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Original Research Article

Methylmercury Biosorption Activity by Methylmercury-resistant Lactic Acid Bacteria Isolated From West Sekotong, Indonesia

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ABSTRACT

Methylmercury has been generally known as a toxic heavy metal for both human and environment. Bacterial-based bioremediation of heavy metal is suggested as an ecofriendly and low-cost bioremediation process. There was limited information regarding the role of lactic acid bacteria (LAB) as detoxification agent for methylmercury addressed for human body. West Sekotong, West Lombok, Indonesia, is one of the newly developed artisanal and small-scale gold mining site with high mercury contamination level. This present study was aimed to isolate the human origin methylmercury-resistant LAB and further evaluate their ability to absorb methylmercury. Methylmercury absorption assay was conducted in broth media. The remaining and absorbed methylmercury was measured using the gas chromatography flame ionization detector. A total of 56 methylmercury-resistant LAB isolates were isolated from 37 feces and 19 breast milk samples from 19 volunteers in West Sekotong. Of them, 10 isolates were further selected based on several basic probiotic characteristics and subjected to methylmercury removal assay. The selected isolates showed different methylmercury absorption ability ranged between 17.375 and 51.597 μg/g of wet mass of cell after incubated for 24 hours. Two isolates from feces showing the best removal activity were identified as *Enterococcus durans* and one isolates from breast milk as *Enterococcus faecium* based on the sequences of 16s rDNA.

1. Introduction

Recent data from the United Nations Environment Programme mentioned that the source of environmental mercury contamination is dominated by artisanal and small-scale gold mining (ASGM) (UNEP, 2000). The number of ASGM rose rapidly during 2006–2009 in Indonesia. District of Sekotong, Located in West Lombok, is one of the newly developed ASGM site in Indonesia that was first started in the middle of 2009 (Ismawati, 2010). Some recent studies had reported the negative output of the ASGM activity in Sekotong on environment and the miner's health (Krisnayanti et al., 2012; Ekawanti & Krisnayanti, 2015).

During the gold extraction, inorganic mercury is commonly used for amalgamation process. Because there is no appropriate waste management in ASGM, the excess mercury directly flows to the soil, ground water, and even sea. Mediated by microbial methylation reaction, that inorganic mercury is transformed into organic mercury, the most toxic form of mercury, such as methylmercury, phenyl mercury, and ethyl mercury and accumulated in living organism including human body (Zhang et al., 2012; Friberg & Mottet, 1989).

Bioremediation using bacterial cell had been introduced for many years and suggested as an ecofriendly and low-cost bioremediation agents (Alluri et al., 2007; Halttunen et al., 2007). However, most published bacterial bioremediation studies were designed for waste management. Lactic acid bacteria (LAB), the major bacteria group used for probiotic, exhibits many beneficial effects for human health. Some strains of LAB were reported to have ability to remove cadmium, arsenic, lead, mercury, and further decrease their toxicity (Halttunen et al., 2007; Bhakta et al., 2010; Abdel-Salam et al., 2012; Zhai et al., 2015; Allam et al., 2015). There was limited information regarding the role of LAB in methylmercury detoxification addressed for human body. To meet the

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future goal for the alternative safety methylmercury detoxification agent for human consumption, we isolated the human origin methylmercury-resistant LAB from breast milk and feces, then further investigated the methylmercury biosorption activity.

2. Materials and Methods

2.1. Sample sources

Ten breast milk and 12 stool samples obtained from 19 subjects from Gawah Pudak Village and Tembowong Village (Figure 1) were used as bacterial sources in this study. All the subjects were apparently healthy adults aged between 18 and 35 years, have been living in Sekotong area for more than 5 years, and did not consume any antibiotic for at least 2 months before sample collection. The procedure of sample collection had been accepted by Medical and Health Research Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada.

2.2. Isolation of methylmercury-resistant bacteria

Isolation of methylmercury-resistant bacteria was divided into two steps: first, isolation was performed using De Man, Rogosa, and Sharpe agar (MRSA) supplemented with 5 μg/mL of mercury chloride and followed by repurification onto MRSA supplemented with 5 μg/mL of methylmercury chloride. Each breast milk (1 mL) and stool samples (1 g) were diluted into 0.85% of sodium chloride to reach the appropriate dilution factor (10^4–10^8), inoculated into the agar plate, and then incubated anaerobically for 24–48 hours at 37°C. The morphologically different colonies were purified into MRSA containing 5 μg/mL of methylmercury, then streaked the growing colony into the fresh MRSA.

2.3. Screening of probiotic potential LAB

Screening of probiotic potential LAB was performed according to several basic criteria of probiotics. Gram staining and catalase test were conducted to screen the LAB. Afterward, all the gram-positive isolates and those showing negative result on catalase test were subjected to further screenings, which were resistance assay in low pH medium, resistance assay in bile salt supplemented media, and antimicrobial activity assay against some pathogenic bacteria. Isolates showing appropriate growth after 6 hours of incubation in low pH and bile salt media and also appropriate antimicrobial activity were selected for further investigation.

2.4. Methylmercury biosorption assay

Methylmercury biosorption activity of the selected isolates was evaluated in De Man, Rogosa, and Sharpe Broth containing 10 μg of methylmercury chloride. Approximately, 10^6 CFU/mL of each isolate culture was inoculated into the tested medium and incubated for 24 hours at 37°C. Thereafter, the culture was centrifuged for 15 minutes at 3,500*g*. The pellet and supernatant were separated and then subjected for methylmercury measurement using the gas chromatography flame ionization detector.

2.5. Identification of selected isolates

Three isolates showing the best methylmercury biosorption activity were further identified according to the sequences of 16s rDNA. The genomic DNA was extracted using microbial DNA isolation kit. Before DNA extraction, the isolate was cultured into De Man, Rogosa, and Sharpe Broth for 20–24 hours at 37°C. DNA amplification was performed using forward primer 5′-AGAGTTTGATCMTGGCTCAG-3′ and reverse primer 5′-GGTTACCTTGTTACGACTT-3′. The amplification condition was as follows: 96°C for 4 minutes as initial denaturation, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1.5 minutes, extension at 68°C for 8 minutes, and a final extension at 68°C for 10 minutes. The amplicons then were sequenced by 1st Base (Singapore). The sequencing results were aligned with some similar sequences obtained from GenBank database. The neighbor-joining analysis was used to construct the phylogeny tree.

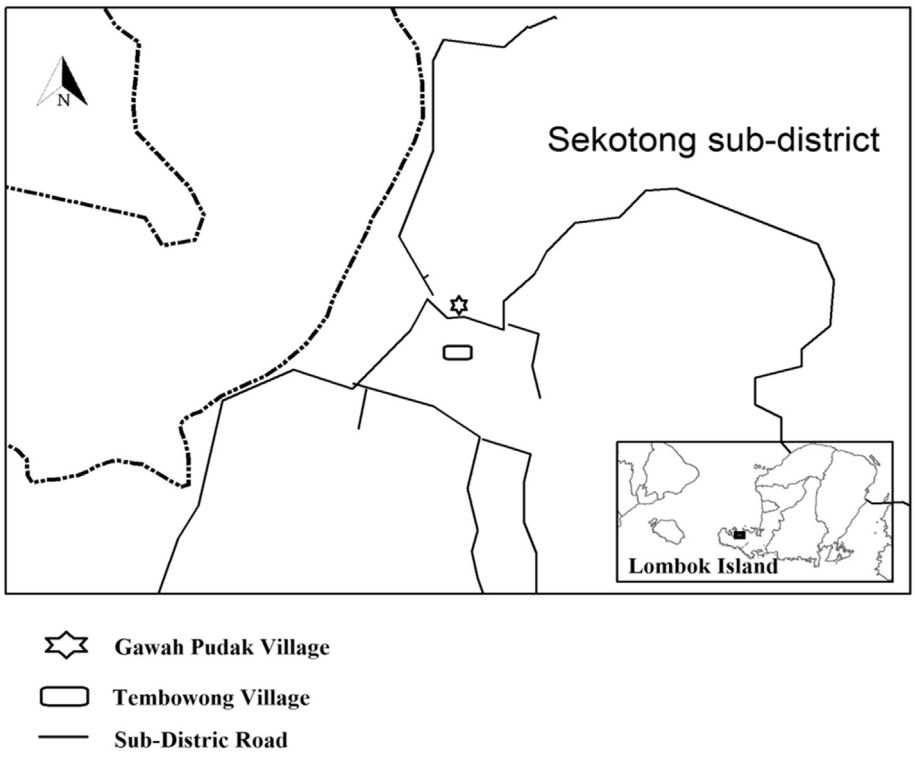

Figure 1. Sampling site location: Gawah Pudak and Tembowong Village.