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Characterization and Hemolytic Activity of Isolated Bacteria from Bats's Saliva Samples on the Menoreh Karst Area

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Abstract: *Bats are carriers of the disease (Reservoir). Bats have differences of diet that has a mouth ecosystems with different levels of toxicity. This study aims to investigate the characteristics of the bacteria and the hemolytic activity of the bat saliva samples as an level indicator of bat saliva's toxicity in the karst region Menoreh. The method to collect the datas is exploration method by conducting isolation bat saliva samples and then isolating the bacteria by using Nutrient Agar (NA) medium, MacConkey agar, and Eosine Methylene Blue Agar and the bacteria is characterized by macroscopic and microscopic characters using reference books Bergey's Manual of Determinative Bacteriology 9th edition (1994). Then the bacteria proceed hemolytic activity test using a Blood Agar. The data analysis was done descriptively.*

The amount of bacteria can isolated from 3 genus of bats Cynopterus sp (sub order Megachiroptera), Miniopterus sp and Hipposideros sp (sub order Microchiroptera) that is 93 isolated bacteria.Five genuses identification results show that the alleged genuses is Streptobacillus, Streptococcus, Staphylococcus, Shigella, and Bacillus. There are different characteristics in the bacteria of the suborder Microchiroptera and Megachiroptra in terms of the number of β-hemolytic, total of 36 isolates in Microchiroptera saliva sample and 1 isolate in Megachiroptera saliva sample,can lyse the blood from the blood agar. So the bat of microchiroptera group has high toxicity values on saliva in the menorah karst region.

Keywords: *bats, bacteria, pathogens, saliva*

1. Introduction

Bats are included in the Order Chiroptera that divided into two sub-orders are Megachiroptera and Microchiroptera. Megachiroptera is a fruit and nectar bats and plays a role as pollinators in the forest while Microchiroptera have feed more varied but most are insect eaters.[1]

Bats are one of the mammals that important to know and learn related to his role as a reservoir of various tropical diseases. Bats in life can carry a wide variety of microorganisms are harmful to humans, especially in health. Microorganisms are most numerous in bat saliva, as saliva is a combination of various kinds of liquids containing various materials both off the bat itself or from its food. A person who is bitten by a bat can lead to certain diseases if known pathogenic microorganisms contained in the saliva of bats. Some diseases caused by bat is Mers, Nipah, and Rabies. [2]

The study was conducted in Menoreh Karst Area, Purworejo, Central Java because this region has a sub-level diversity order Megachiroptera Bats and Microchiroptera high enough. In addition, people in the karst region Menoreh Bats often make arrests and use it as a food needs of the community. Though it is known that bats carry a variety of sources of harmful diseases.

According to the Center for Research and Development Vector and Reservoir disease that began in 2015 to 2017 will be conducted research on various animal reservoirs one of which was a bat. This research involves various research institutions, especially the Ministry of Health, LIPI, health office, Atmospheric Science Technology Center (LAPAN) and other related research agencies. [3] Data from this study can be used as initial data the researchers Reservoir Bat Disease Research Center launched and Vector and Disease Reservoir

development 2015-2017. And furthermore based on the results of this study can be used for public information in order to avoid direct contact with bats and minimize the incidence of diseases by the zoonis animals.

2. Materials and Methods

Sampling was carried out at the cave in Karst Menoreh, Purworejo, Central Java and the tests conducted in the laboratory of Microbiology, Faculty of Mathematics and Science, Yogyakarta State University that took place in May until June 2015

2.1 Sampling and identification of bats

Bats captured using misnet in pairs in front of the mouth of the cave, bats are caught later identified to determine its genuses. The identification is done by morphometric methods or measuring body parts of bats and compare with identification book. [1]

2.2 Isolation of bacteria from the bat saliva

Bat Saliva obtained by physiological saline flow into the oral cavity bat and contain it in a glass beaker. The sample is then diluted with a dilution factor of 10^{-4} to 10^{-6}. A total of 1 ml of each dilution then isolated on a media Nutrient Agar (NA), MacConkey Agar and Eosine Methylene Blue Agar (EMB) with a pour plate method. Isolates then incubated at 37 ° C for 24 to 48 hours until the bacterial colonies grow. Mixed culture of bacteria obtained were then purified on NA media slant streak plate technique in order to obtain pure cultures.

2.3 Hemolytic Activity Test

Each of the bacterial isolates pure culture that has been obtained then grown on Blood Agar media (BA) and incubated for 24 to 48 hours at 37 ° C and observed the changes in the media. Alpha hemolytic indicated by the growth of bacterial colonies and media into a greenish brown color, beta hemolytic indicated by the formation of the clear zone around the colony, and gamma hemolytic causes no discoloration in the media.

2.4 Characterization of bacteria

Macroscopic characterization of bacterial colonies include color, elevation, the edge of the colony and configuration. Microscopic characters include color and shape outcomes gram staining bacterial cells. While the characterization of physiology based on the biochemical test bacteria include oxygen demand using the medium of Nutrient Broth (NB), reduction citrate with media Simon's Citrate (SC), motility with media Sulfide Indol Motility (SIM), catalase test, and test the breakdown of carbohydrates include media sucrose, lactose, fructose and glucose.

2.5 Identification of bacteria

Identification was performed using the book Bergey's Manual of Determinative Bacteriology 9th edition (1994) based on the characterization has been done.

3. Result and Discussion

Bats were found consist of three genera Miniopteros, Hiposideros, and Cynopterus. Miniopteros and Hipposiderostermasuk in order Mikrochiroptera sub order, while the genus Cynopterus is Megachiroptera sub order. Bacteria in bat saliva is grown on three media which is EMB, NA, and MC. The total amount of bacteria that can be isolated from bat saliva samples are 93 bacterial isolates, the number of colonies that vary in each media.

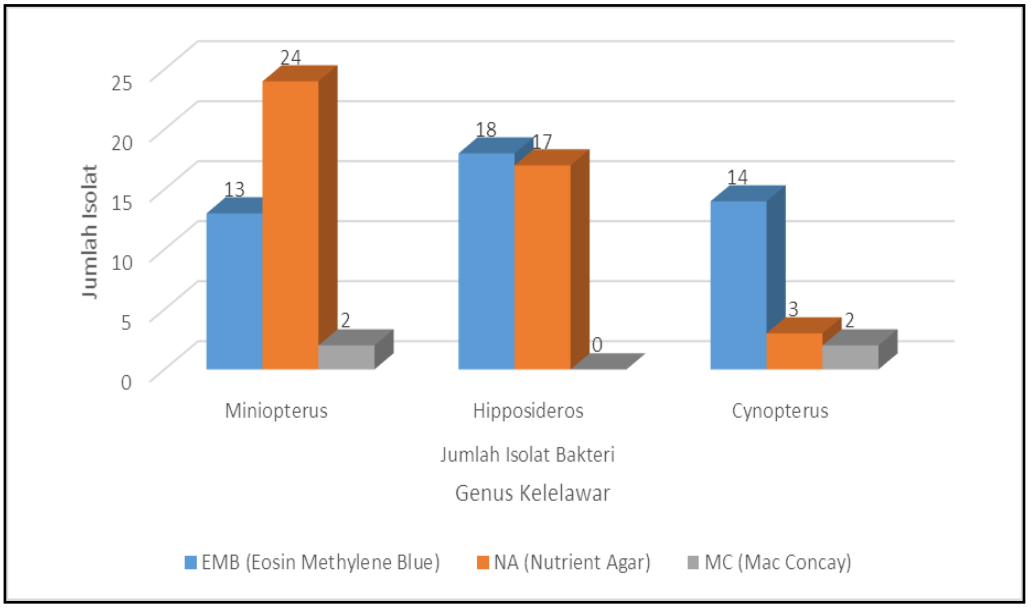

Fig. 1: Bacterial growth on EMB, NA, and MacConkey media

EMB medium is a differential selective media to grow a gram-negative bacteria of the Enterobacteriaceae group. EMB medium has a feature that contains lactose which serves selecting microbes, meaning that only bacteria that can ferment lactose to live. On this medium to grow 13 isolates of bacteria of the genus miniopterus, 18 isolates of the genus Hipposideros and 14 isolates of the genus Cynopterus.

Mac Conckay medium has the smallest number of bacterial isolates were grown that is numbered each two isolates of the genus Miniopterus and Cynopterus genus, the genus Hipposideros while there are no bacteria can grow. This is because only gram-negative bacteria that can grow in the media. Presence of bile salts and crystal on this media will block the growth of gram-positive microorganisms. [5]

When viewed from the three media, the most widely grown bacteria is on NA medium, with the number of genus miniopterus have 24 isolates, 17 isolates from the genus hipposideros and 3 isolates from the genus Cynopterus. This is because NA is a common medium for the isolation of bacteria that most bacteria can grow on this medium.

Based on data from phenotypic characters there are some tests done of growth in NB medium to see the need of the oxygen, where the results of the isolation of the sample is known that bats sub order Microchiroptera mostly facultative anaerobic, aerobic and anaerobic other. While the sub order Megachiroptera has a result that all of the bacteria are facultative anaerobes. Aerobic bacteria showed that the bacteria need Oxygen (O_2) for life, while the anaerobic bacteria indicates that the bacteria cannot live with presence of O_2, and the facultative anaerobic bacteria showed that bacteria require CO2 to survive, but to live well in the presence of O2.

Motility known through the growth of bacteria that spreads on SIM media, besides motility can also be found in the bacterium's ability to reduce sulfite. Positive bacteria media change to black, while the bacteria were not able to reduce sulfite does not change the color of the media. In this case the bacteria in saliva bat isolates were largely able to reduce sulfite yet capable of motility.

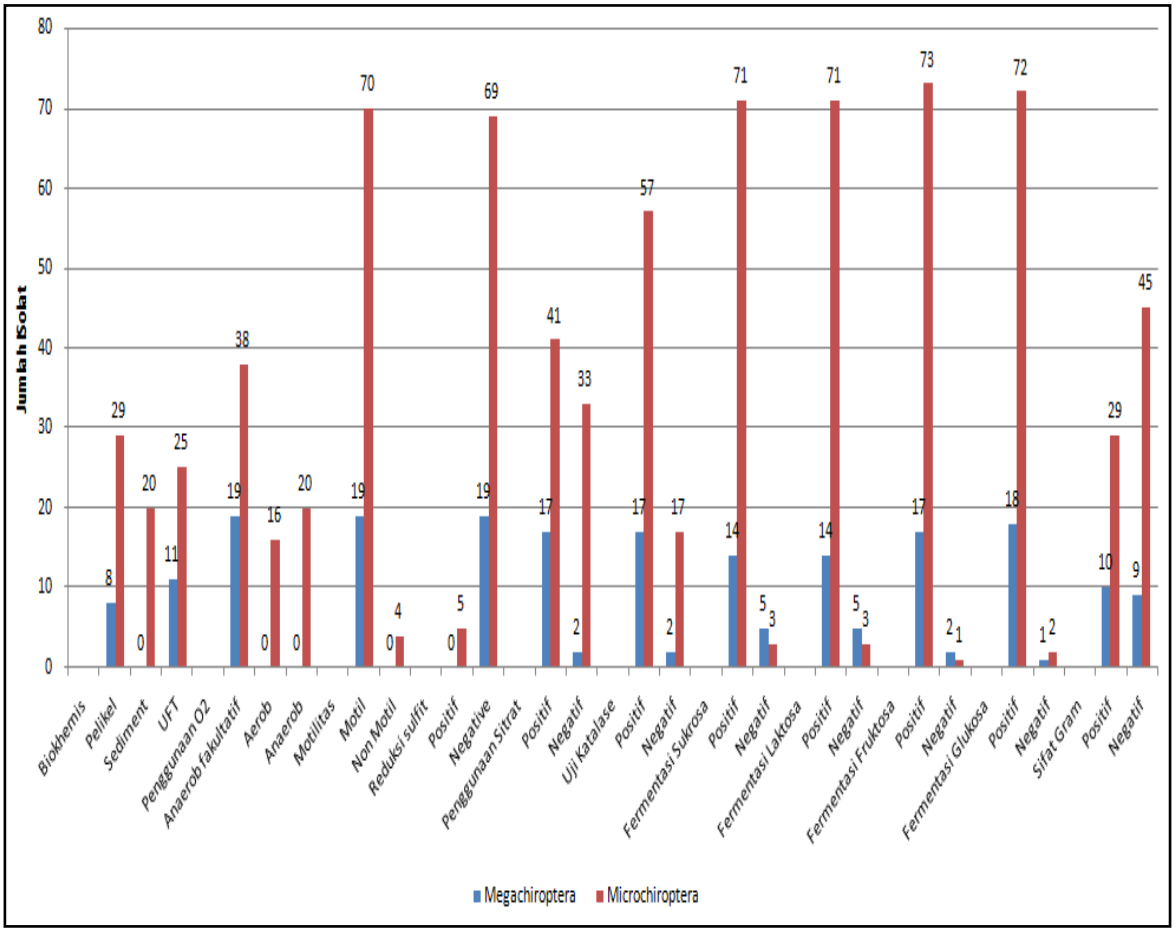

Fig. 2: Biochemical Test Result

Simon citrate test was conducted to determine the ability of bacteria to hydrolyze citrate as a carbon source in the Simon Citrate (SC) medium by the enzyme citrate permease. Citric positive test is indicated by color change from green to blue media. Based on the results obtained, the isolates which is able to hydrolyze citric consisted of 41 isolates from sub order mikrochiroptera and 17 isolates from sub order Megachiroptera. The next test is the test of catalase. Catalase is an enzyme which catalyze the decomposition of hydrogen peroxide (H_2O_2) into water and O_2. Based on data obtained is known that there are 57 positive bacterial isolates Microchiroptera against catalase test is characterized by the formation of bubbles. While the bacteria from the Megachiroptera sub order that produce catalase consist of 17 bacterial isolates.

Fermentation of carbohydrates was done to determine the ability of bacteria to ferment the carbohydrates into alcohol, carbon dioxide, organic acids and energy. Glucose hydrolysis test aims to determine the ability of a bacterium in hydrolyzing glucose. From the test result show that almost all isolates can hydrolyse glucose is 72 isolates from sub order mikrochiroptera and 17 isolates from sub order Megachiroptera. Other carbohydrate fermentation test is shown through the fermentation of sucrose as indicated by the ability of the bacteria that break down sucrose into monosaccharides glucose and fructose. From the fermentation of lactose is all most of the isolates showed positive results at 71 isolates from sub order Microchiroptera and 14 isolates from sub order Megachiroptera.

Based on macroscopic and phenotypic characteristics through biochemical tests were then compared to the reference book[5] can be presumed that the genus of the bacteria is Streptobasilus, Bacillus, Streptococcus, Staphylococcus and Shigella.

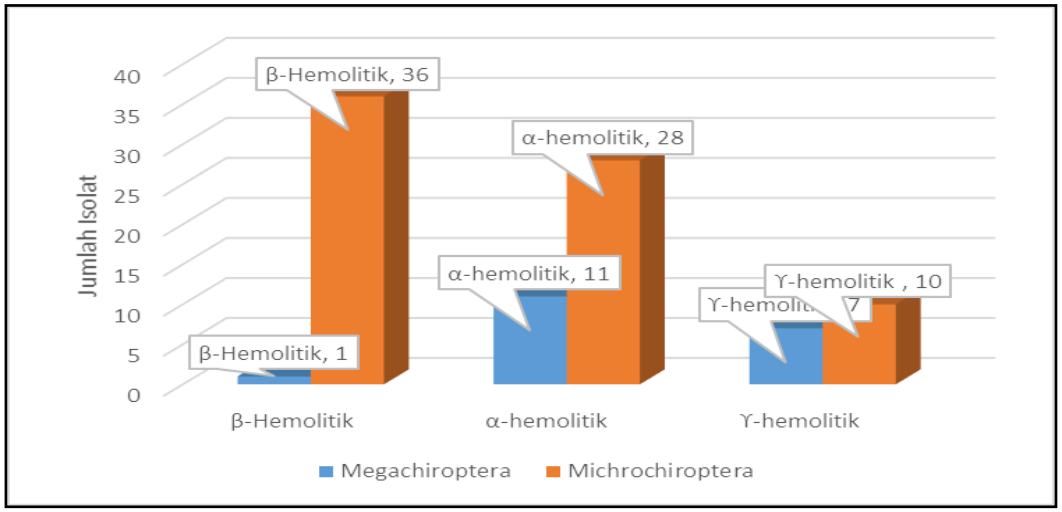

Fig. 3: Hemolytic Activity of The Bacteria

Hemolytic activity testing of bacterial isolates from bat saliva samples Megachiroptera and Microchiroptera conducted using blood media that have varying results. Megachiroptera observed 19 isolates bacteria, which are then inoculated into blood medium in order to be able to see the level of toxicity. After incubated for 48 hours was observed from 19 isolates, one isolate showed results in the form of beta hemolytic characterized by the formation of clear zone on the media, 11 isolates showed the alpha hemolytic characterized by the formation of brownish green color around the colonies of bacteria, and 7 isolates did not process hemolytic called gamma hemolytic. From these data indicate that the bacterial isolates from bat saliva samples Megachiroptera dominated by alpha-hemolytic bacteria with total 11 isolates. As for the bacterial isolates from bat saliva samples Mikrochiroptera there were 36 isolates were observed to be beta hemolytic.

The presence of bacteria with of alpha and beta hemolysis showed that the bacterium has the form of alpha hemolysin toxin and beta-hemolysin. Results of previous studies revealed that the hemolysin toxin can form a zone of hemolysis around colonies of bacteria. Consisting of alpha hemolysin hemolysin, beta hemolysin, and delta hemolysin. Alpha hemolysin toxin that is responsible for the formation of hemolysin zone around bacterial colonies on media Blood Agar. These toxins can cause necrosis of the skin of animals and humans. Beta hemolysin toxin which is mainly produced Staphylococus isolated from animals, which causes lysis of red blood cells to the blood hemoglobin sheep and cattle. While delta hemolysin is a toxin that can lyse human red blood cells and rabbits, but the effect is less lysis of the sheep red blood cells. [6]

These results indicate that bat saliva contains various bacteria which are toxic and harmful to human health. Bat is one of the potential reservoir hosts and vectors of zoonotic pathogens. Bacterial enteric pathogens are also found in bats can be derived from the diet (diet) and foraging habitat for bats, even the transmission cycle involving bats, humans and other animals such as pets and livestock. For some pathogenic bacteria are common in human and animal diseases (eg, *Pasteurella*, *Salmonella*, *Shigella*, *Escherichia* and *Yersinia* spp.), Potential pathogens has been confirmed to bat. [7]

The capacity of the bacteria causing the disease depends on pathogenity. With these criteria the bacteria are grouped into three, namely the causative agent of bacterial, opportunistic pathogen and non pathogen. The causative agent of the disease is a bacterial pathogen that causes a disease (*Salmonella* sp.). Opportunistic pathogen is a bacterial pathogen that is enabled as when a weakened host defense mechanisms (eg *E. coli*) infect the urinary tract when the host defense system is compromised (weakened). Non pathogen is a bacterial pathogen that never become. However, non-pathogenic bacteria may be pathogenic because of the ability of adaptation to the lethal effects of modern therapies such as chemotherapy, immunotherapy, and mechanisms of resistance. [8]

This initial study is expected to trigger further research weighing the lack of studies with similar objects. So as to explore new discoveries and other potential in the field of microbiology, both positive and negative, and the information obtained can be applied to the society for a better life. The results also should be used properly so that this study can provide a positive impact for the community in general.

4. Conclusion

The conclusions from this research were (1) The discovery of three genera of bats that *Cynopterus* sp (sub order Megachiroptera), *Miniopterus* sp and *Hipposideros* sp (sub order Microchiroptera). Isolates of bacteria that can be isolated as much as 93 isolates from all bat saliva samples with medium NB, EMB and MacConkey. (2) There is a difference of character between the 93 isolates in terms of morphology and physiology character bacteria. 5 genus identification results show that the alleged genuses is *Streptobacillus*, *Streptococcus*, *Staphylococcus*, *Shigella*, and *Bacillus.* (3) The amount of bacterial isolates from sub-order Mikrochiroptera bat have more hemolytic activity than the sub-order Megachiroptera. So the bat saliva from Microchiroptera group has a higher potential toxicity and harmful to other living creatures, especially humans.

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