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MICROBIAL IDENTIFICATION OF WATER PEATLAND IN BANJAR DISTRICT, GAMBUT, SOUTH BORNEO

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Abstract: In Indonesia, the largest distribution of peatlands is found in Sumatra, Kalimantan and Papua, which cover an area of around 14.9 million hectares. Rural communities in Borneo who live in peat swamp areas and tidal areas generally have difficulties in obtaining clean water, especially during the dry season, so that people in Borneo used surface water in the form of peat water. Peat water does not require for daily use such as brushing teeth because it has a low pH, brownish red color and high organic content. The quality of water suitable for use in tooth brushing have to require physically, chemically and microbiologically. Microbiological requirements should be free from pathogenic microbial that cause disease, especially diseases in the oral cavity.: This study used Explanative descriptive method. Peat water samples were taken in the Gambut sub-district, Banjar Regency, South Kalimantan using a sterile bottle that was put into Cary-Blair as Transport medium then put into a coolbox and taken to the microbiology laboratory for identified. Rough colony have identified. Color of colony were white (nutrientagar), white-transparent (Man Rogosa medium) and yellow (Mannitol Salt Agar). Gram staining obtained bacteria in the form of bacilli and cocus. Biochemical tests were found Bacillus sp., Lactobacillus sp and Staphylococcus aureus. The bacteria identified in the peat waters of the Peat District of Banjar Regency are Bacillus sp., Lactobacillus sp and Staphylococcus aureus.

Key Words: Peat Water, Peat Water Microbial, Wetland

1. INTRODUCTION

Based on WHO, as much as 76.5% had suffor from caries and the age group under 12 years is most vulnerable to oral dental disease. This means that 24 million children in Indonesia have

experienced tooth and mouth pain 1. Almost 60% of the proportion of dental and mouth problems in South Kalimantan are dental and oral disease. It has a prevalence which is quite high at 36.1%

(RISKESDAS, 2018; Riskesdas 2013).

Caries is a hard tissue disease in the oral cavity. It is caused by four factors, environment, host, bacteria and time. The environmental factor had played a role to caries in people who lived in South Kalimantan. They used surface water sources in the form of peat water for daily activity such as brush their teeth. It happen along hard to obtaining clean water, especially during the dry season **(Kidd, 2020; Sumawijaya, 2016).**

Peat water is surface water which is greatly influenced by the condition of the peatland below. The world's peatlands cover a total area of 420 million hectares and including tropical peat reaches 30-45 million hectares. Indonesia is a country that globally has the most extensive tropical peatlands in ASEAN. According to the development of the national survey and land mapping, the estimated peatland area in Indonesia has fluctuated from 13.2 to 26.5 million hectares. In Indonesia, the largest distribution of peatlands is found in three large islands, namely Sumatra, Kalimantan and Papua, which cover an area of around 14.9 million hectares **(Agus, 2016).** Peat water does not worthy of daily consumption needs such as brushing teeth because it has a low acidity scale, around 3-5 , has brownish red color and high organic substance content **(Febrianty et al, 2018)**. This acidity scale in peat water supports the growth of bacteria that are acidogenic and asiduric which can affect the process of tooth decay in the oral cavity **(Purwandari et al, 2015)**. Besides, the peat water contain high organic substances that allow for amylolytic bacteria being able to live in a state an environment high in organic matter. Water quality should has the physical requirements, chemical requirements and microbiological requirements for brush teeth **(Agus, 2016)** Microbiological requirements of water should be free from pathogenic bacteria that cause disease, especially diseases that exist in the oral cavity.

Microbes had found in peat soil consisted of groups (1) early decomposers such as fungi and bacteria, both aerobic and anaerobic, (2) development or thickening of peat such as fungi or bacteria that are anaerobic in nature, and (3) further dekomposition after the land is drained, such as fungi, aerobic bacteria **(Yanti, 2001; Abbasi et al., 2020)**. According Purwandari, 2015 there are aerobic bacteria colonies in peatland water soil of 656 bacterial colonies. Previous research stated that in peatland water there were bacteria *Clostridium sp* and *Bacillus sp* **(Adhani et al, 2017; Abbas et al., 2020; Ahmad et al., 2018; Purwandari et al, 2015).**

2. METHODS AND MATERIALS

The research begins with the preparation of a research permit and ethical clearance issued by the Faculty of Medicine, University of Lambung Mangkurat No. 295/KEPK-FK UNLAM/EC/IX/2020.

This research method is Explanative descriptive with cross sectional approach. Water sample was taken based on include criteria: 1. Taken in tidal, swampy and lowland areas, 2. The water color is brownish red, 3. The water with pH value 3 - 5. Peat water samples were taken in the peat sub-district, Banjar Regency, South Kalimantan using a sterile bottle that was put into Cary-Blair as Transport medium then put into a coolbox and taken to the laboratory for identified.

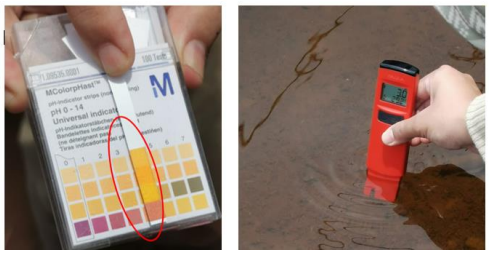

Fig. 1 1pH Measurement of Water Sample

The tools used in this study are: erlenmeyer, dropper pipette, test tube, petri dish, inoculating loop, microscope, object glass, cover glass, tube clamp, test tube rack, coloring rack, refrigerator, bunsen, sterile container, cotton, ice gel and ice box. The materials used in this study were peat water, Handscoon, masks, 70% alcohol, 95% alcohol, sterile distilled water, NaCl solution, Gram stain, BHIB medium, glicerol, sheep blood medium, Blood agar medium, SDA medium, EMB medium, SS medium, MSA medium, TCBS medium, TYCBS medium, Manrogose medium, and biochemical test materials (catalase test, coagulase test, confectionery test, urease test, citrate test)

3. MEASUREMENT AND IDENTIFICATION OF PEAT WATER MICROBES

A sample of 1 ml of peat water was taken aseptically and put into a sterile test tube containing 9 ml of physiological NaCl. The suspension was made homogeneous using a vortex. Furthermore, serial dilutions are carried out to a level of 10-7. Then, three series of dilutions were taken for each of the last series (10-5, 10-6, 10-7). Furthermore, the last 3 dilution series were inoculated, into Nutrient Agar (NA) media and the culture was incubated at 25-300C, for 24 hours. Then the bacterial isolates were observed and their numbers were counted. The bacteria suspected to be present in the oral cavity were then subjected to Gram stain and biochemical tests.

4. GRAM STAINING

Gram staining was carried out on bacterial cultures aged 24 hours grown on Nutrient Agar (NA) medium. Bacterial isolate was taken as much as 1 inoculating loop and flattened on a sterile object glass. Then 1-2 drops of distilled water were added, then it were fixed over a bunsen fire until it dries. Then the crystal violet dye was droped and was waited until 1 minute for the dye to seep into the bacteria. Then it was rinsed with flowing aquadest and was dripped with iodine solution for 30 seconds and it was rinsed again with acetone alcohol. Last, it was droped with safranin dye for 1 minute and was rinsed with aquadest. After the preparation was dry, it was observed using a microscope.

5. BIOCHEMICAL TESTS

Identification of bacterial colonies was carried out by using the biochemical test aims to determine the ability of bacteria to produce H2S gas, presence the ability of bacteria to use other energy sources (urea, nitrate, and citrate tests), sugar fermentation (glucose, sucrose,

mannitol, lactose), production mixed acid (Voges-Proskauer), and ability of bacteria to produce coagulase enzymes

Catalase Test. A total of 1 loop of bacterial isolate was taken using a inoculating loop then immersed in a test tube containing H_2O_2 reagent. A result will be positive if gas bubbles form and negative if no gas bubbles form.

6. RESULTS AND DISCUSSION

Based on the identification results of 11 bacteria, 3 types of bacteria are identified. Sampling was carried out in peat waters in Gambut District, Banjar Regency with a pH measurement characteristic of 3, the water is reddish in color and found in tidal areas, this is according to the inclusion criteria desired by the researcher. The research results can be seen in the following table.

Table 1. Macroscopic Identification Results of Microbe on peat waters in Gambut District, Banjar Regency

Sample	Colony in Medium				Suspected Backteria
	Shape	Consistensi	Colour	Medium	
01	Round	Rough	White	Nutrient Agar	*Bacillus sp*
01	Round	Rough	White-Transparent	Man Rogosa and Sharpe	Lactobacillus sp
01	Round	Rough	Yellow	Mannitol Salt Agar	*Staphylococcus aureus*

Peat waters have a very acidic pH of 3. This is due to the result of the decomposition of organic matter in anaerobic conditions by bacteria that produce phenolic and carboxylate metabolites so the acidity of peatland water had increased **(Adhani et al, 2017; Arshad et al., 2020).**

Table 1 show that *Bacillus sp, Lactobacillus sp.* and *Staphylococcus aures* are identified in this study. *Bacillus sp* was a bacterium that has been identified in peat waters in Gambut District, Banjar Regency. The macroscopic characteristics of this bacteria on this study were round white, medium colony size, have a convex surface and flat edges. Bacillus spp can produce organic acids so that they are more acid-resistant, besides that Bacillus also produces proteases. This is appropriate with study of **Lu et al, 2017** who found colony formed of *Basillus sp* in Nutrient Agar medium was characterized by gray-white, round, opaque, flat and dry with medium-size

Lactobacillus is a bacterium found in this study. Macroscopically, these colonies are round, white, yellowish white, to light brown transparent with smooth, wavy edges, and some are in the form of rhizoids, the elevation of bacterial colonies found is convex, and some are flat. This is supported by Islam's research that identified *Lactobacillus* spp. from Selective Regional Yoghurts. **Islam et al, 2016** state that *Lactobacillus spp* on Man, Rogosa and Sharpe (MRS) medium at pH 6.5 were produced small, irregular and round shape with shiny whitish cream or brownish colored.

Beside Bacillus sp and Lactobacillus sp, cocci bacteria had found in this study. *Staphylococcus aureus* have identified, macroscopically, it resemble a ball with diameter of ± 1 µm. The colony is densely rounded, smooth, prominent and shiny. *Staphylococcus*

aureus formed dark gray to yellow gold colonies. *Staphylococcus aureus* growth at a temperature of 6.5-46º C and at pH of 4.2-9.3, including an acidic pH, so that these bacteria can be found in acidic peat water. *Staphylococcus* colonies grew in seedlings for 24 hours with a diameter of up to 4 mm. *Staphylococcus aureus* forms a lipochromic pigment that causes the colony to appear golden yellow and orange yellow. The yellow pigment distinguishes it from *Staphylococcus epidermidis* which produces a white pigment. Golden-yellow pigments appear on growth for 18-24 hours at 37 ° C, but form the best pigments at room temperature (20-25 ° C). Pigments are not produced in anaerobic cultures or in broth. Staphylococcus aureus is easy to grow in many bacterial hatcheries. Various degrees of hemolysis are produced by *Staphylococcus aureus* and occasionally by other bacterial species **(Jewetz et al, 2005)**.

In this study, *Staphylococcus aureus* on Mannitol Salt Agar (MSA) media seen as a yellow colony growth surrounded by a golden yellow zone due to the ability to ferment mannitol to be acid. The product produced by this bacteria is an organic acid which changes the pH indicator in Mannitol Salt Agar, changing the red color of the Mannitol Salt Agar medium to bright yellow **(Rahmi et al, 2015; Ashraf et al., 2020)**. This is in accordance with Dewi's research in 2013, which carried out the isolation and identification of *Staphylococcus aureus* bacteria, which was marked by the occurrence of mannitol fermentation on Mannitol-Salt agar (**Dewi, 2013)**.

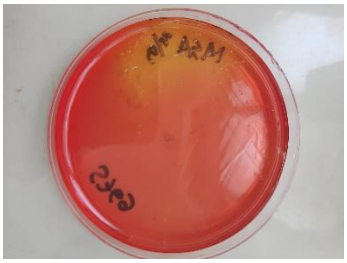

Fig. 2 Staphylococcus aureus Colony on Manitol Salt Agar Medium

Table 2. Gram Staining Identification Results of Microbe on peat waters in Gambut District, Banjar Regency

Sample	Gram Staining			Suspected Bacteria
	Shape	Formation	characteristic	
01	Basil	Clusters	Gram (+)	*Bacillus sp*
01	Basil	Chains	Gram (+)	*Lactobacillus sp*
01	Cocus	Staphylococci	Gram (+)	*Staphylococcus aureus*

Table 2 show gram Staining Identification Results of Microbe on peat waters in Gambut District, Banjar Regency. The Gram stain is a very important preliminary step in the initial characterization and classification of bacteria. It is also a key procedure in the identifi cation of bacteria based on staining characteristics, enabling the bacteria to be examined using a light microscope. The bacteria present in an unstained smear is invisible when viewed using a light microscope. Once stained, the morphology and arrangement of the bacteria may be observed

as well. Gram stain easily divides bacteria into two groups, Gram-positive and Gram-negative, on the basis of their cell wall and cell membrane permeability. The mechanism further implies that solvent decolorization causes signifi cant damage to the cell surfaces of Gram-negative bacteria and only limited damage to Gram-positive bacteria. This suggests Gram-negative bacteria are more "leaky," causing these thin-walled lipid-rich cells to lose their crystal violet stain and appear red from

the counterstain. Gram-positive cells, thick walled and lipid-poor, appear blue from retaining the original stain (crystal violet) **(Thairu et al, 2016)**

Bacillus sp microscopically, basil with cluster formation and gram positive. *Bacillus* grow in the mesophilic temperature range. Starvation and stress are common in this environment. *Lactobacillus sp* in this study microscopically is in the form of bacilli (rods) and are gram-positive, forming short chains. *Staphyloccus sp* forms a grape-like formation and can also be arranged four-by-four (tetrad), forming chains (3-4 cells), in pairs or one by one.

Staphylococcus aureus is a Gram-positive bacterium and cocci-shaped, non-motile, non-spore, facultative anaerobic, catalase positive and oxidase negative. This is in accordance with Irfan's 2014 research regarding the identification of peatlands, which found the presence of Staphylococcus sp bacteria with gram-positive coccus morphology and was found on the surface of peatlands **(Irfan, 2014).**

Table 3. Biochemical Tests Result on Bacteria of Microbe on peat waters in Gambut District, Banjar Regency

Sample	Biochemical Tests				Suspected Bacteria
	Catalase Test	Glucose test	Voges-Proskauer test	Urea Test/ Coagulase test	
01	Negative	Mannitol (+) Glucose (-) Lactose (-) Maltose (-) Sukrose (-)	Negative	Urea = negative	*Bacillus sp*
01	Positive	Glucose (+) Lactose (+) Maltose (+) Sukrose (+)	Negative	Urea = negative	*Lactobacillus sp*
01	Positive	Lactose (+) Maltose (+)	Positive	Koagulase = positive	*Staphylococcus aureus*

Table 3 show biochemical tests result. This test includes Catalase test, Glucose Test, Voges-Proskauer test, Urea Test, and Coagulase test. The catalase test used for differentiate microorganisms with catalase enzyme to degrade toxic hydrogen peroxide. The catalase reaction shows positive results

if air bubble is formed which indicates the formation of O_2 gas and the result is negative if it does not show O_2 gas bubbles. Air bubbles formed on the positive results of the catalase test are oxygen from the reaction catalase enzymes and H_2O_2 **(Dewi, 2013).**

The results show that no air bubbles are formed which indicates catalase test isolate of *Bacillus sp* was negative. This is because *Bacillus sp* are not produces the enzyme catalase which can convert hydrogen peroxide (H_2O_2) into water (H_20) and oxygen (O_2). This is ability of *Bacillus spp* only need a little oxygen to be able to live. Opposite, *Lactobacillus sp* and *Staphylococcus sp* catalase test result are positive because these bacteria are produces the enzyme catalase which can convert hydrogen peroxide (H_2O_2) into water (H_20) and oxygen (O_2) **(Sya'baniar et al, 2017)**.

Glucose test have done as biochemical test to identify microbe. Glucose test is only medium glucose and maltose which form acid which is indicated by changes the color of the media from blue to yellow, meaning that these bacteria form acids from fermentation glucose. On the lactose and mannitol media, negative results were seen which was indicated by no color change on the media. This characterized is identified *Staphylococcus aureus.* The ability of bacteria to form acids from various carbon sources, can be seen by testing other sugars. Positive results are characterized by occurrence change in media color **(Sya'baniar et al, 2017)**. *Lactobacillus sp* is able to ferment various types of sugar into acids, such as glucose, maltose, lactose, arabinose, sorbitol and mannitol. These bacteria are acidogenic and asiduric so that these bacteria can live in an acidic environment. This is in accordance with the statement of **Hiranya et al in 2013** that *Lactobacillus sp* can survive in an acidic environment and produce acid up to 4 pH. 21. In dentistry, *Lactobacillus sp* is considered as one of the bacteria that plays a role in the occurrence of dental caries. *Lactobacillus sp* in the oral cavity produces lactic acid from fermented sugar. Lactobacillus plays a role in the development and continuation of the caries process so that the oral pH decreases and is susceptible to dental caries25, 26.

Another biochemcical test was Voges Proskauer test. This is used to determine acetyl methyl carbinol formation from the sugar fermentation of lactic acid bacteria. According Voges Proskauer test, *Bacillus sp* and *Lactobacillus sp* are negative. The examination was negative with no change in color. *Bacillus sp* and *Lactobacillus sp* ferment non-acetyl methyl carbinol (acetoin). These bacteria isolates by using energy another used the nitrate test. This indicates that the *Bacillus sp* and *Lactobacillus sp* are not using nitrates as another energy source **(Sya'baniar et al, 2017)**.

The coagulase test aims to determine the ability of bacteria to produce coagulase enzymes. Coagulase production is the most commonly used criterion for the temporary identification of. A positive coagulase reaction is very important to differentiate S. aureus from other *Staphylocccous aureus* species. Coagulase is an extracellular protein produced by *Staphylocccous aureus* which can coagulate plasma with the help of factors present in serum. Therefore the role of coagulase produced *Staphylocccous aureus* can be used as a identification tool of bacteria **(Dewi, 2013)**.

This study have identifies three bacteria species, *Bacillus sp, Lactobacillus sp* and *Staphylococcus aureus.* This is in accordance with the research of **Pratiwi et al, in 2018** which identified peat soils, and found many species of Bacillus bacteria, namely Bacillus kribbensis, Bacillus panaciterrae, Bacillus salarius, B. soli, Bacillus vallismortis 22, 23. Also supported by Rustanti's research, 2009, which looked at bacterial species in peat water, found Bacillus sp and Clostridium sp which were also stated in the study that the deeper the peat water was, the more acidic the pH of the peat water was 24.

7. CONCLUSION

From the results of the study it can be concluded that water samples showed positive presence of bacteria *Bacillus sp, Lactobacillus sp, Staphylococcus aureus* based on identification macroscopic (Nutrient agar medium, Man Rogosa medium, and Mannitol Salt agar medium), microscopic (Gram Stain) , biochemical tests (Catalase test, Glucose test, Voges Proskauer test, Coagulase test).

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