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Isolation Of Endophytic Bacteria From *Tithonia Diversifolia* For Biocontrol Of Phytopathogens

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Abstract
Excessive use of synthetic pesticides in agriculture has led to antimicrobial resistance, environmental pollution, and disruption of beneficial soil microbiota. This study explored endophytic bacteria from the Kipahit plant (Tithonia diversifolia) as a potential source of environmentally friendly biopesticides. Kipahit plant tissues (leaves, stems, and flowers) were surface sterilized, then endophytic bacteria were isolated, purified, and characterized morphologically and by Gram staining. Antibacterial activity against Pantoea ananatis was evaluated using the disc diffusion method. A total of twelve isolates were obtained, of which three isolates (BETD 2, BETD 10, and BETD 11) showed strong inhibitory effects. BETD 2 showed the most consistent and broad-spectrum activity, with maximum inhibition. These results highlight endophytic bacteria associated with Tithonia diversifolia, especially BETD 2 as a promising candidate for the development of sustainable biocontrol agents in controlling phytopathogenic bacteria in plants.

Keywords: *Antibacterial activity; endophytic bacteria; biocontrol; phytopathogen; Tithonia diversifolia*

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I. Introduction

The problem of antimicrobial resistance (AMR) in agriculture has become a serious global concern, particularly in crop production. The excessive and continuous use of synthetic pesticides not only contributes to environmental pollution but also leads to the emergence of antibiotic-resistant phytopathogenic microbes, ultimately lead to crop failure up to 100% if left untreated. Furthermore, synthetic pesticides can negatively impact beneficial soil microorganisms that play a crucial role in ecosystem functions such as nitrogen fixation, siderophore production, and the synthesis of plant growth regulators such as indole-3-acetic acid [1].

To solve these problems, biopesticides derived from natural sources have emerged as a safer and more environmentally friendly alternative to synthetic chemicals [2]. One promising plant is *Tithonia diversifolia*, commonly known as the Kipahit plant, which is traditionally used by farmers to control various crop pests. This plants, belonging to the Asteraceae family, is known for its richness in bioactive compounds such as alkaloids, tannins, flavonoids, terpenoids, saponins, and diterpenes that exhibit strong antimicrobial and antioxidant properties [3]. In addition, *Tithonia diversifolia* has a high nutritional content, including nitrogen (0.95–1.55%), phosphorus (0.33–1.5%), and potassium (0.35–0.88%) [4].

Several studies have highlighted the biopesticide potential of *Tithonia diversifolia*. *Tithonia diversifolia* plant extract effectively inhibits *Colletotrichum acutatum*, the causative agent of anthracnose in chili peppers. Although many studies have focused on plant extract itself, exploration of associated microbial communities particularly endophytic bacteria, as a source of biopesticide agents has been limited [5].

Endophytic bacteria are symbiotic microorganisms that inhabit plant tissues without causing harm. These bacteria can produce a variety of secondary metabolites beneficial for plant growth and protection, including antimicrobial compounds, phytohormones, and antioxidant enzymes [6]. Various genera, such as *Bacillus*, *Pseudomonas*, and *Streptomyces*, have been reported to suppress plant pathogens and enhance plant growth [7]. Recent findings indicate that endophytic bacterial isolates from chili peppers and cucumbers can inhibit *Fusarium* spp. and enhance plant growth. Furthermore, isolates from *Tithonia diversifolia* have shown effectiveness in suppressing *Fusarium oxysporum* and enhancing rice growth, demonstrating their potential as biological control agents. Therefore, this study aims to isolate endophytic bacteria isolated from *Tithonia diversifolia* to develop biopesticides with effective biological control activity against plant pathogenic microbes.

II. Materials And Methods

Materials

The materials used were *Tithonia diversifolia*, NA (Nutrient Agar) medium, antibiotic medium, crystal violet, safranin solution, 70% ethanol, 3% H_2O_2, 0.85% NaOCl, 0.5% *McFarland* solution, distilled water, bacteria isolated from *Tithonia* plants, and the plant pathogenic bacteria *Pantoea ananatis*.

Isolation of Endophytic Bakteria

Tithonia diversifolia leaves, stems, and flowers were washed under running water, cut into small pieces, and surface-sterilized using 70% ethanol (2 minutes), sodium hypochlorite (5 minutes), and rinsed twice with sterile distilled water. A 1 mL final rinse was plated on NA medium to ensure sterility. Plant segments were dried on sterile paper and inoculated onto NA medium, then incubated at 37°C for 72 hours. Emerging colonies were purified using the streak method and stored on slants at 20°C. Identification of isolated endophytic bacteria was based on macroscopic characteristics (colony color, shape, margin, height) and microscopic features using Gram staining. All isolated pure bacterial cultures were inoculated onto slants and stored at 4°C as a bacterial isolate collection.

Preparation of Media for Antibiotic Production

The antibiotic media consists of 3 g of KH_2PO_4, 3 g of K_2HPO_4, 3 g of $MgSO_4$, 5 g of NaCl, 30 ml of corn soaking water, and up to 1 liter of distilled water.

Screening of Endophytic Bacteria

Each isolate was inoculated into 150 mL of antibiotic production medium and incubated on a shaker at 150 rpm for 24 hours. Then, 5 mL of the culture was transferred to 95 mL of fresh medium and incubated under the same conditions for 48 hours. The culture broth was centrifuged at 3,000 rpm for 15 minutes, and the supernatant was used for further testing.

Test of Antibacterial Activity

The disc diffusion method was used to test inhibitory activity. A 0.1 mL pathogen suspension was spread on NA medium. Sterile paper discs soaked in bacterial supernatant were placed on the agar surface and incubated at 37°C for 24 hours. The zone of inhibition was measured using a slide anchor.

III. Results

Isolation of Endophytic Bacteria from *Tithonia diversifolia*

Tithonia diversifolia collected from the Talago Koto Baru area, Tanah Datar Regency, West Sumatra, Indonesia was selected as the source of endophytic bacterial isolates. Plant identification was conducted at the Biology Laboratory in Universitas Andalas. Endophytic bacteria were isolated from various plant parts, including leaves, stems, roots, resulting in several bacterial isolates exhibiting diverse colony morphologies, as shown in Figure 1.

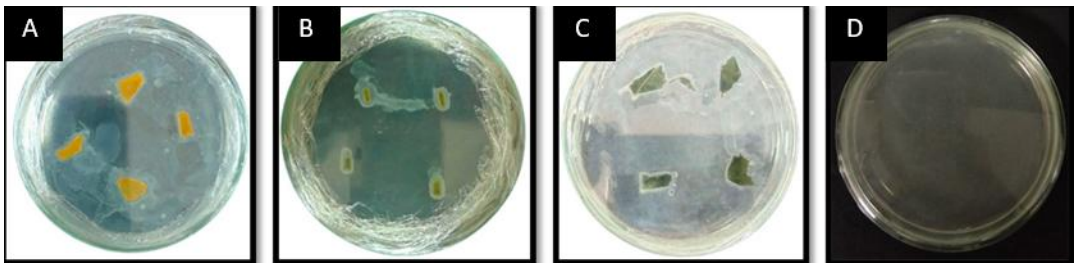

Figure 1 Isolation of endophytic bacteria in kipahit plants, (a) flowers, (b) stems, (c) leaves, (d) control

Endophytic bacteria were isolated from Tithonia diversifolia, surface sterilization was performed on plant tissue to remove epiphytic microorganisms and prevent contamination. After sterilization, plant parts (leaves, stems, and roots) were placed on NA media and incubated. Bacterial colonies emerging from the edges of internal tissues were considered endophytic. These colonies were then purified using the line plate method on fresh NA media to obtain pure single colonies. A total of 12 endophytic bacterial isolates were obtained. These isolates showed varying colony morphology in terms of color, texture, and elevation. Most colonies appeared white to slimy white. Microscopic examination revealed that all isolates had rod-shaped cell morphology [8]. Gram staining results showed that three isolates were Gram-positive and nine were Gram-negative. Catalase activity was tested by adding hydrogen peroxide (H_2O_2) to the bacterial culture. A positive catalase reaction was indicated by the formation of gas bubbles upon contact with H_2O_2, as shown in Table 1.

Table 1 Characteristics of the endophytic bacterial isolate *Tithonia diversifolia*

Isolate	Color	Shape	Gram	Cell Shape	Catalase
BETD 1	Milky white	Spherical	-	Rod-shape	+
BETD 2	Slimy white	Spherical	+	Rod-shape	+
BETD 3	Pale white	Spherical	-	Rod-shape	+
BETD 4	Pale white	Irregular	-	Rod-shape	+
BETD 5	Milky white	Irregular	-	Rod-shape	+
BETD 6	Slimy white	Spherical	-	Rod-shape	+
BETD 7	Milky white	Spherical	-	Rod-shape	+
BETD 8	Pale white	Spherical	-	Rod-shape	+
BETD 9	Pale white	Irregular	-	Rod-shape	+
BETD 10	Slimy white	Irregular	+	Rod-shape	+
BETD 11	Milky white	Irregular	+	Rod-shape	+
BETD 12	Slimy white	Spherical	-	Rod-shape	+

Screening of Endophytic Bacteria as Biocontrol of Plant Pathogens

Table 2 Inhibitory Activity of *Tithonia diversifolia* Endophytic Bacteria Screening Results

Inhibitory Activity (mm)	
Isolate Code	***Pantoea ananatis***
BETD 1	-
BETD 2	8.56
BETD 3	-
BETD 4	-
BETD 5	-
BETD 6	-
BETD 7	-
BETD 8	-
BETD 9	-
BETD 10	8.10
BETD 11	7.89
BETD 12	-

A total of 12 endophytic bacterial isolates were tested against *Pantoea ananatis*, using the agar diffusion method. The results showed varying levels of antibacterial activity (Table 2). Isolate BETD 2 showed the highest inhibition against all tested pathogens. Isolates BETD 10 and BETD 11 also showed broad-spectrum activity, while others showed inhibition against only one or two pathogens. Five isolates (BETD 1, 3, 4, 5, 6, 7, 8, 9 and 12) showed no inhibitory effect. This finding is consistent with previous studies showing that endophytic bacteria are capable of producing antimicrobial compounds that are effective against plant pathogens [9].

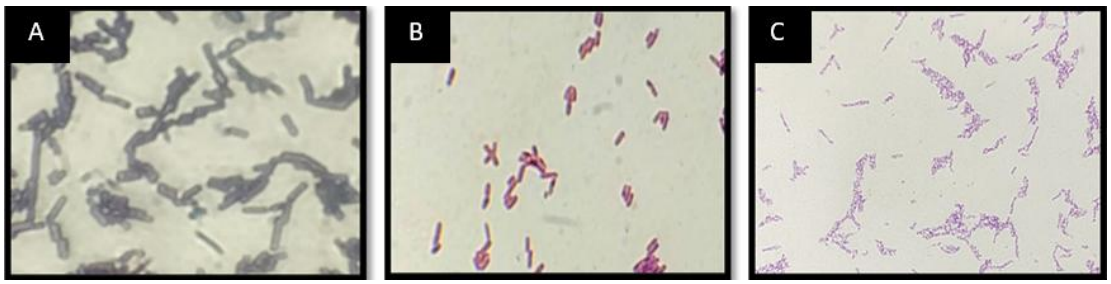

Figure 2 Gram stain screening of endophytic bacteria A : BETD 2, BETD 10, BETD 11

To identify the bacterial species, Gram staining can be performed, as shown in Figure 2. All three bacterial isolates are gram-positive, meaning they appear purple in the Gram stain. Gram-positive bacteria appear purple due to their thick peptidoglycan layer, which retains the crystal violet-iodine complex during decolorization. In contrast, Gram-negative bacteria, which have a thinner peptidoglycan layer and a disrupted outer membrane, lose the primary stain and take up the counterstain, safranin red [10].

IV. Conclusion

A total of 12 endophytic bacterial isolates were successfully obtained from various parts of *Tithonia diversifolia*. Screening results showed that isolates BETD 2, BETD 10, and BETD 11 exhibited strong

antibacterial activity against *Pantoea ananatis*, with BETD 2 exhibiting the most consistent and broad-spectrum inhibition. These findings indicate that endophytic bacteria associated with *Tithonia diversifolia*, particularly BETD 2, have high potential as a source of biopesticides to control phytopathogenic bacteria in plants. Further studies are needed to purify and characterize the bioactive compounds responsible for this inhibitory activity.

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