



# Isolation of Mercury Reducing Bacteria from Gold Mining waste that has the Potential as a Chromium Bioremediation Agent



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## ABSTRACT

Traditional gold mining has been a widely handed down livelihood from the colonial era in Lebong Regency, Bengkulu. Limited resources and low technology used on the gold mining has to produce mercury waste that discharges to the environments directly. Over a long period, a unique bacteria community has been established in the mercury-contaminated area. These bacteria can survive in the toxic metals' environments. Bioremediation can be an alternative in dealing with environmental pollution by heavy metals. The goal of this research was to obtain a morphological characterization of colonies and cell isolates of mercury reducing bacteria from the waste of gold miner for chromium bioremediation. Bacterial isolation was carried out with Nutrient Agar (NA) media containing HgCl<sub>2</sub> concentration of 0.01 ppm, 0.03 ppm, 0.05 ppm, 0.1 ppm, and 0.2 ppm. The isolation results were selected from bacterial isolates that grew at HgCl<sub>2</sub> concentrations. The selected isolates supported the independence of chromium with K<sub>2</sub>CrO<sub>4</sub> concentrations of 10, 100, and 1,000 ppm. Then, the morphology characterization of selected colonies and bacterial cells was carried out. The results of the study obtained 8 pure mercury reducing isolates. Only Sp8 bacterial isolates have the highest resistance to chrome to a concentration of 1,000 ppm. It has the surface morphology of fine colonies, edges of circular colonies, flat elevation, overall appearance and color of yellowish-white colonies, and cell morphology with gram negative properties, basil cell shape, single-cell arrangement, and available endospores.

**Keywords:** Bioremediation, bacterial isolate, mercury, chromium, reducing bacteria.

## INTRODUCTION

Gold mining activities in the village of Lebong Tambang - Lebong Regency have produced the remnants of excavated and tailings containing mercury. Mercury is an element that is very poisonous to animals and humans. Because it is toxic, mercury vapor can be dangerous if inhaled even in small amounts. In addition to mercury, other heavy metals can be found in gold mining water environments such as Cr, Cu, As, Pb, Zn and Fe. Cr or chromium in nature forms Chromium Cr (0), Chromium (III), and Chromium (VI). Hexavalent chromium is more toxic than the trivalent form. The United States Environmental Protection Agency (US-EPA) proves that hexavalent chromium compounds or their reaction products in cells causes genetic material damage (Darmono, 1995; Firdaus, 2019). Mercury and chromium heavy metals will have a bad impact on the ecosystem if left unchecked.

One of the bioremediation efforts against such pollution is using bacteria that can reduce heavy metals. Bacteria isolated from sediment water from gold miners in the district Lebong. Based on

initial research, we found 8 bacterial isolates that can live in media containing mercury. Therefore, the researcher wants to test the level of ability to survive mercury reducing bacteria to reduce other heavy metals such as chromium (chromium bioremediation agent). After obtaining isolates that can reduce chromium, the bacteria will be tested for their characteristics phonetically based on colony and cell morphological observations, carbohydrate fermentation test. Based on this experiment, there arises the problem of how to obtain and characterize the morphology of the colonies and cells of mercury-reducing bacteria (Hg) isolates from waste gold miners without permission as chromium bioremediation agents. So that, this study aimed to obtained and characterized the morphology of the colonies and cell isolates of mercury reducing bacteria (Hg) from waste gold miners without permission as a chrome bioremediation agent.

## RESEARCH METHODS

### *Research Time and Location*

This research was conducted during 2019 in the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Bengkulu University. Sediment and sludge samples were obtained from a gold mining stream in the Lebong Tambang area, Lebong Regency, Bengkulu.

### *Isolation of Mercury reducing bacteria*

Bacterial isolation was carried out by serial dilution techniques, with 1 ml of water sample diluted with 9 ml of distilled water. Dilutions are made from 10<sup>-1</sup>, 10<sup>-3</sup> and 10<sup>-5</sup>. The last dilution was taken as much as 1 ml then poured into a petri dish containing NA medium with a concentration of HgCl<sub>2</sub> respectively 0.01 ppm, 0.03 ppm, 0.05 ppm, 0.1 ppm and 0.2 ppm. Isolation is done by pouring technique. Petri dishes were incubated at 37 ° C for 2x24 hours to obtain mercury resistant bacterial isolates. The results of isolation were selected from bacterial isolates that grew at HgCl<sub>2</sub> concentrations (Lewaru *et al.*, 2012).

### *Bacterial Reduction Test on Chromium*

This stage aims to get bacteria that have a high tolerance ability to Chrome (VI). This process is carried out by inoculating all bacterial isolates into Nutrient Broth (NB) media enriched with K<sub>2</sub>CrO<sub>4</sub> concentrations of 10, 100 and 1000 ppm. Each treatment was then incubated for 24 hours at 37 ° C and then spectrophotometrically observed at 600 nm Optical Density (OD). The isolates which had the highest amount of each subsequent treatment were observed for the morphological character of the colony and its cells (Joutey *et al.*, 2015).

### *Observation of the morphology of the colony and cell morphology*

Bacterial isolates that grew on NA media observed the characteristics of the colony including the surface of the colony, edges, elevation, appearance. Each isolate observed its cell characteristics including cell shape, cell structure, Gram reaction, ability to form endospores, motility.

### *Gram determination*

A 24-hour-old bacterial culture from each Nutrient Agar (NA) media was placed using ose up to ± 1 cm<sup>2</sup> flat on a glass of objects which had sterile distilled water and then dried. The preparations are then fixed by passing on the flame of the spirits 6-7 times. After a cold, given a drop of paint Hucker's crystal violet (Gram A) drops of 2-3 drops, flattened on a glass object, allowed to standing for 1 minute, then washed with distilled water flowing and dried. The

preparations are then added with a solution of Mordan Lugol's iodine (Gram B), allowed to stand for 2 minutes, washed with running distillate water then dried. Then, the alcohol is dropped and rinsed with running distillate water, dried again. Painting is continued with the addition of safranin paint evenly left for 30 seconds, rinsed with running distillate water, and dried. The preparations are then ready to be observed under a microscope that has been smeared with immersion oil with a magnification of 1000 x. observations include the shape, composition and properties of Gram cells.

#### *Cell length and endospores determination*

In observing cell length after Gram staining. Cell length can be seen under a microscope that has been placed on the microscope lens microscope. The glass of the object is cleaned with 96% alcohol and then heated on a spiritus flame. A solid culture was taken, then flattened on a glass of objects that have sterile distilled water and dried, then fixed over a spiritus flame and allowed to cool. Add one drop of 5% Malachite green and heat it over the water vapor until it evaporates for  $\pm$  10 minutes, washed with running distillate water, and dried. Furthermore, the safranin solution is allowed to stand for 1 minute, washed with distilled water then dried. The preparations are then ready to be observed under a microscope that has been applied with immersion oil with a magnification of 1000 times. Spores that are released will look green, spores that are still in the cell will appear transparent, while vegetative cells will look red (Arsyadi, 2016).

## **RESULTS AND DISCUSSION**

### *Isolation of Mercury reducing bacteria*

In this process 8 isolates of mercury reducing bacteria were obtained, namely: Sp1, Sp2, Sp3, Sp4, Sp5, Sp6, Sp7 and Sp8. The isolates were distinguished based on the colony morphology which included the surface of the colony, the edge of the colony, the elevation and appearance of the colony with the dominance of a yellowish white color generally the edges of the irregular colony, elevation of flat domination, the appearance of dominant lobate colonies, smooth dominant colony surface (Khasanah, 2020).

### *Bacterial Reduction Test on Chromium*

All isolates that were successfully grown in NA media containing  $\text{HgCl}_2$  were tested for bacterial reduction of Cr (VI) metal. Enriched with  $\text{K}_2\text{CrO}_4$  concentrations of 0 ppm, 10 ppm, 100 ppm and 1000 ppm. From the results of this study, all bacterial isolates survived to a concentration of 1000 ppm based on the 600nm OD test on a spectrophotometer.

Table 1. Bacterial isolate with the highest OD 600 nm value at 1000 ppm

[Cr] (ppm)	Selected Species Sp8	
	Replicate 1	Replicate 2
0	0.718	0.549
10	0.230	0.425
100	0.395	0.367
1000	0.316	0.301

OD (Optical density) results indicated the level of bacterial density with a wavelength of 600 nm. The value of OD Isolate Sp8 at a concentration of 0 ppm on the first iteration is 0.718 (Table 1) and the second iteration is 0.549. At a concentration of 10 ppm on the first iteration is 0.230 and

the second iteration was 0.425. At a concentration of 100 ppm on the first iteration was 0.395 and the second iteration was 0.367. At a concentration of 1000 ppm on the first iteration was 0.316 and the second iteration was 0.301. at a concentration of 100 ppm has the largest OD value, this was because at this concentration the Sp8 bacterial isolate was able to survive and increased bacterial density against the Cr (VI) concentration.

*Observation of Morphological Colonies*

The results of observations on the morphology of colonies of mercury reducing bacteria that can reduced metal chromium are shown in Table 2 and Figure 1.

Table 2. Morphology of selected bacterial isolates from mercury reducing bacteria that were capable of reducing chromium

Selected Bacterial Isolates	Sp8
Surface	Smooth
Edge	Circular
Color	Yellowish white
Elevation	Flat
Appearance	Entire



Figure 1. Morphology of colonies of mercury-reducing bacterial isolates that were tolerant with  $K_2CrO_4$

*Observation of cell morphology*

The results of observations of selected bacteria are shown in Table 3 and Figure 2, the morphological characters of eight cells of bacterial isolates that can survive in the media containing Mercury. The bacterial isolate has gram-negative properties, bacilli cell shape, single cell structure and contains endospores.

Table 3. Morphological identification

Isolate	Gram	Cell form	Cell Arrangement	Endospore
SP8	Negative (-)	Basil	Single	Positive (+)

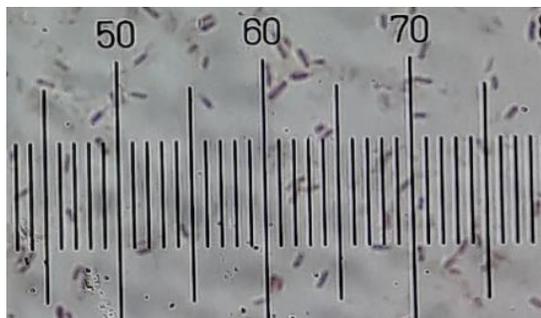


Figure 2. Morphology of colon cells of mercury-reducing bacterial that are able to tolerate  $K_2CrO_4$

Based on the research results obtained by isolation of bacteria that have the potential to reduced heavy metals. One source of isolates was gold mining waste containing mercury levels. Bacteria obtained expected not to require adaptation in the application to treat wastes containing heavy metals. After being isolated NA media containing mercury, the bacteria were able to tolerate with mercury metals to dilute 0.2 ppm. Then proceed with the reduction test of bacterial isolates that have the highest survival ability.

Bacteria can grow on media containing 10 ppm  $K_2CrO_4$ . Bacterial isolates that grow in these media were bacteria that tolerant to chromium (VI) and have the potential to reduce chromium (VI). To be sure, the isolates obtained were further tested with media containing  $K_2CrO_4$  with various concentrations up to 1000 ppm. Then, it was found that Sp8 bacterial isolate had the highest OD value at a concentration of 1000 ppm in the first repetition of 0.316 and the second repetition of 0.301. At a concentration of 100 ppm had the largest OD value, it was because at these concentrations Sp8 bacterial isolates were able to survive and increased bacterial density towards concentration Cr (VI).

According to Yazid *et al.* (2007), the ability of these bacteria to continue to grow was due to the detoxification mechanism for the toxic effects of chrome metal which they have including reduction, bioaccumulation, precipitation (formation of extracellular complexes) and methylation. Because of this, the Sp8 bacterial isolate can be a bioremediation agent to overcome the problem of environmental pollution by chromium-containing waste. The Sp8 bacterial isolate was further observed by the morphological characteristics of the colony and its cells as an initial step in the process of identifying the isolates of chromatic reducing potential. Based on table 2 showed the morphology of Sp8 bacterial isolates was smooth colony surface, circular colony edge, flat elevation, entire appearance and yellowish white colony color and table 3 showed the morphology of gram-negative cells, bacilli cell shape, single cell structure and endospores.

## CONCLUSION

In summary, the isolates of bacteria that have the potential as a chromium reductant can be obtained from mercury-resistant bacteria in sediment (sludge) waste of gold mining in Bengkulu. The selected isolates showed colony and cell morphological characteristics that are gram negative, basil form and single arrangement. It is necessary to continue the research to identify the isolates obtained.

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