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DNA Fingerprinting Among *Bacillus* Isolated From the Mercury Polluted Kalimas River Surabaya

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ABSTRACT

The Kalimas River, Surabaya was contaminated 0.105 ppm mercury. This value was higher than the government acceptable number. It meant the considerable action was needed to clean the river. One of those actions could be potential done by bioremediation using mercury resistant bacteria. Our previous study was successfully isolated and biochemically characterized 3 bacterial isolates, coded S1Hg, SS19Hg and DA11Hg. They tended to affiliate to *Bacillus* strand. This further study was aimed the characterized their molecular fingerprinting after *Alu*I digestion for accomplishing their biochemical characters. The molecular result showed that even they were *Bacillus*, but each had different fingerprinting pattern. This may indicate that they were different *Bacillus* strain.

KEYWORDS: mercury resistant bacteria, DNA genome fingerprinting, *Alu*I digestion.

INTRODUCTION

Up to present time mercury is a considerable environmental importance, since it is potentially toxic causing liver and kidney damage in humans and animals, and effect on neurological and renal disturbances and impartment of pulmonary function [1, 2, 3]. Naturally mercury present in an extremely low concentration of about 1 nanogram per liter [4, 5]. But if the amount is getting higher than environment acceptable value, it meant that environment is already polluted by mercury. The Kalimas River, Surabaya at middle part was detected contained 0.105 ppm mercury [6] as well as 6.3 ppm at downstream area around The Tanjung Perak, Surabaya Port [7]. This was a high number than the acceptable environmental number of 0.001 ppm [8]. Since the most mercury enters the environment as a toxic mercuric ion, ex. Hg^{2+}, the polluted environment needs to be concerned.

Some bacteria are able to transform a toxic mercuric ion Hg^{2+} to an elemental mercuric ion Hg^0 called mercury resistant bacteria. The mercury resistant bacteria express mercuric reductase. Mercuric reductase is an NADPH-linked enzyme for reducing mercuric ion Hg^{2+} to ion Hg^0 [9, 10, 11]. Mercuric ion Hg^0 is a less or even a not toxic and volatile mercury form. *Bacillus* is the most reported mercury resistant bacteria [12, 13, 14], which also had ability to reduce other toxic pollutant metals, ex. cadmium [15], chromium VI [16], cuprum and plumbum [17].

Previous study was already successfully biochemically characterized 3 bacterial isolates from the Kalimas River, coded S1Hg, SS19Hg and DA11Hg which were resistant in a 5 ppm $HgCl_2$ solid agar medium. Based on their biochemical characters, they tended to affiliate to *Bacillus* strand [7]. Since molecular methods also provide an excellent characterization for bacterial isolates [18], this paper was aimed to further molecular characterize those Kalimas bacterial isolates addressing their genome fingerprinting after *Alu*I digestion.

MATERIALS AND METHODS

DNA extraction.

A 24 hours bacterial culture of S1Hg, SS19Hg and DA11Hg was extracted using a commercial genome extraction kit following its manufacturer's protocols with a minor modification. The sample was spinned twice at 14.000 g for 30 seconds and the extracted genomic DNA was diluted twice, each in 25 µl PCR water. Extracted genomic DNA was measured qualitatively with agarose electrophoresis on 1.5% gels. As a reference *Bacillus subtilis* ATCC6633 and *Bacillus cereus* ATCC1178 was used. Both bacterial references were performed in the same way.

Enzyme digestion and agarose electrophoresis.

One µl containing approximately 100 ng of extracted genomic DNA was digested in 20 µl reaction volumes with 10 U of *Alu*I restriction enzyme for 2 hours at 37°C following the manufacturer's protocols. Afterwards the enzyme was inactivated at 65°C for 20 minutes. For about 10 µl digested product was loaded onto 1.5% agarose electrophoresis for restricted fragment separation.

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RESULTS AND DISCUSSION

The Kalimas Surabaya had been contaminated by mercury, therefore maintaining its sustainability must be under serious consideration, as the river is one of water main resources for the Surabaya City. As a scientific participating on that consideration, we were looking for a high potential bioremediation agent; in this study bacteria were isolated directly from the polluted river. We assumed that indigenous resistant bacteria may potential highly applicable in the particular field application.

Previous study we were successfully isolated and biochemically characterized 4 bacterial isolates from the Kalimas River, coded S1Hg, SA1Hg, SS19Hg and DA11Hg. They were very well growing on solid agar medium containing 5 ppm $HgCl_2$, Gram positive bacteria, producing endospora and responding positively to a catalase assay (Table 1). Those biochemical characters were tending them to affiliate to *Bacillus* strain. But among them there were also different biochemical characters (i.e. response to oxidase and manithol fermentation assays) that might be distinguishing their strain [7]. *Bacillus* reported have ability to transform a toxic mercuric ion Hg^{2+} to a less toxic elemental mercuric ion Hg^0 [12, 13, 14] *Bacillus* are also able to reduce other toxic pollutant metals, for instance cadmium [15], chromium VI [16], cuprum and plumbum [17].

Table 1. Biochemical characters among 3 bacterial isolates from the Kalimas Surabaya [7]

Clusters number of strains	S1	SA1	SS19	DA11	B.s*	B.c**
Motility	+	+	+	+	+	+
Cell shape : Rods	+	+	+	+	+	+
Gram stain	+	+	+	+	+	+
Endospore formed	+	+	+	+	+	+
Growth on/at :						
Sodium chloride 5 %	+	+	+	+	+	+
$HgCl_2$ 10 ppm	+	+	+	+	+	+
$PbCl_2$ 25 ppm	+	+	+	+	+	+
$CdCl_2$ 25 ppm	+	+	+	+	+	+
$CuCl_2$ 25 ppm	+	+	+	+	+	+
Ampicilin 10µg	+	+	+	+	+	+
Tetracycline 30µg	+	+	+	+	+	-
Chloramphenicol 30µg	+	+	-	-	-	-
Biochemical characterization						
Aerobic	+	-	+	-	-	-
Facultative anaerobe	-	+	-	+	+	+
Acid production from:						
Glucose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	-	-	-	-	-	-
Mannose	+	-	-	+	+	+
Sucrose	+	-	+	+	-	-
Lactose	-	-	-	-	-	-
Maltose	-	+	+	+	+	+
Mannitol	+	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
Yellow pigment (colony)	+	-	-	+	-	-
H_2S production	-	-	-	-	-	-
Indole production	-	-	+	-	-	-
Methyl Red	-	+	-	-	+	+
Vouges-Proskauer	+	-	+	-	-	-
Citrat, Simmons	-	-	-	-	-	-
Catalase production	+	+	+	+	+	+
Oxidase	-	+	-	+	-	-
Urease activity	+	+	+	+	+	+

***B.c. *Bacillus cereus* ATCC1178, **B.s. *Bacillus subtilis* ATCC6633**

Those biochemical assignments were then accomplished by a molecular study using a DNA genome fingerprinting. After *Alu*I digestion, it was clearly seen that there were differences among *Bacillus* strains (Figure 1). There were DNA fragments, 650 bp, 200 bp, 175 bp, 92 bp and 80 bp that present for all of the 3 *Bacillus* isolates (S1Hg, SA1Hg, SS19Hg and DA11Hg) as well as for bacterial references (*B. subtilis* ATCC6633 and *B. cereus* ATCC1178). Those DNA fragments may general DNA fragments for *Bacillus* strains, or it could mean that *Bacillus* strains must have those DNA fragments.

Unique fragment for a particular bacterial isolate was also detected as well in the gel electrophoresis. For instance isolate SS19Hg had 800 bp, 500 bp, 163 bp and 138 bp DNA fragments, but not for isolate SA1Hg and DA11Hg. Interestingly those fragments were also detected in both bacterial references, unless 163 bp and 138 bp only for *B. subtilis* ATCC6633. The DNA fragment 113 bp was only present for isolate DA11Hg and *B. subtilis* ATCC6633. Thus the unique DNA fragments may indicate the bacterial genomic deference among *Bacillus* strains.

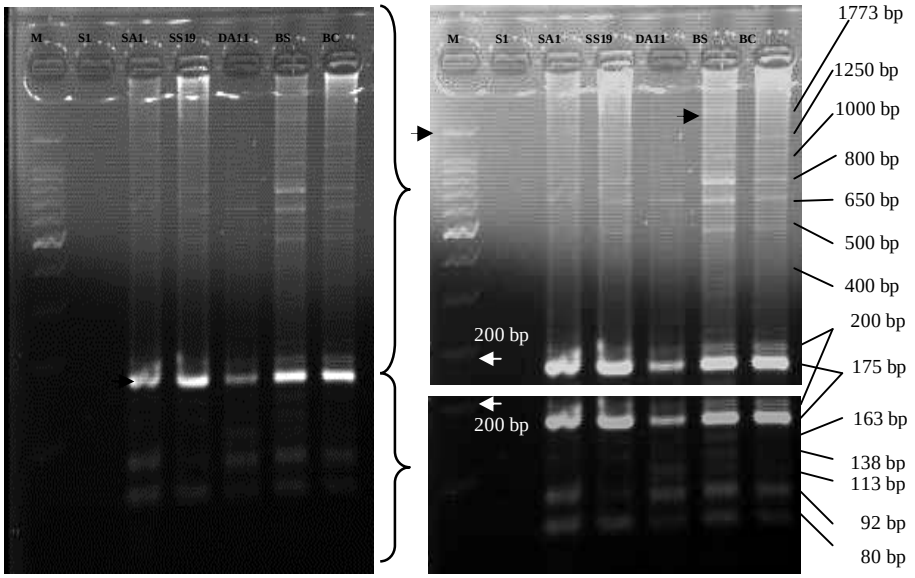

Figure 1. DNA fragment after *Alu*I digestion.

Based on this study, the genome fingerprinting supported the biochemical assays done in the previous study [7]; biochemical and molecular study showed that they were different strain. They were resistant mercury bacteria since they were growing very well in 5 ppm mercury containing solid agar medium. Anyhow another molecular study, 16S rRNA gene sequencing, may absolutely support the exactly bacterial characterization into a species name. But most of it, since those *Bacillus* isolates were mercury resistant bacteria. Another important study must be performed is exploring their ability to reduce toxic mercury ion Hg^{2+} to a less toxic mercury ion (Hg^0) individually or in a bacterial consortium in the laboratory scale or in field scale.

CONCLUSION

The 3 isolated bacteria from the Kalimas river after *Alu*I digestion showed a different DNA genome fingerprinting. This result supported the biochemical characters reported on the previous study. Even they were affiliated to *Bacillus* strand; but they were different strand.

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