SHORT COMMUNICATION

A Study on Mercury-Resistant Bacteria Isolated from a Gold Mine in Pongkor Village, Bogor, Indonesia

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Mercury is one of the major pollutants in the environment which is highly toxic. Bioremediation strategies using bacteria have been proposed as an attractive alternative because this is effective, less expensive and more efficient to remove mercury. *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2 were two highly mercury resistant bacteria isolated from a gold mine in Pongkor village with MIC of 575 ppm. The purposes of the research were to study the effect of mercury on bacterial growth and morphological changes of bacterial colony and to measure the ability of bacterial isolates to accumulate Hg^{2+}. The growth was monitored by measuring optical density at 600 nm, whereas accumulation of Hg^{2+} was measured by mercury vaporisation unit. These present studies revealed that the addition of 50 and 100 ppm HgCl2 in *Brevundimonas* sp. HgP1 resulted in the decreasing of growth rate and the elongation of lag phase in 8 and 16 hours, respectively. The addition of HgCl2 also affected morphological appearance of the bacterial colony to black. *Brevundimonas* sp. HgP1 accumulated Hg^{2+} up to 1.09 and 2.7 mg/g dry weight of cells and removed 64.38 and 57.10% Hg^{2+} from the medium containing 50 and 100 ppm HgCl2, respectively.

Key words: accumulation, bacteria, *Brevundimonas* sp., bioremediation, mercury resistance

INTRODUCTION

Mercury pollution of the environment by mining activities and industrial wastewaters has resulted in worldwide contamination of large areas of soils and sediments and led to elevated atmospheric mercury levels. Contamination of soils by heavy metals such as mercury is the most serious environmental problem and has significant impact on human health (Mathivanan et al. 2010). Mercury has been recognized as one of the most toxic heavy metals in the environment and has been released through natural events and anthropogenic activities (Kiyono & Hou 2006). Mercury and its compounds bind to the sulphydryl groups of proteins and enzymes, thereby inactivating vital cell functions (Mirzaei et al. 2008). Mercury is potentially concentrated through the food chain and if consumed by humans for long term can cause adverse effects to human health (Nascimento & Souza 2003). Due to prolonged exposure to mercury-polluted environment, certain environmental strains of bacteria have acquired highly specific resistance to mercury ion, organo mercury, antibiotics and other heavy metals (Xiao-xi et al. 2010). Many bacterial species have been shown to develop resistance to Hg and other heavy metals (Ravel et al. 2000). The mercury-resistant bacteria were found to belong to *Pseudomonas*, *Proteus*, *Xanthomonas*, *Alteromonas*, *Aeromonas*, and *Enterobacteriaceae* (De et al. 2003). *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Citrobacteria*, *Klebsiella*, and *Rhodococcus* are organisms commonly used in bioremediation mechanisms (Keramati et al. 2011).

Exposure of toxic heavy metals makes the cells of microorganisms develop resistance mechanisms and metal-ion homeostasis (Aspassia et al. 2007). Since heavy metal ions can’t be degraded or modified, there are few mechanisms for a heavy-metal resistance system such as binding to the cell surface, influx and efflux, accumulation, detoxification of toxic metals to less toxic form, the use of *metallothionein* protein, and combination of two or three mechanisms mentioned. Accumulation of the respective ion can be reduced by efflux, an active extrusion of the heavy-metal ion (Wagner-Dobler 2003; Aspassia et al. 2007). Microbes have evolved a mechanism for mercury detoxification based on intracellular reduction of Hg^{2+} to non-toxic Hg^{0} by the mercric reductase enzyme and
Irawati et al. (2012) have isolated two mercury resistant bacteria from a gold mine in Pongkor village, Bogor, West Java. These bacteria have been identified with 16S rDNA as *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2 under the accession number of JX009135 and JX009136, respectively. The purposes of the research were to study the effect of mercury added on growth and morphological changes of colony. The ability of bacterial isolates to accumulate Hg²⁺ were also studied.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Medium.** *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2 were two highly mercury resistant bacteria isolated from soil of Pongkor village gold mining, Bogor, West Java under the accession number of JX009135 and JX009136, respectively. Bacterial isolates demonstrated high resistance to HgCl₂ with MIC of 575 ppm (Irawati et al. 2012). Bacterial isolates were grown in Luria Bertani (LB) agar containing the following (per liter): tryptone: 10 g, yeast extract: 5 g, NaCl 10 g, glucose 0.1 g, and pure agar 0.15 g. Stock of HgCl₂ (50,000 ppm) was added to the autoclaved media.

**Effect of Mercury on Bacterial Growth and Colony Morphology.** Cells were grown in 25 ml LB medium supplemented with 50, 100, 150, 200 ppm of HgCl₂ and without HgCl₂. Cultures were incubated at 37 °C. Growth was monitored by measuring optical density at 600 nm using spectrophotometer. The morphological changes of colony on medium containing various concentration of HgCl₂ and without HgCl₂ were also observed.

**Accumulation of Hg²⁺.** Cells were grown in 25 ml LB medium containing 50 and 100 ppm of HgCl₂ and were collected by centrifugation at 4,000 rpm for 15 minutes. Cells were washed several times with phosphate buffer. About 50 mg of dry weight of cells were digested with 2 ml of HNO₃ at 85 °C for 3 hours (Cha & Cooksey 1991). Accumulation of Hg²⁺ in the cells was determined by Mercury Vaporation Unit (Kopp et al. 1972).

**RESULTS**

**Effect of Mercury on Bacterial Growth and Colony Morphology.** The growth of *Brevundimonas* sp. HgP1 was inhibited by the addition of 50 and 100 ppm of HgCl₂ on the medium. This bacterium survived in the presence of high mercury concentration as shown by the extension of the lag phase (Figure 1). It was observed that *Brevundimonas* sp. HgP1 with the addition of HgCl₂ showed a lag phase and resumed to normal growth after the lag phase, but its cell density did not reach as high level as its cell density on medium without HgCl₂. The duration of the lag phase depends on the concentration of mercury added to the medium. In the medium containing 50 ppm HgCl₂, *Brevundimonas* sp. HgP1 extended the lag phase in eight hours, whereas, this bacteria needed 16 hours of the lag phase when 100 ppm was added to the medium. There was no growth detected in medium containing 150 and 200 ppm of HgCl₂.

The addition of HgCl₂ in the medium affected morphological appearance of the colony to black in *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2. The cells of *Brevundimonas* sp. HgP1 also formed a darker aggregate that precipitated after being centrifuged when 100 ppm of HgCl₂ was added in LB broth (Figure 2). These effects were not observed when the bacteria was grown in medium without any addition of mercury.

**Accumulation of Hg²⁺.** The ability of *Brevundimonas* sp. HgP1 to accumulate Hg²⁺ increased along the increasing of mercury concentration (Figure 3). This bacteria accumulated Hg²⁺ up to 1.09 and 2.7 mg/g dry weight of cells and removed 64.38 and 57.10% Hg²⁺ from the medium containing 50 and 100 ppm HgCl₂, respectively.

**DISCUSSION**

Mercury resistant bacteria can be isolated from various sources contaminated with mercury and can be developed as bioremediation agents in these area (Xiao-xi et al. 2010). Mirzaei et al. (2008) found that high mercury levels in the environment increased the ability of resistance to mercury among the bacterial communities residing in the contaminated sites. Two highly mercury resistant bacteria have been isolated from a gold mine in Pongkor village with MIC of 575 ppm and have been characterized as *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2 (Irawati et al. 2012). This level of resistance was seven times higher than mercury resistant bacteria that was previously reported. *Pseudomonas putida* isolated from waste water in Iran and *Pseudomonas aeruginosa* isolated from soil sample surrounding Hunan Zhuzhou Smelter, China exhibited resistance to HgCl₂ with MIC of 80 ppm (Mortazavi et al. 2005) and 60 ppm (Xiao-xi et al. 2010), respectively. Meanwhile, *Escherichia coli* isolated from water sample of Yamuna river, India, demonstrated
The ability of *Brevundimonas* sp. HgP1 and HgP2 to grow in the presence of highly HgCl₂ would be helpful in the waste water treatment, where microorganisms are directly involved in the decomposition of organic matter in biological processes because the inhibitory effect of heavy metals is often a common phenomenon that occurs in the biological treatment of waste water and sewage (Raja *et al.* 2009). Bacteria with highly resistance to heavy metals develop various resistance mechanism. These mechanisms could be utilized for detoxification and removal of heavy metals from polluted environment.

The addition of HgCl₂ in the medium affected morphological appearance of the colony to black in *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2. This might be due to survival mechanism of bacteria by accumulating Hg²⁺. *Brevundimonas* sp. HgP1 accumulated Hg²⁺ up to 1.09 and 2.7 mg/g dry weight of cells and removed 64.38 and 57.10% Hg²⁺ from the medium containing 50 and 100 ppm HgCl₂, respectively. The cells of *Brevundimonas* sp. HgP1 also formed darker color aggregates that precipitated following the centrifugation in the presence of 100 ppm HgCl₂ (Figure 2e). This result was similar to what has been demonstrated in transgenic bacteria-resistant mercury namely *Escherichia coli*. This bacteria expressing metallothionein and polyphosphate kinase demonstrated cell aggregation, precipitation and darker color when the cells were grown in high mercury concentrations. It was possible that these effects depend on high mercury resistance and accumulation by transgenic bacteria due to the presence of metallothionein. Metallothionein protects bacteria against the harmful effects of mercury
Mercury-resistant bacteria are now considered a potential approach to biological remediation. Bioremediation strategies including biotransformation, biosorption, and bioprecipitation of mercurials have been developed yet rarely been applied to remediation of mercurials in the environment (Wagner-Dobler et al. 2000). Mercury resistant bacteria are extremely important in detoxifying the mercury compounds by two sequentially acting enzymes namely, organomercurial lyase which cleaves the carbon-mercury bonds of certain organomercurials and mercuric reductase, which reduces to the volatile mercury (Wagner-Dobler 2003). More studies are needed to further understand the mechanism of mercury resistance in *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2.

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**REFERENCES**


