RESEARCH PAPER

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Plasmid-borne mercury resistance in aquatic *Escherichia coli*

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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