DISSERTATION

DETERMINING PLASMID MEDIATED HEAVY METAL RESISTANCE OF BACTERIA

Submitted by

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ABSTRACT

Bacteria isolated from tannery effluent waters and soil samples contaminated with wastes of brassware industry were exposed to different concentrations of Chromium and Copper. Their tolerance levels were measured. Seven morphologically different bacteria, isolated from tannery effluent water, were tested for Cr (VI) tolerance. Among the isolates, three bacteria were selected as Cr (VI) resistant based on their MICs, percentage survival and EC_{50} values. Chromium resistant bacteria were identified by biochemical methods as *Pseudomonas* sp. and *Bacillus* sp., while *Psuedomonas* sp., *Alcaligenes* sp. and *Aeromonas* sp. were Cu^{2+} resistant. All heavy metal resistant bacterial isolates were subjected to genomic DNA extraction and plasmid DNA extraction. Their plasmid sizes were also determined. Out of all the bacterial isolates, two Cr (VI) resistant bacteria and one Cu^{2+} resistant bacterium contained plasmids. In order to determine whether their heavy metal resistant mechanism is plasmid borne, the plasmids and genomic DNA of heavy metal resistant bacteria were introduced into competent *E.coli* JM109, using chemical transformation methods. Transformants were tested for Cr (VI) and Cu^{2+} resistance. One transformant could tolerate Cr (VI) in a level close to the resistance level of original Cr (VI) resistant bacterium. The transformant was found to pocess the newly introduced plasmid. There were no transformants which could tolerate Cu^{2+} as much as the original Cu^{2+} resistant bacterium In order to determine genes responsible for the heavy metal resistance, the plasmid DNA of the transformant was subjected to PCR amplification using oligonucleotide primers designed for *chrB*, *merA* and *nccA* genes that are known as genes those encode heavy metal resistance. It was found that the isolated plasmid contained *merA* gene confirming that the plasmid bear mercury resistance. The absence of amplified *chrB* product showed that the Cr (VI) resistant gene carried by the particular plasmid is different from the *chrB* gene, for which the particular primer was designed. Further investigations are needed to determine the exact sequence of Cr (VI) resistant gene elements of the plasmid. The transformant together with the original bacterium were tested for Hg^{2+} and confirmed that it is Hg^{2+} resistant.

Keywords: Percentage survival, Chromium resistant bacteria, transformation, PCR amplification, Hg^{2+} resistant