The engineered phytoremediation of ionic and methymercury pollution

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Graduate students working on EMSP project

Student	Position	% Time Project	Current Status
Scott Bizily	Genetics Graduate Student with RBM	100%	Completed Ph.D. 2001– Currently postdoctoral fellow at Univ. Penn.
Andrew Heaton	Ecology Graduate Student with RBM	100%	Ph.D. candidate
Anne Marie Zimeri	Genetics Graduate Student with RBM	100%	Ph.D. candidate
Raoufa Rahman	Graduate Student - Alexandria Univ., Alexandria, Egypt -RBM is Co-Advisor	100%	Ph.D. candidate - recently joined the laboratory

YEARLY PROGRESS REPORT

RESEARCH OBJECTIVE

Our long-term objective is to enable highly productive plant species to extract, resist, detoxify, and/or sequester toxic organic and heavy metal pollutants (Meagher, 2000) applying scientific strategies and technologies from a rapidly developing field called phytoremediation. The phytoremediation of toxic elemental and organic pollutants requires the use relatively different approaches (Meagher, 2000). **Our current specific objectives are to use transgenic plants to control the chemical species, electrochemical state, and aboveground binding of mercury to a) prevent methylmercury from entering the food-chain, b) remove mercury from polluted sites, and c) hyperaccumulate mercury in aboveground tissues for later harvest.** Various parts of this strategy are being critically tested by examining different genes in model plants and field species and comparing the results to control plants as recently reviewed (Meagher et al., 2000; Rugh et al., 2000).

RESEARCH PROJECT AND IMPLICATIONS

During the last grant period we focused our efforts on plant species that would be more useful along and in aquatic environments where methylmercury is a produced (e.g., rice and cottonwood). Both plant species required great initial effort to transform and propagate, but now are available for testing. Multiple transgenic lines of both species appear to use our highly modified bacterial mercuric ion reductase gene, *merA*, to detoxify ionic mercury (Hg(II)), by reducing it to Hg(0) (Heaton and Meagher, in preparation; Che et al., in preparation a & b). MerA expressing rice and cottonwood plants grow vigorously on levels of Hg(II) that are lethal to normal plants. During the last year (2000/2001) we began to focus on physiological experiments with transgenic cottonwood and rice, determining how they would process mercury in damp or aquatic sediments. MerA expressing cottonwood and rice plants are extremely resistant to mercury even in sediments where it is quite toxic and kills wild-type control cottonwood plants.

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), 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. MeHg has been a major focus of our research for the last two years. Model plants, *Arabidopsis* and tobacco, have been transformed 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). Arabidopsis 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 et al., 2000) as shown in the figure below. However, this research demonstrated conclusively that MerB activity is rate limiting in the coupled MerA/MerB catalyzed reaction. During the last year (2000/2001) we focused on improving the efficiency of MerB activity by targeting the enzyme to subcellular environments (Bizily et al., in prep.; Bizily, 2001, Ph.D. Thesis). In a very significant finding, MerB enzyme targeted to the endoplasmic reticulum (ER) or through the ER to the cell wall processes methylmercury 10 to 20 times more efficiently than cytoplasmic MerB. Subcellular targeting of enzymes is an exciting new breakthrough for

phytoremediation of toxic heavy metals or organics. 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, 2000; Meagher et al., 2000).

During this last grant year (2000/2001) we focused research in three new areas, each with the aim of developing plants that hyperaccumulate mercury and other metals. First, a postdoctoral fellow, Yujing Li, isolated genes encoding the three enzymes in the phytochelatin synthesis pathway γ-glutamylcysteine synthetase (*E. coli*), glutathione synthetase (*E. coli*) and phytochelatin synthetase (fission yeast, *Arabidopsis*, genes in collaboration with Dr. J. Schroeder). They were cloned for constitutive plant expression (all tissues and organs) and for focused expression aboveground in leaves and stems. These constructs have been tested in transgenic *Arabidopsis* and show that plants expressing any one of these three enzymes are more resistant to mercury than control plants (Li et al. in prep. a & b; Dhankher et al., in prep.). In addition, we isolated three specific monoclonal antibodies to monitor the plant expression levels of three of these proteins using a novel rapid immunological protocol developed in our laboratory (Li et al., 2001). Second, a graduate student isolated several of the diverse *Arabidopsis* metallothionein genes and demonstrated their differential binding specificities for Hg(II) and other metal ions (Zimeri et al., in prep).

Third, we examined three new promoter systems for expressing phytoremediating enzymes like MerA and MerB. We developed the ACT2pt (pt = promoter terminator) system, based on *Arabidopsis* actin gene *ACT2* (Balish, in prep.). *ACT2pt/merA* expresses MerA protein at 5 times higher levels than the widely accepted *35Sp/NOSt* constitutive expression system. Dr. Balish has shown that *ACT2pt/merA* appears to be more consistent in being expressed in nearly all transgenic Arabidopsis and tobacco lines tested instead of only 60-70% of the *35Sp/merA/NOSt* lines. Similarly, we developed SRS1pt based on our previous work a decade ago on the light induced SRS1 soybean rubisco small subunit gene (Parkash-Dhankher, in preparation). This new cassette expresses very high levels of a GUS reporter in leaves, but not roots. It will be used to express genes that assist with the hyperaccumulation of metals in above-ground tissue. A third plant promoter system was obtained from a European laboratory for root-specific expression of MerA and MerB, to be used as part of our mercury hyperaccumulation efforts (Heaton, in prep.). This tobacco promoter is active in roots, but further research is needed to see if it is expressed

strongly and specifically enough for efficient expression of *merA* and detoxification of Hg(II) in roots.

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 primarily in rice and cottonwood; (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 primarily with *Arabidopsis* and cottonwood; (3) we will continue to examine targeted MerB protein expression in attempts to improve efficiency of this rate limiting enzyme; and (4) to engineer plants that extract mercury from soil and water and hyperaccumulate mercury and other toxic metals aboveground in leaves and stems. Over a dozen different gene constructs are being examined in transgenic plants to improve mercury resistance and accumulation in plants. Our promising results from this last year's research suggests that progress on the hyperaccumulation of mercury and other toxic metals will be rapid during the next grant period. These experiments are being repeated and data analyzed for several different manuscripts that are in preparation (Li et al., 2001; Meagher, 2002; Bizily et al., in prep.; Heaton and Meagher, in prep.; Heaton et al., in prep.-a; Heaton et al., in prep.-b; Li et al., in prep.; Balish et al., in prep.; Che et al., in prep.-a; Che et al., in prep.-b; Dhankher et al., submitted; Li et al., in prep.; Zimeri et al., in prep.).

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