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Phytoremediation of Ionic and Methyl Mercury Pollution

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Research Objective

The long-term objective of our research is to manipulate single-gene traits into plants, enabling them to process heavy metals and remediate heavy-metal pollution by resistance, sequestration, removal, and management of these contaminants. We are focused on mercury pollution as a case study of this plant genetic engineering approach. The working hypothesis behind this proposal was that "transgenic plants expressing both the bacterial organo mercury lyase (merB) and the mercuric ion reductase gene (merA) will: A) remove the mercury from polluted sites and B) prevent methyl mercury from entering the food chain." The results from our research are so positive that the technology will undoubtedly be applied in the very near future to cleaning large mercury contaminates sites. Many such sites were not remediable previously due to the excessive costs and the negative environmental impact of conventional mechanical-chemical technologies.

Research Progress and Implications

At the time this grant was awarded 20 months ago, we had successfully engineered a small model plant, Arabidopsis thaliana, to use a highly modified bacterial mercuric ion reductase gene, merA9, to detoxify ionic mercury (Hg(II)), reducing it to much less toxic and volatile metallic Hg(0) (Rugh et al., 1996). Seeds from these plants germinate, grow, and set seed at normal growth rates on levels of Hg(II) that are lethal to normal plants. In assays on transgenic seedlings suspended in a solution of Hg(II), 10 ng of Hg(0) was evolved per min per mg wet weight of plant tissue. At that time, we had no information on expression of merA in any other plant species, nor had we tested merB in any plant. However, the results were so startlingly positive and well received that they clearly presaged a paradigm shift in the field of environmental remediation. We have broken our new results in to two sections following from our hypothesis.

A. Removing the mercury from polluted sites— The small size of Arabidopsis plants prevented us from carrying out more complex physiological studies and field tests, so transgenic tobacco, Liriodendron tulipifera (yellow poplar), and Brassica napus (rape seed) plant lines expressing merA derivatives were constructed. In each case expression of merA leads to striking resistance to Hg(II) relative to control plants.

   When grown on highly mercury-contaminated soil, merA transgenic Arabidopsis and tobacco shoot samples accumulated very low levels of mercury, while controls if they grow at all, accumulated substantial levels. We interpret this experiment as follows: expression of the MerA protein catalyzes Hg(0) volatilization and loss from the system and prevents Hg(II) from becoming strongly bound to plant tissues. This is an important result, because it suggests that the MerA-expressing plants will be safer for herbivores to consume than native plants growing at a contaminated site.

   Using transgenic tobacco, a number of experiments have examined the mechanisms of mercury processing by these plants. For example, we demonstrated that when merA9 tobacco plants are grown in hydroponic media with 1 ppm Hg(II) (i.e., 1000 μg total in Hg(II) in 1 liter), they volatilize 80% of the mercury from the system in a week, whereas the control plants have little impact. All the Hg(II) is bound to roots of both control and merA9 plants in the first 24 hrs, but only the transgenic plants efficiently eliminated mercury from the roots over the next few days. As in the above experiments with soil only small amounts of mercury were found in stems or leaves of the merA9 tobacco plants. These data suggest that MerA-expressing

transgenic plants: 1) can be used immediately to process industrial waste streams contaminated with mercury, and 2) work efficiently even at very low levels of Hg(II) (i.e., the EPA limit for water is typically about 1 ppm).

Next we focused on determining if metallic Hg(0) vapor transpired up through the vascular system and out of leaves, as are H_2O vapor, O_2, and CO_2. We also wanted to test models in which the plants were "engineered" to trap mercury instead of volatilizing it. In one set of experiments we demonstrated that transgenic yellow poplar plantlets efficiently volatilized Hg(0) into the air from solid agar growth. These results were quite suggestive of a transpiration mechanism. In a second set of experiments, we grafted all combinations of merA and wild-type tobacco tops and roots together and grew them hydroponically for one week with Hg(II) in their media. Again merA transgenic tissues accumulate little mercury in roots, stems, or leaves relative to control tissues. However, as predicted by the transpiration model, wild-type tops grafted over merA root system accumulate substantially more mercury than any other sample. This is presumably because Hg(0) vapor produced by the merA root system is transpired to the wild-type tops where it is reoxidized to Hg(II) by endogenous catalases and peroxidases and trapped as Hg(II). This suggests the likelihood of a transpiration mechanism and demonstrates the feasibility of phytoextracting mercury from soil and water and trapping it above ground tissues for later removal.

Many of the largest mercury contaminated sites with the most serious environmental impact due to the production of methyl mercury (below) are aquatic or marine ecosystems. Aquatic and marine grasses engineered to express MerA would be the most suitable for complete remediation of such environments. New merA gene constructs are being made for monocot expression using a variety of promoters.

B. Preventing methyl mercury from entering the food chain— Endogenous bacteria at most aquatic and marine sties produce an organic form of mercury, called methyl mercury, which is the most serious threat to the environment. Methyl mercury is biomagnified up the food chain, poses an immediate threat to wildlife and human populations, and is the most common source of mercury poisoning. Gram-negative bacteria living in these environments often encode an organo mercury lyase, MerB, which protonolyzes the carbon mercury bond to produce the less toxic Hg(II). The activity of a partially engineered merB gene was confirmed in E. coli and transformed into Arabidopsis. Seeds from the merB plants germinate and seedlings grow on 0.1-2 μM (0.02-0.4 ppm) phenyl mercury acetate (PMA) or methyl mercury chloride, while the wild-type germinants died. Similar results were obtained with merB transgenic tobacco. When merA9 and merB are combined genetically into the same Arabidopsis plants they are resistant to even higher levels of methyl mercury (10 μM). Our work strongly suggests that native macrophytes (e.g., trees, shrubs, grasses) engineered to express merBpe derivatives could be used to clean methyl mercury from polluted sites and prevent it from entering the food chain.

Planned Activities

The next year focuses on preparing merA/merB tobacco and rice lines, and testing their ability to break down methyl mercury and transpire Hg(0).

Other Access To Information

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