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Biomolecular Engineering of *Escherichia coli* Organo-mercurial Lyase Gene and its Expression

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Studies were carried out to characterize *mer*B gene from five wild type strains of broad-spectrum *Escherichia coli*, collected from five geographically distinct regions of India. Each strain produced 23kb plasmid from which functional *mer*B gene (0.64kb) was PCR amplified. The *mer*B gene from isolate G18, which tolerated highest concentration of organic form (PMA) of mercury was cloned in high expression vector pQE30 and pGEMT-Easy vector. The transformants obtained demonstrated varied results in their appropriate hosts. The transformants (IAxpress) carrying *mer*B gene cloned in pQE30 and negative control having pQE30 without *mer*B insert did not grow on agar plates amended with 1μg/ml PMA. Due to the hyperexpression of *mer*B in pQE30 most of the protein was found in nonfunctional inclusion bodies and did not show any resistance as sensitive strain (Devoid of *mer*B gene) against PMA. On the other hand transformants of *mer*B cloned pGEMT vector tolerated up to 5μg/ml of PMA, which indicates that low expression of *mer*B in this vector produces a functional product and thus tolerates five times more PMA than sensitive strain. The results demonstrate that this gene can be better exploited for bioremediation of toxic form of mercury in polluted water bodies.

Keywords: *mer*B, cloning expression, *Escherichia coli*, bioremediation

Introduction

Pollution due to chemicals, including heavy metals, is a problem that may have negative consequences on the hydrosphere. Heavy metals are the most abundant pollutants to be found in sewage and waste-water (Fillali *et al*, 2000). Mercury is among the most hazardous of the heavy metals, primarily because its charged species have great affinity for the thiol group on cysteine residues of proteins and other important biological molecules.

Although many governments of many countries now require the companies to detoxify/recover mercury from the sludge and liquid wastes but have been less successful regarding the clean up of previously polluted landfills and water wastes by using physical and chemical remediation techniques. Bacteria that have evolved a variety of means of resistance to different forms of mercury can be better exploited for bioremediation of mercury-polluted sites. The most commonly encountered mechanism for mercury detoxification in bacteria is the operon mediated resistance mechanism (Osborn *et al*, 1997). The bacterial enzyme, organo-mercurial lyase, catalyzes the cleavage of C-Hg bond to yield Hg^{2+} which is 100 times less toxic than organic form. Further, mercuric reductase- an inducible NADPH dependent flavin containing disulfide oxido-reductase enzyme completes the reaction by the reduction of Hg^{2+} to Hg^{o} that is chemically inert (Reniero *et al*, 1998). The genes coding mercuric reductase (*mer* A), together with genes coding for Hg^{2+} transport and regulatory functions, comprise narrow spectrum *mer* operon. However, *mer*B gene, if found associated with all genes make it a broad-spectrum *mer* operon (Murtaza *et al*, 2001).

In order to rehabilitate the damaged environment, the most promising alternative is to exploit mercury resistant bacteria. Organomercurials are one to two orders of magnitude more toxic in some eukaryotes and are more likely to get biomagnified across trophic levels than ionic mercury i.e. Hg^{2+} (Scott *et al*, 1999). In our previous reports we have demonstrated that five broad-spectrum wild *E.coli* isolates showed highest tolerance towards both inorganic as well as organic form of mercury by converting both forms of mercury to elemental mercury. The present study, therefore, was carried out to characterize the organomercurial lyase gene from the most efficient isolate of *E.coli*.

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Material and Methods

Strains and Plasmids

The broad spectrum mercury resistant *E.coli* were isolated from five different geographical regions of Indian subcontinent (Murtaza *et al*, 2001). Plasmid DNA of *E.coli* was extracted by established method of Brinbiom and Dolly (1979). DH5α (*E.coli*) and *E.coli* SG 13009 ($pREP_4$) have been used as host for transformations and pGEMT-Easy vector (Promega, USA) and PQE 30 (Qiagen) as vectors.

PCR Amplification and Cloning

The *mer* B gene from each isolate was amplified by PCR using gene specific synthetic sense (IA34) deoxyoligonucleotide primer containing BamHI site at 5′ (TTGGATCCATGAAGCTCGCCCCAT) and antisense (IA35) deoxyoligonucleotide primer having *Kpn*1 at 5′ (TTGGTACCCACGTGTCCTAGATGA) ends to facilitate the directional cloning in pQE30 vector. The amplified genes were subcloned in cloning pGEMT Easy- vector and PQE30 expression vector and transformed into DH5α and SG13009 *E.coli* host cells (IAxpress) respectively.

Mercury Sensitivity Test and Expression Profile of merB

The transformants of both the vectors pQE30 and pGEMT vector possessing *mer*B gene were grown on agar plates amended with different concentrations of PMA (5,10,15,20,40 μg/ml) for 14 hrs at 37°C. Simultaneously transformed *E.coli* cells of SG13009 and DH5α cells harboring the PQE30 and pGEMT vector each devoid of *mer*B were grown under parallel conditions to serve as negative controls. To establish the expression of the recombinant protein, SG13009 transformant cells (IAxpress strain) were grown and induced by 1mM IPTG. The presence of *mer*B was determined by SDS-PAGE and protein was purified to homogeneity under denaturing conditions (Gupta *et al*, 1999).

Results and Discussion

In India, very little work has been carried out to exploit the mercury resistant bacteria for remediation of this toxic form of mercury from water bodies. The main objective of this study was to characterize the organomercurial lyase gene from the most efficient isolate of *E.coli*, which catalyzes the protonolysis of the carbon mercury bond, removing the organic ligand and releasing Hg^{2+} a less mobile mercury form (Scott *et al*, 2000). Since all these previously isolated broad-spectrum *E.coli* strains (D2, D14, G18, K6 & Y5) showed diverse biochemical and physiological response in the form of resistance towards mercury, they were subjected to PCR amplification of *mer*B. Upon DNA sequencing it was revealed that all of them encode *mer*B gene similar to already characterized *mer* B from *E.coli* (Unpublished data).

Plasmids obtained from the above five isolates were able to generate DNA fragment corresponding to the size of the already characterized *mer*B gene on PCR amplification. The expected length of PCR product for *mer*B gene corresponding to 0.64kb was obtained from all of them. Whereas no amplification was observed using narrow-spectrum plasmid used as negative control (Fig. 1). DNA sequencing reports showed that *mer*B gene of two most efficient strains (D14 & G18) had only 75-85% homology with the already characterized *mer*B gene (Murtaza *et al*, 2001).

The *mer*B gene from wild type isolates G18, which tolerated the highest concentration of PMA, was cloned in the appropriate vectors and transformed as discussed in material and methods. It was observed that transformants having *mer*B insert in pGEMT grew vigorously on the wide range of concentrations (1-5 μg/ml) of PMA while the growth of the transformants of pQE30 lacking *mer*B gene were severely inhibited and they died even at the lowest concentration of PMA (1μg/ml). It is evident that cloning of *mer*B gene in pGEMT easy vector

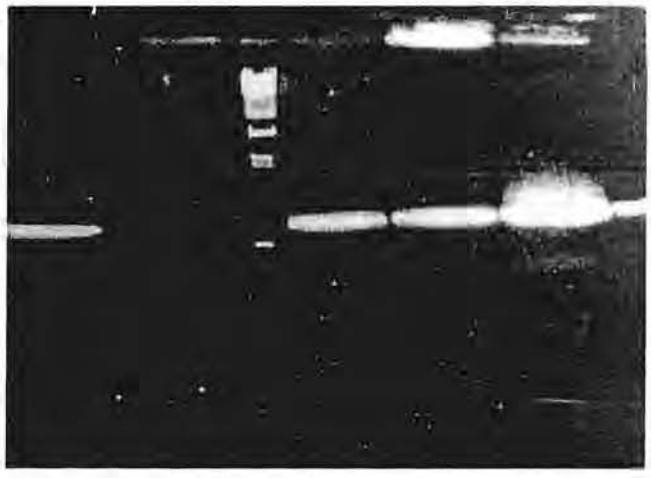

Fig. 1—PCR products of the five wild plasmid DNA, amplified with the *mer*B primers IA34 (forward) and IA35 reverse. Lane 1: PCR product of wild type Plasmids from D2; lane 2: sterile distilled water; Lane3: λ *Hind III* marker; lane 4: PCR product of D14 plasmid; lane 5: from Y5; lane 6: from K6, and lane 7: from G18.

increases the efficiency of the transformant five times than the sensitive strains. The results clearly indicate that the transformants of pGEMT having *mer*B insert in the proper orientation expresses functional *mer*B protein that degrade the toxic form of mercury (PMA) into less toxic form Hg^{2+} more efficiently and the strains thus survived due to the immediate conversion of toxic PMA into subtoxic level of Hg^{2+}. However we were unable to get *mer*B protein from these transformants due to low expression of *mer*B in pGEMT vector. The expression of *mer*B gene in the expression vector pQE30 vector indicate a single expected polypeptide of 22.4kDa from the induced cells of IAxpress on SDS PAGE (Fig. 2). These results are in consonance with the previous reports (Begley *et al*, 1986).

Expression of *mer*B in *E.coli* under the transcriptional regulation of the T5 promoter yielded an insoluble inactive protein aggregating bodies, which may be the good reason for them to be unable to tolerate the PMA levels beyond the limit tolerated by sensitive strain. Gupta *et al* (1999) also observed same sort of bodies during over expressing recombinant protective antigens of *Bacillus anthracis* in pQE30 vector. Most of the protein of *mer*B found in cell pellet fraction in the form of inclusion bodies was purified under denaturation conditions to homogeneity (Fig. 3). Although various strategies were used to avoid the formation of inclusion bodies e.g. reducing the IPTG concentration, incubation temperature, and induction time, these were unable to isolate the protein in native form.

Organic form of mercury has adverse effect on most of the living organisms, mechanical and chemical approaches to clean up the pollution are eco-unfriendly and cost intensive. Bioremediation has, however, emerged as a powerful and environmental friendly technique and should be used to remediate mercury-contaminated sites. Naturally occurring microbial activities are and have been the starting point for all biotechnological application. The use of microbial metabolic potential for eliminating environmental pollutants provides a safe and economic alternative to their disposal in waste dumpsites and to commonly used physiochemical strategies (Pieper & Reinelles, 2000). Thus the *mer*B gene characterized in this study can be better exploited for the bioremediation of mercury polluted sites.

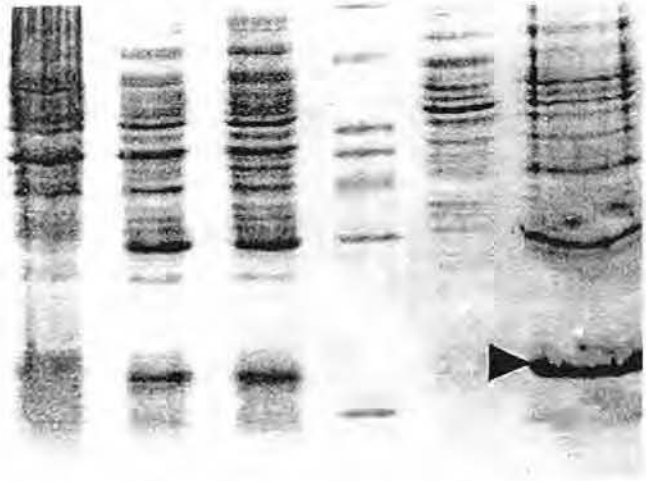

Fig. 2—Localization and molecular weight determination of recombinant MerB protein. Proteins were separated on SDS – 12% PAGE and stained with Commassie brilliant blue. Lanes 1: IAxpress cell extract (Uninduced), 2 & 3: IAxpress sonic pellet (inclusion bodies), (induced), 4: molecular weight standards (Promega, Low molecular weight marker), 5: Sonic supernatant (cytosolic proteins of the IAxpress, induced), 6: total cell extract of IAxpress cells (induced)

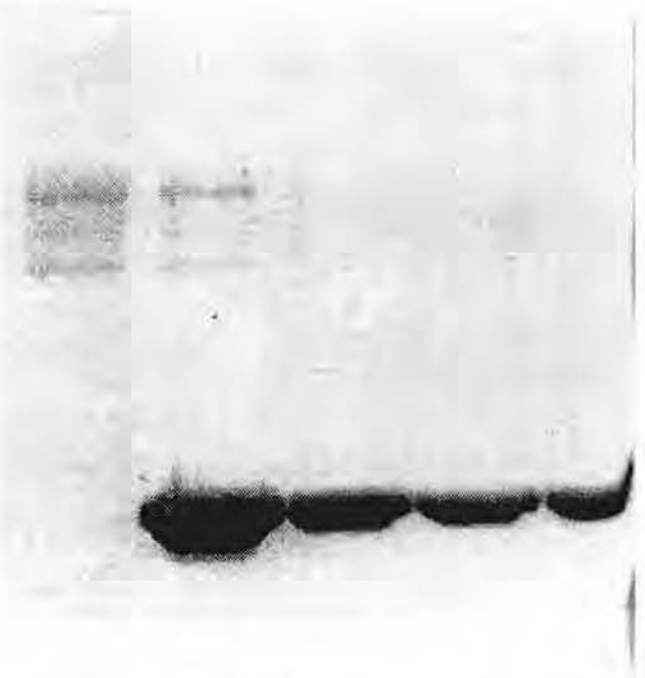

Fig. 3—Purification of MerB from IAxpress cells. The MerB was purified from inclusion bodies of 100ml culture using the NI-NTA resin under denaturing conditions and eluted in buffer at *p*H. 5.9. From each 1ml elan 10 µl were loaded and SDS –12% PAGE was performed. Fractions were visualized by Commassie Brilliant Blue staining. Lane1: Sonic supernatant; lane 2: Sonic pellet; lane 3: First elute; lane4: second elute; lane 5: third elute.

References

Begley T P, Walt A E & Walsh C T, 1986. Bacterial organomecurial lyase: overproduction, isolation and characterization. *Biochemistry*, **25**, 7186-7192.

Brinboim H & Doly J, 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res*, **7**, 1513-1525.

Filali B K, Touufik J, Zeround Y, Dzairi F Z, Talbi H & Blazhew H, 2000. Waste water bacterial isolates resistant to heavy metals and antibiotics. *Curr Microbiol*, **41**, 151-156.

Gupta P, Waheed SM. & Bhatnagar R, 1999. Expression and purification of the recombinant protective antigen of *Bacillus anthracis*. *Protein Expression Purification*, **16**, 369-376.

Murtaza I, Dutt A & Ali A, 2001. Relationship between the persistence of *mer* operon sequence in *Escherichia coli* and their resistance to mercury. *Curr Microbiol* (In Press).

Osborn A M, Kenneth DB, Petre S, Donald A & Richie A. 1997. Distribution, diversity and evolution of the bacterial mercury resistance *(mer)* operon. *FEMS Microbiol Rev*, **19**, 239-262.

Peiper D H & Reinelles W, 2000. Engineering bacteria for bioremediation. *Curr Opinion Biotechnol*, **11**, 262-270.

Reniero D, Mozzon E, Galli E & Barbieri P, 1998. Two aberrant resistance transposons in the *Pseudomonas stutzeri* plasmid pPB. *Gene*, **208**, 37-42.

Scott P B, Clayton L R, Summers A O & Richard B M, 1999. Phytoremediation of methylmercury pollution: *mer*B expression in *Arabidopsis thaliana* resistance to organomercurials. *Proc Natl Acad Sci USA*, **96**, 6808-6813.