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Title of the Ph.D. thesis : **"Mercury volatilization in transgenic *Nicotiana* plants with mercuric reductase (*merA*) gene from environmental *E. coli* isolates"**

ABSTRACT OF Ph.D. THESIS

Mining, agriculture, manufacturing and urban activities, all contributed to extensive contamination of soil and water with heavy metals. Thousands of square miles of land, rivers, lakes and estuaries are contaminated with millions of kilograms of mercury (Hg) in India. Mercury distribution in the environment has been a focus of scientific attention because of the potential health risks posed by mercury exposure and its concentration in organisms. Most of the conventional procedures for cleaning up Hg contaminated sites are all prohibitively expensive and destructive of the natural environment. An alternative approach is the use of plants to remove pollutants from soil and water, known as "phytoremediation". The main objective of this study was to transform merA gene from environmental E. coli to tobacco plants, which has a property of reducing the mercury from Hg^{2+} to least toxic, less reactive elemental Hg^0 and investigate its phytoremediation abilities.

Hg^r E. coli have been isolated from 8 mercury polluted sites of India. Their response for $HgCl_2$/PMA was studied and found that narrow spectrum Hg^r is more prevalent in nature. Antibiotic susceptibility was checked for 7 antibiotics and found that multi-antibiotic resistance genes are present on plasmids. E. coli were identified biochemically and their mer operon was located. merA PCR was performed and cloned into pGEM-T and pBI121, respectively. Sequencing of full length merA from Yamuna-II showed ~98% homology with R100 merA. On the other side, Nicotiana seeds were grown and callus was induced. Construct of merA in binary vector was made and calli transformation was performed with A. tumefaciens GV3101. Transgenics were verifed for merA integration through PCR and Southern transfer. Transgene merA expression was checked by RT-PCR and Northern blot. Over-expression of merA protein was performed with pQE-30UA in E. coli BL21(DE3)pLysS cells and over-expressed protein was purified from 6XHis-tag column for anti-merA polyclonal antibody generation into rabbit. Western blot was performed using rabbit raised anti-merA as probe; high expression of MerA was noticed in 3 lines in comparison to rest 2 lines. Mercury response assay was performed in tissue culture as well as in soilrite conditions to check the volatilization ability of the transgenics, and it was noticed that transgenic lines were 6-7 times more resistant towards inorganic mercury in comparison to wild type tobacco plants. The study is the primary step towards the development of field adapted mercury resistant plants which can be utilized for the detoxification of mercury pollution in near future.

The key finding and novelty of the study is the integration of bacterial native merA gene into nuclear genome of tobacco plants without any codon modification, and merA expression and enhanced mercury volatilization form tobacco transgenics. Based on the results obtained in the present study, it can be concluded that the expression of merA gene in transgenic tobacco plants might provide an effective and superior means for phytoremediation of mercury-polluted areas. The placement of these improved plants around pollution sources and at their point of discharge and harvest could prevent toxic Hg^{2+} accumulation and transport from these locations.