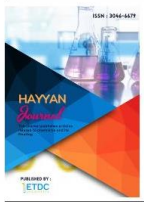

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Exploration of Endophytic Bacteria from Katuk Plants (*Sauropus androgynus L.*)

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ABSTRACT

This study aimed to explore and identify endophytic bacteria from Katuk plants (*Sauropus androgynus L.*) and evaluate their potential as biological control agents. A total of 14 bacterial isolates were successfully obtained from the roots, stems, and leaves of Katuk plants. Safety assessments were conducted through hypersensitivity response (HR) and hemolysis (HL) tests. The HR test on tobacco plants indicated that all isolates were non-pathogenic, as no necrotic symptoms were observed. However, the hemolysis test revealed two isolates capable of forming α-hemolysis zones on blood agar, suggesting potential risks to mammals. Antibacterial activity against the plant pathogen *Ralstonia solanacearum* was evaluated using spot inoculation methods. The results showed that none of the isolates were able to inhibit the growth of *R. solanacearum*. Although the isolates may produce antibacterial compounds, the amounts are likely insufficient or involve unidentified active compounds. Further studies are needed to fully characterize these isolates and explore their potential applications in sustainable agriculture.

Keywords: Endophytic Bacteria, Plant Pathogen, Antibacterial

I. INTRODUCTION

Endophytic microorganisms (Generally fungi and bacteria) colonize plant tissues intercellularly and intracellularly. Endophytic microbes spend part of their life inside plant tissues, where they remain unseen and do not cause symptoms (Schulz and Boyle 2006). The role of endophytic microbes as one of the triggers of plant resistance to pathogens is well established. Endophytic microbes can be fungi or bacteria. Endophytic bacteria are defined as bacteria that colonize healthy plant tissues without causing symptoms or producing visible damage to the host plant. The colonies of endophytic bacteria can be detected or isolated using surface sterilization methods and grown on culture media. Endophytic bacteria actively colonize plant tissues without a specific organ and associate naturally during the plant's life cycle (Bacon and Hinton 2006). According to Tan and Zou (2001), endophytic bacteria can suppress important plant diseases in vitro, as they are suspected to produce antibiotics and secondary metabolites.

The katuk plant is widely consumed in the community and used as a salad, especially in Java. People use katuk as a vegetable and a plant to increase breast milk production for nursing mothers. According to Rahmanisa (2016), administering katuk leaf extract (*Sauropus androgynus*) to breastfeeding mothers can affect the increase in breast milk production. This

is due to the alkaloid and sterol content in the katuk leaf extract, which can influence breast milk production. Based on research by Fatimah et al. (2010), there is an effect of various concentrations of katuk leaf extract (*Sauropus androgynus* L) on inhibiting the growth of *Staphylococcus aureus*. Concentrations of 60% to 100% can inhibit the growth of *Staphylococcus aureus*. At concentrations of 20% and 40%, there was no effect on inhibiting the growth of *Staphylococcus aureus*.

Research on the exploration and isolation of endophytic bacteria from various plants has been widely conducted. Additionally, the use of endophytic bacteria to promote plant growth and their ability to suppress pathogen development in vitro or in greenhouse settings has also been widely reported. However, there have been no reports on the use of endophytic bacteria from katuk plants as biocontrol agents.

II. METHODS

Endophytic Bacteria Isolation

The parts of the plant isolated include the roots. The plants used are young plants with soft stems, which make grinding easier. Endophytic bacteria isolation is performed using a surface sterilization method modified from Munif et al. (2015b). The plant parts to be isolated are first washed under running water, then air-dried and weighed at 5 grams. The roots then soaked in 2% NaOCl for 1 minute, 70% alcohol for 1 minute, and rinsed three times with sterile distilled water. The surface-sterilized plant parts are then ground using a sterile mortar and pestle. One mL of the ground material is taken for serial dilution up to 10^-4. The diluted samples are then plated (0.1 mL) on TSA 20%, NA 20%, and King's B 100% media. The isolated bacteria are subsequently purified to obtain pure isolates.

Hypersensitivity Test

The hypersensitivity test aims to assess the potential of endophytic bacteria as pathogens. The hypersensitivity test method follows Klement and Goodman (1967). The endophytic bacteria isolates to be tested are cultured on tryptic soy broth (TSB) 100% media and incubated for 48 hours. Then, the bacterial suspension of each isolate is injected into the lower part of tobacco plants using a sterile syringe. The tobacco plants are incubated for 24 hours. Observations are made on each tobacco leaf segment that was injected with the bacterial suspension. Symptoms observed include necrosis localized at the segment where the bacterial suspension was injected. If such symptoms are found, the bacterial isolate is excluded from further testing, as it may be pathogenic.

Hemolysis Test

The hemolysis test aims to assess the potential of endophytic bacteria as pathogens to humans and animals. The hemolysis test is conducted following the method of Beutin (1991). Bacterial cultures are grown on blood agar media, then incubated for 24 hours at room temperature, and the formation of hemolytic zones is observed. Endophytic bacteria that do not show the formation of hemolytic zones or color changes in the media are used for further testing (Khusnan et al., 2008).

Antibacterial Activity of Endophytic Bacterial Isolates Against Pathogens (Simarmata et al. 2007)

Endophytic bacteria that have been tested for biological safety are further tested for their ability to inhibit *Ralstonia solanacearum*. The isolate of *Ralstonia solanacearum* is introduced into warm media, homogenized, and the endophytic bacterial isolates are inoculated onto the media containing the pathogen using an inoculation loop. The culture is then incubated at room temperature for 1-2 days. The formation of a clear zone indicates the presence of antibacterial compounds produced by the endophytic bacteria.

III. RESULTS AND DISCUSSION

Selection of katuk plants as the source of endophytic bacteria for isolation was based on a study by Fatimah et al. (2010), which showed that katuk leaves have antibacterial properties that can inhibit the growth of *Staphylococcus aureus*. A total of 5 endophytic bacteria were isolated from the katuk plant. Five different bacterial species were isolated from the roots based on morphological color. The root part is richer in nutrients, thus supporting a higher number of bacteria associated with the plant roots.

To assess the diversity of endophytic bacteria, characterization can be performed. Initial characterization involves morphological observation, including color, shape, and elevation, which can be observed macroscopically. The next step is to purify the bacterial isolates on new media to obtain single isolates (Figure 2). The results of morphological observations in this study are presented in Table 1.

Table 1. Morphological Observation of Root

Source of Isolate	Morphological Observation			Gram Test (KOH)
	Warna	**Bentuk**	**Elevasi**	
Root				
T7	Yellow	Round	Convex	+
T8	White	Oval	Convex	+
T9	Pink	Round	Convex	+
T10	Orange	Round	Flat	+
T11	Cream	Round	Flat	+

Hypersensitivity Test

Endophytic bacterial candidates to be used as bioagents must be safe for plants and mammals. Therefore, the endophytic bacteria successfully isolated must undergo safety testing through hypersensitivity response (HR) and hemolysis (HL) tests. In the hypersensitivity test conducted on tobacco plants, all 14 bacterial isolates tested did not show necrotic symptoms on the tobacco leaves (Figure 3). This was indicated by the absence of lesions on the leaves. Localized lesion symptoms are a plant's response to the presence of pathogens to inhibit the spread of pathogenic microorganisms within plant tissues. In contrast, non-pathogenic microorganisms do not form localized lesions in the tissues (Klement 1982; Fahy & Hayward 1983; Agrios 2005). This indicates that all 14 bacterial isolates are safe and do not have pathogenic potential for plants, making them safe for further testing.

Hemolysis Test

Observation of endophytic bacteria on blood agar media 24 hours after treatment showed that some bacteria were capable of forming clear zones. The formation of a clear zone on the media indicates that the bacteria are hemolysis-positive, while the absence of a clear zone indicates hemolysis-negative and safe for mammals. The results of the study showed that out of 14 bacterial isolates tested, 2 were positive and formed clear zones on blood agar media (Figure 4). The formation of clear zones on blood agar media indicates hemolysin activity. Hemolysin is an important protein in pathogenic bacteria for mammals, capable of degrading the red blood cell membrane (Salasia et al., 2004 in Oktafiyanto, 2017).

Antibacterial Activity of Endophytic Bacterial Isolates Against Pathogens (Simarmata et al. 2007)

Screening of endophytic bacterial isolates capable of inhibiting the growth of pathogenic bacteria was conducted using the spot/streak inoculation method. The formation of a clear zone indicates that the endophytic bacteria possess the ability to produce extracellular compounds with antibacterial properties. Antibiotics can play a role in preventing the synthesis of peptidoglycan, a key component in the cell wall of pathogenic bacteria. Peptidoglycan is an essential part of the bacterial cell wall, responsible for maintaining cell wall flexibility and integrity. The absence of peptidoglycan in bacterial cells can lead to cell lysis and subsequent cell death (Hoff et al., 2008). The results of the study showed that all 5 endophytic bacterial isolates were unable to inhibit the growth of *R. solanacearum*, as no clear zones were formed. This suggests that the endophytic bacteria may produce antibacterial compounds, but in very small amounts, or they may produce other active compounds that are not yet identified (Son & Cheah, 2002).

IV. CONCLUSION

Based on the results of the study, 14 endophytic bacterial candidates were isolated from katuk plants (*Sauropus androgynus* L.). The HR test on tobacco plants showed that all 14 bacterial isolates had a negative response. Meanwhile, the hemolysis test revealed that 2 isolates were capable of forming α-hemolysis on blood agar media; these two isolates were derived from the leaves and roots. In the antibacterial activity test against the pathogen *Ralstonia solanacearum*, all 14 isolates were unable to inhibit the growth of *R. solanacearum*

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