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Our long-term goal is to enable highly productive plant species to extract, resist, detoxify, and/or sequester toxic heavy metal pollutants as an environmentally friendly alternative to physical remediation methods. We have focused this phytoremediation research on soil and water-borne ionic and methylmercury. Mercury pollution is a serious world-wide problem affecting the health of human and wild-life populations. Methylmercury, produced by native bacteria at mercury-contaminated wetland sites, is a particularly serious problem due to its extreme toxicity and efficient biomagnification in the food chain. We engineered several plant species (e.g., *Arabidopsis,* tobacco, canola, yellow poplar, rice) to express the bacterial genes, *merB* and/or *merA*, under the control of plant regulatory sequences. These transgenic plants acquired remarkable properties for mercury remediation. 1) Transgenic plants expressing *merB* (organomercury lyase) extract methylmercury from their growth substrate and degrade it to less-toxic ionic mercury. They grow on concentrations of methylmercury that kill normal plants and accumulate low levels of ionic mercury. 2) Transgenic plants expressing *merA* (mercuric ion reductase) extract and electrochemically reduce toxic, reactive ionic mercury to much less toxic and volatile metallic mercury. This metal transformation is driven by the powerful photosynthetic reducing capacity of higher plants that generates excess NADPH using solar energy. MerA plants grow vigorously on levels of ionic mercury that kill control plants. Plants expressing both *merB* and *merA* degrade high levels of methylmercury and volatilize metallic mercury. These properties were shown to be genetically stable for several generations in the two plant species examined. Our work demonstrates that native trees, shrubs, and grasses can be engineered to remediate the most abundant toxic mercury pollutants. Building on these data our working hypothesis for the next grant period is that transgenic plants expressing the bacterial *merB* and *merA* genes will (a) remove mercury from polluted soil and water and (b) prevent methylmercury from entering the food chain. Our specific aims center on understanding the mechanisms by which plants process the various forms of mercury and volatilize or transpire mercury vapor. This information will allow us to improve the design of our current phytoremediation strategies. As an alternative to volatilizing mercury, we are using several new genes to construct plants that will hyperaccumulate mercury in above-ground tissues for later harvest. 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. The knowledge and expertise gained by engineering plants to hyperaccumulate mercury can be applied to the remediation of other heavy metals pollutants (e.g., arsenic, cesium, cadmium, chromium, lead, strontium, technetium, uranium) found at several DOE facilities.

The following publications resulted from this DOE-EMSP funded research. Some of this work can be viewed more completely by starting at the RBM laboratory home page: www.genetics.uga.edu/RBMSite/rbmlabhome.html.

Bizily, S., C.L. Rugh, and R.B. Meagher. (in preparation) Efficient degradation of the environmental toxin methylmercury by engineered plants. *Nature Biotechnology*.

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Meagher, R.B., C.L. Rugh, M.K. Kandasamy, G. Gragson, and N.J. Wang. 1998. Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In *Fourth International Conference on the Biogeochemistry of Trace Elements* (ed. I.K. Ishndar, S.E. Hardy, A.C. Chang, and G.M. Pierzynski), pp. 203-221. Ann Arbor Press, Inc., Berkeley, CA, USA.

Rugh, C.L., S.P. Bizily, and R.B. Meagher. 1999. Phytoremediation of environmental mercury pollution. In *Phytoremediation of toxic metals: Using plants to clean-up the environment* (ed. B. Ensley and I. Raskin). Wiley and Sons, New York.

Rugh, C.L., G.M. Gragson, and R.B. Meagher. 1998a. Toxic mercury reduction and remediation using transgenic plants with a modified bacterial gene. *Hort. Sci.* 33: 12-15.

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