DEVELOPMENT OF TRANSGENIC YELLOW-POPLAR FOR REMEDIATION OF MERCURY POLLUTION

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Phytoremediation involves the use of plants to stabilize, reduce or detoxify pollutants, including heavy metals. A number of plants, known as hyperaccumulators, have displayed a tolerance for accumulation of high levels of some heavy metals and have generated considerable interest in their use for soil decontamination. However, hyperaccumulator plant species are typically slow-growing, possess low biomass and probably are of limited utility for phytoremediation. Engineering other plant species with the genes involved in hyperaccumulation may be one option for making use of this ability, but the genetic pathways for hyperaccumulation in plants are somewhat complex. By contrast, bacterial heavy metal resistance genes are assembled in discrete operons of small numbers of genes. The expression of bacterial heavy metal genes in transgenic plants could expedite the development of efficient phytoremediative species. Trees in particular are excellent candidates for engineering with heavy metal detoxification genes for use as phytoremediative crops, due to their large biomass, long lifetimes, and abundance of nonliving, woody tissues.

Aerobic bacteria may display broad-spectrum mercury resistance by virtue of a cluster of genes comprising the mercury resistance operon (Summers 1986). One gene of the bacterial mercury resistance operon, *merA,* codes for mercuric ion reductase, which converts Hg++ to elemental mercury, Hg^0. The *merA* gene provides a potential mechanism for mercury removal and detoxification using plant genetic engineering. Rugh et al. (1996) employed a directed sequence modification strategy to develop modified *merA* gene constructs for transformation and analysis in plant species. Overlap-extension PCR (OE-PCR) was used to create gene sequences having codon usage and nucleotide ratios more typical of highly expressed plant genes, though without altering the MerA enzyme sequence or structure. The bacterial gene was modified in a series of stepwise constructions to optimize the flanking regions and coding sequence of the gene, resulting in constructs with only the flanking region optimized *(merAO),* and with 9 percent or 19 percent of the coding region optimized in addition to the modified flanking regions *(merA9* and *merA19,* respectively). *Arabidopsis thaliana* plants transformed with *merA9* displayed high level Hg++ resistance (Rugh et al. 1996).

As discussed by Rugh et al. (1995), our long-term goal is to test the same modified *merA* gene in forest trees, which may someday be applied for actual remediation of mercury-contaminated sites. Using the technique developed by Wilde et al. (1992), *merAO, merA9* and *merA19* were each transformed into embryogenic yellow-poplar *(Liriodendron tulipifera)* cells as inserts in plasmid pVSTI. The same plasmid without inserts of the modified *merA* genes was used to generate transformed controls. Bombarded cells were selected on 100 μg/ml kanamycin and kan-resistant colonies of PEMs began to appear after about 2 months. Between 30 and 45 independent putatively transformed PEM colonies were generated for each *merA* construct. Small clumps of putatively transformed PEMs were tested for survival and growth on semisolid medium with 25 or 5014M $HgCl_2$. Many mercuric ion-resistant lines were found, some able to withstand up to 100 μ
transformants were apparently no longer Hg++-resistant. The loss was especially striking among merAO-transformed lines, compared to those transformed with *merA9* and *merA19*. Genomic DNA PCR was used to detect *merA* transgenes in putative transformant yellow-poplar PEMs. The percentage of lines confirmed by PCR to contain *merA* transgenes that were Hg++-resistant was determined for each merA construct set. This percentage appeared to relate

well to the extent of plant-optimized gene modification for each transformed set. Methylation could have been responsible for this relationship, with the forms of the gene less optimal for plant expression tending to be methylated over time.

Suspension cultures were initiated from the nontransgenic source line, vector (pVSTI) transformed control, and lines transformed with *merAO, merA9* and *merA19*. PEMs grown in suspension were size fractionated as described in Merkle et al. (1990) and plated on basal medium with or without $HgCl_2$ to generate populations of somatic embryos. Only one line transformed with *merA19* produced mercuric ion-resistant embryos from fractionated PEMs following plating on medium with 25 or 50 μM Hg++. Embryos from this line could also germinate on germination medium with up to 50 M Hg++.

We used a mercury vapor analyzer to measure activity of the MerA enzyme in regenerated *merA19* plantlets and untransformed control plantlets. Germinants were planted in 15 mls of half-strength germination medium gelled with 1.5% low melting point agarose, while the medium was still molten, allowing the medium to gel around the root system of each germinant. Tubes were attached via a sampling port to the mercury vapor analyzer and the headspace in each tube was sampled every 12 hours for 6 days. As the end of the mercury evolution assay, two plantlets of each genotype were removed from the tubes and analyzed for mercury content. Hg^0 release rates were dramatically different between the *merA19* plantlets and control plantlets, with *merA19* plantlets displaying up to ten times the Hg^0 evolution rates of control plantlets. Analysis of MerA-yellow-poplar plantlets confirmed that they also accumulated less mercury in their tissues relative to controls.

These preliminary results indicate that the modified merA genes can confer mercuric ion resistance and detoxification on yellow-poplar trees. We are currently preparing experiments t0 examine the ability of merA-yellow-poplar plants to extract and detoxify ionic mercury from prepared soil mixtures as a model for actual site remediation.

Literature Cited

Merkle, S.A., R.J. Sotak, A.T. Wiecko and H.E. Sommer. 1990. Maturation and conversion of *Liriodendrontulipifera* somatic embryos. In Vitro Cell. Dev. Biol. 26:1086-1093.

Rugh, C.L., H.D. Wilde, N.M. Stack, D.M. Thompson, A.O. Summers, and R.B. Meagher. 1996. Mercuric ion reduction and resistance in transgenic *Arabidopsis thahana* plants expressing a modified bacterial *merA* gene. Proc. Natl. Acad. Sci. USA 93:312-3187.

Summers, A.O. 1986. Organization, expression, and evolution of genes for mercury resistance. Ann. Rev. Microbiol. 40:607-634.

Wilde, H.D., R.B. Meagher, and S.A. Merkle. 1992. Expression of foreign genes in transgenic yellow-poplar plants. Plant Physiol. 98:114-120.