtained.

Bacterial isolation was performed using the dilution plate method (serial dilution) as described by Madigan, Michael T., and Martinko (2014). One gram of the homogenized sample was weighed and suspended in 10 mL of sterile distilled water in an Erlenmeyer flask. From this suspension, 1 ml was taken and serially diluted from 10^{-2} to 10^{-9}. Bacterial inoculation was then carried out using the spread plate method, in which 100 µl aliquots from dilutions 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} were spread evenly on Nutrient Agar (NA) plates using an L-shaped spreader. The plates were incubated at room temperature (±28 °C) for 24 hours. Colonies that appeared on NA medium were subsequently purified by streaking on fresh NA plates and incubating for 24–48 hours at room temperature. Each distinct colony was counted and sub-cultured repeatedly until a pure and single isolate was obtained (Dinata, 2018; Dinata, Ariani, et al., 2021; Dinata et al., 2023; Djatnika, 2012; E. Siswadi et al., 2023; Edi Siswadi et al., 2024).

2. Bacterial Characterization

Bacterial colonies were characterized by examining the morphological structures of the microbes grown on different media. The characterization was based on colony shape, margin, elevation, size, and pigmentation. The isolates obtained were further purified until pure microbial cultures were established (Widura et al., 2024).

3. Screening of Antagonistic Bacteria

Screening of antagonistic bacteria was carried out using a modified dual culture method in a confrontation plate assay. The pathogenic isolate *Colletotrichum* sp., the causal agent of anthracnose in edamame, was inoculated on Nutrient Agar (NA) medium and

placed at the intersection point (Dinata, 2018). The antagonistic activity of rhizosphere bacteria against *Colletotrichum* spp. was assessed by the dual culture technique. Mycelial plugs of the fungal pathogen and bacterial isolates, each measuring 0.5 cm in diameter, were inoculated in pairs onto NA medium. A negative control treatment was also prepared as a comparison (Dinata, Aini, et al., 2021; E. Siswadi et al., 2023).

4. Abundance and Diversity of Antagonistic Bacteria

The bacterial population was expressed in Colony Forming Units per gram (CFU/g), which represents the number of bacterial colonies capable of forming visible growth on culture media. The abundance of antagonistic bacteria associated with the host plant was calculated using the following formula:

$$\text{Kelimpahan bakteri (CFU/ g)} = \frac{\sum \quad Number\ of\ colonies\ counted\ \ x\ Y}{leaf\ Dry\ Weight\ (g)}$$

Where: **Y** = dilution factor × volume of sample plated.

3. Results and Discussion

3.1. Abundance of Endophytic Bacteria in Edamame Leaves

The exploration results revealed the number of species and the abundance of endophytic bacteria associated with soybean plants, which were designated with different isolate codes. The number of species and the abundance of endophytic bacteria are presented in Table 1.

Table 1. Abundance of edamame endophytic bacteria

Isolate Code	Abundance of bacterial isolates (CFU)
ED1	10^3
ED2	$200x10^1$
ED3	$30x10^2$
ED4	10^3
ED5	10^2
ED6	$100x10^1$
ED7	$100x10^1$
ED8	$200x10^1$
ED9	$86x10^3$

The diversity observed in the number and abundance of bacterial isolates is presumed to be associated with differences in the ability of each isolate to adapt to the plant's environment. This finding is consistent with the study by Fitriana (2012), who successfully isolated 25 endophytic isolates from the roots, stems, and leaves of edamame plants. Furthermore, the diversity of endophytic populations is supported by the results of Dalal & Kulkarni (2013), who reported that the population of endophytic bacteria in soybean could reach 5.7 $\log_{10}$ CFU/g of fresh tissue during the vegetative stage, and that endophytic communities exhibit high dynamics and diversity across different plant organs. The high abundance indicates a strong colonization capacity within plant tissues.

3.2. Morphology of Endophytic Bacteria

Based on macroscopic morphological characterization, nine distinct endophytic bacteria were identified. These isolates were obtained through serial dilution (dilution plate) isolation. The bacterial morphology was examined with respect to colony shape, color, margin, surface, and size, as presented in Table 2.

Table 2. Macroscopic morphology of endophytic bacteria from edamame leaves

Isolate Code	Shape	Color	Margin	Surface	Size
ED1	Circular	Milky white	Wavy	Flat	Medium
ED2	Circular	Creamy white	Flat	Cembung	Medium
ED3	Circular	White	Irregular	Flat	Small
ED4	Circular	Yellow	Flat	Flat	Small
ED5	Circular	Yellow	Wavy	Convex	Large
ED6	Circular	Yellow	Irregular	Convex	Small
ED7	Circular	White	Irregular	Convex	Large
ED8	Circular	White	Flat	Flat	Medium
ED9	Circular	White	Wavy	Flat	Small

The isolation and morphological characterization revealed nine endophytic bacterial isolates from edamame leaves, each exhibiting distinct colony morphologies. Most colonies were circular in shape with color variations ranging from white to yellow. These color differences indicate the diversity of metabolic pigments produced by each isolate. This finding is consistent with the report of Hung & Annapurna (2004), which stated that variations in colony morphology serve as an initial indicator of endophytic community diversity in soybean and may reflect differences in the metabolic capacity of isolates, including their ability to produce hydrolytic enzymes and bioactive compounds. Thus, the morphological diversity of the isolates obtained in this study suggests potential differences in their physiological functions.

3.3. Screening of Antagonistic Bacteria

The screening of edamame endophytic bacteria against the pathogenic fungus *Colletotrichum* sp. resulted in three isolates exhibiting inhibition percentages above 50%, while the remaining six isolates showed values below 50%. The differences in inhibition ability are presumed to be influenced by the enzymatic activity possessed by each bacterial isolate. *Colletotrichum glycines* is a pathogenic fungus, and one of the mechanisms for inhibiting its growth is through the production of chitinase enzymes by endophytic bacteria. Chitinase is an extracellular enzyme capable of hydrolyzing chitin into monomers or oligomers (Yusuf et al., 2024). This enzyme plays a crucial role in degrading chitin, the main structural component of fungal cell walls, thereby suppressing fungal growth and development (Loc et al., 2020).

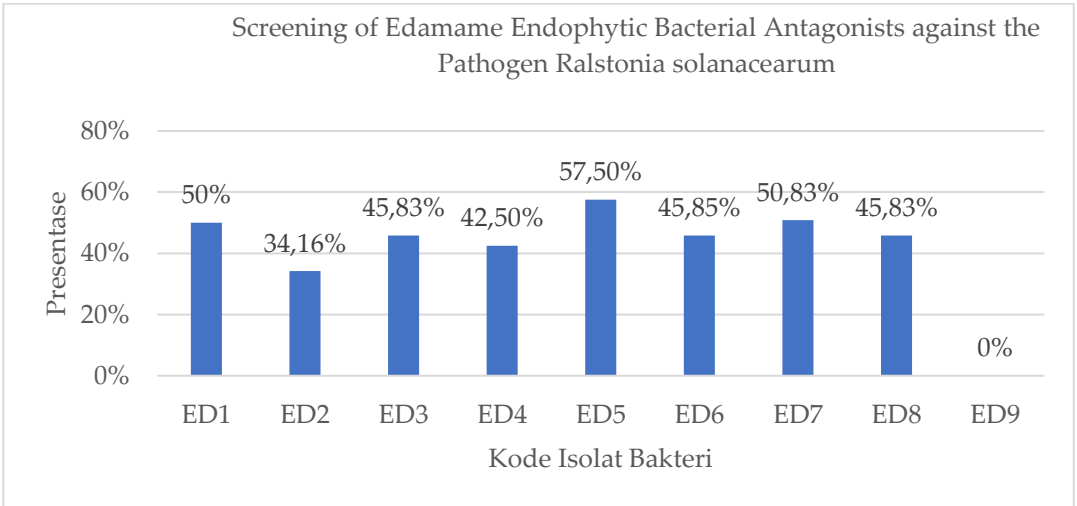

Figure 1. Antagonistic screening results of endophytic bacteria isolated from edamame against the pathogenic fungus *Colletotrichum* sp**.**

Based on the inhibition percentages against the pathogen presented in Figure 1, three isolates, namely ED1, ED5, and ED7, can be considered as potential biocontrol agents against *Colletotrichum glycines*., since they exhibited relatively strong inhibition levels (≥50%). According to Dewi et al., (2020), inhibition values of ≥50% are categorized as strong, thus qualifying the isolates for application as plant disease control agents. The proposed inhibition mechanisms exerted by the endophytic bacteria are illustrated in Figure 2.

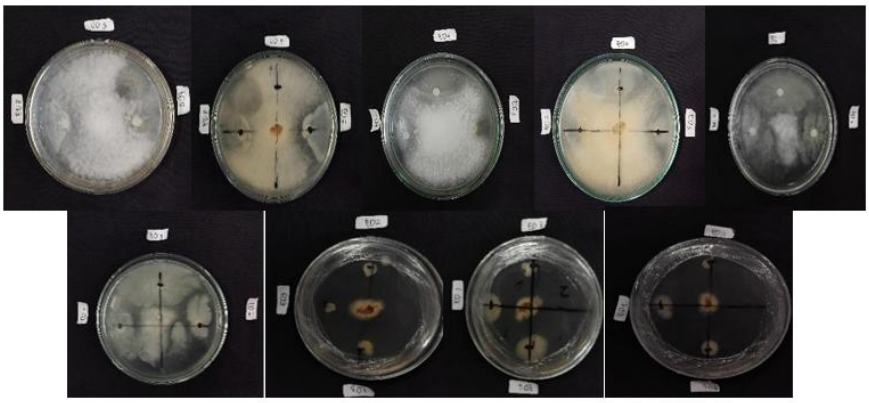

Figure 2. Proposed inhibition mechanisms of endophytic bacteria isolated from edamame against the pathogenic fungus *Colletotrichum* sp.

3.4. Hypersensitivity Test

The hypersensitivity test of endophytic bacteria isolated from edamame revealed that five isolates with the highest inhibition values, namely ED5, ED6, ED7, ED4, and ED1, did not induce necrotic symptoms on the leaf surface. The hypersensitive response is a plant defense reaction characterized by rapid and localized cell death, manifested as necrotic lesions (Ballint-Kurti, 2019). If no necrosis occurs on tobacco leaves up to seven days after infiltration, the bacterial isolates can be considered non-pathogenic to plants (Dinata, 2018). Therefore, these five isolates are regarded as safe and can be advanced to subsequent testing stages.

4. Conclusions

Based on the results of the analysis and discussion, it can be concluded that, successfully isolated nine endophytic bacterial strains from the leaves of edamame (*Glycine max* (L.) Merril), exhibiting diverse morphological characteristics and varying abundance levels. Antagonistic assays revealed that three isolates—ED1, ED5, and ED7—showed strong inhibitory activity against the pathogen *Colletotrichum sp.*, with inhibition percentages of ≥50%. Based on the hypersensitivity test, five promising isolates (ED1, ED4, ED5, ED6, and ED7) did not induce necrotic symptoms, indicating their non-pathogenic and safe nature for plants. Therefore, isolates ED1, ED5, and ED7 are potential candidates for development as effective biocontrol agents against anthracnose disease in edamame, supporting sustainable and environmentally friendly plant disease management.

Suggestion

Further research is recommended to identify and characterize the bioactive compounds and enzymatic activities, particularly chitinase, produced by the potential isolates ED1, ED5, and ED7, to elucidate their specific mechanisms in suppressing *Colletotrichum sp.* infection.

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