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Thesis Title: "MOLECULAR CHARACTERIZATION OF GENETIC DETERMINANT *arsB*, AN ARSENIC RESISTANT GENE, OF ARSENIC RESISTANT BACTERIA"

ABSTRACT

Arsenic is a ubiquitous toxic metalloid released into the environment from natural as well as anthropogenic sources. It occurs in two inorganic forms; arsenate As(V) and arsenite As(III). As(III) is more toxic than As(V). Arsenic, in particular inorganic arsenic, was classified by the International Agency for Research on Cancer and the US Environmental Protection Agency as a known human carcinogen. To survive harsh exposure of arsenic, bacteria have developed a surprising array of genes for arsenic detoxification in an operon referred as "*ars* operon", widely distributed in bacteria, mostly located on plasmids and chromosomes. Arrangement of genes in an operon is *arsRBC* or *arsRDABC* expressed as a single transcriptional unit. ArsB, an integral membrane protein that pumps arsenite out of the cell is often found associated with an ATPase subunit ArsA.

Our study related to estimation of arsenic contamination in different water bodies also showed variable levels of arsenic contamination in different water bodies with Dhaka (Bangladesh) showing (0.075 mg/L) the highest level of contamination and Yamuna river shows almost negligible level. Out of total 80 isolates only 22 were found to be resistant to 10^{-5}M arsenic trioxide in this study.

From our current study the comparative analysis of resistance pattern shown by *E. coli* (ARM-2 and ARM-6) isolates from heavily polluted Yamuna river (Agra) and Coal industry (Faridabad) tolerated highest concentrations of arsenic (10^{-2}M) as compared to others. Over all resistance patterns of all the ten isolates towards other heavy metals like mercury, cadmium and copper and their MIC value were also determined. Our study also demonstrates variable resistance pattern offered by different isolates towards different types of antibiotics. Our results suggest that all the ten isolates resisted the specific concentration of Cephalexin, Ciprofloxacin and Tetracycline used.

In order to check the actual mode of resistance in our collected isolates, the growth curve inhibition studies were carried out which showed that all of the ten isolates give a clue for an inducible *ars* gene expression mechanism operating in these isolates to resist arsenic. The isolates ARM-2 were able to show maximum reduction by almost 2 hours in lag phase. To elucidate the exact mode of arsenic resistance at molecular level plasmid isolation for all ten isolates and their subsequent transformation studies in *DH5α* were carried out. Plasmids isolated from all isolates were found to be resolved at the same position on Agarose gel (26kb). Transformation of *DH5α* with the plasmids isolated from all the 10 wild type *E. coli* rendered the *E. coli DH5α* transformants resistant to arsenic which provides a strong evidence for the plasmid borne arsenic resistance operating in them.

Keeping in view the importance of molecular based approaches, gene specific primers were used for PCR amplification of the arsenite transporter gene (*arsB*) in our collected isolates. All the isolates were found to be able to produce an amplified product that corresponds to approximately 1.28 kb, as was expected, which further strengthens the hypothesis of wide distribution of the *arsB* gene found in all the isolates of this study in nature. Transformation of *DH5α* cells with the PCR amplified products cloned in pGEMT-Easy vector confirmed the presence of insert in the transformants as detected by colony PCR.

DNA sequencing analysis of all the amplified products was carried out in order to study the diversity and distribution of arsenite transporter gene (*arsB*) gene among the collected isolates. All of the ten PCR amplified *arsB* fragments (ARM-1 to ARM-10) showed a good homology with each other as well as with the already characterized *arsB* genes. However, a slight divergence was observed in the nucleotide sequences of ARM-2, while rest of the isolates showed >90% homology with each other. Further *arsB* from these isolates showed maximum homology with other characterized *arsB* gene at amino acid level. Comparative analysis of amino acid sequences of the *arsB* sequences from this study with other well characterized *arsB* reported sequences till date revealed not only the occurrence of a number of conserved DNA stretches but also revealed diversity zones mostly restricted towards N- terminus of amplified *arsB* gene which infers that N- terminus end most probably may act as a non-functional part in *arsB* for arsenic efflux. Phylogenetic analysis of our results suggest that the arsenic resistance for our isolates is mediated as a part by classical arsenic resistance operon operating in these organisms.