Removal of Mercuric Chloride by a Genetically Engineered Mercury-Volatilizing Bacterium *Pseudomonas putida* PpY101/pSR134

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Mercury is known to be one of the most toxic metals. Many areas in the world are contaminated by industrial use of mercury, constituting serious environmental problems (Moreira et al. 1996; Straaten 2000). Removal of mercury from industrial wastewater has been achieved by means of ion-exchangeable resin and several chemical processes (Habashi et al. 1978). However, chemical processes of mercury removal require enormous quantities of chemicals and are very expensive. Therefore, new cost-effective and environmentally friendly technologies for the removal of mercury are needed. There is currently great interest in bioremediation, a technology that is known to be cost-effective and clean. Therefore, the biological removal of mercury has received strong attention. Processes for biological mercury removal include mainly sorption (Chang and Hong 1994; Mamaril et al. 1990), accumulation (Chen and Wilson 1997) and reduction (Chang and Law 1998; von Canstein et al. 1999). It is well known that mercuric ion reduction by mercury-resistant microorganisms is conferred by the *mer* operon which consists of the genes *merR*, *T*, *P*, and *A* (Brown et al. 1986; Foster and Ginnity 1985; Ni'Bhraïain et al. 1983; Misra et al. 1983). The *merR* is a regulatory gene that controls transcription of *mer* operon (O'Halloran and Walsh 1987; Ross et al. 1989; Summers 1992). MerT (inner membrane) and MerP (periplasmic) are mercuric transport proteins (Hamlett et al. 1992). The *merA* gene encodes the flavoprotein mercuric reductase which catalyzes the reduction of mercuric ion (Hg\(^{2+}\)) to elemental mercury (Hg\(^{0}\)) (Fox and Walsh 1982; Brown et al. 1983). While many molecular biological studies on mercury-resistant microorganisms have been reported, there are only a few studies aimed at their application for removal (Chang and Law 1998; von Canstein et al. 1999). The present study was designed to apply a mercury-volatilizing bacterium to the treatment of wastewater containing mercury. We examined the removal of mercuric chloride from aqueous solution by growing cells and resting cells of the mercury-volatilizing bacterium, *Pseudomonas putida* PpY101/pSR134 (Iwasaki et al. 1993).
MATERIALS AND METHODS

Genetically engineered *P. putida* PpY101/pSR134, which can volatilize mercuric ion to elemental mercury was used for experiments (Iwasaki et al. 1993). Plasmid pSR134 (18.6 kb) was constructed by inserting two *EcoRI* DNA fragments, H (4.9 kb) and I (4.2 kb), encoding the mercury resistant gene from the *NRI* plasmid into a broad-host-range vector pSUP104 (9.5 kb). A stock culture of *P. putida* PpY101/pSR134 at -80°C was precultured on L-agar (tryptone 10 g/l, yeast extract 5 g/l, NaCl 5 g/l, glucose 1 g/l, agar 1.5%) containing 20 mg/l of mercuric chloride, and incubated at 30°C for 2 days. One loopful of grown cells was inoculated into 2 ml of L-broth containing 20 mg/l of mercuric chloride and incubated at 30°C overnight with shaking (140 rpm). The overnight culture was inoculated into 100 ml of L-broth containing 20 mg/l of mercuric chloride and incubated at 30°C with rotary shaking (160 rpm) until the late-log phase. Cell growth was determined by optical density measurements (at 600 nm).

For growing-cell studies, 1 ml of late log-phase culture was added to 69-ml serum bottles with 9 ml of sterile 0.1 × L-broth (tryptone 1 g/l, yeast extract 0.5 g/l, sodium chloride 0.5 g/l, glucose 0.1 g/l) containing mercuric chloride. The 0.1 × L-broth was used in removal of mercury experiments, because it showed that mercury concentration decreased in L-broth without bacterial cells (data not shown).

For resting-cells studies, late log-phase cells were harvested (6000 × g, 10 min, 4°C) and washed with 50 mM phosphate buffer (pH 7.0), and then suspended in 50 mM phosphate buffer. The cell suspension added to 155 ml serum bottles with 9 ml of sterile 50 mM phosphate buffer (pH 7.0) containing mercuric chloride. The bottles were sealed with teflon rubber caps and aluminum rings and incubated at 30°C with shaking.

The total mercury concentration was determined with mercury atomizer unit (Model 2538, Sugiyamagen Co., Ltd., Tokyo, Japan) applying heat-vapor atomic adsorption photometry. The mercury concentration in supernatant was determined by mercury analyzer (Model 330, Sugiyamagen Co., Ltd.) applying cold-vapor atomic absorption photometry. To determine the mercury concentration in the supernatant, sampled culture or buffer from the flasks were centrifuged (6000 × g, 10 min, 4°C) to remove cells, and the supernatants were used.

RESULTS AND DISCUSSION

The effect of mercuric chloride concentration on the removal of mercury and cell growth in 0.1 × L-broth was investigated. Figure 1a, b shows that the almost 100% of