RESEARCH PAPER

Asian Journal of Bio Science (April, 2010) Vol. 5 Issue 1 : 80-83

Cloning of mercuric reductase (*mer*A) gene isolated from wild strains of *Escherichia coli*

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(Accepted : February, 2010)

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 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 *mer*A 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 (*mer*A), 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 *mer*A genes form environmental *E.coli* strains were purified and cloned into a suitable plant expression vector like pB1121 or pCAMBIA. The construct will be transformed in calli of *Nicotiana* plants. Expression of *mer*A gene in transgenic plants might provide an ecologically compatible approach for the remediation of mercury pollution.

Key words : Mercury resistance, *E. coli*, PCR amplification, *Nicotiana*

INTRODUCTION

Mercury pollution is a major environmental problem accompanying industrial activities. Mercury, a potent neurotoxin, is one of the most harmful and toxic environmental pollutants. Actually, 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 (Hajela *et al*., 2002). However, its levels have risen due to environmental contamination, such as burning coal and petroleum products, use of mercurial fungicides in paper making and agriculture and mercury catalyst in industry, with a consequent release of mercury into the air and water on the land. These activities can increase local mercury levels several thousand fold above background (Robinson and Tuovinean, 1984). Therefore, environmental pollution is an increasing problem both for developing and developed countries. Industrial use of mercury led to pollution of 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 (Nikiforvo *et al*., 1999). 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 (Summers, 1986). 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 (Summers and Silver, 1972; Brown *et al.*, 1986; Griffin *et al.*, 1987) and chromosomes; they are often components of transposons (Misra *et al.*, 1984) and integrons. A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of (Hg^{++}) to its volatile metallic form Hg^0 (Liebert *et al.*, 1997).The biotransformation is mediated by mercury reductase, an inducible NADPH-dependent, flavin containing disulfide oxidoreductase enzyme. The gene coding for mercury reductase is *mer*A (Nies, 1999). 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 *mer*A 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 form the bacterial cells and its microenvironment. Researchers developed bioremediation as one feasible way to accelerate or encourage the degradation of pollutants. The basis of bioremediation is that all organisms remove substances form the environment to carry out growth and metabolism. Bioremediation does not involve only the degradation of pollutants. Bioremediation can be used to clean unwanted substances from air, soil, water and raw materials from industrial processing. Expression of *mer*A in transgenic plants might provide an ecologically compatible approach for the remediation of mercury pollution. Hyperaccumulation 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 nontoxic form. 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 phyoremediation. The present study aims to transform *E. coli mer*A 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 regenerant. Therefore, this system will be used for introduction of *mer*A gene and these plants will be used for phytoremediation of mercury polluted sites.

Toxicity of mercury in human beings :

Pure elemental mercury is a cumulative heavy-metal that is moderately absorbed through the skin, rather poorly absorbed through the gastrointestinal tract, and readily absorbed as vapor through the lungs. The element is strongly toxic when absorbed as vapor form the respiratory tract, but it is considerably less so when exposure occurs via other routes. Elemental mercury often passes through the GI 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 milliliter. Mercury damages the central nervous system, endocrine system, kidneys and other organs and adversely affects the mouth, gums, and teeth. Exposure over long periods of time or heavy exposure to mercury vapor can result in brain damage and ultimately death. Mercury and its compounds are particularly toxic to fetuses and infants. Women who have been exposed to mercury in pregnancy have sometimes given birth to children with serious birth defects. Some of the toxic effects of mercury are reversible; either through specific therapy or through natural elimination of the metal after exposure has been discontinued. However, heavy or prolonged exposure can do irreversible damage, particularly in fetuses, infants, and young children. Exposure to certainly highly toxic compounds of mercury such as dimethymercury can be fatal within hours or less.

Mercury exposure in very young children can have severe neurological consequences, preventing nerve sheaths from forming properly. Research has been done that demonstrates the inhibitory effect that mercury has on myelin, the building block protein that forms these sheaths. Mercury poisoning in the young is suspected as a possible cause of autistic behaviors, However, there is a lack of quality peer-reviewed work on this matter and the claim of autism as mercury poisoning is considered suspect by mainstream medicine.

Humans or animals poisoned with mercury or its compounds often manifest excessive salivation, a condition called mercurial ptyallsm. Therefore, there is an urgent need to stop the polluted sites by a method called bioremediation/phytoremediation. That will benefit a lot in future.

MATERIALS AND METHODS

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). 100µl of the exponentially growing cultures of each of the three *E.coli* strains was sub cultured 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 lyses method as described by Birnoboim and Doly (1979). *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 (1983). 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.

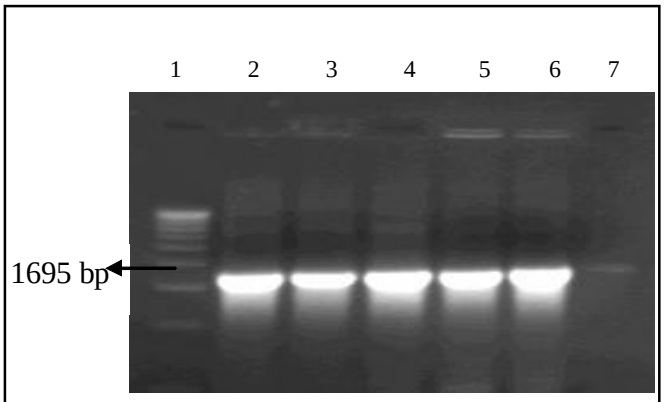
PCR amplification to isolate putative mer A :

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

RESULTS AND DISCUSSION

All the three strains used in this study showed significant levels of tolerance to mercuric chloride. The minimum inhibitory concentration (MIC) form site-I, site-II and site-III of Yamuna river lay in the range of 28-30 µg/ml. 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% 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 + Hindlll marker. Transformation of the plasmid DNA isolated form the wild-type *E. coli* strains into the competent, plasmid-less, mercury-sensitive (Hg) *E.coli* DH5α cells yielded transformation in each case on plates supplemented with different concentrations of $HgCl_2$ to which the donor strains were resistant. All the transformation could tolerate the same concentration of mercury as the wild type strains. The amplicons were detected on 1% agaose gel. The bands are size of 1695 hp (Fig. 1). PCR products of putative *mer*A genes were purified using the Genei PCR purification Kit. Amplicons of putative *mer*A gene was cloned into pRT100 expression vector. The legation reaction was first incubated at 37^0C for blunt end ligation and then at 16^0C overnight for cohesive end ligation prior to transformation into *E.coli* competent DH5α cells. Two types 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 for the presence of the putative *mer*A gene. Plasmid minipreps were performed on recombinant clones using Plasmid Miniprep proteocol (Bangalore Genei).

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.



Lane 1 : 500bp DNA marker
Lane 2 : Amplification of *mer*A gene from mercury polluted site-1 of Yamuna river.
Lane 3 : Amplification of *mer*A gene from mercury polluted site-2 of Yamuna river.
Lane 4 : Amplification of *mer*A gene from mercury polluted site-3 of Yamuna river.
Lane 5 : Amplification of *mer*A gene from mercury polluted site of Hindane river.
Lane 6 : Amplification of *mer*A gene from NRI *E. coli* R100 strain (positive control).
Lane 7 : Negative control

Fig. 1 : PCR amplifications of *E. coli mer* A *gene*

Acknowledgement :

The authors are thankful to the Department of Biotechnology, Govt. of India for funding this project. They are also thankful to the Vice Chancellor, Jamia Millia Islamia, New Delhi for providing the necessary infrastructure to carry this work.

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