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Isolations andAntibacterial Tests of Aloe Vera Endophytic Bacteria (*Aloe barbadensis miller*) towards *Staphylococcus aureus* and *Eschericia coli*

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Abstract. Research in the field of microbiology regarding the identification of endophytic bacteria in aloe vera is still rarely carried out while many people in East Nusa Tenggara often use aloe vera to cure diseases. The purpose *of* this study was to identify the endophytic bacteria *Aloe barbadensis miller* and to determine its antibacterial ability against the growth of *Staphylococcus aureus* and *Escsherichia coli bacteria.* The research procedures included taking samples using purposive sampling method, isolating endophytic bacteria using streak plate method, testing the antibacterial potential against *Staphylococcus aureus* and *Eschericia coli* using the paper disc diffusion method. The results showed that endophytic bacteria found on aloe vera leaves were marked by the growth of several bacterial colonies on *Murashige-Skoog media* with different shapes and colors, then the colonies were separated and 8 isolates were obtained with isolate codes C1B, C2B, C3B, C4B, C5B, C6B, C7B and C8B. The results of the antibacterial potency test showed that isolates C1B, C3B, C5B and C8B had the potential to inhibit the growth of Staphylococcus aureus *characterized* by the presence of a clear zone on the surface of the media with the largest size being 20 mm in isolate C1B and the smallest clear zone being 7 mm in isolate C8B, while isolate C1B, C3B, C5B and C8B have the potential to inhibit the growth of *Eschericia coli bacteria* characterized by the presence of a clear zone on the surface of the media with the largest size of 10 mm in isolate C1B and the smallest clear zone of 7 mm in isolate C8B. The conclusion of this study is that endophytic bacteria have been identified in aloe vera and some of these bacteria have antibacterial abilities against the growth of *Staphylococcus aureus* and *Escherichia coli bacteria.*

Keywords: Aloe vera; endophytic bacteria; *Escherichia coli;* identification*; Staphylococcus aureus*

INTRODUCTION

Endophytes are microorganisms that live in host plant tissues without causing disease symptoms. This microorganism is categorized in the beneficial microorganism group because it can help metabolic processes in host plants (Sianipar et al., 2020). This endophytic community lives mutually beneficial, in this case the endophytes obtain nutrients from the results of plant metabolism and protect the plant against herbivores, insects, or pathogenic tissues while the plant obtains nutrient derivatives and active compounds throughout its life (Yahya et al., 2017).

Endophytic bacteria are present in healthy plant tissue. These bacteria can produce special natural compounds that are exactly the same as compounds produced by their host plants, such as *Cryptocandin*, *Cryptosporiopsis quercina* isolated from the medicinal plant *Tripterigeum wilfordii* which is efficacious as an antifungal pathogen in humans, for example *Candida albicans* and *Trichopyton* sp (Suryani & A'yun, 2022).

Various studies on endophytic bacteria are developing very rapidly because of the benefits and very beneficial role played by these endophytic bacteria. Based on research conducted by (Susilowati et al., 2018) showed the results that the endophytic microbial *Pestalotiopsis microspora* isolated from *Taxus andreanae*, *Taxus brevifolia* , and *Taxuswallichiana plants* was able to produce an anticancer metabolite, paclitaxel. In addition, endophytic microbes are known to produce antimalarial (*Colletotrichum* sp.), antioxidant (*Pestalotiopsis microspora*), antidiabetic (*Pseudomassaria* sp.), and immunosuppressive compounds. (Missa et al., 2022) found 6 isolates of endophytic bacteria from aloe vera that had the antibacterial ability against bacteria *Staphylococcus aureus* and *Escherichia coli* (Safira et al., 2017), found 14 isolates of endophytic bacteria from green betel plants that have antibacterial ability against

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Staphylococcus aureus and *Escherichia coli bacteria*, *Bacillus cereus* and *Salmonella enteritidis* .

Based on the great ability of endophytic bacteria and the diversity of biodiversity in Indonesia, the prospects for research on endophytic bacteria from plants in Indonesia are also very large. *The aloe* vera plant (*Aloe barbadensis miller is widely known for its* enormous health benefits. Since ancient times, people have used aloe vera as a traditional medicine, even though there are many more modern medicines (Desriani et al., 2014).

There are many benefits that can be obtained from aloe vera, one of which is the microorganisms that live in symbiotic mutualism in aloe vera plants. So far there has been no research on the endophytic bacteria *Aloe barbadensis miller* in Kupang City, East Nusa Tenggara, given the great potential of the aloe vera plant, so research on the endophytic bacteria *Aloe barbadensis miller*, which has potential as an antibacterial, really needs to be done. This study aims to identify the endophytic bacteria *Aloe barbadensis miller* and to determine its antibacterial ability against the growth of *Staphylococcus aureus* and *Escsherichia coli bacteria*.

METHODS

This research is a descriptive exploratory method by isolating bacteria from aloe vera (*Aloe barbadensis miller*) and then proceeding with testing the antibacterial ability of endophytic bacterial isolates *Aloe barbadensis miller*. The research results are presented in an explorative descriptive manner covering the following stages:

Sampling

Samples of aloe vera plants (*Aloe barbadensis miller)* were taken in the Liliba Village, East Nusa Tenggara Province. The sampling technique used in this study was a *purposive sampling method*, namely sampling with certain considerations, these considerations were based on research objectives with criteria, sampling in the same habitat taking into account the type of leaves taken were young, not damaged by bacteria. endophytes live on healthy plant tissue. The plant material taken was in the form of Aloe vera leaves which were put in a paper bag and labeled with information on where the samples were taken, then brought to the Biotechnology Laboratory of the Kupang State Agricultural Polytechnic for identification.

Endophytic Bacteria Isolation

Aloe vera leaves were washed in running water then successively soaked in 70% alcohol for 5 minutes, then soaked in 3% sodium hypochlorite solution for 4 minutes and rinsed with sterile distilled water 5 times. The sterile leaves were then mortally crushed under aseptic conditions. As much as 1 gram of dead leaves was diluted with sterile distilled water up to 10^{-3} dilution. The dilution series was taken as much as 0.1 ml and spread in a petri dish containing *Murashige-Skoog media.* **(**MS) and leveled using an L glass rod, then wrapped using paper and incubated upside down at 37^{0} C for 24 hours. After 24 hours, the growth of endophytic bacteria will appear on MS media (Basri, 2016).

After obtaining the growth of endophytic bacteria, endophytic bacteria were isolated on Nutrient Agar (NA) in a petri dish to obtain pure cultures of endophytic bacteria. One ose of the microbes obtained is inoculated on nutrient agar using streak plate, incubated at 37ºC for 24 hours; separate bacterial colonies will be obtained. Bacterial colonies from the last streak were inoculated by streaks into the slanted NA medium for microbial stocks (Parida & Damayanti, 2016).

Antibacterial Potential Test of Endophytic Bacteria Isolates

The test bacteria used in this study were *Staphylococcus aureus* which represented Gram Positive bacteria and *Escherichia coli* which represented Gram Negative bacteria.

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The antibacterial potency test was carried out through the preparation of the test bacterial suspension by taking ± 1 ose of the test bacterial stock, then inoculating it with 9 ml of Nutrient Broth (NB) and incubating it on an *incubator shaker* at 37 ^{0} C for 24 hours.

The obtained pure cultures of endophytic bacteria were tested for their antibacterial potential against *Staphylococcus aureus* and *Escherichia coli* using the *Paper Disc Agar Diffusion* Technique method. The endophytic bacterial cultures were then grown in *Nutrient Broth* medium and shaken using *a shaker* at 37°C at 1,700 rpm for 1-2 days. Separation of cell biomass from *Nutrient Broth* medium containing secondary metabolites of endophytic bacteria (supernatant) was carried out by centrifugation at 5,000 rpm for 1 hour. The supernatant was used to screen endophytic bacteria producing antibacterial compounds. After the supernatant was prepared, 40 µl of the supernatant was inoculated with sterile paper discs. To reduce excess water, the paper discs were dried on a sterile watch glass for approximately 1 hour. The test microbe was grown in NA by pour platting method on a different petri dish, by taking 1 ml of the test bacterial suspension and then inoculating it into 20 ml of Nutrient Agar which was melting at 50 ◦ C then pouring it into a petri dish and shaking it slowly to mix the bacterial culture with agar. After the agar has solidified, a paper disc that has been inoculated with the supernatant is placed on the surface of the medium. Incubation was carried out at 37 ◦ C for 24 hours or until a clear zone formed around the paper disc. The inhibition potential was measured by measuring the diameter of the clear zone using a ruler (Hastuti et al., 2014).

RESULTS AND DISCUSSION

Endophytic Bacteria Isolation

In this study, samples of aloe vera leaves were used. Aloe vera leaves have antioxidant activity, ascorbic acid, and phenolic compounds that have the ability to fight gram-positive and gram-negative bacteria (Desriani et al., 2014). The aloe vera leaves used as samples were sterilized and then mortally crushed under aseptic conditions. Sample sterilization is important so that the sample is not contaminated by other microorganisms. This is in line with research (Fitri et al., 2017) that sterilization must be carried out on leaf samples so that they are not contaminated with other non-endophytic bacteria. After sterilization, it is continued by making multilevel dilutions up to 10^{-3} and inoculating using selective media for bacterial isolation, namely MS media (Murashige Skoog).

Endophytic bacteria were successfully grown on MS media with different bacterial colony morphologies, then purification was carried out to obtain a single colony by conducting macroscopic observations in the form of direct observation to see the shape of the colony from above, the elevation or edge from the side, as well as the color and colony texture to distinguish one colony from another, while microscopic observations were carried out by gram staining. This gram staining uses reagents in the form of crystal violet, iodine, acetone alcohol and safranin. The aim is to see the gram form of bacteria. If the bacterial cell wall can bind crystal violet, then the bacteria are included in the gram positive group and the gram negative group if the bacteria are unable to bind crystal violet.

Bacterial purification was carried out by transferring different bacterial colonies to Nutrien Agar (NA) media repeatedly using the streak method. The goal is to obtain a truly pure bacterial colony. According to (Missa et al., 2022) Endophytic bacterial isolates must be in a pure state and must not be mixed with other bacterial cultures.

The results of the purification of the bacteria found 8 isolates that had different properties, shapes and colors and gram shapes. Data from bacterial observations are presented in table 1.

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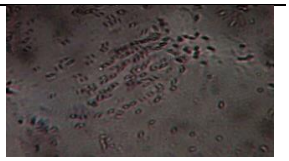
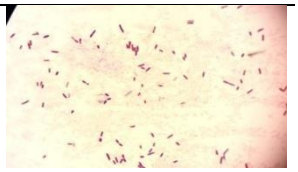
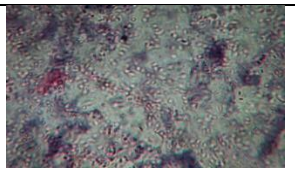
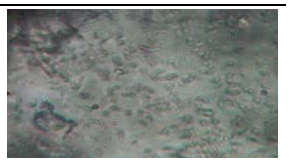
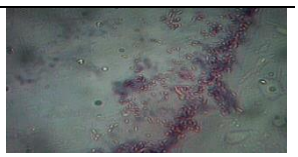
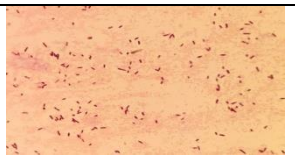
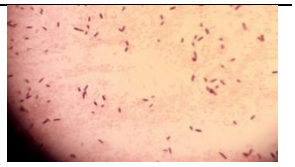
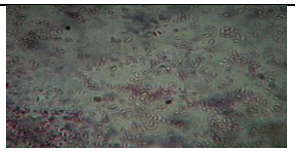
Table 1. Macroscopic and microscopic description of endophytic bacterial isolates *Aloe barbadensis* miller

Isolate Code	macroscopic					Microscopy
	Form	elevation	edge	Color	Cell shape	Grams Group
C1B	Circular	Raised	Entire	Yellow	Basil	
C2B	rhizoids	Raised	Undulant	Pink	Basil	
C3B	Circular	Raised	Entire	Brown	Basil	
C4B	Circular	Raised	Undulat	Brown	Basil	
C5B	Circular	Raised	Undulat	Yellow	Basil	
C6B	rhizoids	Convex	Entire	Orange	Basil	
C7B	Circular	Raised	Undulat	Bright white	Basil	
C8B	rhizoids	Convex	Entire	Milk white	Basil	

Note: C: Researcher's initials, 1-8: Isolate Number, B: First letter Sample

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The table above shows that the 8 bacterial colonies were coded isolates C1B, C2B, C3B, C4B C5B, C6B, C7B, and C8B making it easier to analyze further. The most dominant form of the 8 endophytic bacterial isolates of *Aloe barbadensis Miller* was circular, raised surface, entire margin, brown and yellow in color, bacilli cell shape.

Antibacterial Potential Test of Endophytic Bacteria Isolates

The potency test used positive and negative controls. The positive control used in this study was Ampicillin containing Streptomycin sulfate 4 grams which can inhibit or kill disease-causing bacteria, both gram-positive and gram-negative bacteria, while the negative control used Nutrien Agar (NA).

The purpose of the potency test was to determine the ability of endophytic bacteria *Aloe barbadensis miller* isolated from test bacteria. The test bacteria used were *Staphylococcus aureus* which represented Gram-positive bacteria. This bacterium causes several diseases such as boils, suppuration, inflammation, and poison in food, while the *Escherichia coli bacteria* represent Gram negative which causes mild to severe diarrhea can even be fatal (Safira et al., 2017), therefore this potency test is important to do to see the ability of the endophytic bacteria *Aloe barbadensis miller* in producing antibacterial substance or antibacterial activity.

The results of the antibacterial potency test showed that the endophytic bacteria *Aloe barbadensis miller* had antibacterial ability on the two test bacteria used which were marked by the presence of a clear zone on the surface of the media with isolate codes C1B, C3B, C5B and C8B with the test bacteria being *Staphylococcus aureus* and *Escherichia coli.,* Figure 1. The diameter of the clear zone produced has different sizes where isolate C1B has a greater ability to inhibit the growth of Staphylococcus *aureus* and *Escherichia coli* , this is indicated by the large diameter of the inhibition zone produced by endophytic bacteria code C1B, namely *Staphylococcus aureus* the inhibition zone was 20 mm and *Escherichia coli* was 10 mm, while the isolate code C3B had an inhibition zone diameter of 18 mm for *Staphylococcus aureus* and 12 mm for *Escherichia coli* . In isolate code C5B, it has an inhibition zone diameter of 16 mm for *Staphylococcus aureus* and the smallest diameter of the inhibition zone is found in isolate code C8B, namely 7 mm in *Escherichia coli* and 7 mm in *Staphylococcus aureus*, table 2.

Table 2. Diameter of the clear zone on the test bacteria *S. aureus* and *E. coli*

Code I prayer	Inhibition Zone Diameter(mm)	
	Staphylococcus aureus	***Escherichia coli***
control +	40 mm	30 mm
control -	-	-
C1B	-	-
C2B	20mm	10mm
C3B	18mm	12mm
C4B	-	-
C5B	16mm	7mm
C6B	-	-
C7B	-	-
C8B	7mm	7mm _

Based on table 2, the diameter of the clear zone in the control has the size for the test bacteria *S. aureus* and *E. coli*, namely 40 mm and 30 mm, while the diameter of the

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clear zone in C2B isolate with the test bacteria *S. aureus* is 40 mm, while the diameter of the clear zone is the smallest shown in the isolate code C8B with the test bacteria *S. aureus* while *E. coli* has the smallest diameter of the clear zone with a size of 7cm. The clear zone formed around the paper disc shows that the endophytic bacteria have antibacterial ability against the test bacteria (Nursulistyarini & Ainy, 2011). The larger the diameter of the clear zone produced, the greater the potential of endophytic bacterial isolates as antibacterial (Endah, 2017). The difference in the diameter of the clear zone is because each bacterium has the ability to produce different antibacterial compounds (Fithriyah, 2015) . From the results of this study it can be seen that the endophytic bacteria *Aloe barbadensis miller* can produce extracellular compounds that are antibacterial.

Endophytic bacterial potency test, in this study it can be seen that the isolate codes indicating the zone of inhibition for the two tested bacteria were isolates C2B, C3B, C5B, and C8B. Cup showing the presence of a clear zone formed can be presented in Figure 1.

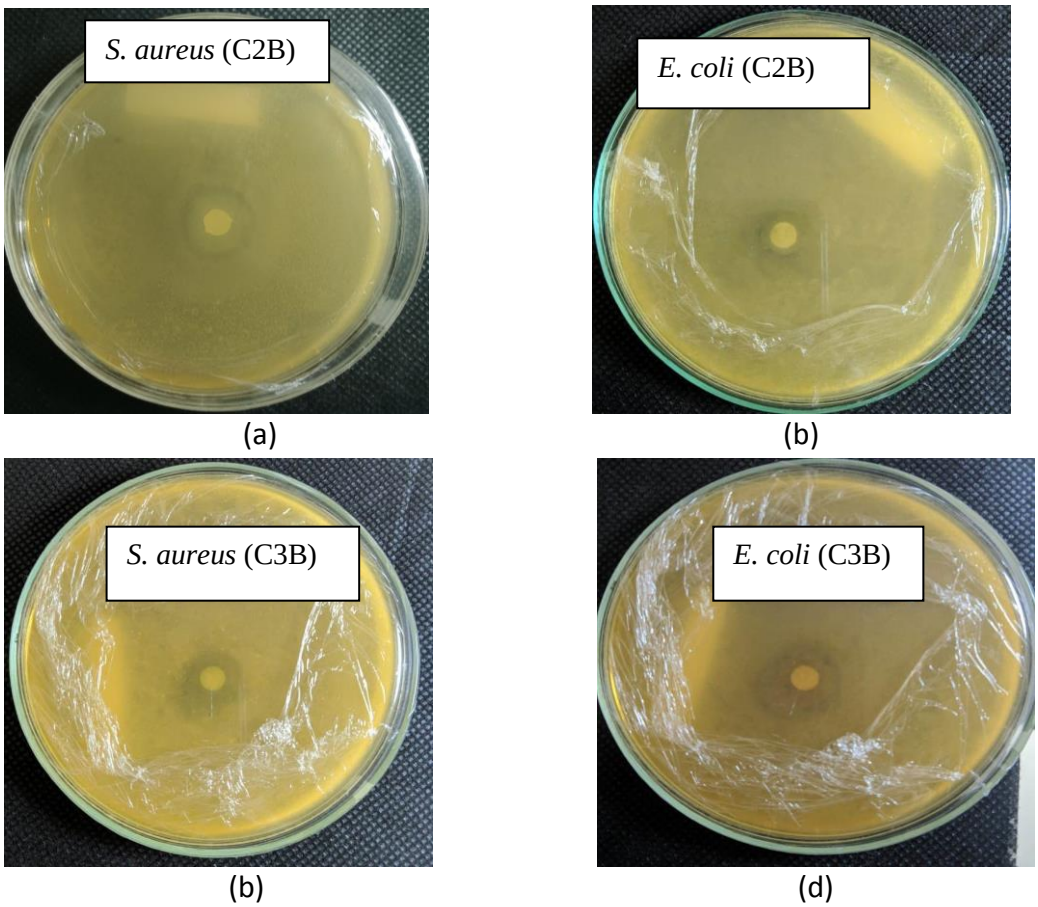

Figure 1. Endophytic bacteria potential test results on *S. aureus bacteria* and *E. coli*. (a) C2B *S.aureus isolates* (b). C2B isolates *E. coli*, (c) C3B isolates *S.aureus*, (b). C2B isolates *E. coli*,

Endophytic bacteria in inhibiting or killing pathogenic bacteria in plants can be done by directly inhibiting growth in plant tissue by carrying out competition or antibiosis in obtaining food (Hikmah, 2018) Endophytic bacteria associated with plants play an important role in plant health. Until now hundreds of endophytic bacteria can be isolated from one type of plant, and the environmental conditions where the host plant grows, the type of plant and the age of the plant will affect the population and profile of endophytic microbes (Hastuti et al., 2014).

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The diversity of endophytic bacteria in plants can be influenced by environmental factors, differences in plant types, tissue types and sampling time (Suryani & A'yun, 2022).

Endophytic bacteria have very unique properties where the physiology of plants originating from the same species but plants in different environments, the resulting endophytic bacteria will also differ according to environmental conditions (Endah, 2017).

Based on research (Nurzakiyah, 2016) shows that endophytic bacteria can act as biological agents associated with host plants. Endophytic bacteria as biological agents have several advantages compared to other bacteria because they are more protected from abiotic stress factors, occupy the same niche as most plant pathogens, have the ability to colonize plant tissues and better translocate metabolic compounds into plant tissues (Arifin Aziz et al. al., 2013).

CONCLUSION

The conclusion of this study is that endophytic bacteria have been isolated from plant leaves and several isolated isolates have antibacterial properties against *Staphylococcus aureus* and *Escherichia coli bacteria.*

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