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Abundance of Endophytic Bacteria in *Sauropus androgynus* and Evaluation of Chitinolytic Activity

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ABSTRACT

This study aimed to isolate and evaluate the biological safety and potential of endophytic bacteria from Sauropus androgynus (katuk) and their Chitinolytic activity for use as biological agents. A total of 14 endophytic bacterial isolates were obtained from different plant parts: 5 from the roots (T1, T2, T3, T4, P14), 5 from the stems (T7, T8, T9, T10, T11), and 4 from the leaves (T5, P6, KB12, T13). The safety of these isolates was assessed through hypersensitivity (HR) and hemolysis (HL) tests. In the HR test, none of the isolates induced necrotic symptoms in tobacco leaves, indicating they are not pathogenic to plants. In the HL test, two bacterial which were T4 from roots and T5 from leaves, isolates were found to produce clear zones on blood agar, indicating α-hemolysin production. However, the other isolates did not form hemolysis zones, suggesting they are safe for 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. Further testing revealed that one bacterial isolate which was T8 obtained from the stem produced chitinase, as evidenced by the formation of a clear zone around the bacterial colony on chitin-containing media.

Keywords: Endophytic Bacteria, Chitinolytic, Plant Pathogen,

I. INTRODUCTION

Katuk (*Sauropus androgynus*) is a plant species that is widely recognized in Indonesia, known for its nutritional benefits, especially its rich content of vitamins and minerals. Additionally, katuk is also utilized in traditional medicine due to its numerous health benefits, such as enhancing breast milk production in nursing mothers, lowering cholesterol levels, and boosting immunity. Given the considerable potential of katuk, it is essential to further understand the factors that influence its growth, one of which involves the symbiotic relationship with endophytic microorganisms.

Endophytic bacteria are microorganisms that reside within plant tissues without causing any direct harm to the plant. These bacteria can provide various benefits to their host plants, such as promoting growth, reducing biotic and abiotic stress, and enhancing disease resistance. One of the significant roles of endophytic bacteria is their ability to produce various enzymes, including chitinase. Chitinase is an enzyme capable of hydrolyzing chitin, a major component of fungal cell walls, which helps plants combat fungal pathogens.

This study primarily focuses on the identification and analysis of the abundance of endophytic bacteria present in katuk plants, along with an evaluation of their chitinolytic activity. The chitinolytic activity can serve as an essential indicator of the bacteria's ability to assist the plant in defending against fungal pathogens, thereby improving the overall health of the plant.

II. METHODS

Endophytic Bacteria Isolation

The plant parts that were isolated include the roots, stem, and leave.Young plants with soft stems were chosen for the process, as their flexibility makes grinding easier. To isolate endophytic bacteria, a modified surface sterilization method from Munif et al. (2015b) was used. The plant parts to be isolated were first rinsed under running water, air-dried, and then weighed at 5 grams. The roots, stem and leave were treated by soaking them in 2% NaOCl for 1 minute, followed by 70% alcohol for 1 minute, and then rinsed three times with sterile distilled water. After surface sterilization, the plant parts were ground using a sterile mortar and pestle. A 1 mL sample of the ground material was taken for serial dilution up to 10^-4. The diluted samples were then plated (0.1 mL) onto TSA 20%, NA 20%, and King's B 100% media. The bacteria that were isolated were subsequently purified to obtain pure cultures.

Hypersensitivity Test

The hypersensitivity test is conducted to evaluate whether endophytic bacteria have the potential to act as pathogens. The method used for the test is based on the procedure outlined by Klement and Goodman (1967). The endophytic bacteria isolates to be tested are cultured in 100% tryptic soy broth (TSB) media and incubated for 48 hours. After incubation, the bacterial suspension from each isolate is injected into the lower part of tobacco plants using a sterile syringe. The plants are then incubated for 24 hours. Observations are made on the tobacco leaf segments where the bacterial suspension was applied. Symptoms, such as localized necrosis at the injection site, are monitored. If such symptoms are observed, the bacterial isolate is excluded from further testing due to its potential pathogenicity.

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

After the biological safety tests, the endophytic bacteria are further evaluated for their ability to inhibit *Ralstonia solanacearum*. The *Ralstonia solanacearum* isolate is added to warm media and homogenized, after which the endophytic bacterial isolates are introduced onto the media containing the pathogen using an inoculation loop. The culture is then incubated at room temperature for 1-2 days. The presence of a clear zone indicates the production of antibacterial compounds by the endophytic bacteria.

Chitinolytic activity test

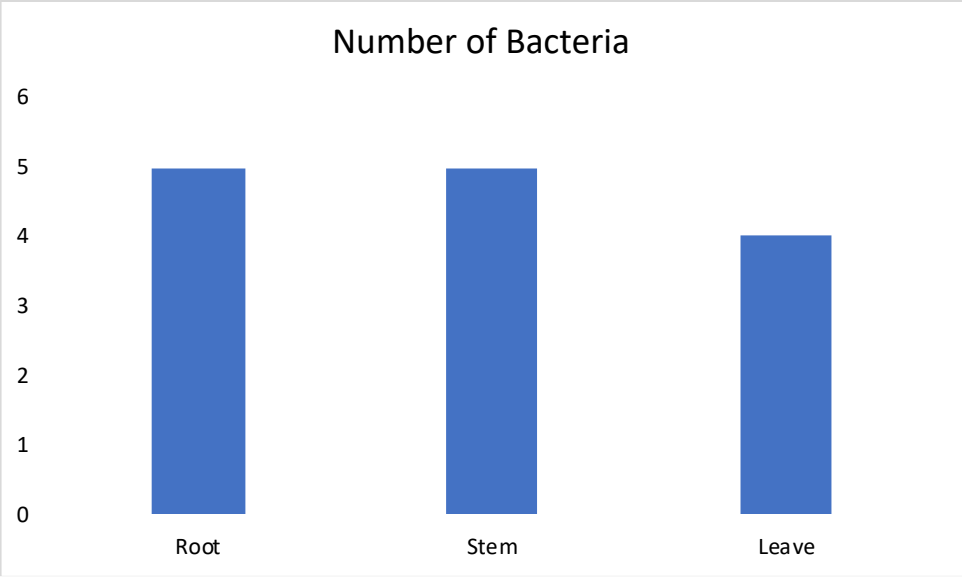
This test aims to determine whether the test bacteria produce chitinase enzymes. The test was performed using chitin medium with the following composition: 15 g of Bacto agar, 5 g of glucose, 2 g of peptone, 10 g of colloidal chitin, 0.5 g of K2HPO4, 0.5 g of MgSO4, and 0.5 g of NaCl in 1 L of distilled water. The formation of a clear zone indicates that the test bacteria are capable of producing chitinase. However, if no clear zone forms, it indicates that the test bacteria do not produce chitinase enzymes (Hariprasad et al. 2011).

III. RESULTS AND DISCUSSION

Endophytic Bacteria Isolation

The isolation of endophytic bacteria from *Sauropus androgynus* resulted 14 bacterials (Figure 1). Five different bacterial were isolated from the roots (T1, T2, T3, T4, P14) based on morphological characteristics, five from the stems (T7, T8, T9, T10, T11), and four from the leaves (T5, P6, KB12, T13). The number of bacterial isolates obtained from the roots was greater than those derived from the leaves, likely due to the higher nutrient availability in the roots, which supports a more abundant bacterial community. In contrast, the leaves, being positioned at the apical regions of the plant, are more susceptible to environmental influences. The number of bacterial isolates found in the stems was comparable to that in the roots. Although, as a general trend, the abundance of endophytic bacteria is typically higher in the roots, with a progressive decrease observed in the stems and leaves (Lamb et al., 1996), there are instances where the bacterial population in the stems may exceed that in the roots (Koomnok et al., 2007, as cited in Kusumawati, 2014). This phenomenon could be attributed to the translocation of photosynthetic products from the leaves to other parts of the plant via the phloem, which may provide a source of nutrition for the endophytic bacteria (Koomnok et al., 2007).

Figure 1. Isolation of bacteria from *Sauropus androgynus*



Hypersensitivity Test

Endophytic bacterial candidates intended for use as biological agents must be safe for both plants and mammals. Therefore, the endophytic bacteria that have been successfully isolated must undergo safety assessments through hypersensitivity (HR) and hemolysis (HL) tests. In

the hypersensitivity test conducted on tobacco plants, the 14 bacterial isolates tested did not exhibit any necrotic symptoms on the tobacco leaves, as evidenced by the absence of lesion formation on the leaves. Localized lesion symptoms are a plant's response to the presence of pathogens, aimed at limiting the spread of pathogenic microorganisms within plant tissues. In contrast, non-pathogenic microorganisms do not induce local lesions within plant tissues (Klement, 1982; Fahy & Hayward, 1983; Agrios, 2005). This indicates that all 14 bacterial isolates are safe and do not pose a pathogenic risk to plants, thus making them suitable for further testing.

Hemolysis Test

The observation of endophytic bacterial candidates on blood agar media, 24 hours after treatment, revealed that some bacteria were able to form clear zones. The formation of clear zones on the media indicates that the bacteria are positive for hemolysis, while the absence of clear zones indicates a negative result for hemolysis, suggesting the bacteria are safe for mammals. The experimental results showed that out of the 14 bacterial isolates tested, 2 were positive for hemolysis and formed clear zones on the blood agar media (Figure 4). The formation of clear zones on the blood agar media indicates the presence of hemolysin activity. Hemolysin is an important protein in pathogenic bacteria for mammals, capable of degrading the red blood cell membrane (Salasia et al., 2004, as cited in Oktafiyanto, 2017).

The results of the blood agar media test indicated that two bacterial isolates were able to alter the color of the media to brown and green, suggesting that these isolates produce α-hemolysin toxins. Both isolates were derived from the roots and leaves of the *Sauropus androgynus* plant. The hemolysis zones formed in samples T4 and T5 differed in their appearance times. The hemolysis zone in isolate code T5, originating from the leaves, appeared after 5 days of testing, while in isolate code T4, originating from the roots, the hemolysis zone appeared 2 days after testing. According to Wahyono et al. (1994), in addition to tannins, *Sauropus androgynus* leaf powder macroscopically shows the presence of alkaloids. According to Padmavathi (1990), *Sauropus androgynus* leaves contain the alkaloid papaverine, which can interfere with health, including causing contraction of the intestines and uterus, lowering blood pressure, acting as an abortifacient, and other effects. The alkaloid content in the leaves of *Sauropus androgynus* is not harmful in small amounts, making them safe for consumption, but not on a frequent basis. According to Supriadi (2006), biological agents should not only focus on their effectiveness but also on their safety for human health, animals, and the environment.

Antibacterial Activity of Endophytic Bacterial Isolates Against Pathogens

The screening of endophytic bacterial isolates for their potential to inhibit the growth of pathogenic bacteria was carried out using the spot or streak inoculation technique. The presence of a clear inhibition zone around the bacterial colony typically indicates the production of extracellular bioactive compounds with antibacterial activity. Such compounds, including antibiotics, can interfere with the synthesis of peptidoglycan—a crucial structural component of the bacterial cell wall that provides mechanical strength and maintains cellular integrity. Disruption of peptidoglycan synthesis can result in cell wall weakening, leading to cell lysis and ultimately bacterial death (Hoff et al., 2008). However, the findings of this study demonstrated that none of the twelve tested endophytic bacterial isolates exhibited inhibitory activity against *Ralstonia solanacearum*, as evidenced by the absence of clear zones. This may

indicate that the isolates either produce antibacterial substances in concentrations too low to be effective or may synthesize other bioactive metabolites whose modes of action are not yet fully identified (Son & Cheah, 2002).

Chitinolytic activity test

Chitinase is an enzyme capable of inhibiting the growth of pathogens. This enzyme catalyzes the hydrolytic degradation of chitin, a linear polymer composed of β-1,4-N-acetyl-D-glucosamine monomers. Chitinase is commonly found in bacteria and fungi that can degrade the cell walls of pathogens, such as fungi and nematodes, which are composed of chitin. The enzyme hydrolyzes acetylglucosamine (β-1,4-N-acetyl-D-glucosamine), breaking down chitin from within and producing short oligomers of N-acetylglucosamine. The final step involves the enzyme N-acetylglucosaminidase cleaving the deacetylchito-biose chain into N-acetylglucosamine monomers (Pratiwi et al., 2014, as cited in Oktafiyanto, 2017). Based on the results obtained in the experiment, one bacterial isolate, code T8, originating from the stem, was found to produce chitinase, as indicated by the presence of a clear zone around the bacterial colony containing colloidal chitin.

The chitinase activity produced by endophytic bacteria is capable of degrading the cell walls of pathogenic organisms, such as fungi, which are primarily composed of chitin (Pratiwi et al. 2015). The chitinase enzyme synthesized by *Serratia marcescens* imparts antagonistic properties to the bacterium, enabling it to inhibit the growth of *Sclerotium rolfsii* (Compant et al. 2005)

IV. CONCLUSION

Based on the results of the study total 14 endophytic bacterial isolates were obtained from different plant parts of *Sauropus androgynus*, the safety of these isolates was assessed through hypersensitivity (HR) and hemolysis (HL) tests. In the HR test, none of the isolates induced necrotic symptoms in tobacco leaves, indicating they are not pathogenic to plants. In the HL test, two bacterial which were T4 from roots and T5 from leaves, isolates were found to produce clear zones on blood agar, indicating α-hemolysin production. However, the other isolates did not form hemolysis zones, suggesting they are safe for 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*. Further testing revealed that one bacterial isolate which was T8 obtained from the stem produced chitinase, as evidenced by the formation of a clear zone around the bacterial colony on chitin-containing media.

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