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Potential of endophytic bacteria from Kailan plant roots in suppressing the growth of *Sclerotium rolfsii* (Sacc.)

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ABSTRACT

The use of biological agents, such as endophytic bacteria, provides an environmentally friendly and safe method for controlling diseases caused by fungi. *Sclerotium rolfsii* (Sacc.), a fungus, causes damping-off disease in various horticultural plants, including chili plants. This study aimed to use endophytic bacteria from the roots of Kailan plants to suppress the growth of *S. rolfsii*. The research involved 1) isolating endophytic bacteria, 2) testing for biosafety, 3) propagating *S. rolfsii*, and 4) testing the antagonistic effects of the endophytic bacteria. The study found 36 isolates of endophytic bacteria. Bacterial colonies isolated from Kailan roots showed diversity in color and shape, with growth rates observed 48 hours post-isolation. Three isolates tested positive for hemolysis, and six isolates were hypersensitive. The antagonism test indicated that isolates grown on Tryptic soy agar (TSA) media demonstrated greater inhibitory effects on *S. rolfsii* than those on Potato dextrose agar (PDA) media, with a clearer inhibition zone appearing each day. In total, six isolates of endophytic bacteria from Kailan roots inhibited the growth of *S. rolfsii*.

1. Introduction

Sclerotium rolfsii (Sacc.) is one of the most important diseases of the fungal group that infects various types of plants. The fungus *S. rolfsii* can cause damping and wilting of seedlings in the nursery. This attack is characterized by the appearance of brown lesions, which then develop into a white, cotton-like mass of mycelium, followed by white, cream, and brown sclerotium granules. Symptoms include yellowing of the leaves, wilting of the leaves, and eventually, the plant falling over. The percentage of disease severity caused by *S. rolfsii* ranges from 40-80% (Samiyarsih et al., 2022).

An alternative control that can be used to control the fungus *S. rolfsii* is the use of biological agents in the form of endophytic bacteria. Biological control of plant pathogens with biological agents is promising because it is safe for the environment. Endophytic bacteria are bacteria that colonize the internal tissues of plants without disturbing the plant, and most endophytic bacteria are beneficial. Marwan et al. (2017) reported that endophytic bacterial isolates were able to inhibit the growth of *S. rolfsii* fungal germination colonies by 37.44 to 49.97%.

Endophytic bacteria benefit their host plants by stimulating plant growth, fixing nitrogen, and increasing plant resistance to plant diseases. This is because endophytic bacteria can produce secondary metabolites, namely alkaloids, saponins, and terpenoids, which have antimicrobial and antifungal activities (Astari et al., 2021; Zulfarina et al., 2022).

The role of endophytic bacteria in increasing plant growth is thought to be supported by their ability to produce growth hormones such as indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA), and cytokinins (CTK). Separately, endophytic bacteria have also been reported to increase plant growth by inhibiting N_2 (diazotrophic bacteria), mobilizing phosphate, and increasing plant resistance to pathogens that cause plant diseases (Abbas et al., 2024). Exploration of endophytic bacteria from different types of plants with different interests has been widely carried out. This is to obtain superior endophytic bacteria, especially in the agricultural sector. Marsaoli et al. (2019) have isolated endophytic bacteria from salawaku (*Falcataria mollucana*) that can inhibit the growth of

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the pathogen *Cercospora* spp. that causes red spot disease on samama plants (*Anthocephalus macrophyllus*), the percentage of inhibition reached 78.04%. Endophytic bacteria from chili plants are able to inhibit the growth of the fungus *Fusarium oxysporum* f.sp. *capsici*, which causes *Fusarium* wilt disease in chili, by 20.41% (Sihombing et al., 2019).

Currently, there is no research on endophytic bacteria isolated from your plants. Kailan plant (*Brassica oleraceae* var *achepala*) is a type of vegetable that has many benefits and is widely consumed as a side dish because it can be useful as a nutritious vegetable food. Apart from being used as food, kailan can also be used to treat various diseases. Endophytic bacteria in kailan plant tissues have not been widely reported. Therefore, this research will isolate endophytic bacteria from the roots of kailan plants to control the disease caused by the fungus *S. rolfsii*.

2. Methods

2.1. Endophytic bacteria isolation

Endophytic bacteria were isolated from the roots of kailan (*Brassica oleracea*) plants obtained at the Faculty of Agricultural Technology, IPB. The kailan plants used as a source of endophytic bacterial isolates were healthy, fertile, and free from exposure to synthetic chemical pesticides.

Isolation of endophytic bacteria was carried out following the modified method of Hallmann (2001) which was modified. Plant root parts were washed under running water until clean from residual soil or other impurities, then weighed as much as 1g. Plant roots were then surface sterilized by immersing in 1% NaOCl (30 seconds) and 70% alcohol (1 minute) and rinsed using sterile distilled water 3 times 30 seconds. Plant roots were then dried with sterile tissue paper, attached to 20% NA media, and incubated for 48 hours to determine the success of surface sterilization as a control.

The surface-sterilized plant roots were then crushed until smooth with the addition of 5 ml of sterile distilled water. The bacterial suspension was then taken in as much as 1 ml and put in a bottle containing 9 ml of distilled water to be diluted. Then, it was taken as much as 0.1 ml and flattened on 20% NA media. Dilutions were carried out up to a dilution level of 10-4. The growth medium was incubated at room temperature for 48 hours.

2.2. Hypersensitivity tests

Hypersensitivity tests were conducted to determine the potential pathogenicity of endophytic bacteria against plants. The test was conducted using Klement and Goodman's (1967) method. One isolate of endophytic bacteria was cultured in 5 mL of nutrient broth (NB) medium and incubated with shaking for 48 hours. A total of 1 mL of endophytic bacterial suspension in NB was infiltrated into the lower part of the tobacco leaf lamina. A positive reaction was indicated if necrosis spots were formed after 24-48 hours, and a negative reaction if no necrosis spots were formed. Endophytic bacterial isolates that did not show a positive reaction were used for subsequent tests.

2.3. Hemolysis activity test

The hemolysis activity test was conducted using the blood agar medium obtained from the bacteriology laboratory of the Faculty of Veterinary Medicine, IPB. Endophytic bacterial isolates aged 48 hours were streaked on blood agar media and then incubated for 24 hours at room temperature. Endophytic bacterial isolates that did not show a zone of hemolysis or discoloration of the medium (λ-hemolysis) were safe for mammals and were used in subsequent tests.

2.4. *Sclerotium rolfsii* isolate propagation

S. rolfsii isolates were obtained from the collection of the Education Laboratory of the Department of Plant Protection, Faculty of Agriculture, IPB. The fungal isolate used was isolated from peanut plants. Propagation of *S. rolfsii* isolates was carried out by taking sclerotia and growing Beutin's (1989) method on new Potato dextrose agar (PDA) media.

2.5. Antagonistic test of endophytic bacteria against *S. rolfsii*

Endophytic bacteria that passed the biosafety evaluation were subsequently used for antagonistic activity testing. The antagonistic potential was assessed using the dual culture method on 100% Tryptic soy agar (TSA) and PDA media. The bacteria were first cultured on 100% TSA media for 24 hours to ensure rejuvenation. The testing involved perforating the TSA medium, which had been pre-inoculated with the endophytic bacteria, and transferring samples into wells (Ø 0.5 cm) placed 3 cm from the edge of the Petri dish. S. rolfsii colonies (Ø 0.5 cm) were then inoculated on the same medium, positioned 3 cm away from the endophytic bacteria. The inhibition of S. rolfsii by the endophytic bacteria was measured using a specific formula:

$$I = (R1 - R2)\,(R1) - 1\,x\,100\%$$

where, I is percentage of inhibition, R1 is radius of fungal colonies that grow to the edge of the petri dish, and R2 is radius of fungal colonies growing towards the endophytic bacteria.

2.6. Data analysis

The research data were analyzed using a completely randomized design with three replications. The observed parameter was the percentage of fungal mycelium growth inhibition of

S. rolfsii. Data analysis was performed using analysis of variance (ANOVA). If significant differences were found, a Tukey test at the 5% significance level was conducted.

3. Results

3.1. Endophytic bacteria isolation

The results of the isolation of endophytic bacteria from the roots of kailan plants were obtained at the Faculty of Agricultural Technology IPB. The Kailan plant used as a source of endophytic bacterial isolates is a healthy, fertile plant that is free from exposure to synthetic chemical pesticides. Endophytic bacteria can generally enter plant tissues through the roots, but plant parts that are directly exposed to air, such as flowers, stems, leaves (through stomata), and cotyledons, can also be the entry point for endophytic bacteria. Endophytic bacteria that invade plants can grow only in one location or spread throughout the plant. These endophytic microorganisms can enter and spread throughout the interior of the plant. They live in cells or intercellular spaces or in the vascular system (Khare et al., 2018; Kumar et al., 2016; Liu et al., 2017). The results showed that 36 isolates of endophytic bacteria were grown on 20% NA media. This medium is rich and consists of yeast extract, peptone, NaCl, and agar. Endophytic bacteria can live on NA media due to the complex nature of the media, and most likely, the media has a composition similar to the conditions in the plant (Bacon and Hinton, 2006). Endophytic bacteria in one host plant generally consist of several genera and species. The diversity of endophytic bacteria in a plant is also influenced by plant growth conditions, especially soil conditions. In some cases, plants of the same type or species have different endophytic bacteria. Endophytic bacteria are known to have non-host-specific properties, so the use of bacteria is not limited to a particular host (Pradana et al., 2022).

Endophytic bacterial colonies successfully isolated from Kailan plants showed diversity in both color and shape and growth rates were calculated 48 hours (2 days) after isolation. The incubation time of at least two days is to ensure that the bacteria incubated for growth are endophytic bacteria and do not contaminate bacteria. In addition, the addition of nystatin (an antifungal agent) to the media also aims to prevent the growth of fungi in the media so that bacteria will appear.

3.2. Biosafety tests

Hemolysis activity testing was conducted to obtain a consortium of endophytic bacteria that are safe and not pathogenic to animals and humans. Testing of 36 endophytic bacterial isolates found only three isolates that formed clear zones on blood agar, namely isolate codes 6, 19, and 22 (Fig. 1), meaning that the hemolysis activity is positive (pathogenic) in humans and animals. A total of 33 isolates tested for hemolysis showed a negative response, meaning they were not pathogenic to humans and animals. Endophytic bacteria used as biological control agents must be microorganisms that are safe for plants, animals, and humans (non-pathogenic). Pathogenic bacteria can produce substances that damage plant cells and red blood cells in animals and humans. Hemolysis testing on blood agar produces discoloration or clear zones around endophytic bacterial colonies, indicating the potential of these endophytic bacteria as pathogens in animals and humans.

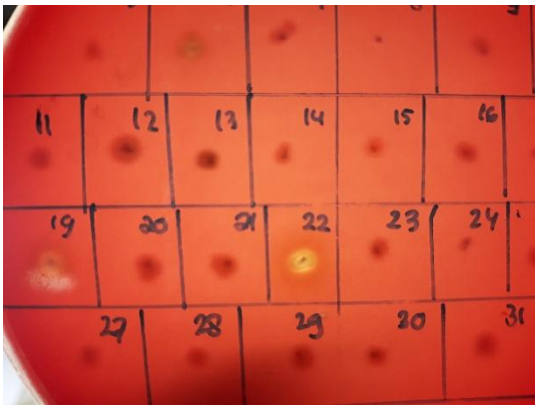

Fig. 1: Hemolysis activity test results on blood agar media

This endophytic bacterial hemolysis test uses blood agar media. The ability of bacteria to degrade red blood cells is divided into three categories, namely, beta hemolysis (β), alpha hemolysis (α), and gamma hemolysis (γ). Beta haemolysis can degrade red blood cell components, resulting in a clear zone around the bacterial colony. Alpha hemolysis occurs when red blood cells are reduced around the colony and form a greenish or brownish color in the medium. Gama hemolysis does not cause a lysis reaction and discoloration of the blood agar medium (Balashova et al., 2006). Bacteria that exhibit β and α hemolysis properties are not used in subsequent tests because they are capable of degrading human blood cell components (Duncan et al., 2007).

The hypersensitivity test results of endophytic bacteria on tobacco plants showed that six isolates (Isolates 3, 10, 11, 14, 15, and 18) exhibited positive hypersensitivity, causing necrosis on tobacco leaves. Meanwhile, 27 isolates did not show hypersensitivity, indicating a negative result (Fig. 2).

The hypersensitive response is defined as a rapid defense reaction of the plant against an incompatible pathogen accompanied by rapid cell death in the tissue in the area injected with bacterial suspense so that its presence does not affect the growth of the host plant, as expressed by Kasi et al. (2015), Endophytic microbes are microbes that live in plant tissue and are able to colonize the plant tissue without having a negative effect on the host.

Balint-Kurti (2019) explained that hypersensitive reactions are triggered by pathogens carrying the *Avr* gene. Localized lesions occur as a hypersensitive reaction when the test plant possesses an *R* gene that

recognizes the *Avr* gene of the pathogenic bacteria. These localized lesions are the plant's defense response to limit the spread of pathogens within the tissue. In contrast, non-pathogenic microorganisms do not cause localized lesions in plant tissues. The invasion of pathogenic microbes increases cell membrane permeability, indicating membrane damage associated with the hypersensitive response. Additionally, plant tissues infected by pathogens undergo desiccation and cell death (Yuniawati and Akhdiya, 2021).

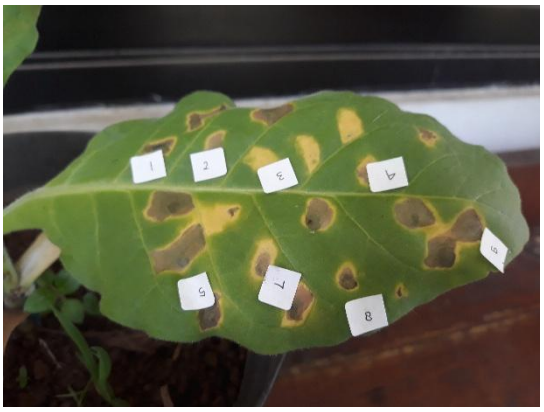

Fig. 2: The results of the hypersensitivity reaction test on tobacco plant leaves

3.3. Antagonism test

Wati et al. (2023) stated that this inhibition could occur due to the influence of bioactive compounds, antibacterial activity, and the influence of chitinolyte activity produced by microorganisms so that they can inhibit the growth and damage the cell walls of pathogens that cause disease in plants so that the metabolic activity of the pathogen is disrupted. Thus, the metabolic activity of the pathogen is disrupted and causes the pathogen cells to die. The effect of antibiotic compounds has a role in the process of cell protein synthesis. Cell protein synthesis can be inhibited when exposed to antibiotic compounds so that cells will be damaged and unable to perform protein synthesis.

Hasani et al. (2014) reported that bacteria of the genus *Streptomyces* can produce various antibiotics, including vancomycin, erythromycin, tetracycline, streptomycin, neomycin, kanamycin, cycloserine, lincomycin, nystatin, sulfonamides, aminoglycosides, aureomycin, chloramphenicol, amphotericin B, actinomycin, fosfomycin, decamycin, rifamycin, avermectin, tobramycin, spectinomycin, clindamycin, daptomycin, puromycin, novobiocin, oxytetracycline, chlortetracycline, ribostamycin, platensimycin, viomycin, dimethyl chlortetracycline, spiramycin, and cephalosporins. These antibiotics inhibit the formation of peptidoglycan, a key component of bacterial cell walls. Peptidoglycan is essential for maintaining the flexibility and integrity of cell walls in both Gram-positive and Gram-negative bacteria. Without peptidoglycan, cell wall formation is disrupted, leading to cell lysis and eventual cell death (Hoff et al., 2008).

In Fig. 3, a clear zone is visible between the endophytic bacteria and the *S. rolfsii* fungus. This indicates that the endophytic bacterial isolates exhibit inhibitory activity against the pathogen, as demonstrated by the clear area formed around the bacterial colonies. The presence of this clear zone suggests the production of compounds that inhibit the pathogen, such as antibacterial substances capable of killing or at least suppressing the growth of pathogenic bacteria. This aligns with Monteiro et al. (2013), who stated that the clear zone observed in dual culture tests results from secondary metabolites, such as antibiotics, released by bacteria into the environment. This represents one of the mechanisms by which biological agents control plant diseases.

Bacteria that have antibiotic abilities can usually interfere with the morphological and physiological growth of fungi. There are several ways that bacteria inhibit the attack of pathogenic fungi. First, bacteria produce bioactive compounds that can degrade the structural components of fungi. Second, bioactive compounds also affect the permeability of fungal cell membranes, thus disrupting the transport of substances needed for metabolism. Third, the compounds produced can function as inhibitors of an enzyme produced by the fungus. Based on the test results, as many as six endophytic bacterial isolates from kailan plants showed inhibition of *S. rolfsii* (Table 1). The six isolates came from the roots.

Table 1: Inhibition of endophytic bacterial isolates against *S. rolfsii* on TSA and PDA media

Isolate name	Inhibition percentage (%)	
	TSA	PDA
Control	0.00 [a]	0.00 [a]
Isolate 3	52.94 [b]	11.11 [d]
Isolate 10	64.71 [d]	13.33 [e]
Isolate 11	57.65 [c]	7.77 [b]
Isolate 14	52.94 [b]	8.88 [c]
Isolate 15	52.94 [b]	10.66 [d]
Isolate 18	57.65 [c]	7.77 [b]

Numbers in the same column followed by the same letter are not significantly different based on the 5% level on the Tukey test

On TSA media, the control treatment was significantly different from the treatment on other isolates using endophytic bacteria. The best treatment was Isolate 1, where the inhibition percentage was 64.71%. Similarly, in the dual culture test using PDA media, the best treatment was also found in isolate 10, but the percentage of inhibition was lower than in TSA media, 13.33%. The percentage of inhibition of endophytic bacteria using PDA media was significantly different from the

control. This is due to the mycelium of *S. rolfsii* fungi on PDA media growing faster so that it covers the colonies of endophytic bacteria. According to Arios et al. (2014), the magnitude of the inhibition zone of endophytic bacteria against pathogenic fungi is influenced by the type, solubility, and stability of metabolite compounds produced by bacteria in the test media and the density and type of test media. In addition to dealing with stress from pathogenic fungi, metabolite compounds are also produced by bacteria when facing environmental and nutritional stress so that these compounds will be produced optimally in an environment with limited nutrients for bacterial growth (Brader et al., 2014).

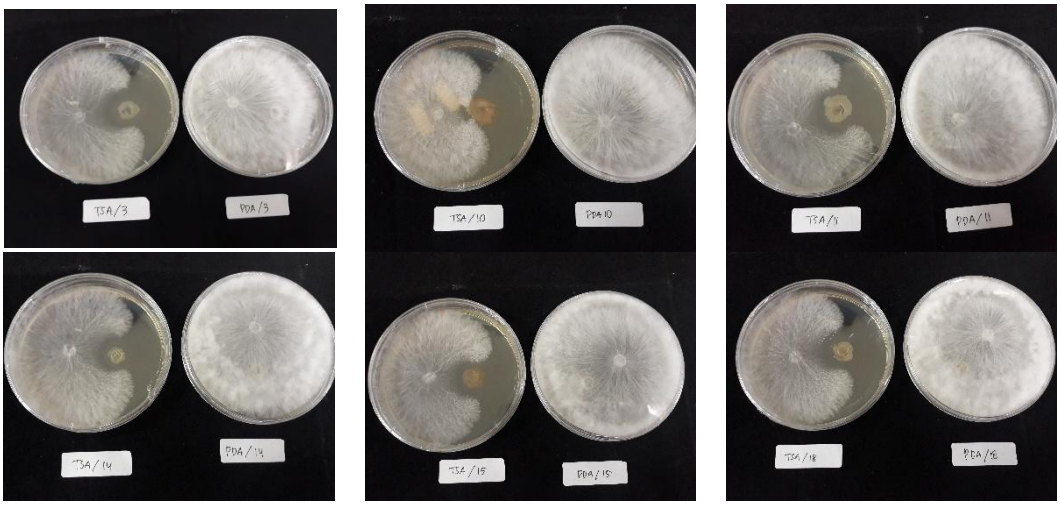

Fig. 3: Inhibition of 6 endophytic bacterial isolates against *S. rolfsii* on 2 types of growth media (TSA and PDA media)

The research results show that endophytic bacteria are able to suppress the growth of the pathogenic fungus *S. rolfsii.* This proves that these endophytic bacteria can be recommended for the biological control of plant pathogens. Biological control is one of the alternative controls that can be used, and it is more environmentally friendly because it does not cause residues and does not damage the environment, so it can be used for sustainable agriculture.

4. Conclusion

Exploration of endophytic bacteria from the roots of kailan plants obtained 36 isolates, three of which are human pathogens and six of which are animal pathogens. A total of six isolates were able to inhibit the growth of *S. rolfsii*, namely isolates 3, 10, 11, 14, 15, and 18. Isolates using TSA media have better inhibitory ability than those using PDA media, and the six isolates have the potential as biological control agents against *S. rolfsii* fungus.

Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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