RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 1 : 103-106 (April to September, 2009)

Plasmid-borne mercury resistance in aquatic *Escherichia coli*

TANVEER A. SHAH AND ARIF ALI
Gene Expression Laboratory, Department of Biosciences, Jamia Millia Islamia, NEW DELHI, INDIA

(Accepted : March, 2009)

Bacteria (*E. coli*) isolated from different aquatic bodies of India were analyzed for their tolerance to mercury ($HgCl_2$). Out of the 30 isolates of *E. coli*, collected from water samples of four geographically distinct regions and hospital settings in India, 8 strains showed significantly high levels of tolerance to the inorganic form of mercury i.e mercury chloride ($HgCl_2$). All the eight strains revealed the presence of a plasmid of approximately 24kb, and transformation of the isolated plasmids into the mercury-sensitive competent cells of *E. coli* DH5α rendered the transformants resistant to the same concentration of mercury as wild type-strains.

Key words : *Escherichia coli*, Mercury resistance, Mercuric reductase (*mer*A) gene.

INTRODUCTION

Mercury has been recognized as one of the most toxic heavy metals in the environment and has been released into environment in substantial quantities through natural events and anthropogenic activities. Mercury pollution is a worldwide problem in aquatic environments, resulting primarily from its industrial use in bleaching operations (*i.e.* chlorine production, paper, textiles etc.), as a catalyst, as a pigment in paints, and in the mining of gold. Its use in seed and bulb dressings directed against bacteria and fungi and in fungicidal sprays on fruit trees has introduced much of the mercury that contaminates agricultural land. If the original source of mercury is large enough even metallic mercury, Hg^0, becomes problematic, since biological systems can re-oxidize it to Hg^{2+} at a low rate (Ogata and Aikoh, 1994). Although the rate of release of mercury into the environment may have slowed in recent years (Adriano, 1986), previously contaminated sites continue to leach large quantities of mercury into adjacent wetlands, waterways, and estuaries (Alberts *et al.*, 1990; Gallagher, 1980; Leigh, 1994). The mercury that is not bound up in insoluble sugar salts, $(RS)_2$ Hg, tends to accumulate in invertebrates and fish as methylmercury (CH_3Hg^+), dimethylmercury [$(CH_3)_2$ Hg] or other organomercury salts. The organomercury compounds are passed on rapidly to local bird, animal, and human populations with tragic consequences (D'ltri and D'ltri, 1978).

The effect of this heavy metal on the ecosystem and health are growing concerns. Several physically and chemically based technologies have been utilized to remove mercury from polluted sites, but these technologies have proved to be expensive. Resistance to mercury is one of the most widely disseminated plasmid- determined phenotypes found in gram-negative and gram-positive bacteria (Summers and Silver, 1972). A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of Hg^{2+} to the volatile form, Hg^0. This biotransformation is mediated by an inducible NADPH-dependent (and in some cases, NADH-independent) flavin containing disulfide oxidoreductase enzyme, mercuric reductase (Summers and Barkay, 1989). The gene encoding mercuric reductase (*mer*A), together with genes coding for Hg^{2+} transport and for regulatory functions, comprises the mer operon (Ni' Bhriain *et al.*, 1983). In a wide variety of gram-negative and positive organisms, mer operons are located on plasmids and transposons (Summers and Barkay, 1989).

The present study was carried out to evaluate the plasmid-borne nature of nercury resistance in eight *E. coli* strains that exhibited maximum tolerance to mercuric chloride ($HgCl_2$) from microbial consortia of our laboratory culture collection of 30 mercury resistant *E. coli* strains.

MATERIALS AND METHODS

Sample collection:

Water samples were collected aseptically from four different metal polluted effluent sink sites in India, namely Yamuna river, Delhi; Hindon river, Ghaziabad; Safdar jung hospital, Delhi and Jehlum river, Kashmir. The fifth sample collected from Dal Lake, Kashmir (pristine type lake) was considered as control. All collected samples were subsequently diluted and plated on Petri dishes containing growth medium *i.e.* Luria Agar (Hi Media, India). The initial screening of *E. coli* was done on Eosin Methyl Blue (EMB) agar (Hi Media, India) plates. The purple colonies with greenish metallic sheen from Eosin Methyl Blue (EMB) agar plates were selected and subjected to

various biochemical tests using the biochemical test kit [Banglore Genei (India)] for their confirmed identification to be as *E. coli*. Total 30 *E. coli* strains were isolated from the above water samples collected from different aquatic sites.

Tolerance to $HgCl_2$:

In liquid medium (Luria broth):

For determining the tolerance limits of the *E. coli* isolates to $HgCl_2$ in liquid media, the *E. coli* isolates were grown overnight at 37^0C in luria broth. Cells from overnight grown cultures were diluted 100 fold in luria broth and grown for approximately 2 hours with gentle agitation. When the absorbance at 600nm (A_{600}) reached 0.3, these were distributed into a series of test tubes. Varying concentrations of $HgCl_2$ (4-60 µg/ml) were added individually to each of the culture strains (in the various test tubes) and incubation continued at a temperature of 37^0C. After 5 hours of growth A_{600} was checked and compared with that of the same strains without stress ($HgCl_2$) under the same conditions. Resistance was determined as the maximum concentrations of $HgCl_2$ that allowed normal growth of the strains.

In solid medium (Luria agar):

Sensitivity of the strains to $HgCl_2$ on luria agar plates was tested by streaking a loopful of the culture on to solidified luria agar plates supplemented with increasing concentrations of $HgCl_2$. The highest concentration of the mercury that allowed growth of different strains was recorded as resistance.

Selection of strains:

Out of 30 *E. coli* strains, 8 strains that showed highest resistance to $HgCl_2$ in both liquid and solid media were selected for further research exploitation. The eight highly resistant strains were designated as MAD4 and MAD6 (Dal lake), MYA 8 and MYA10 (Yamuna river), MAS12 (Safdar Jung Hospital), MAH14 (Hindon river) and MAJ16 and MAJ18 (Jhelum river).

Plasmid screening:

Plasmid DNA was isolated by the alkaline lysis method as described by Birnboim and Doly (1979). The plasmid DNA isolated from the different strains was visualized after electrophoresis on 0.7% agarose gels in 0.5X TBE containing ethidium bromide (1 µg/mL), and the patterns were photographed with a Polaroid camera.

Transformation:

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 in 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 replica plated 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.

RESULTS AND DISCUSSION

Present results revealed that all eight strains used in the study showed significant levels of tolerance to mercuric chloride, MYA8 from the Yamuna River showed the maximal level of tolerance to $HgCl_2$ *i.e.* 48 µg/ml in liquid media and 52 µg/ml in solid media, MAS12 from Safdar Jung Hospital could tolerate the lowest concentration *i.e.* 20µg/ml in liquid media and 22 µg/ml in solid media. The remaining six strains showed tolerance levels ranging from 20-48 µg/ml and 22-52 µg/ml in liquid and solid media, respectively (Fig. 1).

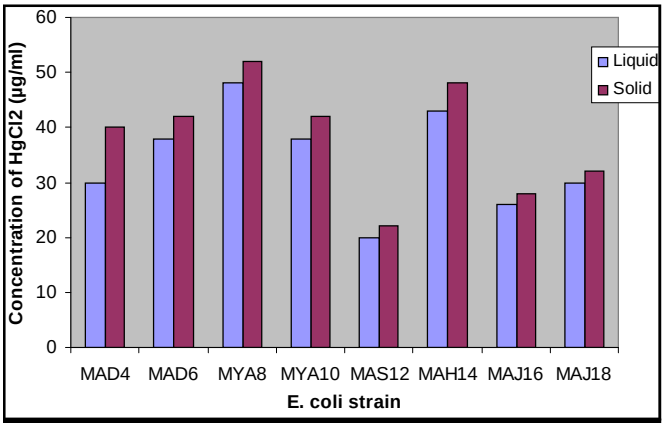
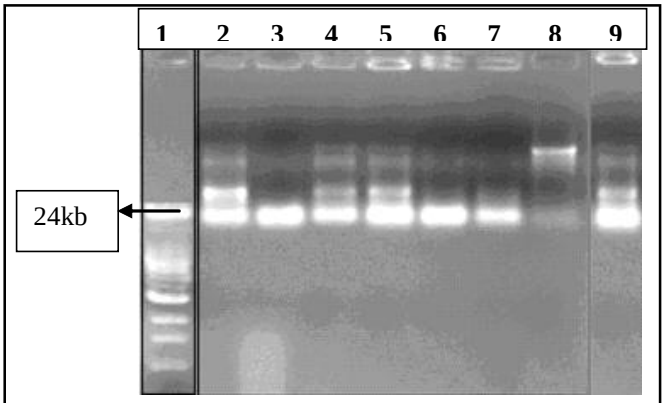


Fig. 1 : Maximum concentration of $HgCl_2$ tolerated by the different E. coli strains in liquid and solid medium

Screening for the presence of plasmids revealed that all eight strains showed presence of at least one detectable plasmid when visualized on 0.7% agarose gels. When all eight plasmids were run with a molecular weight marker, they resolved at apposition that corresponded to a size of approximately 24kb of the DNA/EcoRI + HindIII marker, as shown in Fig. 2.

To confirm the plasmid borne mercury resistance, transformation of the plasmid DNA isolated from the wild type *E. coli* strains into the competent, plasmidless, mercury sensitive (Hg^s) *E. coli* DH5α cells was done. On transformation we were able to get transformant



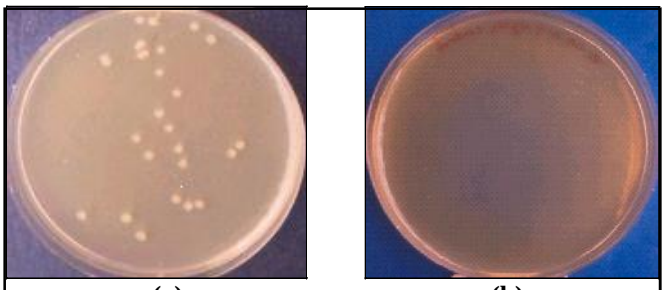
Plasmid DNA isolated from biochemically identified mercury resistant *E. coli* (wild) strains and electrophoresed on 0.7% agarose gel.
Lane 1: lDNA/EcoRI + Hind III marker; Lane 2-9: Plasmid DNA profile of MAD2; MAD6; MYA8; MYA10; MAH14; MAS12, MAJ16 and MAJ18

Fig. 2 : Plasmid DNA isolated from mercury resistant *E. coli* strains

colonies from wild plasmids (Fig. 3 a and b). Transformants were obtained in each case on plates supplemented with different concentrations of $HgCl_2$ to which donor strains were resistant. It was noted that all the transformants could tolerate the same concentration of mercury as the wild type strains. Three transformants from each plate were also analyzed for their plasmid DNA content, and visualization of the plasmids isolated from the transformants showed that they conformed to size approximately 24Kb of the λDNA/EcoR1+HindIII marker (data not shown) clearly identifying them to be the same as those that were transformed.

Present observation of high levels of mercury resistant bacteria from polluted water confirm those of previous studies (Nelson and Colwel, 1975). The frequent occurrence of mercury resistant bacteria and the wide range of genera showing this phenotype indicate the widespread nature of mercury resistance in the environment.

The present study was carried out on eight strains



(a) **(b)**

(a) Transformed DH5~ cells with wild plasmid DNA
(a) Non-transformed DH5~ cells used as control

Fig. 3 : Transformation of plasmid DNA

that exhibited maximum tolerance to the inorganic form of mercury, from the microbial consortia of our laboratory culture collection of 30 mercury resistant *E. coli* strains. Resistance pattern of the strains to $HgCl_2$ showed that the strains isolated from the Yauna river, Delhi and Hindon river, Ghaziabad could tolerate comparatively higher concentrations of $HgCl_2$ than the strains from the other sites. The presence of mercury-resistant bacteria in regions distant from mercury deposits suggest that pre-exposure to mercury may play a role in the adaptive response of bacteria by developing resistance mechanisms. This is in consonance with the earlier reports of mercury-resistant bacteria in our laboratory (Murtaza *et al.*, 2001).

The question of whether and how theses bacteria protect their hosts from heavy metals is also important. Two basic mechanisms of resistance by cells against toxic ions can be envisaged:

(1) Specific alteration of ion transport (inward, preventing entry into the cell or out ward pumping out of the cell) of the toxic ion and (2) by chemical modification or by binding to the cellular factors, resulting in a form that is no longer toxic to the cell (Jobling *et al.*, 1988).

Although bacteria have developed a number of mechanisms to counteract the toxic effects of mercury, the biochemical mechanism of mercury resistance by microbial populations in sea water and fresh water environments has been found to be the plasmid-mediated reduction of toxic, volatile, elemental form Hg^0 (Misra, 2000). This detoxification system is highly specific to mercury, being catalyzed by a modular cluster of genes-the mer operon usually found on plasmids, transposons, and sometimes on the chromosome. The chromosomal location of the mercury resistance operon has been observed mostly in Gram positive bacteria (Kholodii *et al.*, 1997). In an attempt to localize the mercury resistant determinant, present results confirmed the presence of this multifaceted operon, on a plasmid approximately 24kb in size. The evidence for the plasmid-borne nature of the mer operon in our strains stems from two lines of evidence:

– The presence of at least one detectable plasmid in all eight wild-type *E. coli* strains (Fig. 2)

– The transformation of plasmid isolated from the wild-type *E. coli* cells into the mercury-sensitive, competent cells of *E. coli* DH5α which rendered the *E. coli* transformants resistant to mercury (Fig.3).

The frequent occurrence of mercury-resistant bacteria and the wide range of genera showing this phenotype indicate the widespread nature of mercury resistance in the environment (Nakahara *et al.*, 1977). In many cases, mercury resistance has been found to be

associated with conjugative plasmids and/or transposons (Davey and Reanney, 1981), which can facilitate the horizontal transfer and dissemination of mercury resistance genes through the bacterial population. This capacity for genetic exchange among bacterial species has resulted in the general dissemination of plasmids encoding metal and antibiotic resistance (Bass *et al.*, 1999). Wastewater and water bodies that receive various effluents and discharge are very rich nutrient locations existing outside the laboratory, representing potential sites for the exchange of genetic material by conjugative plasmids, such as the R plasmids, which mobilize between bacteria in wastewater (Alcaide and Garay, 1984).

Conclusion:

The isolation of mercury resistant *E. coli* isolates, which could tolerate high levels of mercury, has provided an opportunity to investigate the mechanism of mercury resistance in *E. coli*. The two most efficient strains *i.e.* MYA8 and MAH14 encountered in the present study offer excellent potential for bioremediation and can be utilized for the amelioration of water quality and for reducing the pollution load in water bodies.

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.

REFERENCES

Adriano, D.C. (1986). *Trace Elements in the Terrestrial Environment* (Springer, New York)

Alberts, J.J., Price, M.T. and Kania, M. (1990). Estuarine Coastal Shelf. *Sci.*, **30**, 47-58.

Alcaide, E. and Garay, E. (1984). R-plasmid transfer in Salmonella sp. Isolated from wastewater and sewage-contaminated surface waters. *Appl. Environ. Microbiol.*, **48**: 435-438.

Bass, L., Liebert, C., Lee, M.D., Summers, A.O., White, D.G., Thayer, S.G. and Maurer, J.J (1999). Incidence and characterization of integrons, genetic elements mediating multi-drug resistance, in avian *Escherichia coli*. *Antimicrob Agents Chemother*, **43** : 2925-2929.

Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.*, **7**: 1513-1523.

Davey, R.B. and Reanney, D.C (1981). Extra chromosomal genetic elements and the adaptive evolution of bacteria. In: Health MK, Steere, WC, Wallace R (eds) Evolution biology. New York: *Plemum Publishing Corp*, **13**: 113-147.

D'ltri, P.A. and D'ltri, F.M. (1978). *Environ. Manage.* (NY) **2** : 3-16.

Gallagher, J.L. (1980). In : *Field Biassays for the Role of Plants as Vectors in Contaminant Transfer from Degraded Material*, ed. Baker R.A (Science, Ann Arbor, MI), pp. 445-463.

Hanahan, D. (1983). Studies on transformation of *E. coli* with plasmids. *J. Mol. Biol.*, **166** : 557-580.

Jobling, M.G., Peters, S.E. and Ritchie, D.A (1988). Plasmid-borne mercury resistance in aquatic bacteria. *FEMS Microbiol Lett.*, **49**: 31-37.

Kholodii, G.Y., Yurieva, O.V., Gorlenko, Z.M., Mindlin, S.Z., Bass, I.A., Lomovskaya, O.L, Kopteva, A.V. and Nikiforov, V.G. (1997). Tn5041: A chimeric mercury resistance transposon closely related to the toluene degradative transposon Tn4651. *Microbiolgy,* **143**: 2549-2556.

Leigh, D.S. (1994). *Water Res. Bull.*, **30** : 739-748.

Murtaza, I., Dutt, A. and Ali, A. (2001). Relationship between the persistence of mer operon sequences in *E. coli* and their resistance to mercury. *Curr. Microbiol.*, **44** : 178-183.

Misra, T.K (2000). Heavy metals, bacterial resistance. *Encyclopaedia of Microbiolgy,* **2** : 618-627.

Nakahara, H., Isikawa, T., Sarai, Y., Kondo, I., Isamu, K.I., and Mitsuhashi, S. (1977). Mercury resistance and R plasmids in *E. coli* isolated from clinical lesions in Japan. *Antimicrob Agents Chemother,* **11**: 999-1003.

Nelson, J.D. and Colwell, R.R. (1975). *Microb. Ecol.*, **1** : 191-218.

Ni Bhriain, N., Silver, S. and Foster, T.J. (1983). *J. Bacteriol.*, **155** : 690-703.

Ogata, M. and Aikoh, H. (1984). *Biochem. Pharmacol.*, **33** : 490-493.

Summers, A.O. and Silver, S. (1972). Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. *J. Bacteriol.*, **112** : 1228-1236.

Summers, A.O. and Barkay, T. (1989). Metal resistance genes in the environment, p 287-308. In S. Levy and R. Miller (ed.). *Gene transfer in the environment.* McGraw-Hill Book Co., New York.