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Isolation and Antibacterial Test of Indigenous Bacterial Isolate from The BeeHive of Trigona Itama Against Bacteria Staphylococcus aureus and Escherichia Coli

Assyifa Syafrita[1)] Anthoni Agustien[1)*] Yetti Marlida[2)] Miftahul Zovia[1)] Feskaharny Alamsjah[1)]

[1)]Departement of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, 25163, West Sumatera, Indonesia

[1)]Departement of Animal Science, Faculty of Animal Sciences, Andalas University, Padang, 25163, West Sumatera, Indonesia

[*]Email: anthoniagustien@sci.unand.ac.id

Abstract — This Trigona itama which is a type of stingless bee (stingless bee) is also commonly found in Indonesia. This study aims to isolate and characterize indigenous bacteria found in Trigona itama beehive and to perform antibacterial testing on pathogenic bacteria S. aureus and E. coli and continued with morphological characterization, gram staining and catalase test. Two indigenous bacterial isolates were found from Trigona itama beehives and partial characterization was carried out based on morphological observations, Gram staining and catalase testing. Of the two isolates, isolate ISL-1 was able to inhibit S. aureus with an inhibition zone of 2.84 mm. Meanwhile, ISL-2 isolate was able to inhibit E. coli with an inhibition zone of 2.22 mm. The results of macroscopic observations show that the indigenous Trigona itama bee hive bacterial colony with the code ISL-1 has a circular shape, entire margin, raised elevation, cream color, and has a small size, while the ISL-2 bacterial isolate has a circular shape, curled margin, raised elevation, white, and small in size. Microscopic observation of cell morphology of ISL-1 and ISL-2 isolates showed that the bacteria were Gram negative and had the shape of bacilli and coccus cells. The catalase test of ISL-1 and ISL-2 showed positive results.

Keywords — Antibacterial; Beehive; Trigona itama; Staphylococcus aureus; Eschericia coli

I. Introduction

In Indonesia, several species of bees from the genus Apis have been cultivated to produce honey. In addition, *Trigona itama* which is one type of stingless bee is also widely found in the Indonesian area. Products from *Trigona* spp. bees such as honey and propolis have been widely utilized. Propolis is part of the *Trigona* spp. honeybee hive which consists of compounds that are complex resinous materials produced by honeybees from plant exudates (Selvan & Prabhu, 2010). Previous research on propolis or hive cover which is part of the *Trigona* spp beehive proved that propolis has potential as a source of antimicrobials such as antibacterial activity capable of inhibiting the growth of *Campylobacter* spp. Various studies have been conducted to obtain natural antimicrobials from *Trigona* spp farm products other than propolis, one of which is beehives. The beehive is a place of protection for bee colonies from attacks by bacteria, fungi, viruses and predators, as well as a place for the production of honey, bee pollen, and a place for the growth and development of bee eggs. The condition of the beehive greatly affects the quality of the honey and the honey produced. Honey is generally free from pathogenic microbes. In addition to honey, *Trigona* spp. beehives are also found propolis which serves to strengthen the hive and is used for the *Trigona* spp. defense system (Fatoni *et al*., 2008).).

The types of antimicrobials produced by honey beehives include the antibiotics tetracycline, streptomycin, sulfonamide, tylosin, erythromycin, lincomycin, and chloramphenicol (Reybroeck *et al*., 2012). This shows that honeycomb has the potential to

be used as an antibiotic to suppress to kill various pathogenic bacteria so that the quality of honey is maintained. *Trigona* spp. beehives antimicrobial agent compounds can be used as a source of natural antimicrobials of natural origin. The uniqueness of *Trigona* spp. beehives is that it is always in a sterile condition. This is due to the antimicrobial compounds contained in it. The compounds contained in *Trigona* spp. *beehives* that have the potential as natural antimicrobials can be used as natural alternative medicine in addition to the types of commercial antibiotic drugs on the market. This study aims to determine the antimicrobial power released by *Trigona* spp beehives against microbial growth and examine the chemical components contained in *Trigona itama* beehive as a source of antimicrobial agents.

Honey and propolis produced by *Trigona* spp. bees can be used as a source of probiotics because they contain lactic acid bacteria (LAB). Lactic acid bacteria can help maintain the health of human digestive organs through the synthesis of exopolysaccharides that can inhibit the growth of pathogenic bacteria. Several studies related to the ability of honey and propolis from *Trigona* spp. as a source of probiotics and as an antimicrobial agent have been conducted. Kimoto-Nira and Amano (2008) reported that honey collected from *Trigona* bees in various regions was shown to contain LAB such as *Enterococcus faecalis, E. faecium, Lactococcus lactis ssp. Lactis, and L. Lactis ssp. Cremoris*. and can inhibit the growth of pathogenic bacteria. Research by Chanchao (2009) proved that LAB in *Trigona laeviceps* honey can inhibit the growth of *S. aureus, Escherichia coli, Candida albicans, Auriobasidium pullulans,* and *Aspergillus niger*. The addition of propolis taken from *Trigona* spp. beehives can increase the growth of probiotic bacteria such as *Lactobaccilus casei* subsp. *rhamnosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*. Research by Saleng *et al.* (2016), showed that bacterial isolates taken from *Trigona incisa* propolis were able to inhibit the growth of *Klebsiella pneumonia* and *Staphylococcus aureus* bacteria. This study aims to isolate and characterize indigenous bacteria found in *Trigona itama* beehive and conduct antibacterial testing on pathogenic bacteria. *S. aureus* and *E. coli*. This was done with the aim of finding and exploring new antibacterial potential.

II. METHOD OF RESEARCH

2.1. Materials

The materials used in this research were *Escherichia coli* and *Staphylococcus aureus* cultures, *Trigona itama* beehive, 0.9% NaCl solution, the antibiotic Chloramphenicol (Control +), distilled water (Control -).

2.2. Bacterial Isolation

Isolates were obtained from *Trigona itama*. from Pariaman city. A total of 1 gram of bee hive was taken and then added to 9 mL of physiological salt solution (0.9% NaCl). Isolates were incubated for 24 hours at 37°C. Next, a dilution series of 10^{-1} to 10^{-6} was made, then inoculated on Nutrient Agar (NA) media and incubated at 37°C for 24 hours.

2.3. Morphology Test, Gram Staining, and Catalase Test

Bacterial colony morphology tests include shape, color, edges, elevation, size, appearance and texture. Gram staining was performed using standard methods. The catalase test is carried out using 3% hydrogen peroxide (H_2O_2). Positive results are indicated by the formation of air bubbles.

2.4. Antibacterial Test

Bacteria were cultured on NB media for 24 hours. Secondary metabolite extracts are obtained by taking supernatant from bacterial liquid isolate cultures.

2.5. Bacterial Seeding

One end of the Escherichia *coli* and *Staphylococcus aureus* bacterial colonies from each subculture medium was taken, suspended in 9 ml of 0.9% NaCl salt water (Soemarno, 2000). Next it is homogenized (turbidity).

2.6. Bacterial Growth Inhibition Test (Diffusion Method)

Bacterial Inoculation on Nutrient Agar (NA) Media. The bacterial suspension, which has been standardized for turbidity, is dipped in a sterile cotton swab and then pressed by the cotton to the side of the tube so that the liquid does not drip from the

tube. Then swab it onto the surface of the NA media until it covers the entire surface. The NA medium was left for 5 minutes so that the bacterial suspension seeped into the agar medium. Then dip the disc paper into each concentration that has been prepared. Each paper disc was attached to the NA media using tweezers. Then the disc paper is pressed using tweezers so that there is good contact between the disc paper and the NA media (Lay, 1994).

2.7. Measurement of the Bacterial Inhibition Zone

After incubation for 24 hours, the diameter of the barriers formed in the NA medium was measured using a caliper on a black cloth. The diameter of the inhibition zone measured is the clear area around the disc paper (no bacterial growth), measured from one end to the other through the middle of the disc paper (Soemarno, 2000). Then the average inhibition zone is calculated.

III. RESULT AND DISCUSSION

3.1. Morphological Test, Gram Staining, and Catalase Test Results

Morphological Test, Gram Staining, and Catalase Test Results of bacterial isolation in *Trigona itama indigenous* beehives. 2 isolates were obtained. Based on morphological tests on the two isolates, different results were obtained as shown in Table 1, each isolate coded ISL-1 and ISL2. The results of the Gram staining test and catalase test were obtained as shown in Table 1.

Figure 1. Isolation and purification of indigenous beehive bacteria *Trigona itama*

Bb

Table 1. Partial characterization of the indigenous beehive bacterial isolate *Trigona itama*

Isolate Character	Bacterial Isolate	
	ISL-1	ISL-2
Colony Morphology		
Shapeq	Circular	Circular
Margin	Entire	Curled
Elevation	Raised	Raised
Pigment	Cream	White
Size	Small	Small
Cell Morphology		
Gram Stain	Negative	Negative
Cell Morphology	Bacillus	Coccus

Parija (2012) said that macroscopic characterization of bacterial colony morphology can be done by observing the shape, elevation, edges and color of the colony. The results of macroscopic observations show that the indigenous Trigona itama honeycomb bacterial colony with the code ISL-1 has a circular shape, entire margin, raised elevation, cream color, and has a small size, while the ISL-2 bacterial isolate has a circular shape, curled margin, raised elevation, white, and small in size. Microscopic observation of cell morphology of ISL-1 and ISL-2 isolates showed that the bacteria were Gram negative and had the shape of bacilli and coccus cells.

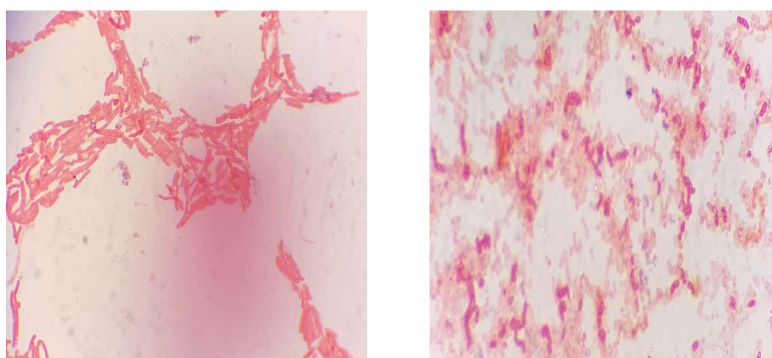


Figure 2. Gram staining of bacterial isolates (a) ISL-1 and (b) ISL-2

Gram staining is used to differentiate gram-positive bacteria from gram-negative bacteria. Lay (1994) stated that the difference in gram staining results was caused by differences in the cell wall structure of the two groups of bacteria which caused differences in the permeability reaction of the dye. Gram-positive bacteria are bacteria that resist decolorization and retain the primary iodine dye complex which appears purple. These bacteria have relatively thick amorphous walls and more protoplasmic acid which is believed to be able to retain violet dye and iodine complexes in the cells (Parija, 2012). Gram-negative bacteria are decolorized by organic solvents and absorb the counterstain so that they appear red. Decolorizing agents, such as acetone or ethanol, used during staining disrupt the membrane envelope of gram-negative bacteria, as a result of which the dye and iodine complex are leached from the walls of gram-negative bacteria.

The catalase test showed positive results as indicated by the appearance of air bubbles when the colony was dripped with H_2O_2 solution (Figure 3). According to Cappuccino and Sherman (2013), the presence of bubbles in the catalase test indicates that the bacteria belong to the aerobic group. Aerobic bacteria use oxygen to produce energy. Hydrogen Peroxide (H_20_2) is a toxic substance for aerobic bacteria. Aerobic bacteria can deal with this poison by using the enzyme superoxide dismutase, which converts superoxide poison into water and oxygen. Therefore, in the test, aerobic bacteria will produce bubbles.

Figure 3. Catalase test of bacterial isolates (a) ISL-1 and (b) ISL-2

3.2. Antibacterial Test

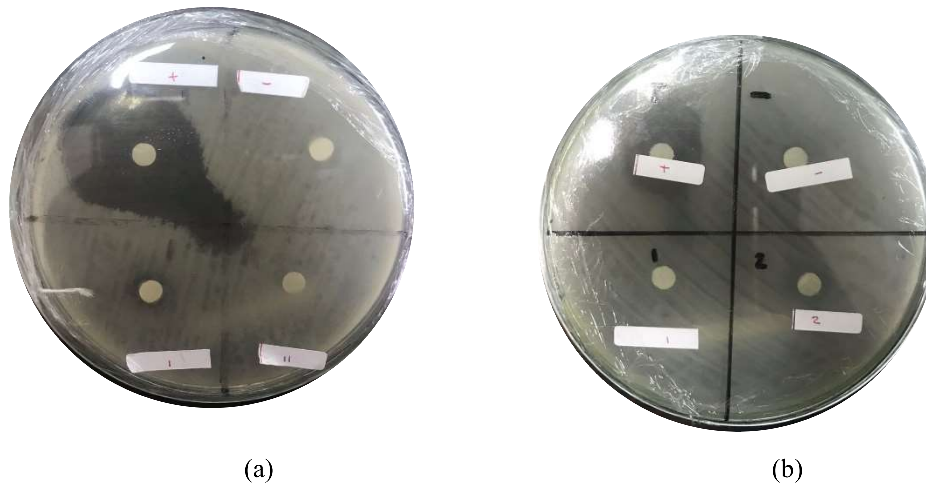
Isolate Code	Inhibition Zone Diameter (mm)	
	S. aureus	*E. coli*
ISL-1	2,84	-
ISL-2	-	2,22
Postive control	65,46	32
Negative control	-	-

Antibacterial tests were carried out to determine the activity of indigenous *Trigona itama* honeycomb bacterial isolates in inhibiting the growth of *S. aureus* and *E. coli bacteria*. This test is carried out by growing isolates of indigenous bacteria and target bacteria in the same petri dish. The activity of indigenous bacteria in inhibiting the growth of *S. aureus* and *E. coli* is shown by the presence of a clear zone that appears away from the paper discs treated with indigenous bacteria. This test uses two controls, namely a positive control using the antibiotic chloramphenicol 0.01 g/10 ml and a negative control using sterile distilled water. Chloramphenicol was used as a positive control because this antibiotic has a broad spectrum that is able to inhibit gram-positive and negative bacteria. The target bacteria used in this antibacterial test are *S. aureus* and *E. coli*. *E. coli* bacteria are gram-negative bacteria, so they were chosen in this study to represent the group of gram-negative bacteria, while the group of gram-positive bacteria was represented by *S. aureus*. This is also supported by Fitri *et al*. (2010), who said that in testing the group of gram-positive bacteria could be represented by *S. aureus* while gram-negative bacteria were represented by *E. coli.*

In this study, the inhibition zone produced by the ISL-1 bacterial isolate was 2.84 mm against *S. aureus*, while the ISL-2 bacterial isolate produced an inhibition zone of 2.22 against *E. coli* and was included in the weak category. The clear zone is measured using a caliper to measure the strength of its resistance. The strength of bacterial inhibition is categorized based on Davis and Stout, namely, very strong with a clear zone result of >20 mm, strong with a clear zone result of 10–20 mm, moderate with a clear zone result of 5–10 mm, and a clear zone <5 mm which is in the weak category. Antibacterial compounds can inhibit the growth and even kill bacteria because these compounds can inhibit cell synthesis and metabolism. The inhibitory mechanisms of antibacterial compounds generally include disrupting cell wall synthesis, disrupting membrane function, protein synthesis, nucleic acid synthesis, and antimetabolites (Hugo and Russel, 2004).

This shows that gram-negative bacteria have better defense capabilities when exposed to growth inhibitors compared to gram-positive bacteria. Fitri *et al*. (2010) said that gram-negative bacteria have better protection against antimicrobial compounds compared to gram-positive bacteria because they have different cell wall components. The cell walls of gram-positive bacteria are relatively thinner because they are only composed of peptidoglycan compared to the cell walls of gram-negative bacteria which are not only composed of peptidoglycan but also composed of lipoproteins, outer membranes and lipopolysaccharides. The structure of the cell wall means that substances produced by bacterial isolates cannot enter gram-negative bacterial cells.

Gram positive bacteria tend to be more sensitive to antibacterial compounds. This is because the cell wall structure of Gram-positive bacteria is simpler than the cell wall structure of Gram-negative bacteria. The differences in cell wall structure in the two pathogenic bacteria can make it easier for antibacterial compounds to enter gram-positive bacterial cells optimally (Fithriyah, 2015). The cell wall structure of Gram-positive bacteria consists of 2-3 layers of cytoplasmic membrane which is composed of teichoic acid and teichourenic acid in the form of water-soluble polymers making it easier for antibacterial compounds to penetrate from the outside (Vitasari, 2012). Meanwhile, the cell wall structure of Gram-negative bacteria has a more complex cell wall structure because it contains less peptidoglycan, but outside this layer there are three polymers, namely lipoprotein, outer membrane and lipopolysaccharide (Amalia and Nursanty, 2018).



(a) (b)

Figure 4. Antibacterial test of ISL-1 and ISL-2 bacterial isolates against

(a) *S. aureus* and (b) *E. coli*

IV. CONCLUSION

Units Isolates of indigenous bacteria were found from *Trigona itama* bee hives and partial characterization was carried out based on morphological observations, Gram staining and catalase tests. Of the two isolates, ISL-1 isolate was able to inhibit *S. aureus* with an inhibition zone of 2.84 mm. Meanwhile, the ISL-2 isolate was able to inhibit *E.coli* with an inhibition zone of 2.22 mm.

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