Identification And Testing Resistance Against Bacteria Isolated Mercury From Gold Mining In Gogorea Buru

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Abstract: Gogorea is one of the villages in the area Buru island that serve as the site of gold. Most of the people of the island rush to mine the use of mercury in the amalgamation process. Mercury is harmful chemicals and cause adverse effects to living beings and the environment, to overcome it can be utilized microbes that are resistant to mercury. The purpose of this study is to obtain a bacteria that is resistance. This research is a qualitative descriptive study. To get the bacteria resistant to mercury initial phase was isolated, then tested the sensitivity of bacteria to mercury, and the next stage of bacterial identification. Based on the results of the samples obtained four isolates Gogorea village, which when tested sensitivity to 10ppm, 20ppm, 30ppm and there is no clear zone so that the four isolates are considered resistant to mercury. Of the four isolates were identified by a type of bacteria the sample G1.1 Chryseobacterium sp.

Keywords: Identification of Bacteria, Resistance, Mercury, Gogorea, Buru

1. INTRODUCTION

Buru Island is one of the areas of livelihood people are panning for gold. In 2011 from various areas in Indonesia came to Bald Mountain Buru district located in the province of Maluku. This increases the economy in the local area, but over time the gold processing process is done by the community to use chemicals that are harmful to living beings and the environment. Until finally the bald mountain is closed today because of the impact of the use of chemicals such as mercury. Bald Mountain closure causes people to seek a new location for mining, the area Gogorea that until now people still active in gold mining. Gold processing generally performed by people using the two methods is the method of cyanide and amalgamation. But in this gold mining area, the method is done in gold processing is a method of amalgamation. Amalgamation method is the process of extracting the ore by mixing gold ore with mercury [1]. Mercury is a heavy metal that is toxic and pervasive as pollutants generated by industrial pollution that settles in the ecosystem and has a density of more than 5 mg/L[2]. Heavy metal pollution results of mining waste using chemicals may degrade the quality of natural resources and land productivity and negatively impact our health. In the body of heavy metals such as Se, Cu, Zn, Ni, Mn needed by the cell but in a certain amount and if the amount exceeds the requirement, it can be toxic to aquatic organisms and humans. While non-essential heavy metals such as Hg, Cd, As, Pb in certain concentrations can be toxic to humans.

One environmental friendly method that can be used to address the dangers of mercury pollution by making use of the bacteria that can degrade toxic waste in the environment [3]. But to get the bacteria necessary isolation, selection and continued with the characterization and identification process so that the goal of this research is to get the bacteria that are resistant from Village Gogorea through a process of isolation, selection and identification.

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2. LITERATURE REVIEW

Discussing the small-scale mining processes or traditional miners use mercury kinds of chemicals in the amalgamation process to separate gold grain this case also occurred in Bald Mountain. Mercury is a heavy metal kind of chemicals derived from Cinabar stone processing with oxygen. Disposal of mercury into the environment would provide a serious threat to health and ecosystems and have consequences reaction complexes containing a combination of physical, chemical and biological. Gogorea village is a village located in the district Waeapo, Buru regency of Maluku province[4]. Based on the news from east news seven miners arrested for mining using mercury. Society thinks the use of mercury will further accelerate the process of amalgamation without thinking about the adverse effects of the use of chemicals[5]. Generally mercury being in nature is divided into three basic forms namely, metallic mercury, inorganic mercury and organic mercury which level of toxicity depends on the form in nature. Mercury that enters in the body will accumulate into the organs of the body that can cause organ damage. Metallic mercury is able to dissolve in fat so it can be distributed throughout the body[6], and is able to penetrate the Blood Brain Barrier and cause the mercury to accumulate in the brain while the Placental barrier can damage fetal growth and development[7]. Mercury only bad for humans, plants contaminated with mercury will show symptoms of chlorosis, root hood damaged, and causes a decrease in the size and number of roots[8]. Not only that, the mercury in the form of Hg (II) capable of binding to the human protein residue system which causes the protein to lose its activity. In contrast to other organic compounds, mercury can change into a form that is not harmful and various forms of mercury stable in nature, the ability of mercury to mercury can accumulate in different proportions in the food chain, and cause problems for human life[9]. Various efforts have been developed to address the problem of mercury pollution one of which utilize biologically based technologies that take advantage of natural materials of

biological origin such as bacteria to clean up the environment contaminated with mercury. Bacteria resistant to mercury can be used as biological tools and have an important role in eliminating mercury pollution on the environment[10]. To get the bacteria that resistance against mercury wastes necessary bacterial isolation and identification of bacterial species. Special identification of bacteria in this study using molecular stages based identification using 16SrRNA gene. Identification of gene sequence analysis method 16SrRNA considered to provide satisfactory results and can be used as a reference in the clinical application of diagnosis methods, which this analysis can answer a variety of issues relating to microbiology one bioremediation activity. Resistant genes found in bacteria capable of changing the ionic form of mercury into a form that is not stable by an enzyme. The enzyme that plays a role in this change, namely, eorganomecurial reductase mercurial mercurial lyase and reductase that work sequentially[11][12]. Organomecurial lyase has a way of working with cutting-carbon chain mercurial mercury reductase enzyme then change the form of water-soluble ions (Hg +) into metallic mercury which can not be dissolved (Hg +). Bacteria which only has a mercury reductase gene (MerA) is called a narrow spectrum mekruri resistant bacteria, while bacteria have genes besides MerA, also genes MerB then these bacteria are called broad spectrum of mercury-resistant bacteria[13].

3. METHOD

Method used in this research that each sample of 250 ml was taken from two different mining locations, namely in the area Gogorea, Savanajaya Village Buru and Buru On Bald Mountain. Samples were inserted into the reagent bottle dark. Samples were obtained from each bottle was taken as much as 50 ml and added 450 ml of Nutrient Broth were subsequently propagated with the aim of multiplying culture for about 5 days at room temperature. Cultures have been obtained subsequently isolated by media Nutrient order with additional HgCl2 30 ppm with dilution pour plate method of multilevel 10-3-10-51. Tested isolates were obtained by using cup-plate sensitivity technique, which each bacterium was suspended in NaCl with 1 MCF turbidity levels were subsequently streaked onto media Mueller Hinton Agar[14]. Each media that made 4 wells containing HgCl2 30 ppm, 20 ppm, 10 ppm and distilled water as a positive control. Bacteria that have a high sensitivity to resume on bacterial screening stage to determine the type of bacteria[15].

4. RESULT

4.1 Total Bacteria Each sample

Samples were obtained from local Gogorea village miners were coded, and grown in NB media. After the bacteria grew on the media and then the bacteria were isolated, counted the total number of colonies. Prior to the calculation of total bacteria, the bacteria must first be isolated by dilution of 10-3-10-5. Total bacteria is an overall number of bacteria present in the media, which is $7.3x10^8$

4.2 Resistance Bacteria Morphology Characteristics Mercury

Of the four samples were obtained and have been calculated total bacteria then acquired 4 colonies of bacteria, as follows: Morphological Characteristics Table 5.2 Bacterial Colonies

No	Isolate code	Color	Shape	margin	elevation	Size
1.	G1.1	Yellow	Round	Slippery	arise	Small
2.	G1.2	white milk	Round	Slippery	arise	Small
3.	G1.3	white milk	Round	Slippery	arise	Small
4.	G1.4	white milk	Round	Slippery	arise	Small-medium

Overall the bacterial isolates showed shape, margin elevation and size are the same, just different colors. In G1.1 isolates have a yellow tint while the other isolates had a milky white color.

4.3 Mercury Resistant Bacteria

Based on research that has been done, the result that the overall isolates have potential as mercury degrading bacteria as evidenced by its ability to grow in the treatment with mercury concentrations of 10 ppm, 20 ppm, 30 ppm not found a clear zone (0 mm). Here is a table of measurement results zones

Table 5.3 Results of measuring the diameter of clear zone (mm) as an indicator of bacterial resistance to mercury

No.	Isolate code	Diameter Zone			
		Control	10ppm	20ppm	30ppm
1.	G1.1	0	0	0	0
2.	G1.2	0	0	0	0
3.	G1.3	0	0	0	0
4.	G1.4	0	0	0	0

Based on the results of measurements of the diameter of the mercury degrading bacteria inhibition seen that the overall isolates could be degrading bacteria mercury, it is seen from the bacteria that can grow at different concentrations of mercury stress about not finding a clear zone. This is due to their mercury resistance gene in each isolate. This resistance gene is usually influenced by the activity of bacterial response in the form of adaptation to the environment so that genes that are expressed are resistant genes. The workings of the mercury resistance genes (mer operon) that is changing the toxic Hg2 + into a form that has a volatile nature Hg0, so bacteria can still grow in the mercury. The results showed that the overall isolates even able to grow at a concentration of 30 ppm of mercury, thus overall isolate genes and gene MerA overall MerB which isolates a broad spectrum of mercury-resistant bacteria. This is in line with the opinions Tanumiharja et al (2017) in his research that states that a bacterium regarded as mercury resistant bacteria if such bacteria can live in the levels of mercury (HgCl2) 5 ppm on nutrient agar media. Bacteria are able to live in the environment that contains mercury (HgCl2) with levels of 20 ppm or more can be classified into high mercury resistance bacteria. Besides bacteria only have mercury reductase gene (MerA) is called a narrow spectrum of bacterial resistance to mercury

4.4 Resistance Bacteria Screening Mercury

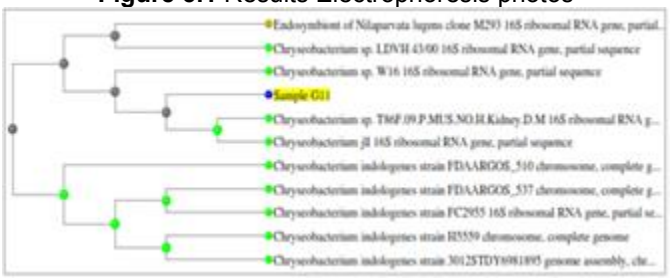
Based on the inhibition zone test results, it appears that the overall isolates capable of degrading mercury. However, based on the consideration of morphology and total bacterial isolates G1.1 then followed the screening stage of the bacteria, this is due to the morphology of different isolates G1.1 with other isolates, namely yellow, as well as the total bacteria in the samples G1 tends to be more. The results of DNA isolation

from G1.1 isolates were analyzed by PCR to amplify 16SrRNA gene using universal primers to amplify genes capable 16SrRNA 16SrRNA throughout 1376 bp gene which then electrophoresis with 0.8% agarose and do photograph under UV light. The display of the images and the images of electrophoresis of PCR as follows

Figure 5.1 The results of PCR

No	Sample Name	Sequences
1.	G1.1	**Sequence Assemble 1376 bp** 1 CTCCTGTTAC GGTCACCGAC TTCAGGTACC CCAGACTTCC ATGGCTTGAC GGGCGGTGTG 61 TACAAGGCCC GGGAACGTAT TCACCGCGCC ATGGCTGATG CGCGATTACT AGCGATTCCA 121 GCTTCATAGA GTCGAGTTGC AGACTCCAAT CCGAACTGAG ACCGGCTTTC GAGATTTGCA 181 TCACATCGCT GTGTAGCTGC CCTCTGTACC GGCCATTGTA TTACGTGTGT GGCCCAAGGC 241 GTAAGGGCCG TGATGATTTG ACGTCATCCC CACCTTCCTC TCTACTTGCG TAGGCAGTCT 301 CACTAGAGTC CCCAACTTAA TGATGGCAAC TAGTGACAGG GGTTGCGCTC GTTGCAGGAC 361 TTAACCTAAC ACCTCACGGC ACGAGCTGAC GACAACCATG CAGCACCTTG AAAAATGTCC 421 GAAGAAAAGT CTATTTCTAA ACCTGTCATT TCCCATTTAA GCCTTGGTAA GGTTCCTCGC 481 GTATCATCGA ATTAAACCAC ATAATCCACC GCTTGTGCGG GCCCCCGTCA ATTCCTTTGA 541 GTTTCAAACT TGCGTTCGTA CTCCCCAGGT GGCTAACTTA TCACTTTCGC TTAGTCTCTG 601 AATCCGAAAA CCCAAAAACG AGTTAGCATC GTTTACGGCG TGGACTACCA GGGTATCTAA 661 TCCTGTTCGC TCCCCACGCT TTCGTCCATC AGCGTCAGTT GTTGCTTAGT AACCTGCCTT 721 CGCAATTGGT GTTCTAAGTA ATATCTATGC ATTTCACCGC TACACTACTT ATTCCAGCTA 781 CTTCAATAAC ACTCAAGACC TGCAGTATCA ATGGCAGTTT CACAGTTAAG CTGTGAGATT 841 TCACCACTGA CTTACAGATC CGCCTACGGA CCCTTTAAAC CCAATAAATC CGGATAACGC 901 TTGCACCCTC CGTATTACCG CGGCTGCTGG CACGGAGTTA GCCGGTGCTT ATTCGTATAG 961 TACCTTCAGC TACCCTCACG AGGGTAGGTT TATCCCTATA CAAAAGAAGT TTACAACCCA 1021 TAGGGCCGTC GTCCTTCACG CGGGATGGCT GGATCAGGCT CTCACCCATT GTCCAATATT 1081 CCTCACTGCT GCCTCCCGTA GGAGTCTGGT CCGTGTCTCA GTACCAGTGT GGGGGATCAC 1141 CCTCTCAGGC CCCCTAAAGA TCGTTGACTT GGTGAGCCGT TACCTCACCA ACTATCTAAT 1201 CTTGCGCGTG CCCATCTCTA TCCACCGGAG TTTTCAATGT CGAATGATGC CATCCAACAT 1261 ATTATGGGGT ATTAATCTTC CTTTCGAAAG GCTATCCCCC AGATAAAGGC AGGTTGCACA 1321 CGTGTTCCGC ACCCGTACGC CGCTCTCTCA TTTCCGAAGA AACAATACCG CTCGGC

Figure 5.1 Results Electrophoresis photos



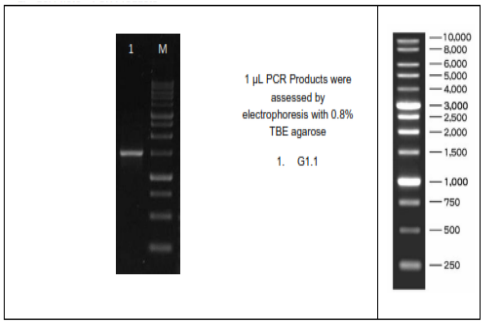
In 16SrRNA gene sequencing results on isolates G1.1, BLAST done online through https: //www.ncbi.nlm.nih.grov/nuccore/JX287899.1,AY278484.2,KC 853182.1,CP033828.1,JQ9758.1,AY468476.1,LR215967.1,CP 033930.1,CP033760. 1, MH628236.1, BLAST results found so that has indent of 99% and coverage of 100% on the sample that is Chryseobacterium sp G1.1 with accession number JX287899.1, Chryseobacterium sp. JLL with accession number AY278484.2, Chryseobacterium sp. W16 with accession number KC853182.1, Chryseobacterium indologenes with accession number CP033828.1, Endosymbiont of Nilaparvata lugens by accession number JQ975885.1, Chryseobacterium sp. LDVH 43/00 with accession number AY468476.1, Chryseobacterium indologenes with accession number LR215967.1, Chryseobacterium indologenes with accession number CP033930.1, Chryseobacterium indologenes with accession number CP033760.1, Chryseobacterium indologenes with accession number MH628236.1. to the results of BLAST 5.2 can be seen in the figure below:

Figure 5.2 Results Against Database NCBI BLAST

No	Sample Name	Result Links
1.	G1.1	https://www.ncbi.nlm.nih.gov/nuccore/JX287899.1,AY278484.2,KC853182.1,CP033828.1,JQ975885.1,AY468476.1,LR215967.1,CP033930.1,CP033760.1,MH628236.1

The next stage is to do a search of kinship between samples with other bacterial genes online throughhttps://www.ebi.ac.uk/Tools/msa/clustalo, The result of this is shown through the trees kinship filogenic. Results filogenic tree can be seen in the picture below.

Figure 5.3 Tree Filogenic



Based on this research can be seen kinship filogenic tree closest sample is Chryseobacterium sp G1.1 T86F.09.P.MUS.NO.H.Kidney.DM Chryseobacterium JLL also has a kinship with the isolate G1.1, but it is not as close as Chryseobacterium sp T86F.09.P.MUS.NO.H.Kidney.DM it is also the same as the bacterial species other Chryseobacterium. This shows that the sample of the bacteria is likely G1.1 Chryseobacterium sp. Based on these results, the bacteria Chryseobacterium sp T86F.09.P.MUS.NO.H.Kidney.DM is a bacteria that is resistant to mercury that can be developed in the process of bioremediation.

5. DISCUSSION

Based on the results obtained by the four isolates which together are considered capable of degrading mercury, it is because at the time of testing the resistance to the cup-plate technique method does not form clear zones around the wells. This is in line with the results of Ulfa et al 2015 study stating that the smaller the inhibition zone formed stronger indicates that the bacteria are able to transform complex metallic mercury compounds into other compounds simpler with lower toxicity that can be tolerated by the bacterial cell. Ability to withstand stress isolate the mercury caused by the presence of genes for resistance to mercury. Resistant genes found in bacteria capable of changing the ionic form of mercury into a form that is not stable by an enzyme. The enzyme that plays a role in this change, namely, eorganomecurial reductase

mercurial mercurial lyase and reductase that work sequentially. Organomecurial lyase has a way of working with cutting-carbon chain mercurial mercury reductase enzyme then change the form of water-soluble ions (Hg +) into metallic mercury which can not be dissolved (Hg +). Bacteria which only has a mercury reductase gene (MerA) is called a narrow spectrum of mercury-resistant bacteria, while bacteria have genes besides MerA, also genes MerB then these bacteria are called broad spectrum of mercury-resistant bacteria[16]. The existence of this resistance gene is affected by the adaptation to the environment so that the superior properties of the gene can be expressed in the form of cellular activity that is done by bacteria. This gene is expressed after threats from the surrounding environment so that the mercury in the form of stress with the maximum adaptation of genes as a means to degrade toxic mercury into the nature of which is not toxic[17]. he bacteria are obtained from this research that bacteria Chryseobacterium sp. T86F.09.P.MUS.NO.H.Kidney.DM, Chryseobacterium sp bacteria are gram-negative bacteria and have habitats on land and water, this is in accordance with the location of soil and water sampling. Chryseobacterium sp bacterium is a family of Flavobacteriaceae and the indigenous bacteria based on research results from Aviani 2016[18]. Indigenous bacteria are bacteria that naturally live freely in nature and have various benefits for humans one of them is a waste bioremediation agent[19]. So from the results of this study bacteria Chryseobacterium sp. T86F.09.P.MUS.NO.H.Kidney.DM, can be used as a bioremediation agent mercury wastes.

6. CONCLUSION

Based on the research results, obtained four isolates are considered resistant to mercury because at the time of testing does not form inhibitory zone around the area stress mercury isolate code G1.1, G1.2, G1.3, G1.4. among the four isolates that have a different color morphology is G1.1 so proceed to the stage of identification with 16SrRNA testing with bacteria Chryseobacterium name sp T86F.09.P.MUS.NO.H.Kidney.DM

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