Construction and Characterization of Escherichia coli Genetically Engineered for Bioremediation of Hg\(^{2+}\)-Contaminated Environments

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Escherichia coli strains were genetically engineered to express an Hg\(^{2+}\) transport system and metallothionein. Overexpression of a glutathione S-transferase fusion protein of Saccharomyces cerevisiae or pea metallothionein significantly increased the bioaccumulation of Hg\(^{2+}\) transported by MerT and MerP and protected the cells from the accumulated Hg\(^{2+}\). The recombinant strains have excellent properties for bioremediation of Hg\(^{2+}\)-contaminated environments.

Mercury is one of the most toxic heavy metals in the environment. Adsorption methods for removing Hg\(^{2+}\) with either ion-exchange resins or biosorbents have been shown to be sensitive to ambient conditions, e.g., pH, ionic strength, and the presence of other inorganic and organic components (4, 13). They also fail to remove and recover metal ions when they are complexed or adsorbed on soil. Finally, adsorption methods lack specificity in metal binding, which may cause difficulty in the recovery and recycling of the desired metal(s).

Intracellular-bioaccumulation processes with microorganisms optimized by genetic engineering could overcome the deficiencies of common metal cleanup processes and may be an alternative for removal and recovery of heavy metals such as Hg\(^{2+}\) from contaminated water or soil. In this study, genetically engineered Escherichia coli strains were constructed to simultaneously express an Hg\(^{2+}\) transport system and overexpress a metallothionein (MT) as a carboxyl-terminal fusion to glutathione S-transferase (GST-MT), and the strains’ ability to accumulate Hg\(^{2+}\) was investigated.

Overexpression of MT. MTs are a class of low-molecular-weight metal-binding proteins rich in cysteine residues (8). They are capable of binding a variety of heavy metals, including Hg\(^{2+}\) and Cd\(^{2+}\). A cloned and overexpressed MT would allow a microorganism to hyperaccumulate the metal transported by a metal transport system. Though a number of MTs have been expressed in E. coli, their stability has been a problem (2). In this study, a GST fusion system (16) was used for overexpressing and stabilizing MT. Figure 1 shows the construction of plasmid pGYMT, which codes for Saccharomyces cerevisiae MT (YMT) fused to GST (GST-YMT). The YMT gene coding sequence was isolated as a 0.2-kb HI digestion and ligated into the BamHI site of pGEX-2T (16) to yield pGYMT and pGRYMT, in which a gene coding sequence was isolated as a 0.2-kb BamHI fragment and ligated into the BamHI site of pGYMT (Fig. 2, lane 5). This was expected due to an in-frame stop codon at the 5’ end of the reversed YMT sequence. While GST-YMT is soluble in E. coli, the GST-HMT fusion protein (Fig. 2, lane 1) formed inclusion bodies after induction. Therefore, GST-HMT was not tested for Hg\(^{2+}\) bioaccumulation. GST-YMT, GST-HMT, and GST-pea (Pisum sativum L.) MT (GST-PMT) (17) was expressed at approximately 25% of the total protein in E. coli, as determined by laser densitometry of stained gels.

Construction of strains expressing MerT-MerP and GST-MT. The products of the merT and merP genes catalyze Hg\(^{2+}\) transport across the cell membrane (10). To express the Hg\(^{2+}\) transport system and GST-MT simultaneously, the ColE1-compatible vector pCL1921 (9) was used to clone the merT and merP genes from pDU1358. Plasmid pCLTP (Table 1), containing merT and merP, was constructed by digesting plasmid pDH1 (11) with HindIII and EcoRI. The 1.8-kb fragment containing merT and merP was inserted between the HindIII and EcoRI restriction sites of pCL1921, producing pCLTP. E. coli strains harboring pCLTP or pCL1921 were transformed separately with plasmid pGEX-2T, pGYMT, pGRYMT, pGHMT, or pGPMT3 (17) by electroporation (6). Transformed cells were selected on Luria-Bertani agar (LB) plates containing ampicillin and spectinomycin.

Hg\(^{2+}\) resistance. To test Hg\(^{2+}\) resistance, E. coli cells harboring various plasmids were grown in LB containing the appropriate antibiotics. When the optical density at 600 nm (OD\(_{600}\)) reached 0.5 to 0.7, isopropyl-\(\beta\)-D-thiogalactopyranoside (IPTG) (Sigma) was added to 1.0 mM and Hg\(^{2+}\) was added to various final concentrations. Cells were then incubated at 37°C and the OD\(_{600}\) was read at various times. The expression of the merT and merP genes in pCLTP caused E. coli cells to be hypersensitive to Hg\(^{2+}\), leading to cell lysis (data not shown). As shown in Fig. 3, the growth of induced JM109(pCLTP/pGRYMT) cells, expressing the Hg\(^{2+}\) transport system but not GST-YMT, was inhibited by 5 \(\mu\)M Hg\(^{2+}\).
while the growth of JM109(pCLTP/pGYMT) cells, expressing both the Hg$^{2+}$ transport system and GST-YMT, was not. pET3a-MT0 and pGPMT3, containing the YMT and GST-PMT genes, respectively, also caused \textit{E. coli} cells to be resistant to Hg$^{2+}$ after induction, while pGHMT, encoding the production of GST-HMT as inclusion bodies, did not. The resistance to Hg$^{2+}$ of the recombinant strains is significant since it would allow the cells to be retained inside a reactor when the strains are used for bioremediation of Hg$^{2+}$-contaminated environments and would make it possible to eventually recover the accumulated Hg$^{2+}$.

\textbf{Hg$^{2+}$ bioaccumulation.} To measure Hg$^{2+}$ bioaccumulation, induced cells were harvested by centrifugation, washed, and resuspended at a final OD$_{600}$ of 1.0 in either LB (pH 7.0) containing 30 \textmu g of chloramphenicol per ml, MOPS [3-(N-morpholino)propanesulfonic acid] minimum medium (pH 7.3) (12), or phosphate buffer (pH 7.0), all containing 5 \textmu M Hg$^{2+}$; cells were then incubated at 37°C for an additional hour, harvested by centrifugation, and washed twice with LB containing chloramphenicol. The Hg$^{2+}$ content of the harvested cells was determined by cold-vapor atomic absorption spectroscopy (7). To assay accumulation with time, cells were harvested at various times by filtration with 0.45-\textmu m-pore-diameter nitrocellulose filters (Millipore), and each entire filter with cells was subjected to Hg$^{2+}$ analysis. A 5 \textmu M Hg$^{2+}$ concentration was

\begin{table}
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\caption{Plasmids and strains}
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Strain or plasmid & Description & Reference or source \\
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\textbf{Strains} & & \\
JM109 & recA1 supE44 endA1 hisD17 merA96 relA1 thiA1 Δ(lac-proAB) F' (traD36 proAB lacI2 lacZΔM15) & 18 \\
XL1-blue & supE44 hisD17 recA1 endA1 hisD17 relA1 lac F' [proAB lacI2 lacZΔM15] Tn10 (Tet') & 3 \\
\hline
\textbf{Plasmids} & & \\
pCL1921 & Cloning vector; pSC101 origin; Spt'; lac promoter & 9 \\
pDH1 & Ap'; Contains the entire broad-spectrum mer operon of pDU1358 except merD & 11 \\
pCLTP & Spt'; pCL1921 with the 1.8-kb HindIII-EcoRI fragment, containing the merT and merP genes, of pDH1 & This work \\
pGEX-2T & GST gene fusion vector; ColEl origin; Ap'; lacI2; lac promoter & 16 \\
pET3a-MT0 & Ap'; Contains the YMT gene & 15 \\
pGYMT & Ap'; pGEX-2T containing the GST-YMT gene & This work \\
pGRYMT & Ap'; pGEX-2T containing the YMT gene in the reverse orientation & This work \\
pING1-HMT & Ap'; Contains the HMT-II gene & 14 \\
pGHMT & Ap'; pGEX-2T containing the GST-HMT gene & This work \\
pGEX-3X & GST gene fusion vector with a multiple-cloning site different from that of pGEX-2T & 16 \\
pGPMT3 & Ap'; pGEX-3X containing the GST-PMT gene & 17 \\
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used for the bioaccumulation assay since it is in the concentration range of Hg^{2+}-contaminated groundwater monitored in industrial sites of the United States (1).

As shown in Fig. 4, E. coli cells without an Hg^{2+} transport system yielded no significant accumulation of Hg^{2+} (bar A). In the presence of an Hg^{2+} transport system, intracellular accumulation of Hg^{2+} was significantly increased (bar B). Cells expressing an Hg^{2+} transport system and either GST-YMT (bar C) or GST-PMT (bar D) accumulated approximately five-fold more Hg^{2+} than cells expressing merT and merP but not MT (bar B). These results indicate that both an Hg^{2+} transport system and MT were required for E. coli cells to accumulate the highest level of Hg^{2+}.

We further tested Hg^{2+} bioaccumulation by strains expressing either GST-YMT or GST-PMT and expressing MerT-MerP under various conditions. It was found that bioaccumulation from phosphate buffer containing an initial concentration of 5 \mu M Hg^{2+} was fast, as over 90% of the maximum accumulation was reached within the first 10 min of incubation, and at the maximum accumulation, over 80% of the total added Hg^{2+} was removed (data not shown). Bioaccumulation was not enhanced in MOPS minimum medium, which contains various nutrients, or by the addition of a carbon source such as glucose (data not shown). These features are significant for engineering the bioaccumulation process for Hg^{2+} removal and recovery. In another paper (5), we documented the bioaccumulation of Hg^{2+} at various initial concentrations and the resistance of the recombinant strains to ambient conditions, such as pH, ionic strength, and the presence of metal chelators, as well as their selectivity against other metals, such as sodium, magnesium, and cadmium. Metal chelators and complexing agents, such as EDTA and cyanides, have been used in a broad range of industrial processes and have been found to interfere with metal cleanup processes. Our studies indicate that neither EDTA, citrate, nor cyanide (data not shown) affected Hg^{2+} bioaccumulation. Hg^{2+} bioaccumulation was also found to be unaffected by Na^{+}, Mg^{2+}, and Cd^{2+}. Besides high affinity and selectivity, the Hg^{2+}-bioaccumulating strains exhibited resistance to extremes of pH and ionic strength. As common Hg^{2+} cleanup processes, e.g., ion exchange, are sensitive to the presence of copollutants and ambient conditions, these features of the Hg^{2+}-bioaccumulating strains are significant. The strains may be used to remove Hg^{2+} selectively from contaminated waters which are resistant to common treatments. The high specificity of the Hg^{2+} bioaccumulation process may make it feasible to recover and recycle the accumulated Hg^{2+}. Further studies are under way to improve the specificity and affinity of the Hg^{2+} bioaccumulation process and to design and test bioreactors for the Hg^{2+}-bioaccumulating strains.

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