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Bacterial Mercury Resistance of Tn*MERI1* and Its' Application in Bioremediation

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Abstract—Mercury resistance is the only bacterial metal resistance system whose mechanism leads to a delicate transformation of its toxic target. The mechanism involves the reduction of the highly reactive cationic form of mercury into metallic vapor. The genes, which are responsible for this resistance, are organized in an operon called *mer* operon. The mechanisms of other cation and oxoanion resistances are mainly based on efflux pumps or extracellular sequestration. Thus, there has been interested in *mer*'s role in the global cycling of mercury and in employing the resistance mechanism for remediation efforts. In our previously study, Tn*MERI1* was found as the first mercury resistance transposon identified from Gram-positive bacteria and was harbored by a Minamata bay sediment isolated bacterial strain which was designated as *Bacillus megaterium* MB1. The *mer* operon encoded by Tn*MERI1* has *merR*, *merT*, *merP*, *merA* and *merB* gene, which code for metal specific activator-repressor, transporting, extra cellular metal ion binding, mercuric reductase and organomercurial lyase, respectively. These genetic elements provide us tremendous resources for bioremediation of heavy metals. The fundamental studies and applications of the operon genes have demonstrated by our laboratory. The membrane component MerP has been used as biosorbents for heavy metals in both bacterial and plant hosts. We also found that MerP can act as free radical scavenger and the MerP transgenic plant was enhanced in abiotic stresses resistance. Furthermore, *merA* gene was transformed into *Chlorella* to remove mercuric ion from aqua environment and the transgenic *Chlorella* may useful in mercury removal of flue gas from coal-fired power plant.

Keywords: bioremediation, mercury resistance, *mer* operon, Tn*MERI1*

INTRODUCTION

As a response to toxic mercury compounds globally distributed by geological activities from the ancient, microbes have developed some astonishing arrays of dedicated resistance systems to overcome the poisonous environment (Barkay *et al*., 2003). Among those resistance systems, one based on clustered genes in an operon (*mer* operon) to detoxify mercuric ion into volatile metallic mercury by enzymatic reduction has been intensively studied (Osborn *et al*., 1997; Barkay *et*

al., 2003). The *mer* operons, with some genetic variation in structure, are constituted by genes encoded the functional proteins for regulation (*merR*), transport (*merT*, *merP*) and reduction (*merA*). In some cases, known as broad-spectrum mercury resistance, additional *merB* genes are required to confer resistance to many organomercurials, such as phenylmercuric acetate (PMA), by cleaving the C–Hg bond before mercuric ion reduction. Although *mer* operons are found in a wide range of bacteria, only a few examples exist for *mer* operon in Gram-positive bacteria (Laddaga *et al*., 1987; Wang *et al*., 1989; Bogdanova *et al*., 1998). However, a *mer* operon found in a Gram-positive bacterium isolated from Boston Harbor, USA, *Bacillus cereus* RC607, is spread globally and represents a typical resistance system of Gram-positive bacteria (Nakamura and Silver, 1994; Osborn *et al*., 1997; Bogdanova *et al*., 1998; Huang *et al*., 1999a, b). In our previous study, we showed that the *B. cereus* RC607-type mercury resistance module is encoded in a class II transposon from Gram-positive bacteria that confers a broad-spectrum mercury resistance on the host strain *B. megaterium* MB1 (Huang *et al*., 1999a). This result indicates that the horizontal spread of *mer* operons among the Gram-positive bacteria is mediated by transposons, as also shown for the Gram-negative bacteria. We designated the class II transposon encoded in *B. megaterium* MB1 as Tn*MERI1*, and it offers a good example to understand the complete structure of the typical mercury resistance module of Gram-positive bacteria and to clarify the function of each gene (Huang *et al*., 1999a, b). This bacterial mercury resistance system represents not only a model for biological detoxification of heavy metals but also offers valuable genetic elements for approaches in bioremediation.

STRUCTURE ANALYSIS OF Tn*MERI1*

Genetic variation of *mer* operons was also studied by sequence analysis of both the operon structure and the corresponding genes in several *mer* operons (Osborn *et al*., 1997). An interesting structural heterogeneity between Gram-negative and Gram-positive bacterial *mer* operons is the transcriptional directions of *merR* and other operon genes. In general, *merR* of Gram-negative bacteria is separated from the remaining genes by the *mer* operator/promotor region and is divergently transcribed. These resistance operons have been found in class II (Tn*3*-like) typified by encoding inverted repeats (IRs) of about 38 bp DNA sequence, transposase (*tnpA*), resolvase (*tnpR*), and the *res* site at which resolvase acts (Grinsted *et al*., 1990). However, *merR* of Gram-positive bacteria is the first gene in the operon and is transcribed in the same direction as other genes (Laddaga *et al*., 1987; Wang *et al*., 1989). The dissemination of *mer* operons in Gram-positive bacteria has not been thoroughly studied and only a few examples of *mer* operons exist that demonstrated the involvement of transposons (Bogdanova *et al*., 1998). In our previous study, a unique transposon was found in the chromosome of *Bacillus megaterium* MB1, a Gram-positive bacterium isolated from mercury-polluted sediments of Minamata Bay, Japan. The transposon region of a 14.5 kb DNA fragment was amplified by PCR using a single PCR primer designed from the nucleotide sequence of an inverted repeat of class II

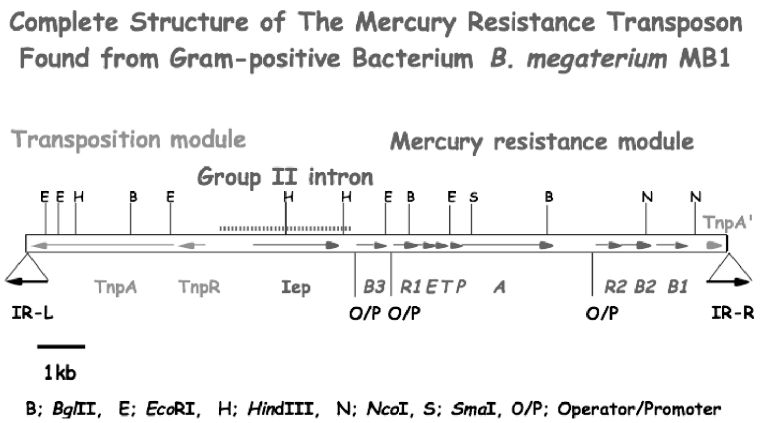

Fig. 1. Complete genetic structure of Tn*MERI1* (Huang *et al*., 1999a).

transposons. The molecular analysis revealed that the PCR-amplified DNA fragment encodes a transposition module similar to that of Tn*21*. The transposon also encodes a broad-spectrum mercury resistance region having a restriction endonuclease map identical to that of *Bacillus cereus* RC607, a strain isolated from Boston Harbor, USA. The result of a phylogenetic analysis of the amino acid sequence of putative resolvase of the transposon showed that the transposon is phylogenetically closer to the transposons of Gram-positive bacteria than those of Gram-negative bacteria. The complete genetic structure of the mercury resistance module is organized as P/O_{merB3}–*merB3*–P/O_{merR1}–*merR1*–*merE*-like–*merT*–*merP*–*merA*–P/O_{merR2}–*merR2*–*merB2*–*merB1* (Fig. 1) (Huang *et al*., 1999b). Besides the transposition module and *mer* operon, the transposon encodes a mobile genetic element of bacterial group II introns between the resolvase gene and *mer* operon. The intron, however, does not intervene in any exon gene (Chien *et al*., 2008, 2009). The discovery of this newly found combination of the complex mobile elements offer a clue to understanding the horizontal dissemination of broad-spectrum mercury resistance among microbes.

APPLICATION OF MerP AS HEAVY-METAL BIOSORBENT

MerP protein possesses a highly conserved domain with two cysteine residues for mercuric ion binding. The Gram-positive baterial originated MerP protein locates on outer membrane of bacterial cell and functions for recognition and transporting of mercuric ion. The MerP protein originated from *mer* operon of Tn*MERI1* possesses adjoining two cysteines while that of Gram-negative bacterium *Pseudomonas* sp. K-62 contains two cysteines separated by spacing two other residues. Both heterogeneous *merP* genes were cloned and over-expressed in *Escherichia coli* BL21 hosts. The resulting recombinant strains exhibited a six- to eightfold increase in Hg^{2+} resistance and 10% increase in Hg^{2+}

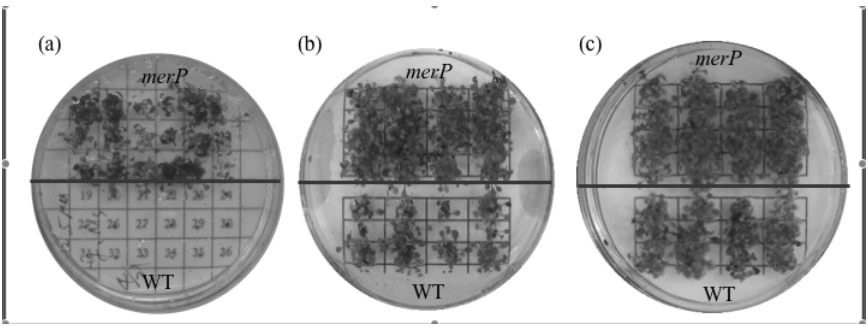

Fig. 2. Heavy metal tolerance assays. Growth comparison of transgenic (*merP*) and wild type (WT) plants grew in the MS medium agar plate containing: (a) 10 μM mercuric chlorides, (b) 15 μM cadmium chlorides, and (c) 32.5 μM lead chlorides after 2 weeks (Hsieh *et al.*, 2009).

adsorption capacity. The *merP* over-expressed strain harboring Gram-positive *merP* had 142, 84 and 33% increase for Cu^{2+}, Cd^{2+} and Pb^{2+} adsorption capability, respectively, over that of *merP*-free host cells. The strain carrying Gram-negative *merP* also increased 47, 55 and 12% for Cu^{2+}, Cd^{2+} and Pb^{2+} adsorption, respectively. Multi-metal biosorption experiments showed that the affinity of metal biosorption by the recombinant strains decreased in the order of Cu > Pb > Cd. Peptides containing the amino acid sequences of metal-binding motif for both heterogeneous MerP proteins were chemically synthesized and covalently immobilized on Celite carriers to examine their metal-binding ability. For *Bacillus* MerP-originated peptides, the binding capacity was 0.72, 0.45 and 0.36 mol/mol peptide for Cu, Cd and Pb, respectively, while the capacity was 0.51, 0.45 and 0.31 mol/mol peptide for Cu, Cd and Pb, respectively, for the peptide containing *Pseudomonas* MerP metal-binding motif. These results may show that MerP with adjoining cysteines seems to be more effective in binding metals than that with two separated cysteines (Huang *et al.*, 2003).

EXPRESSION OF *merP* GENE IN PLANT FOR PHYTOREMEDIATION OF HEAVY-METALS

While phytoremediation has recently been proposed as a low cost and environmentally friendly way to remove heavy metals from contaminated soils, genetic manipulation of plants would allow us to both optimize natural plant processes and further supplement them with novel traits from organisms outside the plant kingdom. Although phytovolatilization via *merA* reductase has been intensively studied for the control of mercury (Rugh *et al.*, 1996, 1998; Bizily *et al.*, 2003; Ruiz *et al.*, 2003), the aforementioned Gram-positive MerP protein has not been expressed in plant to study the metal accumulation ability. In addition, a bacterial heavy-metal transporter, ZntA, has been successfully expressed in plasma membrane of the transgenic *Arabidopsis* (Lee *et al.*, 2003). The information encouraged us to further express the bacterial MerP protein in plant for heavy

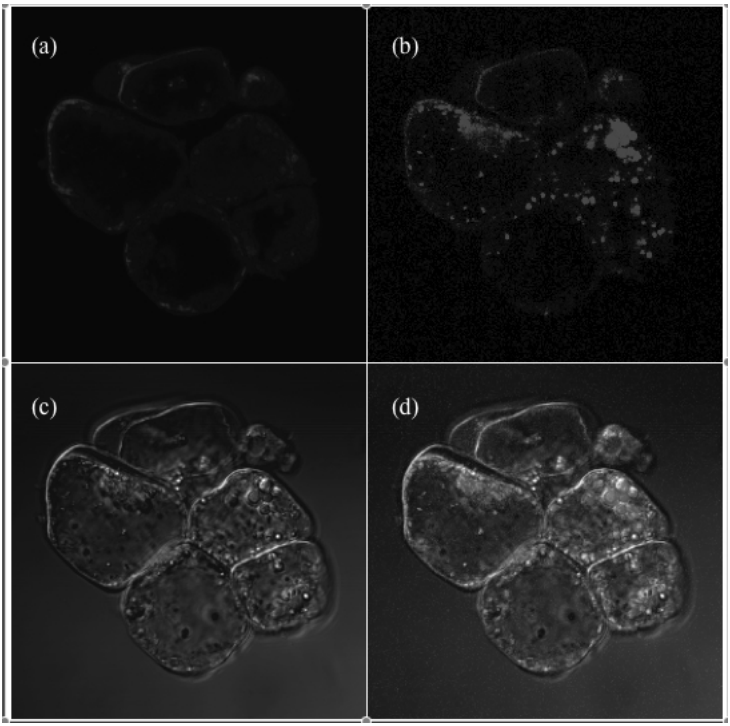

Fig. 3. Localization of MerP in transgenic plant: (a) Callus membrane was stained with Nile red (red), (b) MerP with antibody detected by Fluorescein (FITC)-conjugated AffiniPure Goat Anti-Rabbit IgG (H + L) (green), (c) white light, (d) colocalization of fluorescet signals (Huang *et al*., 2009).

metal removal from soil environment. Since no natural plants have been identified reproducible to hyperaccumulatemercury, our transgenic plantmay provide an optional strategy to tackle the mercury-contaminated problem. The Gram-positive MerP protein was expressed in transgenic *Arabidopsis* to create a model system for phytoremediation of heavy metals. Under control of an actin promoter, the transgenic *Arabidopsis* showed higher tolerance and accumulation capacity for mercury, cadium and lead when compared with the control plant (Fig. 2). Results from confocal microscopy analysis also indicate that MerP was localized at the cell membrane and vesicles of plant cells (Fig. 3). The developed transgenic plants possessing excellent metal-accumulative ability could have potential applications in decontamination of heavy metals.

EXPRESSION OF MerP PROTEIN AS ROS SCAVENGER

Since the thiol groups could be oxidized to form disulfide bond, and release electrons to eliminate free radical, we examined that MerP protein could act as reactive oxygen species (ROS) scavenger or not. Our result showed that MerP

performed better ROS scavenging ability when it was compared to glutathione. The result showed that MerP transgenic plant showed higher tolerance to salinity, drought and UV-B than wild type. In 100 mM NaCl-mediated stress, the SOD activity of transgenic plant was 9.67% lower than wild type. Furthermore, the total protein from transgenic plant performed free radical scavenging ability was 2.29% higher than wild type in DPPH• solution. The ROS production detection using H_2DCF-DA probe proved that transgenic plant produced weaker fluorescence when compared to the wild type in 100 mM NaCl mediated stress. The results suggested that transgenic plant possess the higher ability for scavenging radicals. These studies prove that the MerP protein not only plays an important role in the heavy metal detoxication, when plants experience oxidative stress, but also could scavenge reactive oxygen species, improving the plant growth in the environmental stress (Chen, 2009).

EXPRESSION OF MERCURIC REDUCTASE IN EUKARYOTIC MICROALGA *CHLORELLA*

A significant accumulation of Hg^{2+} by the eukaryotic green microalgae *Chlorella* was reported (Hawkins and Nakamura, 1999). Because of its fast growth rate, inexpensive culturing on a large scale, and minimal negative environmental influence, *Chlorella* may be a suitable bioreactor if the process of removing Hg^{2+} can be promoted by molecular biological means (Leon-Banares *et al*., 2004). Therefore, transferring *merA* gene into the microalga *Chlorella* for the exploitation of MerA-mediated removal of Hg^{2+} from the aquatic environment was proposed.

The *merA* gene, encoding mercuric reductase (MerA), mediates the reduction of Hg^{2+} to volatile elemental Hg^0. The transformed *Chlorella* cells were selected first by hygromycin B and then by $HgCl_2$. The existence of *merA* gene in the genomic DNA of transgenic strains was shown by polymerase chain reaction amplification, while the stable integration of *merA* into genomic DNA of transgenic strains was confirmed by Southern blot analysis. The ability to remove Hg^{2+} in *merA* transgenic strains was higher than that in the wild type. The *merA* transgenic strains showed higher growth rate and photosynthetic activity than the wild type did in the presence of a toxic concentration of Hg^{2+}. Cultured with mercuric ion, the expression level of superoxide dismutase in transgenic strains was lower than that in the wild type, suggesting that the transgenic strains faced a lower level of oxidative stress. All the results indicated that *merA* gene was successfully integrated into the genome of transgenic strains and functionally expressed to promote the removal of Hg^{2+} (Haung *et al*., 2006).

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