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MERCURY REDUCTASE ACTIVITY OF AN INDIGENOUS MERCURY RESISTANT BACTERIAL ISOLATE (*Bacillus* sp. S1) FROM KALIMAS-SURABAYA AS A POTENTIAL REDUCING AGENT FOR MERCURIAL ION (HG^{2+})

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ABSTRACT

Mercury reductase activity and Hg^{2+} lowering capacity of a Mercury Resistant Bacteria (MRB) (*Bacillus* sp. S1) which was isolated from Kalimas River of Surabaya, Indonesia were studied. The activity was determined by Mercury Reductase Assay System (MRAS) in a solution mixture contained 50 mM PBS (pH ± 7.0), 0.5 mM EDTA, 200 µM MgSO4, 0.1% (v/v) ß-merchaptoethanol, 200 µM NADH2 and 25 mg/L $HgCl_2$ and one volume of crude extract incubated at room temperature for various interval period of time. Mercury reductase activity was measured spectrophotometrically at 340 nm. One unit of reductase activity was defined as one molar of oxidized $NADH_2$ produced per total cell per minute. Results of study showed that, the isolate could resist the concentration of $HgCl_2$ up to 11 mg/L. At 30 minute incubation period at room temperature, the highest mercury reductase activity and the Hg^{2+} lowering capacity was found to be 0.006 unit/109cell and 1.48 mg/L/109cell/minute, respectively with the reduction efficiency of Hg^{2+} to Hg^0 of 0.18% per minute. Therefore, it can be concluded that the *Bacillus* sp. S1 isolate could be assumed to be exellent mercury bioremediation agent since it was found to be highly mercury resistant and very efficient to reduce cationic mercury (Hg^{2+}) to elemental mercury (Hg^0).

Key words: Kalimas-Surabaya, *Bacillus* sp. S1, Mercury resistant, Mercury reductase

INTRODUCTION

Kalimas River in Surabaya, East Java province of Indonesia has been contaminated by mercury as indicated by the measured levels of mercury of 6.3 mg/Kg in the samples taken from downstream sediments of the river (Zulaika *et al*., 2012). The phenomenon was very worrying because mercury may cause neurotoxic effects on humans. However, in natural habitats, there are bacteria that might be able to resist mercury which are known as Mercury Resistant Bacteria (MRB). Such bacteria, possess enzymatic mechanism to reduce divalent mercury (Hg^{2+}) into volatile mercury of Hg^0 (Nascimento & Chartone-Souza, 2003) due to their possession of mercury resistant gene known as *mer-operon,* and *mer*A is one of *mer-operon* groups that code for mercury reductase (Kiyono & Pan-Hou, 2006.). Usually, *mer-operon* gene is located within plasmid or transposon (Davis *et al*., 2005). Plasmid is likely to carry a certain gene or more that might code for some special characters in bacteria, such as resistance and viability to antibiotics and heavy metals (Nithya *et al*., 2011).

Mercury reductase is an oxydoreductase which involves $NADPH_2$ or $NADH_2$ coenzyme in catalyzing electron replacement (Murray, 1996). As an electron source in oxidation-reduction process, $NADH_2$ or $NADPH_2$ are involved to change Hg^{2+} to be volatile Hg^0 or *vice versa* (Zeroual *et al*., 2003). The efficiency rate of changing Hg^{2+} to be volatile Hg^0 by *Pseudomonas putida* SP-1 MRB was reported to be 89% (Zhang *et al*., 2012) whereas *Hydrogenivirga* sp. 379 128 R1-1 was reported to reduce 150 ìmole $Hg^{2+}/10^6$cell/hour (Freed-

man *et al*., 2012). A bacterial isolate belong to the genus *Bacillus* isolated from Kalimas-Surabaya was found to be dominant among the MRB isolates, and *Bacillus* sp. S1 was one of them which was highly resistant to $HgCl_2$ concentration of 11 mg/L (Zulaika., 2013). This research aimed at measuring mercury reductase activity as well as Hg^{2+} lowering capacity of *Bacillus* sp. S1 as an indigenous MRB isolate from Kalimas-Surabaya Indonesia.

MATERIALS AND METHODS

Preparation of enzyme extracts

After 12 hour of incubation periods of culture, the culture of *Bacillus* sp. S1 was harvested by centrifugation at 15000 rpm for 20 minute at 4^{o}C. Crude cell extract was re-suspended in 30 ml Phospate Buffer Saline (PBS), pH ± 7.0. Cells were then disrupted by sonication with ultrasonic processor at 600 watt and 50% amplitude for 60 seconds, and the resultant homogenate was centrifuged at 15000 rpm for 30 minute at 4^{o}C. The supernatant was collected as crude enzyme extract and then removed into black and dark vial tube (Ogunseitan, 1998) to be used in further experiments.

Mercury Reductase Assay System (MRAS)

Mercury reductase activity was measured in a Mercury Reductase Assay System (MRAS) solution in dark tube contained 50 mM PBS (pH ± 7.0), 0.5 mM EDTA, 200 ìM $MgSO_4$, 0.1% (v/v) ß-merchaptoethanol, 200 ìM $NADH_2$ and 25 mg/L $HgCl_2$ (Ogunseitan, 1998). One volume of crude extract was added into MRAS solution and incubated at room temperature for various interval durationof 30, 60, 90, 120 and 150 minutes. Mercury reductase activity was measured spectrophotometrically at 340 nm (Takeuchi *et al*., 1999). A one unit of mercury reductase activity was defined as one molar of oxidized $NADH_2$ produced per total cell per minute in the assay condition (Zeroual *et al*., 2003).

RESULTS AND DISCUSSION

The MRB isolate was identified to be member of the genus *Bacillus* and named as *Bacillus* sp. S1 based on key characters (Holt *et al*., 1994) which were rod cell shape, gram positive, motile, forming endospore, aerobes, chemo-organotroph and catalase positive. (Table 1).

Table 1. Identification of *Bacillus* sp. S1 characters based on key characters of the genus *Bacillus*

No	Characters	Genus *Bacillus*	*Bacillus* sp. S1
1	Road	+	+
2	Gram	+	+
3	Motility	+	+
4	Endospore	+	+
5	Aerobes/ facultative anaerobes	+/+	+/-
6	Chemoorganotroph	+	+
7	Catalase	+	+

The genus *Bacillus* contains 18 sub-groups which form endospore, gram positive, and road cell shape (Holt *et al*. (1994). According to Maughan dan Auwera (2011), member of the genus *Bacillus* has abundant number in nature. Those strains are very adaptive to

environments by means of character variation. They might be antagonistic, synergistic, pathogenic or even beneficial. Certain *Bacillus* strains could produce antibiotic, insecticide, hence they could be used as bioremediation agent. Another *Bacillus* strain member may be tolerant to higher osmotic pressure. Their optimum growth temperature is around 30 - 45ºC (mesophilic).

Bacillus sp. S1 isolate was found to be tolerant to antibiotic, such as ampicillin, tetracycline and chloramphenicol (Zulaika *et al*., 2012), indicated that *Bacillus* sp. S1 contained a plasmid. In MRB, it has been clear that plasmid codes for one amongst genes coding for heavy metal resistance, and *mer*A is the gene which codes for mercury reductase and therefore they are resistant to mercury and able to reduce Hg^{2+} into Hg^{0} (Kiyono & Pan-Hou, 2006). Mercury reductase activity of *Bacillus* sp. S1 isolate in various incubation period is shown in Table 2.

Table 2. Mercury reductase enzyme activity, oxidized NADH and reduced Hg^{2+}

Periods (minutes) *	Enzyme activity (unit /10^9cell)	Oxidized $NADH_2$ (µM/10^9cell /minute)	Reduction of Hg^{2+} * (mg/L/10^9cell /minute)	% Reduction of Hg^{2+} /minute
30	0.0060 ± 0,0003	0.179 ± 0,028	1.480^{a} ± 0,19	0.18
60	0.0033 ± 0,0002	0.146 ± 0,023	0.487^{b} ± 0,08	0.12
90	0.0023 ± 0,0001	0.109 ± 0,018	0.243^{c} ± 0,04	0.09
120	0.0019 ± 0,0003	0.100 ± 0,030	0.167^{d} ± 0,05	0.08
150	0.0016 ± 0,0002	0.087 ± 0,025	0.117^{e} ± 0,03	0.07

***) Incubation periods affected to Hg^{2+} reduction. Numbers with different alphabet on beside shown significant differs ($p < 0.05$; a > b > c > d)**

Based on Table 2, the highest mercury reductase activity appeared to be at 30 minute incubation period which was 0.0060 unit/10^9cell either expressed as oxidized $NADH_2$ amount 0.221 µM/10^9sel/minute or as the amount of reduced Hg^{2+} 1.480 mg/L/10^9sel/minute as shown in Figure 1. This might be due to the fact that the longer the incubation period, the lower substrate availability and the less intact enzyme quantity, and therefore autaomatically decreased the oxidized $NADH_2$ production as well as lowered the Hg^{2+} reduction.

Bacillus sp. S1 as MRB was isolated from downstream of Kalimas River of Surabaya with 6.3 mg/Kg level of mercury (Zulaika *et al*., 2012) hence it was ecpected that it could adapt to survive in medium containing mercury. It was reported that indigenous MRB isolate from contaminated mercury area were found to be much more adaptive to Hg^{2+} than the isolate originated from incontaminated area due to its effectiveness in reducing Hg^{2+} (Ghoshal *et al*., 2011). Therefore, the isolate of *Bacillus* sp. S1 could be expected to be very potential to be used as mercury bioremediation agent. This isolate was exellent in terms of mercury reductase enzyme productivity, and capacity to reduce Hg^{2+} to Hg^{0} by means of oxidation of $NADH_2$. The isolate could resist the concentration of $HgCl_2$ up to 11 mg/L. The highest mercury reductase enzyme activity (0.006 unit/10^9cell) and the Hg^{2+} lowering capacity of 1.48 mg/L/10^9cell/minute with 0.18% per minute reduction efficiency of Hg^{2+} to Hg^{0} was observed at 30 minute incubation period at room temperature.

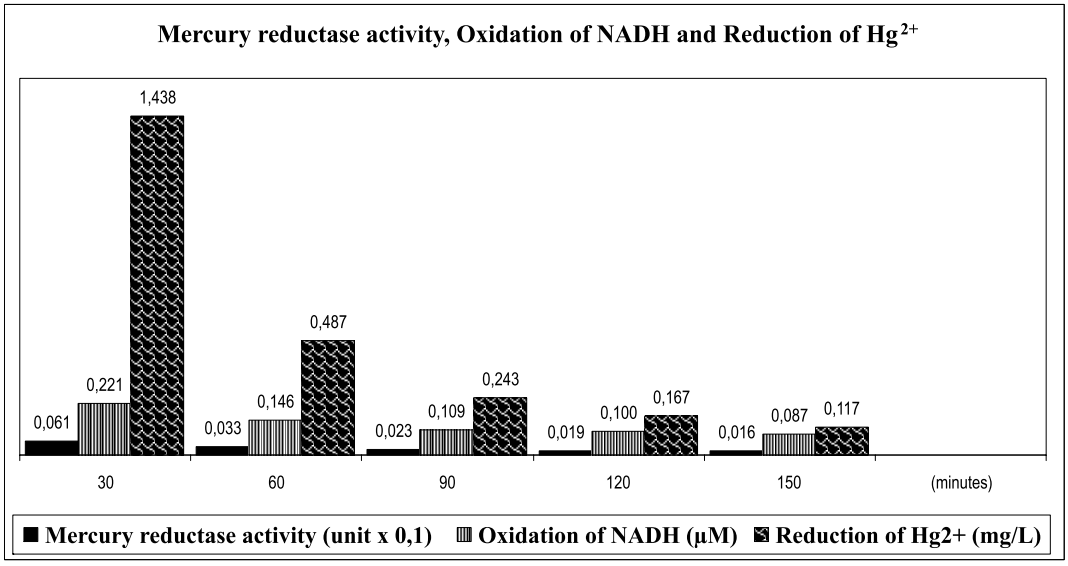

Figure 1. Mercury reductase activity (unit x 0,1), Oxidation of $NADH_2$ (µM) and Reduction of Hg^{2+} (mg/L/10^9cell/minute) by *Bacillus* sp. S1 isolate.

CONCLUSION

Bacillus sp. S1 isolate could be assumed to be exellent mercury bioremediation agent since it was found to be highly mercury resistant. The isolate could resist the concentration of $HgCl_2$ up to 11 mg/L. At 30 minute incubation period at room temperature, the highest mercury reductase activity and the Hg^{2+} lowering capacity was found to be 0.006 unit/10^9cell and 1.48 mg/L/10^9cell/minute, respectively with the reduction efficiency of Hg^{2+} to Hg^0 of 0.18% per minute.

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