Phytoremediation of ionic and methymercury pollution

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Graduate students working on EMSP project

Student	Position	% Time Project	Current Status
Andrew Heaton	Ecology Graduate Student with RBM	100%	Ph.D. candidate, Finished Dec., 2002
Anne Marie Zimeri	Genetics Graduate Student with RBM	100%	Ph.D. candidate, Finishing Early 2004
Raoufa Rahman	Graduate Student - Alexandria Univ., Alexandria, Egypt -RBM is Co-Advisor	100%	Ph.D. candidate – Finishing 2003

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 we recently reviewed (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.

RESEARCH PROJECT AND IMPLICATIONS

During the last grant period we focused our efforts on examining transgenic plant species that would be more useful along and in aquatic environments where methylmercury is a produced (e.g., rice and cottonwood) and expanding the genetic capabilities of model plants to hyperaccumulation. We completed many physiological experiments with transgenic tobacco, cottonwood and rice. MerA expressing tobacco (Heaton et al., in prep.), cottonwood (Che et al., submitted) and rice (Heaton et al., 2003) 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 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 as we modeled earlier (Meagher, 2000; Meagher et al., 2000).

During this last grant year 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 □-glutamylcysteine synthetase (*E. coli*)(Li et al., submitted), glutathione synthetase (*E. coli*) (Li et al., in prep.) and phytochelatin synthetase (fission yeast, *Arabidopsis*, genes in collaboration with Dr. J. Schroeder) (Li et al., in preparation). They were cloned for constitutive plant expression (all tissues and organs) with an actin promoter and for focused expression aboveground in leaves and stems with a rubisco promoter. These constructs have been tested in transgenic *Arabidopsis* and show that plants expressing any one of these three enzymes are more resistant to mercury and arsenic than control plants. 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., submitted).

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 (Dhankher et al., 2002; Li et al., submitted). 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.

Fourth, we completed our first field testing with transgenic *merA* expressing tobacco plants for the remediation of mercury. Two cycles of planting these plants reduced the mercury concentrations in their root zone in the field from 350 ppm to 250 ppm in a six month period. A second field test with transgenic *merA* expressing cottonwood is planned for this spring. We

have propagated large numbers of plants to this effort and confirmed their stable expression of MerA protein.

Fifth, we examined plants expressing the bacterial arsenic reductase *ArsC* gene. These plants were hypersensitive to growth on arsenate due to formation of the more toxic oxyanion arsenite. When ArsC protein expression was combined with overexpression of □-ECS (above) plants became highly resistant to arsenic and accumulated three time the normal level of arsenic in leaf tissues (Dhankher and Meagher, 2003). This is because We screened the ArsC expressing lines for activities to other metal ions such as Hg(II) and Cd(II) and surprisingly found significant levels of Cd(II) resistance associated with high levels ArsC protein expression (Dhankher et al., 2003).

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; (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 been quite successful); (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 (a significant reduction of mercury from sediment was demonstrated). 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.

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