RESEARCH PAPER

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Cloning of mercuric reductase (*mer*A) gene isolated from wild strains of *Escherichia coli*

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Bacterial plasmids encode resistance systems for toxic metal ions including Hg^{2+} 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^0. Phytoremediation involves the use of plants to extract, detoxify and/or sequester environmental pollutants from 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^{+2}) and mercurous (Hg^{+1}) ions into non-toxic elemental mercury. PCR products of putative *mer*A genes form environmental *E.coli* strains were purified and cloned into a suitable plant expression vector like pB1121 or pCAMBIA. The construct will be transformed in calli of *Nicotiana* plants. Expression of *mer*A gene in transgenic plants might provide an ecologically compatible approach for the remediation of mercury pollution.

Key words : Mercury resistance, *E. coli*, PCR amplification, *Nicotiana*

INTRODUCTION

Mercury pollution is a major environmental problem accompanying industrial activities. Mercury, a potent neurotoxin, is one of the most harmful and toxic environmental pollutants. Actually, mercury and its compounds when released into the environment are highly toxic to living cells because of their strong affinity for the thiol groups of proteins (Hajela *et al*., 2002). However, its levels have risen due to environmental contamination, such as burning coal and petroleum products, use of mercurial fungicides in paper making and agriculture and mercury catalyst in industry, with a consequent release of mercury into the air and water on the land. These activities can increase local mercury levels several thousand fold above background (Robinson and Tuovinean, 1984). Therefore, environmental pollution is an increasing problem both for developing and developed countries. Industrial use of mercury led to pollution of environment. Consequently, mercury removal is a challenge for environmental management. Most of the mercury released ends up and retained in the soil as complexes of the toxic ionic mercury (Hg^{2+}), which then can be converted by microbes into the even more toxic methylmercury which tends to bioaccumulate. Mercury detoxification of the soil can also occur by microbes converting the ionic mercury into the least toxic metallic mercury (Hg^0) form, which then evaporates. Microorganisms in contaminated environments have developed resistance to mercury and are playing a major role in natural decontamination (Nikiforvo *et al*., 1999). An extensively studied resistance system, based on clustered genes in an operon (*mer* operon) allows bacteria to detoxify Hg^{2+} into volatile metallic mercury by enzymatic reduction (Summers, 1986). Mercury–resistance determinants have been found in a wide range of Gram-negative and Gram- positive bacteria isolated from different environments. They vary in the number and identity of genes involved and are encoded by *mer* operons, usually located on plasmids (Summers and Silver, 1972; Brown *et al.,* 1986; Griffin *et al.,* 1987) and chromosomes; they are often components of transposons (Misra *et al.,* 1984) and integrons. A widely employed mechanism of bacterial resistance to mercurial compounds is the reduction of (Hg^{++}) to its volatile metallic form Hg^0 (Liebert *et al.,* 1997).The biotransformation is mediated by mercury reductase, an inducible NADPH-dependent, flavin containing disulfide oxidoreductase enzyme. The gene coding for mercury reductase is *mer*A (Nies, 1999). The bacterial *mer* operon encodes a cluster of genes involved in the detection, mobilization and