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Research Article

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The highest mercury resistant bacteria as a mercury remediator from gold mining soil in West Sumatera, Indonesia

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ABSTRACT

Mercury resistant bacteria isolated from gold mining soil in Kabupaten Sijunjung, West Sumatera, Indonesia. Our prior study found 5 bacteria isolates with highest resistant capability. The present study is to evaluate all five isolates abilities in Nutrient Broth medium with three variant additional of mercury ($HgCl_2$): 150 ppm, 200 ppm, and 250 ppm. The result shows all five isolates grow well in the media indicated by the increase of optical density value observed once in three days for twelve days. The highest OD value achieved by MRB 5, which is 1.2. Mercury reduce analyzed on day 12. Mercury reduce ($HgCl_2$) by all five isolate reach the highest value, within range 70.69-85.44%, with highest mercury reduce percentage achieved by isolate MRB5 and the lowest one by isolate MRB2. This research may become the basic concern in bioremediation of mercury contaminated environment, particularly in West Sumatera, Indonesia.

Key words: bacteria, resistant, mercury, remediation, gold mining

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INTRODUCTION

One of the location known as a gold deposit in West Sumatra is Kabupaten Sijunjung. Gold mining activities have been found along the river flows and around the farm soil. The mining activities generally involve the extensive use of mercury to recover gold through the amalgamation process. Amalgamation process could lead to mercury contamination in surround environment [1] [2][3]. Most of this mining operation in Sijunjung happen to be illegal, which makes it difficult to record mercury utilization data accurately.

Most of the mercury (Hg) in the atmosphere is in the form of elemental Hg (Hg^0), which is volatile and is oxidized to the mercuric ion ($Hg2^+$) as a result of its interaction with ozone in the presence of water [4]][5]. When both inorganic mercury forms (Hg^{2+} and Hg^0) are present in aquatic systems, they are converted into highly toxic organic mercury (methylmercury /MeHg) that is subsequently bioaccumulated through all levels of the food chain [6]. This bioaccumulation will give certain risks to consumers at the upper trophic levels [7]][8] [9]

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A study by Essa *et al.*[10] indicated that certain different mechanisms can make microbes capable to survive in the presence of Hg in high concentration. By having evolved resistance mechanisms to detoxify several chemical forms of mercury, resistant microbes may play an important role in mercury bioremediation in mercury-contaminated environments [11] [12] [8].

Our prior study has found some bacteria isolates from former gold mining soil in West Sumatera. After screening of the capability, five superior mercury resistant bacteria isolates selected. This study is the continuation of our prior

study, which aim to evaluate all five isolates abilities in Nutrient Broth medium with mercury ($HgCl_2$) in laboratory scale.

EXPERIMENTAL SECTION

This study is an experimental study to evaluate the capability of superior mercury resistant bacteria isolates in reducing mercury growth in Nutrient Broth medium with additional of 150, 200, and 2500 ppm $HgCl_2$. Cultivation of bacteria isolate measured with optical density using spectrophotometer of λ 600 nm once in three days for 12 incubation days at room temperature (27-30^{0}C).Mercury reduce percentage measured with ICPE 9000 Shimadzu (Inductively Coupled Plasma) after twelve incubation days.

RESULTS AND DISCUSSION

The growth of all five selected bacteria isolates in Nutrient Broth with additional of 150, 200, and 250 ppm $HgCl_2$ in laboratory scale shown in Figure 1.

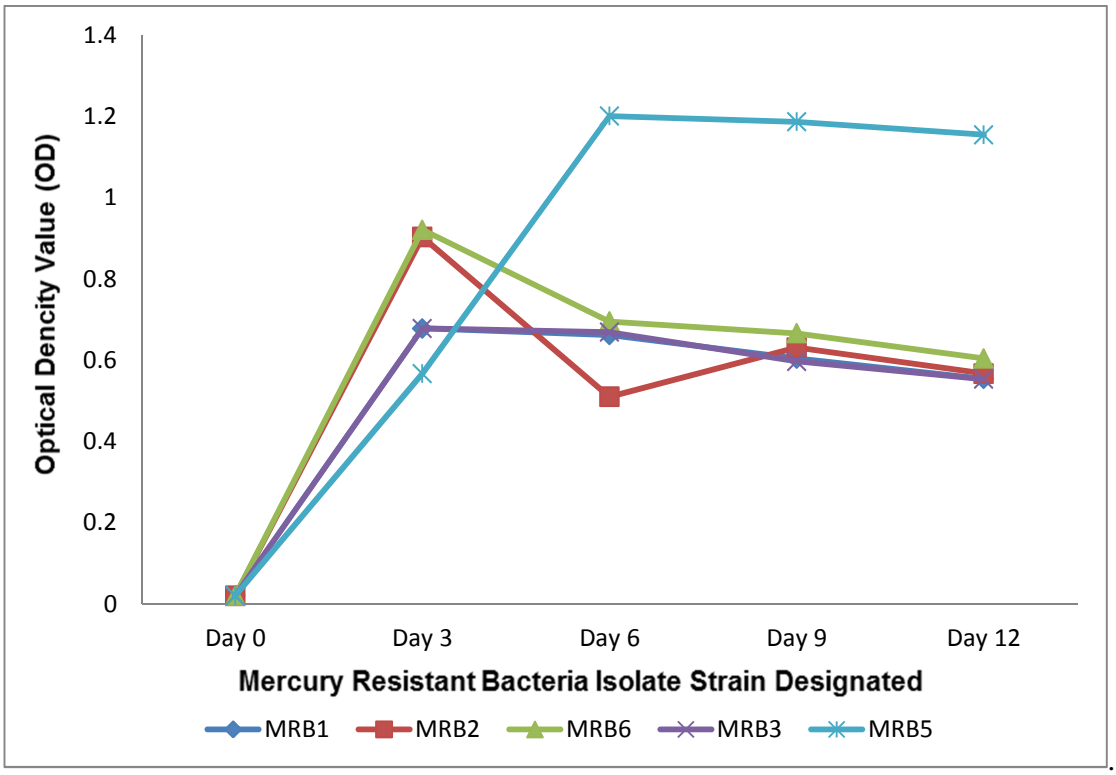

Figure 1. Superior mercury resistant bacteria isolate growth in Nutrient Broth medium with additional of $HgCl_2$ in laboratory scale based on optical density (OD) value taken once in three days for twelve incubation days

Figure 1 showed all bacteria isolates could grow well in Nutrient Broth within laboratory scale evaluated once in three days in twelve incubation days. This indicated by the increasing OD value. There is no prolong lag phase found since the isolates gone through recultivating processand the activation of isolate used the same medium with prior study (nutrient broth with additional of $HgCl_2$ in scale up volume) so isolates has coped with the medium. The highest OD value achieved by isolate MRB5 which is from 0.02 to 1.201. The growth in medium reach its peak on day three (logarithmic phase), only isolate MRB 5 has growth peak up to sixth incubation day. The logarithmic phase will continue as long as cells have adequate nutrients and the environment is favorable. After which, the bacteria enters stationary growth phase, where cells stop growing or grow slowly. The decline in the growth rate is caused by depleted nutrients and oxygen, excretion of organic acids, and other biochemical pollutant into the growth medium, due to the increased density of cells. As the limiting factors intensify, cells begin to die in exponential numbers, in which the death phase begins [13] [14] [15].

Bacteria is able to live in an environment with mercury contamination since it has *mer operon* (mercury resistant gene) [16], but each of bacteria species has different *mer operon* structure. In general, *mer operon* consists of metaloregulator gene (*mer*R), mercury transport gene (*mer*T, *mer*P, *mer*C), mercury reductase gene (*mer*A), and organomercury liase (*mer*B). Bacteria with *mer*A gene is called narrow spectrum mercury resistant bacteria. Some

bacteria not only has mercury reductase protein (*mer*A) but also organomercury liase (*mer*B) which catalyze mercury-carbon bond break thus produce organic compound and Hg ion as a thiol salt. Bacteria with *mer*A and *mer*B is called broad spectrum mercury resistant bacteria [16] [17] .

The capability of isolates in reducing mercury in Nutrient Broth in laboratory scale is shown in Figure 2.

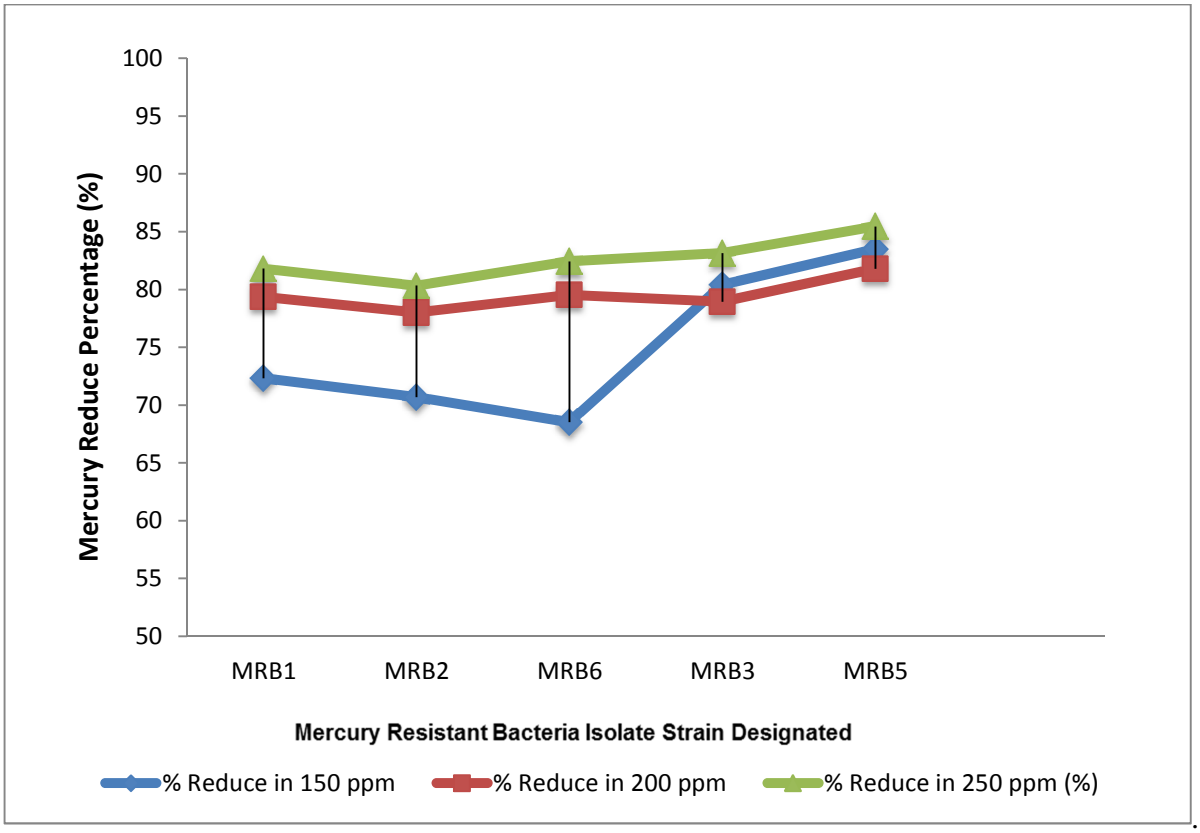

Figure 2. Mercury level reduce percentage ($HgCl_2$) with superior mercury resistant bacteria isolat both as a single culture and as a consorsium in a liquid medium within laboratorium scale

Figure 2 shows all five mercury resistant bacteria isolate has the ability to reduce mercury level ($HgCl_2$) within 70.69-85.44% in 150-250 ppm $HgCl_2$. The highest percentage achieved by isolateMRB5 and the lowest one by MRB2. Bacteria could detoxify mercury because it has mercury resistant gene, *operon mer* [16].

Each bacteria species has different *mer operon* structure. In general, *mer operon* consists of metaloregulator gene (*mer*R), mercury transport gene (*mer*T, *mer*P, *mer*C), mercury reductase gene (*mer*A), and organomercury liase (*mer*B). Bacteria with only *mer*A gene is called narrow spectrum mercury resistant bacteria. Some bacteria not only has *mer*A but also *mer*B gene which is called broad spectrum mercury resistant bacteria. *mer*A protein functions to reduce toxic mercury ion, converting it into less toxic mercury Hg(0) which could evaporate at room temperature, whereas *mer*B protein functions in catalyzing breakage of mercury-carbon bond which will produce organic compound and ion Hg(II) [18]. Thus, MRB could be used as bioremediation agent in mercury contaminated environment.

CONCLUSION

From this study, some things concluded:
1) All five isolates could grow well in liquid medium with additional of 150, 200, and 250 ppm mercury ($HgCl_2$) in laboratory scale, which indicated by growth measurement with optical density value. The highest OD level reached by isolate MRB5 with maximal OD 1.201.

2) All five superior mercury resistant bacteria isolate has the ability to reduce mercury level ($HgCl_2$) 70.69-85.44% within 150-250 ppm $HgCl_2$. The highest mercury reduce level is achieved by isolateMRB5 and the lowest one by MRB2

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