**DIVERSITAS HAYATI**
Volume 3, Number 1, Juni 2025
Pages: 6-11

ISSN: 3025 - 2237

# IDENTIFICATION OF ENDOPHYTE BACTERIA FROM RED BETEL LEAVES (*Piper crocatum*) WITH 16SrRNA GENE AND ITS ANTIBACTERIAL ACTIVITY

**Diza Sartika[1♥], Miftahur Rahmi[2], Queen Anugrahi Rahmadira[1]**

[1]Bachelor of Pharmacy Study Program, Universitas Perintis Indonesia
[2]Biology Education Study Program, UIN Sulthan Thaha Saifuddin Jambi
♥email: dizasartika@gmail.com

Manuscript received: 25 June 2025. Revision accepted: 30 June 2025.

**Abstract.** Endophytic bacteria are bacteria that live in plants and produce compounds similar to their hosts, one of which is antimicrobial compounds. Several studies have shown that certain endophytic bacteria can produce chemical compounds that have health effects, especially endophytic bacteria isolated from medicinal plants. Red betel leaves are known to be able to cure various types of diseases. This plant contains secondary metabolites of the flavonoid, alkaloid, tannin, essential oil, and saponin groups where these compounds have antibacterial activity. This study aims to isolate and test the antibacterial activity of endophytic bacteria from red betel leaves against *Staphylococcus aureus* and *Escherichia coli bacteria* , as well as to conduct molecular identification using the 16S rRNA gene. The number of endophytic bacteria that were successfully isolated was 4 isolates, namely 1 shoot leaf isolate (PM), 2 young leaf isolates (MM1 and MM2) and 1 old leaf isolate (TM). The results of antibacterial activity testing were carried out on isolates that had been purified using the *Kirby-Bauer method* , which showed that each isolate had inhibitory activity against the test bacteria *Staphyloccocus aureus* , namely: PM: 4.03mm, MM1: 12.1mm, MM2: 3.84mm, all of which were classified as weak. While against the test bacteria *Escherichia coli* did not have antibacterial activity. The results of molecular identification with the 16S rRNA gene showed that the MM1 bacterial isolate was *Bacillus thuringiensis* .

**Key words:** Red betel leaf, endophytic bacteria, antibacterial

## INTRODUCTION

Red betel leaf is a climbing plant that is cultivated because it is one of the potential medicinal plants that is empirically known to have properties to cure various types of diseases such as coughs, asthma, nasal inflammation, and sore throats. The benefits of red betel have been widely discussed, but research on red betel is still very limited. In previous studies on the content of red betel leaves, the results of chemical content screening showed that red betel leaves contain compounds of the flavonoid, alkaloid, tannin-polyphenol, essential oil, and saponin groups [1]. Where some of the chemical contents of red betel such as flavonoids are suspected to function as antibacterials by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. Based on the characteristic data obtained, it was concluded that the flavonoid compound is an aurone group. Red betel also contains flavonol and quercetin compounds [2]. In addition, the chemical content of red betel is Alkaloid which has the ability as an antibacterial, which has a mechanism by disrupting the components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes cell death [1]. In red betel leaves there are also essential oils that act as antibacterial by disrupting the process of membrane or cell wall formation so that they are not formed or formed imperfectly, terpenoid compounds detected in phytochemical screening are very likely to come from essential oils which are compounds commonly found in the Piper genus. In general, the use of bioactive compounds from a medicinal plant is achieved by extracting plant parts, but this method is less effective because plant parts must be extracted in large quantities, so that if consumed continuously, the availability of plants in the environment will decrease [3]. Endophytic bacteria are beneficial microorganisms that interact with host plants without causing disturbance or damage to plants. Endophytic bacteria are symbiotic mutualism or mutually beneficial where endophytic bacteria utilize nutrients from the metabolism of the plants they inhabit to live. Endophytic bacteria can produce the same bioactive compounds as their host plants [4]. Endophytic bacteria in red betel plants are also likely to produce one of the active compounds or other antibiotic compounds. So far, there have been no reports on the isolation of endophytic bacteria from red betel leaf plants and testing their antibacterial activity. In this study, the PCR method and 16s rRNA gene identification were used. The PCR (Polymerase Chain Reaction) method is an enzymatic method for DNA amplification in vitro. The PCR process requires double-stranded DNA. For the 16s rRNA gene identification method, namely the analysis of the 16s rRNA encoding gene which can be used to identify bacterial phytogenicity [5]. Based on the explanation above, this study was conducted with the aim of isolating endophytic bacteria from red betel leaves and determining their antibacterial activity, as well as molecular identification of endophytic bacteria that have the greatest antibacterial activity using the 16S rRNA gene with a PCR (Polymerase

Chain Reaction) tool so that the species of endophytic bacteria in red betel leaves can be identified.

## MATERIALS AND METHODS

### Time and Place of Research

This research was conducted from February to June 2022 at the Microbiology Laboratory of the Faculty of Pharmacy, Perintis Indonesia University and the Microbiology Laboratory of the Faculty of Medicine, Andalas University, Padang.

### Tools and materials

Tools: Masks, gloves, Erlenmeyer flasks, measuring cups, test tubes, test tube racks, dropper pipettes, analytical scales, knives, petri dishes, ose needles, tweezers, bunsen burners, refrigerators, laminar air flow, autoclaves, microtubes, micro pipettes, calipers, label paper, plastic wrap, aluminum foil, tissue, cotton, gauze, light microscope, object glass, newspaper, stove, ependorf tubes, spin column tubes, thermoblocks, PCR equipment and electrophoresis equipment.

Materials: Red betel leaves ( *Piper crocatum* ), test bacteria Escherichia coli and Staphylococcus aureus, sterile distilled water, 96% ethanol, 0.9% NaCl, 1%, 1% H2SO4, 1.175% BaCl22H2O, iodine, fuchsin, Nutrient Agar media, crystal violet solution, 5.25% Na-hypochlorite, chloramphenicol disk, lugol, safranin solution, 16S rRNA Forward Primer (5' CCAGCAGCCGCGGTAATACG 3'), 16S rRNA Reverse Primer (3'-ATCGG (C/T) TACCTTGTTACGACTTC5'), gel red, 1 Kbp DNA ladder, loading dye, agarose powder, and TBE buffer.

### Procedures

1. **Sample Collection and Identification**
   The samples used were red betel leaves (Piper crocatum) with 3 different categories, namely leaf tips, young leaves and old leaves. Red betel leaves (Piper crocatum.) were selected based on the size, color and position of the leaves. The leaf tips have a lighter red leaf color than the young leaves, the size is smaller than the young leaves, the position is at the top. The young leaves have a red leaf color mixed with green, the size is smaller than the old leaves, the position is in order 1-4 from the leaf tips. Old leaves are wider than young leaves, the color is dark green and the position is in order 5 and so on (Mulangsri, 2018). Sampling was carried out in Lubuk Minturun, Koto Tangah District, Padang City, West Sumatra. Samples were identified at the Herbarium of the Faculty of Mathematics and Natural Sciences, Andalas University, Padang.
2. **Sterilization of Tools and Preparation of Media**
   Sterilization of Tools: The tools used are washed and dried first before being sterilized. The petri dish is wrapped with newspaper, the test tube and the dropper are closed with cotton and then wrapped one by one with newspaper. All tools are sterilized in an oven at a temperature of 160oC for 1 hour. The mouth of the Erlenmeyer flask is closed with cotton and the measuring cup is closed with cotton and wrapped one by one with newspaper and then sterilized in an autoclave at a temperature of 121oC for 15 minutes with a pressure of 15 lbs, then the tweezers, ose needles and object glass are sterilized by burning using a spirit lamp.
   Media preparation: Making Nutrient Agar Medium Nutrient Agar is made by dissolving 20 gr of Nutrient Agar into 1000 ml of sterile aquades in an Erlenmeyer flask. This solution is then heated on the stove while stirring for 10-15 minutes, then sterilized in an autoclave at a temperature of 121 $^0$C for 15 minutes.
3. **Isolation and Purification of Endophytic Bacteria from Red Betel Leaves**
   Leaf samples were washed with running water until clean, then cut into pieces of about 1-3 cm each. The sample pieces were surface sterilized in stages. The sample pieces were soaked in 96% ethanol for 30 seconds, then in 5.25% Na-hypochlorite for 30 seconds, then rinsed again in 96% ethanol three times. The sterilized samples were then planted on Nutrient Agar isolation media, then incubated and observed until colony growth occurred. Purification of bacterial colonies was carried out by transferring 1 loop of colonies into a petri dish containing new Nutrient Agar media and incubated at 37 $^o$ C for 24 hours. After obtaining a pure culture, endophytic bacteria were transferred to Nutrient Agar slants.
4. **Identification of Endophytic Bacteria**
   a. Macroscopically
   Colony shape (seen from above): round, oval. Colony surface (seen from the side): flat, flat, raised. Colony edge (seen from above): wavy and spreading curve.
   Colony color (pigmentation): Some bacteria produce pigments when they grow in media, for example: white, yellowish white, or thick like milk (Faradiska, 2012).
   b. Microscopically
   Microscopic observation of bacterial cell morphology is done by gram staining. 1-2 drops of sterile distilled water are placed on the object glass, the bacterial colony is taken one loop from the media placed on sterile distilled water and spread evenly, let the smear dry in the air. After the smear is completely dry, then do the object glass several times over the flame until the object glass feels a little hot when attached to the back of the hand. Then drip with crystal violet solution (Gram A), and let stand for one minute, then wash using distilled water in a spray bottle and dry. Next, drip with iodine solution (Gram B) and let stand for 2 minutes, wash using distilled water in a spray bottle and dry. Then drip with 96% ethanol solution (Gram C) for 30 seconds, wash using distilled water in a spray bottle and dry. After that, drip with safranin solution (Gram D) or covering agent and let stand for 30 seconds, then wash using distilled water in a spray bottle and dry. Next, observe using a microscope at high magnification.
5. **Antibacterial Activity Test**
   a. Making Mac Farland Solution 0.5
   10 ml of 1% H2SO4 solution was mixed with 0.05 mL of 1.175% BaCl22H2O solution in an Erlenmeyer flask. Then shaken until a cloudy solution is formed. This

turbidity is used as the standard turbidity of the test bacterial suspension [6].

b. Preparation of Test Bacterial Suspension

The test bacteria, namely *Escherichia coli* are gram-negative bacteria and *Staphylococcus aureus* are gram-positive bacteria that have been inoculated, taken ± 1 loop then suspended in a tube containing 5 mL of 0.9% NaCl solution so that the same turbidity is obtained as the standard turbidity of the Mac farland solution 0.5. The bacterial suspension was pipetted as much as 200 µL and swabbed into the seed media [6].

c. Preparation of Endophytic Bacteria

Endophytic bacterial isolates from each sample were taken with an ose needle and then each was inoculated into 9 ml of 0.9% NaCl and then vortexed until homogeneous.

d. Antibacterial Activity Testing

*Staphylococcus aureus* and *Escherichia coli* test bacteria was taken 200 µL and spread evenly on the surface of the Mueller Hinton Agar media. The surface of the media was given 3 disc papers dripped with endophytic bacterial isolate suspensions from leaf tips, young leaves, old leaves and chloramphenicol discs as positive controls, then incubated in an incubator at 37°C for 24 hours, in this test it was repeated 3 times.

e. Observation and Measurement of Inhibition Zone

Observations were made after 1 x 24 hours of incubation. The clear zone formed around the disc paper was observed. The diameter of this clear zone was then measured using a caliper. The diameter of the clear zone was measured based on the CLSI classification. Based on CLSI (Clinical Laboratory Standards Institute) it is grouped into three categories, namely sensitive, intermediate, and resistant. A bacterium is said to be sensitive to antibiotics if the bacteria can be inhibited well and a clear zone is formed when tested (sensitive to antibiotics), an intermediate category if the bacteria can be inhibited but with weaker inhibition, and a resistant category if the bacteria can be inhibited but show very weak inhibition or no inhibition at all.

**Table 1**. Classification of Microbial Inhibitory Responses

| Diameter Zone Resistance (mm) | Growth Barrier Response |
|---|---|
| ≤14 | Weak |
| 15-18 | Currently |
| ≥19 | Tall |

## 6. Bacteria

a. DNA Isolation

Endophytic bacterial isolates that have the greatest antibacterial activity in slant media are taken using a micro pipette and inserted into a microtube containing 1 ml of Phosphate Buffer Saline (PBS). Then the sample in the microtube is inserted into a centrifuge at a speed of 10,000 rpm for 5 minutes. After centrifugation, the supernatant is discarded and then 1 ml of TE buffer is added, then vortexed, then incubated with a heating block for 10 minutes at a temperature of 950C, then the bacterial suspension is centrifuged for 5 minutes at a speed of 10,000 rpm. Furthermore, the supernatant is transferred into a 1.5 ml microtube, then stored in the freezer [6]. Amplification of the 16S rRNA Gene with PCR The isolated DNA sample is continued with the PCR method consisting of 3 stages of denaturation, annealing, and extension which can be seen in table 4, using the 16S rRNA primer (Rahmani, et al., 2006). This DNA amplification method uses Taq DNA Polymerase using Go Taq Mastermix.

**Table 2.** Components and mixtures for 16S rRNA primers and sample DNA

| Reagent | Amount of reactant (µL) |
|---|---|
| DNA Templates | 3 µL |
| Go Taq Mastermix | 25 µL |
| Primary forward | 1 µL |
| Primary reverse | 1 µL |
| Nuclease free water | 20 µL |
| Total volume | 50 µL |

**Table 3.** PCR machine cycle

| Number of Cycles | Temperature ($^0$C) | Time (minutes) |
|---|---|---|
| 1 | 95 | 3 minutes |
| 35 | 95(denaturation) | 30 seconds |
| | 55(annealing) | 30 seconds |
| | 72(extension) | 30 seconds |
| 1 | F extension 72 | 5 minutes |
| 1 | Cooling 12 | Until finish |

## 7. Agarose Gel Electrophoresis and Visualization

0.2% agarose gel is made by dissolving 0.2 grams of agarose powder in 20 ml of TE buffer, then heated in (microwave soup) for 1-2 minutes until dissolved until clear, then add 3 µl of gel red then pour into the Casting tray mold that has been fitted with a comb. After the gel solidifies for ± 30 minutes, then enter 5 µl of PCR product amplicon sample. To determine the size of the PCR amplification product, 3 µl of 1Kbp DNA ladder and 3 µl of loading dye are inserted into the first gel well, then followed by the amplified sample DNA in the next gel well. Furthermore, the electrode is connected to the power supply then turned on for 40 minutes. After that, the electrophoresis device 6 is turned off, then the gel from the device is taken and then transferred to the UV transilluminator for visualization, then the results are observed and documented [6].

## 8. Data analysis

The analysis was carried out using experimental and descriptive analysis, including by looking at the results of the isolation and identification of bacteria using rRNA-based molecular techniques using the PCR method, where all data was obtained from isolation and observations that had been carried out during the work process, namely by collecting data, direct observation,

and documentation to be used as evidence of research results.

## RESULTS AND DISCUSSION

Following results observation morphology in a way macroscopic bacteria endophyte.

**Table 4**. PCR machine cycle

| Identification Macroscopic | Results Observation | | | |
|---|---|---|---|---|
| | PM | MM1 | MM2 | TM |
| Form | Round | Round | Round | Round |
| Surface | arise flat | arise flat | arise flat | arise flat |
| Edge | Arch | Arch | Arch | Arch |
| Color | White clear | Yellowish white | White clear | White clear |

Note: PM = Top Red, MM1 = Young Red 1, MM2 = Young Red 2 TM = Old Green

**Table 5**. Results Observation Endophytic Bacteria Based on Coloring Gram

| Isolate Code | Gram Staining | Form |
|---|---|---|
| PM | Positive | Basil |
| MM1 | Positive | Basil |
| MM2 | Positive | Basil |
| TM | Positive | Basil |

Note: PM = Top Red, MM1 = Young Red 1, MM2 = Young Red 2 TM = Old Green

**Table 6.** Results Testing Activity Antibacterial Bacteria Endophyte Leaf Betel Green

| Isolate code/ Control | Clear Zone Diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | S. aureus | | | Average | E. coli | Average |
| | Repetition | | | | Repetition | |
| | 1 | 2 | 3 | | 1 | |
| PHM | 0 | 12.1 | 0 | 4.03 | 0 | 0 |
| MM1 | 12.1 | 12.3 | 11.9 | 12.1 | 0 | 0 |
| MM2 | 11.6 | 0 | 0 | 3.84 | 0 | 0 |
| TM | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 20.33 | | | 20.33 | 15.45 | 15.45 |

**Results from electrophoteris from MH isolate**

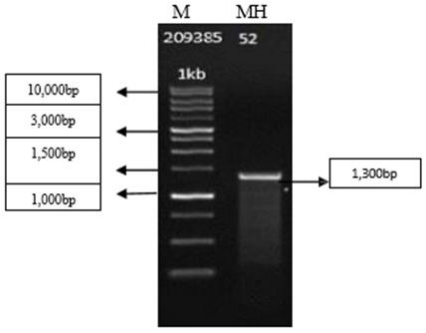


**Figure 1.** Electrophoresis Results of 16S rRNA Gene Amplification Products of endophytic bacteria isolates of young green leaves (MH) with DNA band size ±1300bp. M (DNA marker) 1 kb DNA ladder.

The following are the results of the BLAST analysis of the MH isolate as shown in the image. following :

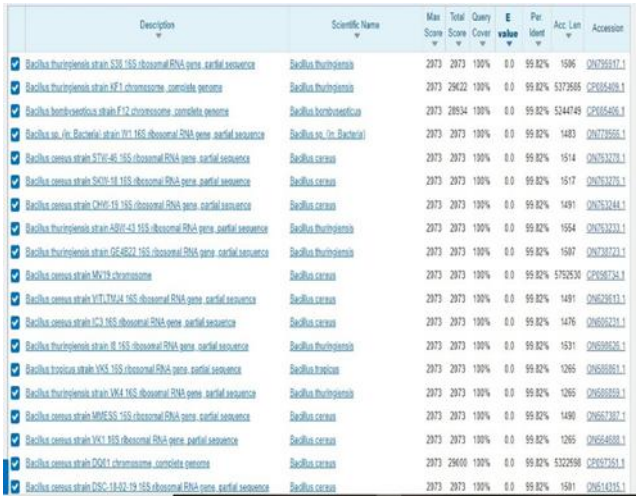


**Figure 2.** Analysis Results BLAST from Bacterial Isolates MH Endophyte

This study aims to determine the characteristics and antibacterial activity produced by endophytic bacteria from red betel leaves ( *Piper crocatum* ) against *Staphylococcus aureus* and *Escherichia coli bacteria.* The samples used in this study were red betel leaves ( *Piper crocatum* ), which were taken in the Lubuk Minturun area, Koto Tangah, Padang, West Sumatra, then identified in the ANDA Herbarium with Identification No.: 491 / K-ID / ANDA / XI / 2021. This identification was carried out to identify the plant in question as the same plant as red betel ( *Piper crocatum* ). This red betel leaf sample has the species name *Piper crocatum* . The samples taken were leaf tips (PM), young leaves (MM), and old leaves (TM), because to find out the leaves which produces endophytic bacteria containing active antibacterial compounds the biggest.

Surface sterilization was performed on the three samples used, then incubated in *Nutrient Agar* (NA) media in a petri dish. After that, colony purification was carried out on bacteria that had grown on NA media, then macroscopic identification of endophytic bacteria morphology was carried out. For macroscopic observations can be seen in Table 4. Based on Table 4. above, it can be seen that the results of observations of the morphology of endophytic bacteria obtained round shapes, flat embossed, curved, clear white, and yellowish white. This is in accordance with Faradiska (2012) the characteristics of endophytic bacterial colonies are that they have a round, oval shape, and the color of the colony is white, yellowish white, or thick like milk, and the edges of the colony have wave-like curves and spread. Furthermore, the isolates were identified microscopically by performing gram staining which showed that the results of observations of endophytic bacterial

isolates were all gram-positive bacteria as indicated by purple colonies and bacillus-shaped. The following are the results of observations of endophytic bacteria based on gram staining.

For the results of microscopic tests based on gram staining can be seen in Table 5. Based on Table 5. above, it can be seen that all isolates have gram-positive bacilli. Gram-positive bacteria are characterized by the presence of purple colonies, because these gram-positive bacteria are able to retain the main dye in Gram staining, namely crystal violet so that they appear purple when observed because the cell walls of this group of bacteria are composed of mostly thick peptidoglycan which is able to bind dyes and does not damage when washed with alcohol. . These gram-positive bacteria are more complex in protecting defenses from physical disturbances or pathogens in tissue host, If seen from structure And composition wall cell, bacteria grams positive relatively simpler compared to more complex gram-negative bacteria. While gram-negative bacteria are characterized by the presence of red colonies because gram-negative bacteria This negative has a cell wall composition that is mostly composed of lipid layers, so that in gram staining it is less able to retain the main dye, especially when washed with alcohol (lipids are damaged when washed with alcohol) so that the pores and cell walls will enlarge and cause the release of the previously absorbed crystal violet complex and the bacteria will turn red after being given safranin [7]. Endophytic bacteria are abundant in gram-positive bacteria because gram-positive bacteria have a low lipid content of 1-4%. When compared to gram-negative bacteria of 11-12%. Gram-positive bacteria also only have one layer of thick peptidoglycan membrane.

After conducting the morphological test of endophytic bacterial isolates, an antibacterial activity test was then conducted to determine the ability of bacterial inhibition against test bacteria which was carried out using agar diffusion against 2 types of bacteria, namely gram-positive Staphyloccocus aureus bacteria and gram-negative Escherichia coli bacteria. Staphyloccocus aureus bacteria are normal floral bacteria in the human body and are pathogenic bacteria in humans if their numbers increase in the body, they can cause disease, and can cause skin pus. While Escherichia coli bacteria are pathogenic in humans, causing digestive disorders in humans and disrupting the working system of the stomach organs.

Antibacterial activity testing was carried out on 4 endophytic bacterial isolates PM, MM1, MM2, and TM which were tested with *Staphylococcus aureus* and *Escherichia coli bacteria* by the diffusion method using disc paper on Mueller Hinton Agar (MHA) media. The following are the results of the antibacterial activity test of endophytes from red betel leaves against *Staphylococcus aureus* and *Escherichia coli bacteria.* Based on Table 6, it can be seen from the results of the average measurement of the clear zone diameter obtained, the antibacterial power with codes PM, MM1, MM2, and TM is (0.00mm) with a category that cannot inhibit *Escherichia colii bacteria* because there is no clear zone around the disc paper. While the antibacterial power against the test bacteria *Staphyloccocus aureus* from the isolate code PM is (4.03 mm) which is included in the weak category, the MM1 code is (12.1 mm) which is included in the weak category, the MM2 code is (3.86 mm) which is included in the weak category, and the TM code is (0.00 mm) where there is no inhibition against the test bacteria. Because according to *the Clinical and Laboratory Standards Institute* (CLSI), weak inhibition response when the diameter of the antibacterial inhibition zone is ≤ 14mm, moderate inhibition response when the diameter of the antibacterial inhibition zone is 15-18 mm, strong inhibition response when the diameter of the antibacterial inhibition zone is ≥ 19mm. This shows that red betel leaves ( *Piper crocatum* .) contain antibacterial substances that can inhibit bacterial growth against *Staphylococcus aureus bacteria* even though the inhibitory power is relatively weak. The formation of a clear zone indicates that the endophytic bacteria produce bioactive compounds such as compounds saponins, tannins, flavonoids, terpenoids, polyphenols, and steroids that can be used as antibacterials. In previous studies on the use of red betel leaf extract in antibacterial activity tests, a large inhibition zone value was obtained. This is likely due to the active substance released during the extraction process which allows secondary metabolites to develop more [8].

The mechanism of bacterial growth inhibition by secondary metabolites can occurs through inhibition of the formation of bacterial wall compounds, increased cell membrane permeability, so that cells lose cell components [4]. From the average calculation, the diameter of the inhibition zone produced by endophytic bacterial isolates is greater in inhibiting the growth of Gram-positive bacteria *Staphylococcus aureus* , this shows that the antibacterial activity of red betel leaf isolates ( *Piper crocatum)* is more effective. Gram-positive bacteria have a gram-positive cell wall structure with more peptidoglycan, little lipid and cell walls containing polysaccharides (teichoic acid). Teichoic acid is polymer Which late in water, Which functioning as transportation ion positive for exit or entry. Because of this water-soluble nature, it shows that the cell wall of gram-positive bacteria more polar in nature. Flavonoid and tannin compounds are polar parts so they are easier to penetrate the polar peptidoglycan layer than the non-polar lipid layer. This causes the inhibitory activity on gram-positive bacteria to be greater than gram-negative bacteria. Because the structure of the cell wall causes the two types of bacteria to respond differently, it shows that bacteria with MM1 isolates have a larger average inhibition zone diameter calculation value compared to PM, MM2, and TM isolates. So that the MM1 endophytic bacterial isolate will be continued for molecular identification using the 16S rRNA gene to determine the type of bacteria.

Next, identify bacterial isolates with MM1 which have the greatest inhibitory power by molecular identification using the 16S rRNA gene to determine the type of bacteria. In the process of molecular identification of microorganisms, there are 4 main processes, namely DNA isolation, PCR, electrophoresis, and Sequencing. The DNA isolation process uses the boiling method which is a simple isolation method by providing physical disruption to bacterial cells in the form of heating at high temperatures (95 $^{0}$ -100 $^{0}$ C). Heating at high temperatures can increase the

ermeability of cell walls, resulting in the entry of fluids and molecules around the cells and the release of materials from within the cells, then the DNA is separated for further use as DNA in the PCR process [9].

Next, the DNA extraction results will be amplified by the PCR method using the universal primer of the 16S rRNA gene. The amplification stage by PCR causes the DNA band to denature (the DNA template separates into single strand). The materials used are H2O , Taq Master mix, forward primer (338F) and reverse primer (1525R). Master Mix functions as a component or mixture of DNA template that will be amplified using a PCR machine. The Forward Primer functions to initiate the synthesis of DNA strands from the 5' end -------- 3' while the Reverse Primer functions to initiate the synthesis of DNA strands from the 3' end ---------- 5'. The function of DNA template in the PCR process is as a mold for the formation of new, identical DNA molecules [10].

The next stage is DNA electrophoresis, which is a technique that measures the rate of movement of charged particles in an electric field. Technique This can used For utilise load electricity that There is on molecules such as DNA which are negative. Molecules that can be separated include DNA, RNA, or protein. If a negatively charged molecule is passed through a medium, such as agarose gel, then an electric current is passed from one pole to the opposite pole, the molecule will move from the negative pole to the positive pole. The results of electrophoresis can be seen in Figure 1. Based on Figure 1. above, it can be seen that after the results of the amplification process is visualized in electrophoresis, then the results of the electrophoresis of the isolate with the MH code contain bands, known to be separated and parallel to the 1300 bp marker. It can be seen in the image that the length of M (DNA marker) of 1kb is marked by the brightness of 1000 bp and 3000 bp as DNA ladder. This This indicates that fragment gene Which amplified own size ±1300 bp which corresponds to the nucleotide size of the 16S rRNA gene, which is around 1500 bp, so it can be concluded that the bacterial amplification process was successful. The success of this PCR technique is based on the suitability of the primers to the isolates to be used and optimization during the PCR process [11].

Furthermore, the isolate was determined using the 16S rRNA sequence which was analyzed completely at 1 st BASE Singapore through CV. Crow Biotechnology. At The sequence is done with the BLAST-N (Basic Local Alignment Search Tool- Nukelotide) program. Based on Figure 2. above, it can be seen that the information from the BLAST results is in the form of *Query Coverage* and *Maximum identity* . *Query coverage* is the percentage of nucleotide length that is aligned with the database contained in BLAST. *Maximum identity* is the highest value of the percentage of identity or match between the query sequence and the aligned database sequence. This BLAST analysis was carried out with the aim of comparing the results obtained with the results of DNA sequences from around the world that were deposited in the Gen Bank database.

*After* the sample sequence with the MM1 code is matched with the database sequence in BLAST (*Basic Local Alignment Search Tool* ) which is accessed online via the NCBI website ( http://blast.ncbi.nlm.nih.gov/Blast.cgi ). The results of the MM1 isolate were obtained namely *Bacillus thurngiensis* . Where the MM1 isolate has a nucleotide length similarity level in the BLAST database reaching 100% and has a sequence match reaching 100% with *Bacillus thurngiensis* .

## CONCLUSION

In this study, endophytic bacteria were successfully isolated from red betel leaves. The number of 4 bacterial isolates, namely 1 isolate from Pucuk Merah (PM), 2 isolates from Muda Merah (MM), and 1 isolate from Tua Merah (TM). Endophytic bacterial isolates from red betel leaves showed the presence of antibacterial activity against Staphyloccocus aureus bacteria with low inhibition, and in Escherichia coli bacteria , all of them could not inhibit the test bacteria. In the PCR test or molecular identification, the results of the MM1 isolate were found to have a molecular match of 99.82% with Bacillus Thuringiensis

**Suggestion**

It is recommended that other researchers identify the bioactive compounds produced by endophytic bacteria from red betel leaves using different methods, as well as testing antibacterial activity using different test bacteria.

## REFERENCES


[1] Fitriyani A, WL (2021). Anti-inflammatory test of methanol extract of leaves. *Traditional Medicine Journal* , 16(1):34-42.

[2] Daniel. (2020). Isolation and identification of flavonoid compounds in the ethyl acetate fraction of red betel leaves (Piper crocatum Ruiz & Pav.). *Mulawarman Sciantie* , 9(1):17-26.

[3] Kusumawati, DE (2024). *Antibacterial Activity of Endophytic Bacterial Isolates from Miana Plants (Coleus Scutellariodes [L.] Benth.) Against Staphylococcus Aureus and Escherichia Coli*. 1(1), 37–44.

[4] Sepriana, CJ (2017). Endophytic Bacteria of Clove Plant Bark (Syzygium Aromaticum L.) and Its Ability as an Antibacterial. *Journal of Science Education Research* , 3(2).

[5] Dinnur, H. (2020). Identification of Multiresistant Bacteria of the Genus Streptococcus (Isolate WK 45) Using the PCR Method Using Universal Primer 16s rRNA. *Muhammadiyah University* .

[6] Sihombing, MC (2018). *Molecular Identification Isolation Using Endophytic Symbionts of Algae Padina sp*. 7(2) : 41–52.

[7] Imawati, R. (2020). *Isolation and Identification of Endophytic Bacteria from Javanese Turmeric Rhizome (curcuma xanthorizza) as a Producer of Antibacterial Compounds for Pseudomonas aeruginosa and Staphyllococcus epidermidis.* 1(9).

[8] Nabila, A. a. (2021). *Antibacterial Activity Of Ethanol Extract Of Red Betel Leaves (Piper Crocatum Ruiz & Pav.) Against The Growth Of Staphylococcus epidermidis and Staphylococcus aureus*. UMY

[9] Afif, R. (2019). 16S rRNA Gene Amplification Of Endophytic Bacteria Which Produces Antimicrobial Compounds. Department of Biology, Faculty of Mathematics and Natural Sciences. *Current Biochemistry*.

[10] Harvianti, Y. (2017). Molecular Identification of Endophytic Bacteria Producing Phytase from Corn Plants (Zea Mays L.) Based on 16S rRNA Gene. *UIN Alauddin Makkassar* .

[11] Rinanda, T. (2015). *16S rRNA Sequencing Analysis in Microbiology.* Banda Aceh: UNSYIAH