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Title of Thesis - **Detection of Bioavailable Arsenic by Whole Cell Based Biosensor**

ABSTRACT

The present research study was aimed to detect the bioavailable fraction of arsenic ions in the water samples after constructing sensor bacteria through genetic engineering. The natural resistance mechanism of *Escherichia coli* against arsenic was implicated and a commonly used laboratory strain was genetically modified to light emitting bacteria using bacterial *lux* genes.

To start up, first the seven water samples were collected for isolation of different *E. coli* strains and from them most efficient promoter for creating fusion with the promoterless *luxCDABE*. The confirmation for *E. coli* was done using biochemical tests and then the MIC with arsenic salts were performed along with growth monitoring and antibiotic susceptibility studies. The *op-arsR* genes were amplified from the plasmid DNA isolated from the *E. coli* strains. Then the amplicons of op-arsR genes were cloned into expression vector pET-28 (a). On the other hand, the Photobacteria *Vibrio fischeri* was collected and its bioluminescent studies were done. Next, the promoterless *luxCDABE* genes were amplified from its genomic DNA. The amplicons were then cloned with the plasmid in which *op-arsR* has already been cloned. Thus, a whole new arsenic inducible operon was created which emitted bioluminescence when the cells harbouring this plasmid were induced with different concentrations of arsenic. Light emission was recorded as Relative Light Units (RLU) using luminometer.

- Water samples collected showed varying physico-chemical properties. Samples from Yamuna river (Delhi), Hooghly river (West Bengal) and Buriganga river (Bangladesh) was more turbid than the other four samples viz., Hindon river (Ghaziabad), Yamuna River (Agra), Coal Industrial area (Faridabad) and Kalu River (Mumbai).
- *E. coli* strains was initially screened on EMB agar and confirmed by biochemical tests. Total 32 strains were isolated.
- The minimum inhibitory concentration of arsenic for solid medium ranged between 27-49µg/mL and for liquid medium ranged between 31-54µg/mL.
- Multimetal resistance was shown by all isolates and AST showed ampicillin resistance was frequent than other 7 seven antibiotics used. Also, multiple antibiotic resistances were observed.
- Plasmid DNA of approx. 54kb was found in all seven highest arsenic resistant strains.

- Amplicons of *op-arsR* (478 bp) from plasmid DNA was cloned and confirmed.
- Genomic DNA (4.2 Mbp) from Photobaterium was isolated and from it the promoterless *luxCDABE* (5.712 kb) was amplified. The amplicons were cloned in the vector containing *op-arsR* from *E. coli*. The ligation was confirmed by transformation into DH5α cells and colony PCR.
- The cells containing *op-arsR* form *E. coli* and *luxCDABE* from *Vibrio fischeri* have now become the sensor cells or bacterial bioreporter.
- The biosensor cells were then calibrated with solutions of known arsenic concentrations. The calibration graphs were plotted according to the amount of RLU emitted when cells exposed to different concentrations at different time periods. The non-linearity in response for both the sensors was observed above the concentration of 0.8µM or above. The lowest concentration which can be measured by our sensor bacteria was 0.74µg As/L and the maximum was 60 µg As/L.
- The concentrations of arsenic in unknown samples were determined by correlating the emitted light units with the known concentrations at standard incubation time of 2 h because at this incubation time always the best linearity in results was obtained.
- Out of total 18 samples, 3 samples collected from tap water of Munirka, Malviya Nagar, Yamuna river (Delhi) and Hooghly river (West Bengal) were found to have arsenic concentration more than the guideline limit of 50 ppb recommended by Indian government and 10 ppb recommended by WHO. Nine samples from Janak Puri, Satya Niketan, Vikas Puri, Patel Nagar, Model Town-II, Naraina, Okhla Phase I, Tuglakabad Extension and Hindon river were found to have concentration of arsenite below 50 ppb but more than 10ppb. Samples from Shaheen Bagh and Sarojini Nagar were having arsenic level of 48 ppb and 50 ppb, which is certainly alarming. Only three samples from Peera Garhi, Pamposh Enclave and Karol Bagh were found to have arsenic concentration below 10 ppb.

Overall the biosensor cells which were constructed were found to be specific and sensitive even to measure the lower concentrations of arsenic than that the prescribed safe limits of WHO and can be a powerful tool for monitoring of environment. But the drawback of this approach lies in their sensitivity to temperature and their cultivation outside the laboratory. Nevertheless, whole cell biosensors have great future with new developments because of their genetic tailoring possibilities.