Reduction Mercury-Polluted Water in Gold Mine with Anaerobic Bacteria

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Abstract: To develop biotechnology for biological treatment of mercury-contaminated wastes or for bioremediation of mercury-polluted sites, mercury-resistant microorganisms have been isolated and characterized. However, understanding of bioremediation mechanism by anaerobic bacteria. In this study, we tried to isolate anaerobic mercury-resistant bacteria from mercury-polluted water in Bombana Gold Mine, Indonesia. One strain of the bacterial isolates, was used for the identification and for the growth capability of mercurials. We also analyzed genetic characteristics of mercury resistance genes (merA gene) from this strain. The strain Bombana, which was isolated from Bombana Gold Mine water, was identified as Flavobacterium sp. and showed resistance to both inorganic mercury. Furthermore, nucleotide sequence analysis showed that merA genes of the strain Bombana were identical of Staphylococcus aureus dan Streptomyces coelicolor an aerobic mercury-resistant bacterium in the nucleotide sequence level. The study describes a mercury bioaccumulating bacteria from Bombana, which remediated mercury from the medium simultaneous to its growth. The applicability of the bacterial cells in removing mercury from synthetic effluent in a batch mode was amply demonstrated. The initial load of 50 ppm mercury in effluent was completely removed in 6 days.

Keywords: Anaerobic bacteria, bioremediation, merA gene, mercury resistant

1 Introduction

Mercury (Hg) pollution of soil and water is a world-wide problem [1]. Among the nineteen heavy metals, arsenic, cadmium, mercury and lead have no known essential biological function and are extremely toxic [2]. Residual effects of most of these heavy metals on aquatic biota are long lasting and highly deleterious as they are not easily or rapidly eliminated from these ecosystems by natural degradative processes. These metals tend to accumulate in sediment and move up the aquatic food chain ultimately reaching human beings where they produce chronic and acute, including a number of DNA mutations [3]. The harmful effects of these heavy metals in the environment therefore tend to be permanent. Consequently, mercury removal is a challenge for environmental management.

Elemental mercury, its ore cinnabar and a variety of its compounds enter the aquatic environment through natural leaching and washing of soils, rocks and atmosphere by rain as well as by anthropogenic chloro-alkali production, instrument manufacturing, chemical laboratories and dentistry [4]. In marine environments, addition and dispersion of dissolved mercury is continuous as both natural and manmade causes lead to its increased concentrations. Mercury can bind to organics leading to formation of generally recalcitrant and highly toxic organomercurial complexes[5].

Some bacteria living in such a mercury polluted environment are often resistant to mercurials. Microorganisms in contaminated environments have developed resistance to mercury and are playing a major role in natural decontamination. An extensively studied resistance system, based on clustered genes in an operon (mer operon)[6]. Mercury-resistance determinants have been found in a wide range of gram-negative and gram-positive bacteria.
isolated. The bacterial mercuric reductase enzyme (MerA) affects mercury mobility and bioavailability by converting water-soluble inorganic mercury and methyl mercury to the volatile elemental form. This is a detoxification process as evidenced by the resumption of microbial growth after the removal of the gaseous form of Hg(0) [7, 8].

Therefore, isolation and characterization of mercury-resistant bacterial have been investigated to develop biotechnology for biological treatment of mercury-contaminated wastes or for bioremediation of mercury-polluted sites [9]. However, research into the mercury-resistant bacteria has been done only for the aerobic microorganisms. In many cases, anaerobic environments commonly exist as mercury-contaminated wastes, such as wastewater sludge, and mercury-polluted sites, such as soil and sediments. Therefore, although an understanding of the characteristics of anaerobic bacteria is important, anaerobic mercury-resistant bacteria have not been surveyed sufficiently and those biological features remain to be understood. It is considered that the anaerobic mercury-resistant bacteria possess the possibility of developing a new anaerobic treatment system and of bioremediation in the anaerobic sites.

For this study, we have tried to isolate and identify the anaerobic mercury-resistant bacteria from the water and sediment of Bombana River, Indonesia. One of the obtained anaerobic isolates has been used for this study. The strain microbs was identified and its growth ability was characterized in the presence of mercury. Analyzed genetic characteristics of mercury resistance genes (merA gene) of the strain by using polymerase chain reaction (PCR) and nucleotide sequencing. Furthermore, we develop a bioremediation method for a mercury-polluted water using mercury resistant bacterial.

2 Problem Formulation

Sampling sites and sample collection. Samples were taken from the Bombana gold mine of Southeast Sulawesi, Indonesia. Types of samples were collected water. All containers used for sampling were cleaned and rinsed following trace metal protocols.

Bacterial Isolates. Bacterial isolates used in this study were isolated from the Bombana gold mine environment. All isolates highly resistant to mercury (i.e., those capable of growth in Luria agar with Hg²⁺). Their biochemical characteristics have been studied for proper identification. Luria Agar used in this study had the composition: 33% pepton; 16% yeast extract; 33% NaCl; 1.9% MgSO₄.7H₂O; 2% Bacto agar (final pH 7.0).

Mercury sensitivity test. All samples were subsequently diluted and plated on Luria agar. The selected strains were subjected to differential and selective growth media, followed by various biochemical tests for their identification. The determination of sensitivity HgCl₂ was also performed for various concentration HgCl₂.

DNA extraction and PCR amplification of merA sequences. Total DNA was extracted from bacterial samples, using DNA isolation kit (Fermentas) according to the manufacturer’s guidelines. The presence of merA genes in DNA extracts was detected using a nested PCR approach using primers specific for merA genes, fragment was amplified using the forward primer A1s-n.F: TCCGCAAGTNGCVACBGTNGG and reverse primer A5-n.R: ACCATCGTCAGRTARGGRAAVA[8]. Amplification conditions were as follows: 30 cycles of 30 sat 94°C, 60 s at 54°C, and 60 s at 72°C. The products of these reactions were separated by electrophoresis on a agarose gel.

Cloning and sequencing of merA gene PCR products. The merA amplicon was cloned into the vector using a Fermentas cloning kit according to the manufacturer’s instructions. Plasmid DNA was isolated from clones using Ferentas isolation plasmid kit and the plasmids screened for inserts of the correct size by restriction digestion. Clones were sequenced.
using the A1s-n.F primer and ABI dye terminator chemistry.

**Mercury bioremediation in synthetic effluent.** The synthetic effluent media (LB) consisting of 33% pepton; 16% yeast extract; 33% NaCl; 1.9% MgSO₄·7H₂O (pH 7.0) was used to check the efficiency of mercury bioremediation by cells. Cells were grown in culture medium (LB) and amended with 50 ppm of HgCl₂, incubated at 30°C. The samples were withdrawn at regular time intervals and centrifuged at 14,000g for 10 min. The supernatants were analyzed for residual mercury content using spectrophotometer.

**Analysis of mercury from mercury-polluted water.** A test tube containing 5 mL mercury-polluted water to 2 mL of a solution containing HCl and HNO₃ (1:1), and add 5 mL deionized water. After the reaction mixture was boiling. The concentration of Hg trapped in the solution was measured by spectronic.

### 3 Problem Solution

Isolation and identification of anaerobic mercury-resistant bacteria 72 strains of mercury-resistant bacteria were anaerobically isolated from the mercury-polluted water of Bombana gold mine, Indonesia (Fig 1). Since these bacterial isolates could grow under anaerobic conditions for 3-5 days at room temperature. Table 2 shows the taxonomic characteristics of these anaerobic isolates according to Bergey’s Manual. From these results, all isolates were classified into the genus *Clostridium*.

**Table 2 Taxonomic characteristics of the anaerobic mercury-resistant isolate**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Anaerobic</th>
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<tbody>
<tr>
<td>Growth</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Morphology</td>
<td>Coccus</td>
</tr>
<tr>
<td>Gram staining</td>
<td>Negative</td>
</tr>
<tr>
<td>Sulfate reduction</td>
<td>Reduction</td>
</tr>
<tr>
<td>Fermentation carbohydrate</td>
<td>Positive</td>
</tr>
<tr>
<td>Gelatine reduction</td>
<td>Negative</td>
</tr>
<tr>
<td>Metyle red test</td>
<td>Negative</td>
</tr>
<tr>
<td>Nitrate test</td>
<td>Positive</td>
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<tr>
<td>Indol test</td>
<td>Positive</td>
</tr>
<tr>
<td>Citrate test</td>
<td>Positive</td>
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</tbody>
</table>

**3.1 Amplification and cloning of *merA* from DNA samples**

Previously described primers for *merA* were used to amplify the *merA* gene from DNA extracts of microbial biomass samples collected from river Bombana gold mine. Fig. 2 show the PCR amplification results of the *merA* genes of isolates Bombana-1 and Bombana-2. The PCR products of 0.3 kb DNA fragments were cloned and sequenced. The MerA phylotypes were obtained which encompassed the known diversity of MerA among bacteria (Fig. 2). Nucleotide sequences of these PCR products showed extremely similarity and were perfectly identical to the corresponding regions of *Staphylococcus aureus* (isolate Bombana1) dan *Streptomyces coelicolor* (isolate Bombana2). The phylotypes observed clustered with a putative MerA from the genome sequence of *Staphylococcus aureus* (isolate Bombana1) dan *Streptomyces coelicolor* (isolate Bombana2).

It has been thought that anaerobic bacteria play roles in converting mercury to either methylated or sulfide compounds. Thus, the mercury resistance of isolates Bombana-1 and Bombana-2 may mainly be acquired by vaporizing the elemental mercury (Hg⁰).
reduced from mercuric ions (Hg\(^{2+}\)). This new finding suggests that the isolates Bombana-1 and Bombana-2 may be used for biological treatment of mercury-contaminated wastes in the anaerobic treatment system and for bioremediation of mercury-polluted sites in the anaerobic environments.

![Image of gel electrophoresis](Lane 1; size marker, Hind III digested λ phage DNA. Lane 2, 3; PCR product of merA gene)

**Fig. 2** The results of PCR amplification of merA gene of *Clostridium*

### 3.2 Analysis of merA

Analysis using the Basic Local Alignment Search Tool (BLAST) revealed that the isolate Bombana-1 sequence corresponded to a species pertaining to the *Staphylococcus aureus* (98% maximum). Isolate Bombana-2, analyzed by another classmate, was identified as a *Streptomyces coelicolor* species. The sequence was then compared in the class using ClustalW, and a phylogeny tree was obtained for all organisms, as shown in Fig. 3.

![Phylogeny tree](Fig. 3 Phylogeny tree (neighbor-joining tree) of all species obtained in class, as returned by ClustalW)

### 3.3 Mercury bioremediation in synthetic effluent

It was possible to recover accumulated mercury by celllysis. The recovery of remediated mercury is another major advantage for sustainability of bacteria mediated mercury detoxification process. The bioaccumulation of heavy metals is a known phenomenon in certain bacterial species when challenged with the toxic environment [10, 11]. Metal uptake is generally a preceding step to metal detoxification by microbial cells. Because microbial metal uptake and detoxification process being species specific, different mechanism of uptake and detoxification could operate simultaneously or individually.

The mercury resistance has been previously noted in Enterobacteria [12]. Pseudomonads have rather been used for mercury bioremediation. The mercury remediation by Pseudomonads has been accorded to the presence of well defined meroperon consisting of a predominant mercury reductase (mer A) responsible for the conversion of Hg\(^{2+}\) to Hg\(^0\) [8, 13]. Other associated proteins are mercury binding protein (merP) and a transporter protein (mer T) responsible for facilitating entry of mercury inside the cell where inorganomercurial lyase (mer B) or mercury reductase (mer A) further act on it. The resultant Hg\(^0\) finally diffuses out of the cells.

![Mercury bioremediation](Fig. 4 Mercury bioremediation by microbes and microbes-carbon active)
Use of microbes cells anaerobic offers viability to bioprocess in terms of better operational controls and easy scale-up. Cells exhibited better remediation efficiency. The initial drop in the mercury concentration in the solution within 1 day may thus be due to quick adsorption of mercury. Microbes cells and microbes cell-carbon active was tested in repetitive batch mode, wherein 99.9% mercury was removed. The applicability of the microbes cells in removing mercury from complex effluent was amply demonstrated. The initial load of 50 ppm mercury was completely removed in 6 days.

4 Conclusions

The results in the present study demonstrate the mercury remediation and bioaccumulation by Flavobacterium sp. cells from Bombana Gold Mine. The novel features were: (i) that the remediated mercury remained confined to the cells without vaporizing back to the environment and (ii) the possibility of recovering the bioaccumulated mercury. The feasibility of mercury bioremediation. Thus, Flavobacterium sp. based mercury remediation has a potential to develop into a green viable process.

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References