Molecular studies of *E. coli* mercuric reductase gene (*merA*) and its impact on human health

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ABSTRACT

Bacterial plasmids encode resistance systems for toxic metal ions including Hg^{2+} functioning by energy-dependent efflux of toxic ions. The inducible mercury resistance (*mer*) operon encodes both a mercuric ion uptake and a detoxification enzymes. In Gram-negative bacteria especially in *E. coli*, a periplasmic protein, *MerP*, an inner- membrane transport protein, MerT, and a cytoplasmic enzyme, *mercuric reductase* (the *MerA* protein), are responsible for the transport of mercuric ions into cell and their reduction to elemental mercury, Hg^0. Phytoremediation involves the use of plants to extract, detoxify and/or sequester environmental pollutants from soil and water. Transgenic plants cleave mercury ions from methyl-mercury complexes; reduce mercury ions to the metallic form; take up metallic mercury through their roots; and evolve less toxic elemental mercury. PCR were performed to detect 1695 bp of *mercuric reductase* gene (*merA*), which is mainly responsible for the conversion of mercuric (Hg^{+2}) and mercurous (Hg^{+1}) ions into non-toxic elemental mercury. PCR products of putative merA genes from environmental *E. coli* strains were purified and cloned into a plant expression vector *pRT100*. The construct will be transformed in *calli* of *Nicotiana* plants.

Keywords: mercury resistance (*mer*) operon, *E. coli*, *mercuric reductase*, PCR Amplification, *Nicotiana*.

INTRODUCTION

Mercury, a potent neurotoxin, is one of the most harmful and toxic environmental pollutants. Mercury and its compounds when released into the environment are highly toxic to living cells because of their strong affinity for the thiol groups of proteins.[1] However, its levels have risen due to environmental contamination from human activities, such as burning coal and petroleum products, use of mercurial fungicides in paper making, agriculture and mercury catalyst in industry, with a consequent release of mercury into the air, water and on the land. These activities can increase local mercury levels several thousand fold above background. Therefore, environmental pollution is an increasing problem both for developing and developed countries. Industrial use of mercury led to pollution of the environment. Consequently, mercury removal is a challenge for environmental management. Most of the mercury released ends up and retained in the soil as complexes of the toxic ionic mercury (Hg^{2+}), which then can be converted by microbes into the even more toxic methylmercury which tends to bioaccumulate. Mercury detoxification of the soil can also occur by microbes converting the ionic mercury into the least toxic metallic mercury (Hg^0) form, which then evaporates. Microorganisms in contaminated environments have developed resistance to mercury and are playing a major role in natural decontamination. An extensively studied resistance system, based on clustered genes in an operon (mer operon), allows bacteria to detoxify Hg^{2+} into volatile metallic mercury by enzymatic reduction.[2,3] Mercury-resistance determinants have been found in a wide range of Gram-negative and Gram-positive bacteria isolated from different environments. They vary in the number and identity of genes involved and are encoded by mer operons, usually located on plasmids.[4-6] chromosomes.[7,8] they are often components of transposons.[9,10] and integrons.[11] A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of Hg^{++} to its volatile metallic form Hg (0).[12] The biotransformation is mediated by mercury reductase, an inducible NADPH-dependent, flavin containing disulfide oxidoreductase enzyme. The gene coding for mercury reductase is merA.[13] The bacterial mer operon encodes a cluster of genes involved in the detection, mobilization and enzymatic detoxification of mercury. Ionic mercury (Hg^{++}) is transported into the cytoplasm by a set of transport genes, where the merA gene, which encodes mercuric ion reductase, reduces this highly toxic ionic mercury (Hg^{++}) to the much less toxic volatile Hg^0. Elemental Hg^0 is gaseous at ambient conditions and evaporates away from the bacterial cells and its microenvironment.

Expression of merA in transgenic plants might provide an ecologically compatible approach for the remediation of mercury pollution.[14] Hyper-accumulation and hyper tolerance of Hg is the characteristic of few

plants but they haven't shown the ability to detoxify the toxic form of Hg to non-toxic form.[15] Improvement of plants by genetic engineering by modifying characteristics like metal uptake, transport and accumulation as well as metal tolerance, opens up new possibilities for phytoremediation. The present study aims to transform *E. coli* merA gene in Nicotiana species and its expression. Tobacco is a well-used system for development of transgenic as it is amenable to tissue culture and easy to get regenerants.

Mercury damages the central nervous system, endocrine system, kidneys, and other organs, and adversely affects the mouth, gums, and teeth.[16] Exposure over long periods of time or heavy exposure to mercury vapor can result in brain damage and ultimately death.

METHODS AND MATERIALS

Bacterial strains, their tolerance to inorganic mercury, plasmid screening and transformation studies: Bacterial strains used in this study were three wild-type, mercury-resistant *E. coli* isolates that were from three different sampling sites in the Yamuna River (New Delhi) and one sample collected from the Dal Lake, Srinagar, Kashmir, which is a pristine-type lake, was considered as the control. 100µl of the exponentially growing cultures of each of the three *E. coli* strains was subcultured on Luria agar plates supplemented with increasing concentrations of mercuric chloride. The plates were incubated at 37^0C for 24h. The minimal inhibitory concentration (*MIC*) to $HgCl_2$ was determined as the lowest concentration of mercury that allowed no visible growth of the organism. The highest concentration of mercury that allowed growth of the different strains was recorded as resistance of the strains to $HgCl_2$. Plasmid DNA was isolated by the alkaline lysis method as described by Birnboim and Doly.[17] *E. coli DH5α* was used as the host for transformation of plasmid DNA isolated from the wild-type *E. coli* strains. Transformation was carried out as described by Hanahan[18]. Transformants were selected on Luria agar plates supplemented with different concentrations of $HgCl_2$ to which the donor strains were resistant. Two transformants were picked randomly from each selection plate and replicated on plates containing the same stress parameters. They were also analyzed for their plasmid content by the alkaline lysis method and compared with the plasmid profile of the wild-type strains.

PCR amplification to isolate putative *merA:* Primer combinations of MerA-FJ (5/ *CGGGATCC ATG AGC ACT CTC AAA ATC ACC* 3/) and MerA-RJ (5/ *TCCCCCGGG ATC GCA CAC CTC CTT GTC CTC* 3/) were used for the detection of *merA* gene. The expected PCR products are bands of 1695bp.

RESULTS

All the three strains from three different sampling sites of Yamuna river; used in this study showed significant levels of tolerance to mercuric chloride. The minimum inhibitory concentration (*MIC*) from site-1, site-II and site-III of Yamuna river lay in the range of 28-30 µg/ml. Yamuna river showed mercury content three times more than the limit ($1 \mu g l^{-1}$) prescribed by WHO.[19-23] Screening for the presence of plasmids revealed that all the three strains showed the presence of at least one detectable plasmid when visualized on 1.0% agarose gel. When all the three plasmids were run with a molecular weight marker, they resolved at a position that corresponded to a size of approximately 24 kb of the *λDNA/EcoRI + HindIII* marker. Transformation of the plasmid DNA isolated from the wild-type *E. coli* strains into the competent, plasmid-less, mercury-sensitive (Hg^s) *E. coli DH5α* cells yielded transformants in each case on plates supplemented with different concentrations of $HgCl_2$ to which the donor strains were resistant. All the transformants could tolerate the same concentrations of mercury as the wild-type strains. The amplicons were detected on 1.0% agarose gel. The bands are size of 1695 bp (shown in Fig.1) PCR products of putative merA genes were purified using the Genei PCR purification kit.

Amplicons of putative *merA* gene was cloned into *pRT100* plant expression vector. The ligation reaction was first incubated at 37^0C for blunt end ligation and then at 16°C overnight for cohesive end ligation prior to transformation into *E. coli* competent *DH5α* cells. Two type of colonies were seen on Luria agar plate supplemented with 100µg/ml ampicillin. The recombinant colonies from the transformation reaction were selected and screened by PCR and restriction endonuclease digestion for the presence of the putative merA gene. Plasmid minipreps were performed on recombinant clones using Plasmid Miniprep protocol (*Bangalore Genei*). *Agrobacterium* construct will be made for the transformation of *Nicotiana tabaccum* plants. The disarmed Ti-binary vector in *Agrobacterium tumefaciens* (GV3101) will be used in calli transformation to produce transgenic *tobacco* plants. After transgenics being screened out by PCR amplification of 1695 bp product, their first level of expression will be checked by isolating RNA from leaves of transgenic *Nicotiana tabaccum* plants. The expression studies will be done after cloning *mercuric reductase* gene into a shuttle vector *pQE30* and the level of protein expression may be checked by SDS PAGE after its transformation into *BL21* cells.

DISCUSSIONS

The water samples collected in this study from different sites of Yamuna river and Dal Lake had different physical properties such as pH, temperature and turbidity etc. The mercury content in these samples were also found to be variable, with the Yamuna river, showing the highest level. In this paper, we have concentrated our studies on Yamuna river. Yamuna river showed mercury content (3.76ppm) three times more than the limit (1µg/l) prescribed by WHO.[23] Therefore this water is not safe for human consumption and needs an immediate attention for some remedial measures.

We have characterized this gene of mer operon (merA) which are mainly responsible for environmental mercury detoxification. The gene product will convert the dangerous methylmercury and other organic mercury derivatives to ionic mercury; which can then be reduced to Hg^0 by merA gene.[24]

Transgenic *tobacco* plants with *mercuric reductase* gene will be an excellent example of bioremediation. Transgenic plants carrying the individual mercury metabolic genes can be crossed to create a universal mercury removing plant for areas where methylmercury and ionic mercury pollution is simultaneously present.[25] *Tobacco* is especially attractive and preferred for this purpose because of the ease of hybridization. The use of bacteria for rehabilitation of polluted environments may provide an ecologically sound method for abatement of pollution and a natural solution for recovery of contaminated soil and water.[25]

Elemental mercury often passes through the gastrointestinal tract without being absorbed, and historically mercury has occasionally been used for mechanical relief of intestinal obstructions. Compounds of mercury tend to be much more toxic than the element itself, and organic compounds of mercury are often extremely toxic. Dimethylmercury, for example, is a potent neurotoxin that is lethal in amounts of a fraction of a millilitre. Humans or animals poisoned with mercury or its compounds often manifest excessive salivation, a condition called mercurial ptyalism. There is an urgent need to stop the polluted sites by bioremediation; which will be beneficial for human health. Bioremediation is one of the most feasible ways to clean unwanted substances from air, soil, water and raw materials from industrial processing.

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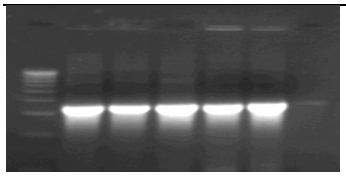

2.0kb
1.5kb

1695bp

Lane 1: 500bp DNA Marker
Lane 2: PCR amplification of *E. coli* merA gene from site-1 of Yamuna river.
Lane 3: PCR amplification of *E. coli* merA gene from site-II of Yamuna river.
Lane 4: PCR amplification of *E. coli* merA gene from site-III of Yamuna river.
Lane 5: PCR amplification of *E. coli* merA gene from site of Hindon river.
Lane 6: PCR amplification of *E. coli* merA gene from NRI plasmid of *E. coli* R100 strain.
Lane 7: PCR amplification of *E. coli* merA gene, when only 2 µl of PCR product loaded onto a gel from Yamuna sample.

Fig. 1. PCR amplification of *E. coli mer*A, a mercury reductase gene of mer operon of size 1695bp.