Phytoremediation of Ionic and Methyl Mercury Pollution

Richard B. Meagher
Genetics Department
University of Georgia
Life Science Building/Room B402A
Athens, CA 30602-7223

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phone: 706-542-1444
fax: 706-542-1387
email: Meagher@arches.uga.edu
http://www.genetics.uga.edu/rbmlab/

Graduate and undergraduate students who have work on this EMSP project in the last grant period and/or will continue to work on this project in the next period. Andrew Heaton, Ecology Graduate Student with RBM and now a postdoctoral fellow,100% time, Ph.D. Finished 2003 Anne Marie Zimeri, Genetics Graduate Student with RBM,100%, Ph.D. candidate, Finishing mid 2004 Raoufa Rahman, Graduate Student, Alexandria Univ., Alexandria, Egypt -RBM was Co-Advisor,100% Ph.D. Finished mid 2003 Rebecca Rogers, UGA Genetics Presidential Scholar beginning her undergraduate thesis project. 50%,B.S. candidate, sophomore.

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 by applying scientific strategies and technologies from a rapidly developing field called phytoremediation. The phytoremediation of toxic elemental and organic pollutants employs a variety of different approaches (Meagher, 2000). Our current specific objectives are to use transgenic plants to control the chemical species, electrochemical state, transport, 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 and waste disposal. 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 we reviewed previously (Meagher et al., 2000; Rugh et al., 2000). A positive spin-off from this work on mercury has been a strategy for the phytoremediation of arsenic (Dhankher et al., 2002) and cadmium (Dhankher et al., 2003).

RESEARCH PROJECT AND IMPLICATIONS

During the last grant period we focused our efforts on examining transgenic plant species that would be more useful adjacent to and in aquatic environments (e.g., rice and cottonwood) where methylmercury is a produced, and expanding the genetic capabilities of model plants (e.g., tobacco and Arabidopsis) for hyperaccumulation. MerA-expressing tobacco (Heaton et al., in prep.), cottonwood (Che et al., 2003) and rice (Heaton et al., 2003) plants are extremely resistant to mercury even in sediments where it is highly toxic and kills wild-type control 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, and therefore, 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). However, this research demonstrated

conclusively that MerB activity is rate limiting in the coupled MerA/MerB catalyzed reaction. During the past two years (2000-2002) we focused on improving the efficiency of MerB activity by targeting the enzyme to subcellular environments (Bizily et al., 2003). 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 that we modeled earlier (Meagher, 2000; Meagher et al., 2000). During this last grant period we focused research in several areas, each with the direct or indirect aim of developing plants that detoxify and/or hyperaccumulate mercury more efficiently. First, a postdoctoral fellow, Yujing Li, finished our research on three enzymes in the phytochelatin synthesis pathway (ECS, GS, PCS). This work included setting up a new fluorescent HLPC assay and examining hundreds of plant samples by for levels of the peptide products of this pathway (Li et al., in prep.-a; Li et al., in prep.-b; Li et al., submitted). Expression of any one of these enzymes confers mercury resistance to plants and combinations of these enzymes provide even higher levels of resistance. Second, and perhaps most new and exciting for this specific mercury project are our results identifying two strong Arabidopsis transporters for mercury among nine zinc transporters we examined. The transporter genes were supplied to us by Mary Lou Guerinot. We believe that these zinc pumps are responsible for accidentally bringing mercury into native plants and causing increased toxicity. By enhancing and ultimately modifying their activity we plan to elevate mercury uptake in engineered hyperaccumulators. To balance this increased uptake of toxic mercury we have several engineered mechanisms to confer high-level mercury resistance. Third, we were able to engineer a strong root-specific promoter system that expresses the target transgene in all root cell types. Both a GUS reporter construct and a merA18 gene construct were expressed at 20 to 1000 fold higher levels in roots than in leaves of several independent transgenic plants. The data on this new expression system are now being assembled into a manuscript (Balish et al., in prep.). Previous research on two root-specific promoters from other laboratories, as described in our original EMSP proposal, met will disappointing results. The new root-specific promoter system is needed to elevate MerA activity in roots only, which will helps transport mercury more efficiently to aboveground tissues for hyperaccumulaiton. In addition, we plan to express mercury transporters using this new promoter system to enhance mercury uptake from sediment. Fourth, we have our first data on a novel conditional male female sterility system to be used for phytoremediation. The long-term release of transgenic plants into the environment for phytoremediation would require less management and cost less if the plants were both male and female sterile. These sterile plants would not be able to pass transgenes into wild plant populations. Based on our published data on actin gene promoters that are expressed strongly, but almost exclusively, in reproductive tissues (An et al., 1996; Huang et al., 1997) we developed simple expression vectors to targeted essential steps in biosynthetic pathways for knockdown by RNA interference (RNAi) or dominant-negative suppression by expression of a mutant protein with negative activities. Many pathways could be targeted by this approach, but we chose to knockdown a vitamin pathway. One of the first two RNAi transgenes examined produced the desired lethality in reproductive tissues. While the T1 generation heterozygotes plants were viable only 25% of the Arabidopsis T2 seeds are viable. These viable seeds turned out to be only those that were homozygous wild type and lacked the transgene by normal Mendelian segregation. The remaining 75% of the seeds turned black and died during development within the fruit (siliques). Again, no T2 seeds developed containing even a single copy of this sterility gene, but the T1 generation plants themselves developed normal stature and set the normal number of siliques. In contrast, when the required vitamin was added to the substrate during growth of the T1 plants, viable seeds developed and the sterility gene was passed on. This system will be used to make sterile plants for long term planting and phytoremediation. The plants will be propagated vegetatively or sexually in the presence of the required vitamin. It should only take another 12 to 18 months to finish the basic research on this important tool in Arabidopsis. At that time, we would begin to move these sterility genes into conservation species like cottonwood and develop their field application. Our research on cottonwood are performed collaboratively with Professor Scott Merkle in the School of Forest Resources at UGA.

PLANNED ACTIVITIES

In order to advance this mercury phytoremediation strategy, our research has focused on the following Specific Aims and we have made the initial progress indicated: (1) to increase the transport of mercury to

aboveground tissue through the root expression of MerA and a mercury transporter; (2) to identify small mercury binding peptides that enhance hyperaccumulation aboveground (initial results are positive with EC and MerP peptides); (3) to test the ability of multiple genes acting together to enhance resistance and hyperaccumulation (several new gene combinations have shown promise conferring higher levels of mercury resistance); (4) to construct a simple molecular system for creating male/female sterility, allowing engineered grass, shrub, and tree species to be released indefinitely at contaminated sites (initial data are positive on model plants engineered with remediable nutrient-based sterility); and (5) to finish testing the ability of transgenic cottonwood and rice plants to detoxify ionic mercury and prevent methymercury release from contaminated sediment (merA cottonwood have been planted in the field). The results of these experiments will enable the phytoremediation of methyl- and ionic mercury by a wide spectrum of deep-rooted, fast-growing plants adapted to diverse environments.