Isolation and Characterization of Bacteria from River Origin in Mandailing Natal North Sumatera

by Mhd Yusuf Nasution

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Mhd. Yusuf Nasution, Ahmad Shafwan S. Pulungan, Eko Prasetya, Salwa Rezeqi
Biology Department, Universitas Negeri Medan

Yusufnasution1963@gmail.com

Abstract. The indicator of water pollution according to microbiological standards is the presence of microbiological pollutants. To see the presence of bacteria in the waters, it is necessary to study the isolation and characterization of these bacteria. The existence of the river in the Mandailing Natal area has experienced a change in character due to gold mining activities along the river flow. The purpose of this study was to determine the types of bacteria in the river in the Mandailing Natal area of North Sumatra. This research was conducted at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Medan. The research period was from June to October 2020. The population in this study was the water of the Batang Gadis Mandailing Natal river, North Sumatra. The sample in this study is the Batang Gadis river water around the Mandailing Natal gold mine, North Sumatra. The research was carried out by taking samples and isolating bacteria which included planting the samples on media enriching the planting of the samples on various growing media, purifying bacterial colonies and observing the morphology of bacterial colonies. The results showed that there were 6 bacterial isolates living in river waters in Mandailing Natal, North Sumatra.

1. Introduction

The mining sector, especially gold mining, needs special attention by the public because it has a high risk of environmental damage. One of the problems that is still a problem is the large number of illegal mining activities (without permits).The main problem in gold mining without a permit is the use of hazardous materials and substances in its processing. The waste produced generally still contains mercury [1]. Mercury which is often used in gold ore mining is elemental mercury, which is mercury in its original form [2].

Metallic mineral content (especially gold) has long been stored in the Mandailing Natal Regency area. The reserves of gold mining in Mandailing Natal (Madina) Regency, North Sumatra Province are quite large and reach 1.5 million ounces (Au) with a content of 2.2 grams tonnes of Au.

Gold mining in Madina has been around since 2008. The rise of illegal mining using hazardous materials has resulted in various diseases caused by the dumping of hazardous heavy metal waste into the environment. One of the alternatives for handling mercury-polluted environment is bioremediation technique, which is one of the uses of microorganisms to improve the polluted environment.

The role of microbes in wastewater treatment has provided many encouraging results [3]. Organic compounds found in wastewater are a source of nutrition for microbes. Microbes will break down these compounds into simpler and more stable forms so that the levels of pollutants contained in the waste decrease. Enzymatic reactions by bacteria are the key to implementing a gradual transformation

process in wastewater management from substrates, which are generally organic materials with complex molecular arrangements, into simple elements.

Several types of bacteria are known to have the ability to reduce or absorb heavy metals. Bacteria that are resistant to mercury stress are called mercury resistant bacteria (BRM). One of the mechanisms contained in BRM to reduce mercury is by converting Hg2 + to Hg0 with the enzyme mercury reductase encoded by the merA gene [4].

The potential possessed by bacteria in reducing heavy metals (mercury) is an environmentally friendly technique in improving the environment [5-6]. As an initial effort to determine the types of bacteria that are capable of reducing mercury, it is necessary to conduct a preliminary study to determine the types of bacteria that are resistant to mercury content in the gold mining area in Mandailing Natal, North Sumatra.

2. Materials and Method

This research was conducted in the microbiology laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Medan. The time of this research is planned to start from June to October 2020. The population in this study are all bacteria obtained from soil and water from the Mandailing Natal gold mine, North Sumatra. The sample in this study was a total sample, that is, all bacteria that are resistant to mercury obtained from the Mandailing Natal gold mine, North Sumatra.

The main materials used in this study were soil and waste water samples obtained from the Mandailing Natal gold mine, North Sumatra, tissue, umbrella paper, rubber, cotton, plastic wrap, aluminum foil, masks, gloves, markers, standard solutions. mercury, distilled water, spirits, 70% alcohol, thick adhesive plastic, HCl, NaOH, HNO3. In addition, the media needed are BHI (Brain Heart Infusion) Media, LDS (Lactose Double Streng), EMBA (Eosin Methylen Blue Agar), Cled Agar, MSA (Manitol Salt Agar), MCA (Mac Conkey Agar), Blood Agar , NaCl, HgCl2 produced by Merck Germany, as well as reagents for Gram positive and negative tests.

The tools used in the research included Laminar Air Flow with a local brand, autoclave with the Hirayama HVE50 brand, an oven with the Memmert brand, Petri dishes with the pyrex brand, test tubes with the pyrex brand, incubators with the Memmert Churt brand, measuring pipettes with the pyrex brand, cupboards ice with the LG brand, ose needle, digital balance with the sartorius brand, Beaker glass with the pyrex brand, spatula, Erlenmeyer with the pyrex brand, measuring cup with the pyrex brand, measuring flask with the pyrex brand, micropipette with the Accumax pro brand.

2.1. Sampling

Samples were taken from the Mandailing Natal gold mine, North Sumatra. 250 ml of samples from each shelter were then put into dark reagent bottles and carried using transport media. Next, 50 ml of samples from each bottle were taken and mixed into bottles, each containing 450 ml of Nutrient Broth. The mixture is then propagated at room temperature for 5 days. Propagation aims to increase bacterial culture.

2.2. Isolation of Bacteria

- Planting samples on enriched media

The samples were planted on enriched media
BHI and LDS.

- Planting samples on various growing media

Samples were grown on EMBA, Blood Agar, Cled Agar, MCA and MSA media.

- Purification of Bacterial Colonies

Bacterial colonies that grew on the growing media of Blood Agar, EMBA, Cled Agar and MCA were observed to determine the differences in the characteristics of the growing bacterial colonies. Furthermore, each colony with different characteristics was replanted in the appropriate planting medium to obtain a pure colony, then incubated at 37oC for 24 hours.

2.3. Observation of Bacterial Colony Morphology

Observation of pure bacterial colony morphology was carried out by looking at the colony shape, colony edge shape, and colony color.

2.4. Morphological Characterization of Bacterial Colonies

All pure isolates were observed maxroscopically by observing the shape, edges and colony color. Then microscopic observations were made through gram staining. Gram staining is done by making sample preparations from various growing media on a slide to be fixed over a fire, adding a solution of crystal violet for 1 minute, flowing distilled water over the slide, adding a lugol solution for 1 minute, adding a bleaching solution (acetone alcohol solution) for 10-20 seconds, flowing distilled water on the slide, adding safranin solution for 15 seconds, flowing distilled water on the slide then drying it with filter paper and examining the object with a microscope [7].

3. Results and Discussion

BHI and LDS media are used as media to support the growth of microorganisms so that they can grow well before planting into specific media. The results of bacterial growth on LDS and BHI enriched media were planted 1 ose on general media, namely Blood Agar and Cled Agar, gram-negative selective media specifically EMBA and MCA, and gram-positive selective media specifically MSA. The results of bacterial colony growth after being incubated for 24 hours at 37 0C showed the colony growth with various characteristics.

Planting samples on various media such as MCA, Blood Agar, Cled Agar, EMBA and MSA shows the results in Table 1 below.

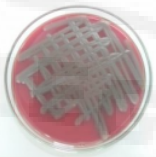
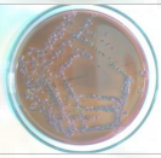
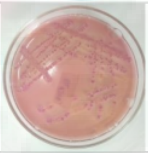
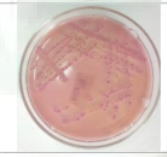
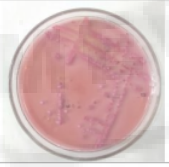
Table 1. Observations of bacterial colonies on various growing media

No	Medium Selective	Medium	Bacterial Morphology		
			Form	Elevation	Colour
1	BHI	MCA	Circular	Flat	red
2	BHI	Blood Agar	Circular	umbonate	silver
3	BHI	Cled Agar	Circular	umbonate	White and yellow
4	BHI	EMBA	Circular	Flat	red
5	BHI	MSA	-	-	-
6	LDS	MCA	Circular	Flat	red
7	LDS	Blood Agar	Circular	Flat	silver
8	LDS	EMBA	Circular	Flat	green
9	LDS	CledAgar	Circular	umbonate	yellow
10	LDS	MSA	-	-	-

Macroscopic morphological characterization was carried out by looking directly at the morphology of bacterial isolates that grew on the medium [8]. Visually, the characteristics that can be observed from the colony include the form of the colony, the shape of the edge and the color of the colony [9-10].

Based on the results of observations on the macroscopic characteristics of bacterial colonies on MCA, Blood Agar, EMBA and Cled Agar media, which had previously been planted on BHI and LDS enriched media, there were 6 bacterial colonies with different characteristics. Of the six colonies, all the characteristics of the colonies that were successfully planted on various media can be seen in table 3 as follows.

Table 2. Characteristics of bacterial colonies on various media.

No.	Colony Characteristic	Picture
1.	Circular, flat and silver	
2.	Circular, flat and green	
3.	Circular, flat and purple	
4.	Circular, flat and red	
5.	Circular, flat and red	
6.	Circular, flat and red	

Bacterial growth on common media, namely Blood Agar, shows colonies with round shapes, flat edges, and dry gray color. Blood Media In order to be able to distinguish hemolytic and non-hemolytic bacteria based on their ability to lyse red blood cells. The growing bacterial colonies show the formation of alpha hemolysis, namely the lysis of some red blood cells and hemoglobin on Blood Agar media. Then on Cled Agar media, bacterial colonies were obtained with round shapes, wave edges and yellowish white. The presence of lactose and pH indicators, namely bromtimol blue in Cled Agar media, is fermented by bacteria to form yellow colonies.

Furthermore, in gram-negative selective media, namely EMBA, colonies were obtained with round shapes, flat edges and dark red in color. EMBA media contains lactose, peptone, eosin and methylene blue. Colony growth is dark red due to the ability of microbes to ferment laksota. Eosin and methylene blue serve to sharpen colony color differences. Whereas on MCA media, bacterial colonies were found to be round, flat edges and pink in color. MCA media contains peptone, NaCl, bile salts, neutral red pH indicator and crystal violet. The red colony growth indicates the ability of bacteria to ferment lactose.

After that, on gram-positive selective media, MSA, there was no bacterial colony growth. MSA media contains NaCl, mannitol and phenol red pH indicator. The growing bacterial colonies are able to ferment mannitol to acid so that it changes the color of the phenol red indicator from red to yellow. While in this sample, there was no bacterial growth.

Then the results of microscopic bacterial characterization observations through gram staining indicated that the six isolates were Gram negative bacteria. In Gram staining, the positive charge of crystal violet passes through the cell wall and cell membrane and then binds to the components in the cell that are negatively charged. The addition of iodine to enhance the crystal violet coloration by forming a crystal violet-lugol complex. Then in Gram negative bacteria, alcohol can increase the porosity of the cell wall by dissolving lipids in the outer membrane layer and dissolving the crystal violet-lugol complex from the cell resulting in discoloration. When safranin is dropped, Gram negative bacteria cells absorb a counter dye which causes Gram negative bacteria to show a red color [11-12].

4. Conclusion

Based on the research results, 6 pure bacterial isolates were found in Batang Gadis River Water. Based on microscopic characterization, all pure isolates were gram-negative bacteria.

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