“Molecular Characterization of Genetic Determinants of Arsenic (As) Resistance in Bacteria”

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ABSTRACT

Over the last few decades contamination of environment with heavy metals has increased drastically. These metals are highly genotoxic as environmental contaminants, whereas in low concentration they are initially important elements. Toxic heavy metals cause DNA damage and their carcinogenic effects in animals and humans are most probably caused by their mutagenic ability. Among the heavy metals which are toxic include Mercury, cadmium, arsenic, selenium etc. Among these heavy metals, arsenic is one of the most important global environmental pollutants and a persistent bioaccumulative carcinogen. Considering these aspects, present investigations were undertaken to identify the strains that were resistant to arsenic heavy metal toxicity and it will be interesting to describe the divergence of the arsenic resistant determinants of arsenic-resistant bacteria. Water samples collected from different geographical sites of India as well as from Bangladesh had varying physical properties such as temperature, pH and turbidity. Total twenty-five *E. coli* isolates were obtained from different water samples, the isolated *E. coli* strains when tested for their tolerance to As_2O_3 exhibited similar resistant patterns both in liquid and solid medium. Therefore seventeen strains exhibiting the highest levels of tolerance to the arsenic were selected for further research exploitation in the present study. The strains were designated as ARSC-1, ARSC-2, ARSC-3, ARSC-4, ARSC-5, ARSC-6, ARSC-7, ARSC-8 (from West Bengal), ARSC-9, ARSC-10(from Ludhiana), ARSC-11(from Yamuna Delhi), ARSC-12(from Yamuna Agra), ARSC-13(from Hindon), ARSC-14 (from Coal industry Faridabad), ARSC-15(from Dal Lake Kashmir), ARSC-16 and ARSC-17(from Bangladesh). All selected strains showed minimum inhibitory

concentration value of 198 µg/ml of 1M As_2O_3. No visible growth was seen above this concentration in any of the strains studied in this study. But prominent growth was there below this MIC value in all the strains. The Yamuna and the Faridabad samples were found to have pH 6.54 (±0.05) whereas samples collected from Hoogly, Bangladesh and Ludhiana showed pH 6.90 (±0.05), 7.20 (±0.05) and 6.50 (±0.05) respectively. Our main effort was to characterize *ars* genes from different isolates so that we could find out the divergence between them and then they can be further used for bioremediation of arsenic. Having established the plasmid borne nature of *ars* operon in the selected strains, PCR amplification of *arsC* and *arsA* genes were carried using specific primers, the expected length of PCR products for *arsC* gene corresponding to 370bp was obtained from all the wild type isolated *E. coli* strains when checked on 2% agarose gel and approximately 1700bp amplified product of *arsA* gene was obtained , when electrophoresed on 0.8% agarose gel from only three strains (one from Hoogly river ARSA-1, the second one from Yamuna-Delhi ARSA-2 and the third from Bangladesh sample ARSA-3) out of selected seventeen isolated strains confirming that *arsRBC* operon is more dominant as compared to *arsRDABC* operon in the strains selected in present study. DNA sequencing was performed in order to check the diversity and distribution of *ars* genes. In the present study we found that all the nucleotide sequences for *arsC* gene fragments from selected strains were showing a great diversity (75-92%) but the sequences for all the three *arsA* gene fragments showed high level of similarity (up to 98%) with other already reported sequences of that gene. Our results clearly indicate that there is a major divergence between the sequences of *arsC* genes of selected strains but *arsA* sequences are very much similar to the already reported sequences of *arsA* genes both at nucleotide sequences as well as at amino acid levels. From this we can conclude that *arsC* gene fragments used in this study from different strains of *E. coli* show more diversity among bacteria as compared to *arsA* gene fragments of this study. Out of seventeen selected strains, only three were positive for the presence of *arsA* gene fragments, and confirmed that most of the selected strains of *E. coli* used in this study, had *arsRBC* operon on the plasmids.