The engineered phytoremediation of ionic and methymercury pollution

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Graduate students working on EMSP project

Graduate	Position	% Time	Current Status
Scott Bizily	Genetics Graduate Student with RBM	100%	Ph.D. candidate in final year / Genetics Training Grant Slot
Andrew Heaton	Ecology Graduate Student with RBM	100%	Ph.D. candidate
Anne Marie Zimeri	Genetics Graduate Student with RBM	100%	Recently joined – Ph.D. candidate

YEARLY PROGRESS REPORT

RESEARCH OBJECTIVE

Our long-term objective is to enable highly productive plant species to extract, resist, detoxify, and/or sequester toxic heavy metal pollutants (Meagher, 2000). We have focused our research on the phytoremediation of soil and water-borne ionic and organic mercury (Meagher and Rugh, 1996; Meagher et al., 2000). Mercury pollution is a serious world-wide problem affecting the health of human and wild-life populations. The Department of Energy's Oak Ridge National Laboratory and Brookhaven National Laboratory have sites with significant levels of mercury contamination that could be cleaned by applying the scientific discoveries and new phytoremediation technologies described in this proposal. In the near future, the experience gained through engineering plants that hyperaccumulate mercury, can be applied to extraction or accumulation of various toxic heavy metal and radionucleide contaminates at dozens of DOE sites.

RESEARCH PROJECT AND IMPLICATIONS

During the first three-year EMSP grant period (1996-1999), we successfully engineered several diverse plant species (e.g., *Arabidopsis*, tobacco *Brassica napus*, yellow poplar) by using a highly modified bacterial mercuric ion reductase gene, *merA*, to detoxify ionic mercury (Hg(II)) by reducing it to Hg(0) (Rugh et al., 1996; Heaton et al., 1998; Rugh et al., 1998a; Rugh et al., 1998b). MerA expressing tobacco and *Arabidopsis* seeds germinate and plants grow and set seed at normal growth rates on levels of Hg(II) that are lethal to normal plants. During the last year (1999/2000) we focused on physiological experiments with transgenic yellow poplar and tobacco. Our recent results suggest these plants extract ionic mercury (Hg(II) from contaminated soil and water, reduce it to Hg(0), translocate Hg(0) up the vascular system, and transpire Hg(0) from leaves. Grafting experiments with various combinations of wild-type and MerA expressing tobacco shoots and roots suggest plants can be engineered to reoxidize Hg(0) to Hg(II) in leaves to trap and hyperaccumulate mercury above ground. Two additional accomplishment finished this year were completing the construction of an entirely synthetic 1695 bp long *merA* gene that is optimized in GC composition and codon usage for expression in plants and demonstrating that MerA expressing rice detoxify mercury in aquatic sediments.

Methylmercury (MeHg) produced by native bacteria at mercury-contaminated wetland sites is a far more serious problem than Hg(II). MeHg is inherently more toxic than Hg(II). It is efficiently biomagnified by several orders of magnitude in the food chain and poses the most immediate threat to animal populations. MeHg is responsible for the vast majority of cases of mercury poisoning from mercury contaminated fish. In order to eliminate the threat from this toxin in the environment, we have engineered two model plant species, *Arabidopsis* and tobacco with a modified bacterial organomercurial lyase gene (*merB*) to degrade methylmercury and other forms of organic mercury (PMA) to the less toxic Hg(II) (Bizily et al., 1999). Many of the plants examined are highly resistant to levels of MeHg far beyond the levels ever recorded in the environment. During the last one year period (1999/2000) we focused on confirming our results on the activity of MerB alone in plants and on experiments combining the *merB* and *merA* genes in a single plant species. Plants expressing both *merA* and *merB* are resistant to even higher levels of MeHg and are capable of efficiently converting MeHg to Hg(0) at levels 1000 times faster than control plants (Bizily, in preparation). Initial results suggest that MerB activity is rate limiting in the coupled MerA/MerB catalyzed reaction. An extensive array of molecular physiological experiments are underway to further characterize the expression of *MerA* and

MerB in transgenic plants. Our work suggests that native macrophytes (e.g., trees, shrubs, grasses) can be engineered to thrive on and detoxify the most abundant forms of ionic and organic mercury at polluted sites (Meagher et al., 2000; and in preparation). In addition, our research suggests that phytoremediation is an environmentally friendly and affordable technology that can be applied globally to massive pollution problems in Eastern Europe and the Third-world (Meagher, 1998).

PLANNED ACTIVITIES

Our working hypothesis for future research is that transgenic plants expressing the bacterial *merB* and *merA* genes will **a)** remove mercury from polluted sites and **b)** prevent MeHg from entering the food chain. This hypothesis is being tested by focusing our research on three Specific Aims: (1) to test the mechanisms and efficiency of ionic mercury removal from media and soil by transgenic plants (expressing various *merA* gene constructs; (2) to test the ability of transgenic plants expressing *merB* and/or *merA* to degrade MeHg and prevent the release of MeHg from mercury-contaminated sediment; and (3) to engineer plants that extract mercury from soil and water and hyperaccumulate mercury and other toxic metals aboveground in leaves and stems. The emphasis on hyperaccumulation of mercury and other toxic metals in Specific Aim #3 represents a major new direction for our research effort that complements previous work on mercury transformation and detoxification. Several new bacterial, fungal, animal, and plant genes are being isolated and tested in transgenic plants for various mercury hyperaccumulation strategies.

INFORMATION ACCESS (PUBLICATIONS AND WEB SITE)

www.genetics.uga.edu/RBMSite/Abstracts.html#anchor-40901557

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