Mercury resistant *E. coli* strains have been isolated from different mercury polluted sites of India namely Yamuna River (YR), Delhi; Hindon River (HR), Ghaziabad; Kalu River (KR), Bombay; Guru Tegh Bahadur Hospital (GTB Hospital), Delhi; Floodwater (FW), Delhi; Hoogly River (H_gR), Kolkata; and Coal Industry (CI), Faridabad; whereas the sample collected from Dal Lake (DL), Srinagar, Kashmir (which is a pristine-type lake) was taken as control. Their MIC levels were determined as the lowest concentration at which no growth was found on incubation at 37^0C for 18-24 hours and the determination of resistance was also performed for various antibiotics by disk inhibition test. Once, biochemically identified; plasmid DNA was isolated by the alkaline lysis method. The location of *mer* operon was determined by transforming the isolated plasmids into mercury sensitive host *E. coli* DH5α cells. The corresponding transformants were grown on Luria Agar plates amended with 100 µM concentrations of mercury. Plasmid isolated from transformed DH5α cells were also analyzed and compared with the plasmid profile of the wild-type strains. Primer combinations of *mer*A-FJ (5′ CGG GAT CCA TGA GCA CTC TCA AAA TCA CC 3′) and *mer*A-RJ (5′ TCC CCC GGG ATC GCA CAC CTC CTT GTC CTC 3′) having *Bam*H1 site on antisense primer and *Sma*1 site on sense primer were used for the amplification of *mer*A gene of size 1695 bp. *E. coli* R100 was used as a positive control for *mer*A. PCR product of putative *mer*A gene was purified and cloned into pBI121 (13.0 kb) plant expression vector. The construct (pZA04) had been transformed into competent *Agrobacterium tumefaciens* cells. They had been further screened by PCR and restriction endonuclease digestion for the presence of the putative *mer*A gene.

Uniform sized calli (*Nicotiana tabaccum* cv Xanthium) of size 0.2 - 0.3 mm were prepared by cutting the undifferentiated mass of cells. The calli were then immersed in

Agrobacterium culture containing the construct pZA04 (1:5 dilution of overnight grown culture in Yeast Extract Mannitol medium) for 15-20 min. Calli were taken out, blot dried and placed upside down on the MS plates. The explants were allowed to co-culture with *Agrobacterium* for 2-3 days in dark. After five weeks, callus was induced and further transferred on MS half strength medium (pH 5.8) supplemented with NAA (0.5 mgL^{-1}) and kinetin (1 mgL^{-1}). *Agrobacterium* construct (pZA04) have been made for the transformation of *Nicotiana tabaccum* plants. The disarmed Ti-binary vector in *Agrobacterium tumefaciens* (GV3101) had been used in calli transformation to produce transgenic *tobacco* plants. Integration of the transgene in transformed plants were analyzed by Polymerase Chain Reaction (PCR) and Southern blotting.

Total RNA was extracted from leaves of transgenic *Nicotiana tabaccum* plant using RNeasy plant mini kit (Qiagen, Germany). Complementary DNA (cDNA) were synthesized from the plant mRNA purified from total RNA and expression of the integrated gene in transgenic plants was analyzed by RT-PCR. The bands were found of the expected size of 1695 bp. The purified *mer*A gene was cloned in pQE30UA (3.5 kb) expression vector and transformed in BL21(DE3)Plys. host cells at *Sma*1- *Bam*H1 site to over express this protein as 6X His tagged proteins. The expressed protein will be purified by column chromatography. This purified protein will be used to raise polyclonal antibody that will further be used for western blotting (high level expression of mercuric reductase gene (*mer*A) in transgenic *Nicotiana* plants).