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Development of mercury resistant transgenic *Nicotiana* plants and their environmental impacts

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SUMMARY

Bacterial plasmids encode resistance systems for toxic metal ions including Hg^{++} functioning by energy-dependent efflux of toxic ions. The inducible mercury resistance (*mer*) operon encodes both a mercuric ion uptake and detoxification enzymes. In Gram-negative bacteria especially in *E.coli*, a periplasmic protein, MerP, an inner-membrane transport protein, MerT, and a cytoplasmic enzyme, mercuric reductase (the *mer*A protein), are responsible for the transport of mercuric ions into cell and their reduction to elemental mercury, Hg^{o} . Phytoremediation involves the use of plants to extract, detoxify and/or sequester environmental pollutants form soil and water. Transgenic plants cleave mercury ions from methyl-mercury complexes: reduce mercury ions to the metallic form; take up metallic mercury through their roots; and evolve less toxic elemental mercury. PCR were performed to detect 1695 bp of mercuric reductase gene (*mer*A), which is mainly responsible for the conversion of mercuric (Hg^{++}) and mercurous (Hg^{+}) ions into non-toxic elemental mercury. PCR products of putative *mer*A genes form environmental *E.coli* strains were purified and cloned into a plant expression vector pB1121. The recombinant vector had been further transformed in calli of *Nicotiana taboccum* plants and inoculated on Murashige and Skoog medium. Transgenics are being screened out and their molecular analysis is under process. Expression of *mer*A gene in transgenic plants might provide an ecologically compatible approach for the remediation of mercury pollution. In future, these transgenic plants will be used for trial to measure the mercury volatilization.

Key words : *mer* operon, *E. coli*, *mer*A gene, Phytoremediation, *Agrobacterium tumefaciens, Nicotiana tabaccum*

Bacteria have evolved a variety of means of resistance to heavy metal (Silver, 1996) especially to different forms of mercury found near the polluted sites that include water bodies and landfills. A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of Hg^{++} to its volatile metallic form, Hg^{0} (Ali *et al.*, 2002). The biotransformation is mediated by mercuric reductase and inducible NADPH-dependent. Flavin-containing disulfide oxido-reductase enzyme. The gene encoding mercuric reductase (*mer*A), together with genes coding for Hg^{++} transport and regulatory functions comprises a narrow spectrum *mer* operon (Scott *et al.*, 1999). The *mer*B gene product called organomercurial lyase cleaves the mercuric ion from the organic moiety, allowing subsequent reduction of Hg^{++} to Hg^{0} by mercuric reductase. Available data also indicate that plasmid-encoded resistance to mercury (Misra *et al.*, 1988) is as common as antibiotic resistance. In India, it is estimated that about 180 tons of mercury salts are discharged into the environment annually. In view of the toxicity of mercury and the harmful effects that it inflicts upon the biological community, there is a need to decrease the mercury load in water bodies, particularly in the river system. The present study was carried out to evaluate the resistance offered by several; multimetal-resistant *E. coli* isolates towards mercury and antibiotics. Further, the occurrence and distribution of *mer* genetic determinants was investigated in mercury-resistant as well as mercury-sensitive *E. coli* strains. The mercury resistance genes are clustered in the form of operon, which are mostly associated with plasmids or transposes in Gram-negative bacteria (Brown *et al.*, 1986) and involves inducible mercurial detoxifying enzymes, organonmercurial lyase and mercuric reductase. The transformation studies carried out with the wild plasmids of these isolates confirmed plasmid borne mercury resistance among them as the corresponding transformants showed almost the same pattern of resistance towards the 10^{-4} M concentration of mercury ($HgCl_2$) as their wild type strains. The results suggest that in the collected *E. coli* isolate a broad spectrum *mer* operon possessing both *mer*A and *mer*B genes embedded in a large plasmid is responsible for conferring the resistance towards inorganic form of mercury.

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