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Department - Biotechnology
Title of Thesis - Construction of luminescence based sensor for sensitive detection of mercury ions in water samples.

ABSTRACT

The objective of this research study was to construct the sensor bacteria through recombinant DNA techniques which can sense the presence and quantify the amount of mercuric ions present in the water samples. The environmental isolates of *E. coli* and photobacterium *Vibrio fischeri* were used and the non-luminescent DH5α cells were genetically modified to produce bacterial luciferase producing light in proportion to its exposure to the bioavailable mercuric ions.

- In order to start up with this research study six water samples were collected from different geographical metal polluted effluent sink sites of India. Variation in physico-chemical properties like pH, temperature and turbidity was found for different water samples and it was found that the water sample collected from Yamuna river was much more turbid than the others and only the water from Narmada river exhibited least turbidity.
- After initial screening and biochemical identification the all selected *E. coli* strains were subjected to MIC studies showed significant level of tolerance against mercuric chloride on evaluation, Yamuna river sample showed highest resistance 55µg/ml while Narmada River *E. coli* shows least tolerance of 25µg/ml. The minimum inhibitory concentrations of mercuric chloride for 6 mercury resistance isolates were range from 25-55µg/ml.
- The antibiotic susceptibility test (AST) showed ampicillin resistance was frequent than other 7 seven antibiotics used. Also, multiple antibiotic resistances were observed for all strains.
- 24 kb of plasmid was isolated from all the strains and the amplification and cloning of *merR-op-T* genes (873bp) in pET-28(a) was carried out and confirmed by transformation studies.
- The genomic DNA (4.2Mbp) from photobacterium (*V. fischeri*) was isolated and the amplification of promoterless *luxCDABE* (5.712 kb) was done. The amplicons were cloned in pET-28(a) vector in which *merR-op-T* genes have already been cloned. The

ligation was confirmed by transformation into DH5α cells and colony PCR for amplification of *luxCD* (2.129 kb) and *luxCDABE* (3.583 kb). Thus, the whole new inducible operon was generated which will produce bioluminescence on exposure to mercuric ions. The cells harbouring this plasmid have become the biosensor cells.

- Responses of these biosensors were examined with different concentrations of mercuric chloride. The relative light units were measured when these biosensor cells were exposed to mercuric concentrations for different time periods.

- The concentrations of mercury in unknown samples were determined by correlating the emitted light units with the known concentrations at standard incubation time of 150 mins because at this incubation time always the best linearity in results was obtained.
- The results obtained shows that Yamuna river sample had 3.8 ppb of mercuric ions, in Hindon river sample, the concentration was found to be 1.2 ppb, Hooghly river sample had 0.52 ppb, Ganga river sample collected from Rishikesh shows the mercury content of 0.081 ppb. In groundwater of Jamia Nagar (Okhla, New Delhi) shows the presence of 0.7 ppb whereas groundwater from Jasola (Okhla, New Delhi) had mercury concentration of 1.1 ppb.
- The concentration of mercuric ions in Yamuna river and in ground water of Jasola (New Delhi) was found to be quite more than the prescribed safe limits by WHO which is 1ppm or 1µg/L. Therefore, the water from these sources is not safe for human intake and requires a serious concern for the removal of mercury and other heavy metals from these waters.

Thus the biosensors which we had construct showed a good sensitivity to Hg^{2+}, beside some drawbacks such as restricted use of whole cell sensor bacteria in fields, longer incubation time requirement and temperature sensitivity, but still biosensors are powerful tools for monitoring bioavailable metals.