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# POTENTIAL OF ENDOPHYTIC BACTERIA FROM LIVERWORT (*MARCHANTIA POLYMORPHA* L.) WHICH PRODUCES ANTIBIOTICS IN *STAPHILOCOCCUS AUREUS* L.

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## ABSTRACT

*Marchantia polymorpha* is a type of liverwort that is often used by the Indonesian people, especially the Maninjau West Sumatra community as a source of traditional medicine in dealing with infections of the skin. This moss is known to have simple terpenoids and phenolics (flavones and flavones glycosides) which are antimicrobial compounds. To obtain these compounds, of course requires large amounts of moss, this has the potential to disrupt ecological stability. To avoid this problem, endophytic bacteria that have the potential to produce antibiotics are used. The stage of this study starts from sampling, isolation and screening of antibiotic-producing endophytic bacteria and macroscopic, microscopic and biochemical tests. The purpose of this study was to obtain endophytic bacterial isolates that could potentially produce antibiotics in *S. aureus* test bacteria. Six isolates of endophytic bacteria that have the potential to produce antibiotics were successfully isolated. Isolates with code BEMP-09 were the best isolates in inhibiting the growth of *S. aureus* pathogenic bacteria in the medium category.

**KEYWORDS:** Endophytic Bacteria, *Marchantia polymorpha*, Antibiotics, *Staphylococcus aureus.*

## 1. INTRODUCTION

Infection is a disease condition due to the entry of pathogenic germs or other microorganisms into the body causing certain symptoms. One of the skin infections by bacteria that often occurs in tropical areas, especially Indonesia is honey pox. Honeypox or in medical terms

*impetigo krustosa* is an infectious disease by *Staphylococcus aureus* and or *Streptococcus pyogenes* bacteria that occurs in the skin of the epidermis.[1,2]

Someone who contracted chickenpox will usually be given antibiotics by medical personnel. Antibiotics are the most widely used synthetic drugs in the world. It is estimated that one quarter of the total hospital budget is used for antibiotic needs[3], so researchers are competing to find new sources of antibiotics, since there are already many antibiotic resistant bacteria.

Maninjau district community District. Agam, has a unique way of dealing with skin infections, one of which is honey pox, by rubbing moss on the infected skin. The result is that the part of the skin that is rubbed with moss dries and heals quickly without the help of antibiotics, although it is necessary to prove the scientific properties. Research on the efficacy of liverworts as raw material for medicines has not been done too much. One study of the content of moss plants shows that *Marchantia* sp. contains secondary metabolites in the form of ethanol, flavonoids, and terpenoids which usually can inhibit the growth of *S. aureus, Pseudomonas aeruginosa,* and *Staphylococcus tiphymurium* bacteria.[4] To get secondary metabolites directly, of course requires large amounts of moss, this has the potential to disrupt ecological stability. For that purpose specific endophytic bacteria are used from inside the liverworts which are expected to be able to produce these secondary metabolites.

Endophytic bacteria are bacteria that can grow and develop in plant tissues. These bacteria live in mutual symbiosis with their host plants and can jointly produce certain secondary metabolites with their host plants.[5] Therefore, it is necessary to do research on the potential of endophytic bacteria from antibiotic-producing liverworts in *S. aureus* bacteria.

## 2. MATERIAL AND METHODS

### 2.1 Sample Collection

Sampling of liverworts was carried out by *purposive random sampling* method in the Nagari Sigiran Maninjau area, Tanjung Raya District, Agam Regency, West Sumatra. Moss samples were washed with sterile distilled water, put into plastic and taken by the laboratory. Processing of samples in the laboratory is carried out aseptically. Fresh moss is washed clean with running water. Next, the surface disinfection was done by soaking moss with 70% alcohol for 1 minute, then rinsing with sterile aquadest 3 times.[6]

### 2.2 Isolation and Purification of Endophytic Bacteria

Isolation and purification of endophytic bacteria was carried out by sterile moss samples were crushed with sterile mortar and pestle, then put into physiological NaCl solution 0.85% and homogenized, after homogeneous then 0.1 ml of solution was inoculated into a petri dish containing NA medium with "*spread plate*" method, then incubated at room temperature for 48 hours. Different bacterial colonies were taken and inoculated on the medium so that by "*streak plate*" and incubated for 48 hours at room temperature. Single colonies are inoculated on oblique and labeled media[7]

### 2.3 Screening for antibiotic-producing endophytic bacteria

An antibiotic production medium was prepared with a composition of 3% corn soaking water, 3% sucrose, 0.5% CaCO3, 0.1% FeSO4, 0.2% MgSO4, 0.01% ZnSO4 and 50 ml sterile distilled water at 50 ml at Erlemeyer 100 ml sterilized. Inoculated 2.5 ml of the inoculum in the production medium, then shaking at room temperature (2C) for 24 hours, then the production medium was centrifuged at a speed of 5000 rpm for 15 minutes. The supernatant obtained was tested for potential antibiotics using the Kirby-Baurer method, also known as the paper disc method. Incubation at room temperature for 48 hours. Bacterial isolates that have antibiotic-producing metabolites will form inhibitory zones around the disc paper. The diameter of the resistance formed is measured with the help of the calipers.[8]

### 2.4 Characterization of Isolates of Antibiotic-Producing Bacteria

Characterization of bacterial isolates observed included macroscopic character in the form of colony color, colony shape, colony edge, colony elevation. This observation was carried out after 24 hours of incubation time. Furthermore, microscopic observations in the form of Gram type bacteria and bacterial cell forms were carried out. Finally, biochemical tests of endophytic bacterial isolates have the potential to produce antibiotics.[8]

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and screening of antibiotic-producing endophytic bacteria

Isolation of endophytic bacteria from liverworts from Nagari Sigiran, Maninjau, Kab. Agam, West Sumatra with spread plate technique obtained an average of 151 bacterial colonies. The morphological observations of the bacterial colonies were obtained by 12 isolates of endophytic liverworts. Tests for 12 antibiotic isolates of endoffytic bacteria from liverworts obtained six isolates that formed inhibitory zones, namely isolates coded BEMP-03, BEMP-04, BEMP-07, BEMP-08, BEMP-09, BEMP-11 and six other isolates not forming inhibitory

zones, namely isolates with the code BEMP-01, BEMP-02, BEMP-05, BEMP-06, BEMP-10 and BEMP-12.

The formation of clear areas around the colonies of endophytic bekteri indicates the possibility of antibacterial compounds capable of killing or at least inhibiting the growth of pathogenic bacteria.[9] The formation of inhibitory zones in the colonies of bacterial isolates tested indicated that the tested bacterial isolates carried out antimicrobial activity.[10] The greater the established inhibition zone, the greater the activity of existing antibiotics.[11]

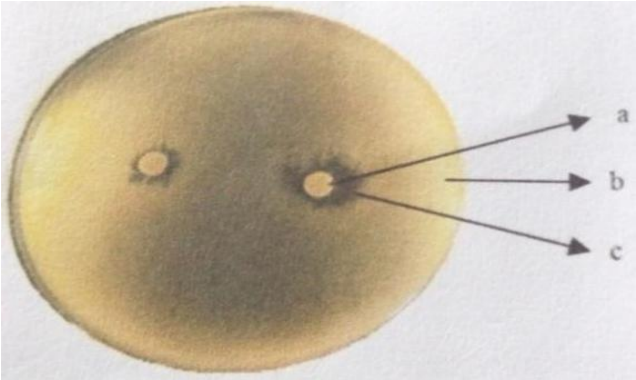


**Figure 1: Testing of antibiotic activity (a) disc paper, (b) test bacteria, (c) inhibition zone).**

**Table 1: Average diameter of the inhibition zone after incubation for 48 hours.**

| No. | Code of isolates | Zone of inhibition of *S.aureus* bacteria (mm) |
|---|---|---|
| 1 | BEMP-01 | 0 |
| 2 | BEMP-02 | 0 |
| 3 | BEMP-03 | 6,5 |
| 4 | BEMP-04 | 6,5 |
| 5 | BEMP-05 | 0 |
| 6 | BEMP-06 | 0 |
| 7 | BEMP-07 | 7,3 |
| 8 | BEMP-08 | 8,1 |
| 9 | BEMP-09 | 9,4 |
| 10 | BEMP-10 | 0 |
| 11 | BEMP-11 | 9 |
| 12 | BEMP-12 | 0 |

Six isolates of endophytic bacteria were isolated, BEMP-09 isolates were the isolates that had the largest inhibitory zone diameter of 9.4 mm, while BEMP-03 and BEMP-04 were isolates that had a small transient inhibition zone of 6.5 mm. Each bacterial colony has the ability to produce different inhibitory zones. The difference in diameter formed is estimated because of

the concentration and different types of antibiotics. If the diameter of the inhibition zone is the same or close to the same, then the possibility of the antibiotics that it produces is the same.[12]

Microorganisms can produce antibiotics if these microorganisms contain genes that encode antibiotic or biosynthetic antibiotics and those genes are expressed.[13] Microbes that do not show antibiotic activity in this study, may contain very little or possibly other antibiotic compounds.[14] Endophytic bacteria are generally able to produce one or several compounds that have antibiotic activity.[15] The difference in diameter formed is estimated because of the concentration and different types of antibiotics. If the diameter of the inhibition zone is the same or close to the same, then the possibility of the antibiotics that it produces is the same.[12]

### 3.2 Characteristics of endophytic bacteria

Characterization of antibiotic-producing endophytic bacteria was carried out through macroscopic, microscopic characterization and biocomia test. Macroscopic observation of endophytic bacteria from fresh extracts of liverworts, found isolates that have varied colors, ranging from white, yellowish white and yellow. There are round and irregular forms of colonies. The margins or edges of bacterial colonies isolated are entire, undulate, irregular and lobate. Whereas the elevation of the colonies can be seen convex, flat and raised (Table 2). Differences in bacterial morphological characteristics indicate different types of bacteria.

Microscopic observations of endophytic bacteria isolates found four Gram-negative bacterial isolates and the other two had Gram-positive types. Whereas the endophytic bacterial colonies obtained five shaped bacilli and one shaped cocci.

**Table 2: Macroscopic and Microscopic Observations of Endophytic Bacteria from Liver Moss.**

| Code of isolates | Macroscopic observation | | | Microscopic observation | | |
|---|---|---|---|---|---|---|
| | Colour | Shape | Margin | Elevation | Gram type | Cell shape |
| BEMP-03 | White | Irregular | Undulate | Flat | - | Basil |
| BEMP-04 | White | Irregular | Entire | Flat | - | Basil |
| BEMP-07 | Yellowish white | Irregular | Irregular | Flat | + | Basil |
| BEMP-08 | Yellowish white | Round | Entire | Raised | + | Basil |
| BEMP-09 | yellow | Round | Entire | Convex | - | Basil |
| BEMP-11 | yellow | Round | Lobate | Convex | - | Cocous |

The color difference between Gram positive and Gram negative bacteria is caused by the ability of the cell wall to base dyes (crystal violet) after 96% alcohol washing. Gram positive bacteria appear purple because the cell wall binds to crystal violet more strongly, while Gram negative bacteria contain more lipids so that the pores are easily enlarged and crystal violet is easily dissolved during washing with alcohol. When dissolved with the opposite dye (safranin) the bacterial cell will capture the red color.[16] The subsequent characterization process was carried out by observing the results of biochemical tests of endophytic bacterial isolates. This test is done to determine which group of bacteria is more specific.

**Table 3: Observation of biochemical tests of endophytic bacterial isolates.**

| No. | Treatment | Code of isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | BEMP-03 | BEMP-04 | BEMP-07 | BEMP-08 | BEMP-09 | BEMP-11 |
| 1 | Aerob/ anaerob | A | A | A | A | A | A |
| 2 | TSIA | K/K | K/K | M/K | K/K | K/M | K/M |
| 3 | Gas | + | + | - | - | - | - |
| 4 | $H_2S$ | - | - | - | - | - | - |
| 5 | Catalase | + | + | + | + | + | + |
| 6 | Oxidase | - | - | - | - | - | - |
| 7 | Motilitas | + | + | - | + | - | + |
| 8 | Indol | - | - | - | - | - | - |
| 9 | Urea | + | + | - | - | + | + |
| 10 | Citrate | + | + | - | - | + | + |
| 11 | Laktosa | + | + | - | - | + | + |
| 12 | Glukosa | + | + | - | - | + | + |
| 13 | Sukrosa | + | + | - | - | + | + |
| 14 | Mannitol | + | + | - | - | + | + |
| 15 | MR | - | - | + | - | + | - |
| 16 | VP | + | | - | - | - | - |
| 17 | OF | + | + | - | - | + | + |
| 18 | Nitrat | - | - | + | - | - | - |
| 19 | Gelatin | - | - | + | + | - | - |
| 20 | KCN | + | + | | | + | + |
| 21 | Arginin | - | - | | | - | - |
| 22 | Lysine | - | - | | | - | - |
| 23 | Ornitin | - | - | | | - | - |
| 24 | Phenylalanin | - | - | | | - | - |
| 25 | Arabinose | + | + | | | + | + |
| 26 | Xylose | + | + | | | + | + |
| 27 | Dulcitol | - | + | | | + | - |

Description: A = aerob, K / K = Yellow / yellow, M / K = Red / Yellow, K / M = Yellow / Red. (+) = the test in question is positive, (-) = the test in question is negative, empty () = test is not done.

The series of characterizations that have been carried out either macroscopically, microscopically or biochemical tests show that six isolates potentially produce antibiotics are different types of bacterial isolates. This can be concluded from the differences in the results of the characterization that has been done. The diversity of endophytic bacteria colonies that grow on the medium is influenced by the diversity of endophytic bacteria that live in their host plants. Factors that influence the diversity of endophytic bacteria include plant species[17], habitat and environmental factors, as well as conditions for plant growth, especially soil conditions. Some plants have specific endophytic bacteria that inhabit these plants.[18]

## CONCLUSION

Six endophytic bacterial isolates were successfully isolated from antibiotic-producing liverworts which have the potential to inhibit the growth of *S. aureus* bacteria in the medium category.

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