Annual Report for EMSP Project DE-FG07-96ER20256

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1. Research Objectives

Our research objectives are as follows:

- Transform poplar and other tree species to extend and optimize chlorinated hydrocarbon (CHC) oxidative activities.
- Determine the mechanisms of CHC oxidation in plants.
- Isolate the genes responsible for CHC oxidation in plants.

2. Research Progress and Implications

Metabolism of TCE by Plant Cells As of our 2002 annual report, we have made significant progress in our understanding of the pathway of TCE degradation by plant cells. We had previously demonstrated that plant cells metabolize TCE, producing chloral and trichloroethanol as early metabolites. When the cells are removed from TCE, the trichloroethanol levels, both free and conjugated, decrease, indicating further metabolism. Dechlorination of trichloroethanol is difficult to detect due to the prevalence of chloride ion; therefore, we are using tribromoethanol as a surrogate. Poplar cells metabolize tribromoethanol, releasing bromide ion. As shown in Figures 1 and 2, only living poplar cells metabolize tribromoethanol and the production of bromide ion is linear over time. Next we are going to do competitive inhibition studies to verify that trichloroethanol and tribromoethanol are metabolized by the same enzyme. The implications of these experiments are that poplar cells do not leave TCE in its more hazardous trichloroethanol state, but that they metabolize it more completely to non-hazardous compounds.

Since Arabidopsis is fully sequenced and has a smaller genome than poplar (four times smaller), it would be easier to construct a cDNA library and clone the genes with our activities of interest. As a first step, we needed to determine if Arabidopsis has the same pathways for TCE metabolism that poplar does. We exposed Arabidopsis culture cells to either TCE or tribromoethanol, and assayed for the production of trichloroethanol and bromide ion, respectively, as indicators of metabolism. Indeed, Arabidopsis and poplar share the same pathways. These results are important because they provide us with a system to more fully study TCE metabolism by using the genome sequence, P450 libraries, and T-DNA mutant libraries.

Introduction of Rabbit P450 2E1 into Poplar We have successfully introduced rCYP2E1 (driven by the 35S promoter) into two poplar hybrids. We are in the process of multiplying these transgenic plants for metabolic studies. We have also developed a method for transformation of poplar with *Agrobacterium rhizogenes* to produce hairy root cultures for metabolic studies. This method offers the advantage of being able to assay for metabolism of TCE in an enclosed system with reduced variability compared to using whole plants. We have successfully made hairy root cultures with rCYP2E1 and are allowing them to propagate further before analysis.

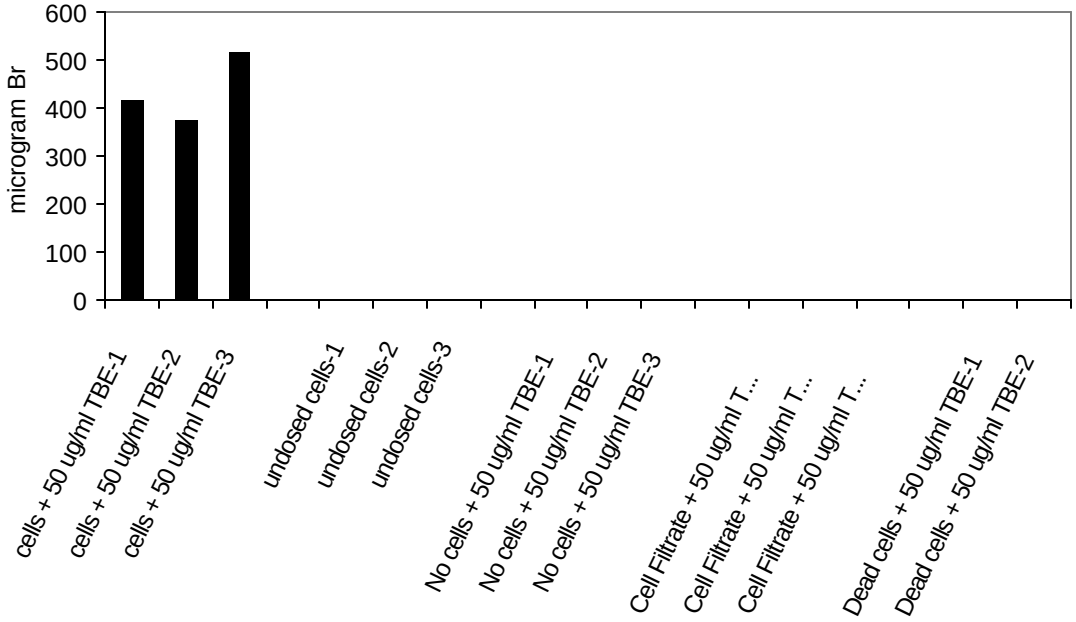

Figure 1 – Bromide production from dehalogenation of tribromoethanol by poplar cell suspensions

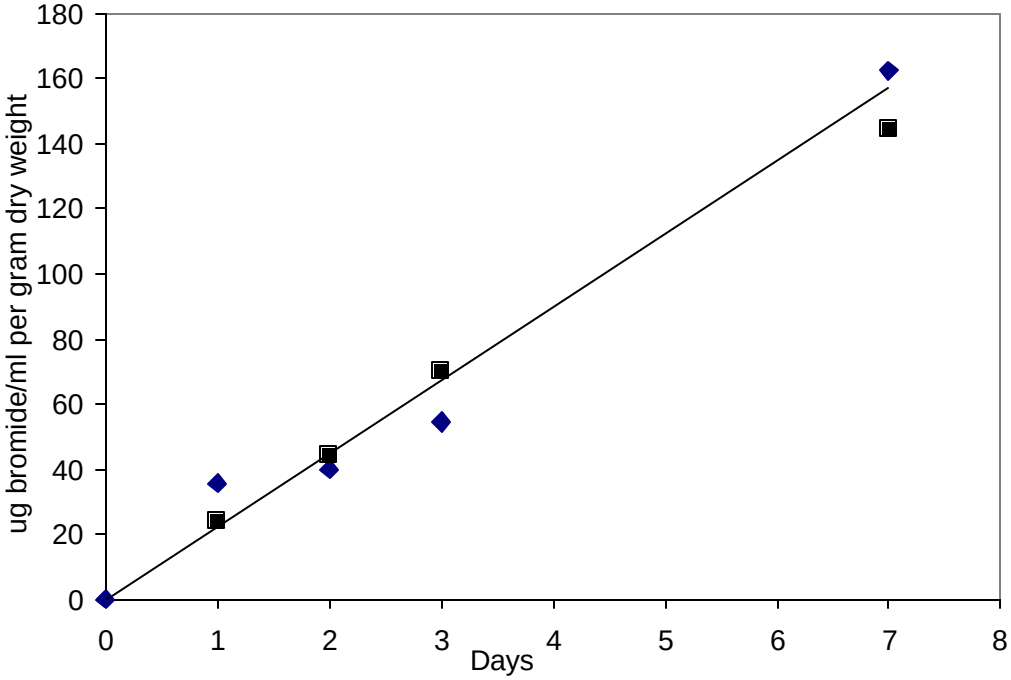

Figure 2 -- Time Course of Debromination of Tribromoethanol by Poplar Suspension Cells

cDNA Library Construction We had purified RNA from poplar culture cells dosed with TCE, and had isolated mRNA. cDNA was produced for ligation into a vector designed for expression of the library in yeast. However, the yield was insufficient and the transformation frequency too low for cDNA library development, so we are turning to the more efficient lamda library system. Given our results described above, it is advantageous to also produce an Arabidopsis cDNA library from TCE-exposed cells. Once these libraries are constructed, we will screen them using our assays for TCE metabolism. Isolation of the genes involved in TCE degradation in plant cells will be highly valuable both by increasing our understanding of the pathway in plants and also by giving us the ability to overexpress these genes in transgenic poplar, most likely leading to enhanced phytoremediation of TCE.

Poplar Endophyte We had previously identified a bacterium within the stems of poplar as a type of Rhizobium tropici. We have since shown that it is prevalent in all of our poplar hybrids both on and off site. Since Rhizobium is known to fix nitrogen in root nodules of legumes, it was intriguing to test if this endophytic Rhizobium was providing fixed nitrogen for poplar. We have demonstrated that poplars can grow well in medium lacking nitrate and ammonium while control plants die of nitrogen deficiency in this medium. We are about to test Koch's Postulates on freshly collected poplar seeds and sterilized shoot tips of hybrid poplar. If we can demonstrate that an endophytic rhizobium can fix atmospheric nitrogen within stems of this non-legume, it will open up a whole new field of symbiosis research.

3. Planned Activities

We continue experiments to demonstrate the metabolism of tribromoethanol (as a surrogate for trichloroethanol) by plant cells. We are preparing to publish these experiments this year.

A major focus will be the production of cDNA libraries from TCE-exposed Arabidopsis and poplar culture cells. The cDNA expression library from Arabidopsis can then be screened for indicators of TCE metabolism. The poplar genomic sequence is due to be published in October 2003. Genes identified in Arabidopsis can then be cloned from poplar cDNA library based on homology.

We hope to complete our studies into the suspected nitrogen fixation of poplar endophytes in the next few months for a first publication of the subject.

We are also involved in collaborations with Steve Strauss at OSU to increase our ability to handle large numbers of transformed poplar callus cultures, and Ron Sederoff at North Carolina State University to produce CYP2E1 transformants of pine.

4. Information Access

Two graduate students (one PhD and one MS) were supported on this project and graduated during the last year. Four peer-reviewed papers were published with this support:

Doty, S. L., Shang, T. Q., Wilson, A. M., Moore, A. L., Strand, S. E., Oda, C. and Gordon, M. P. Metabolism of the halogenated hydrocarbons, TCE and EDB, by the tropical leguminous tree, Leuceana leucocephala. 2003. Water Research 37(2):441-449.

Banerjee, S., Shang, T. Q., Wilson, A. M., Moore, A. L., Strand, S. E., Gordon, M. P., and Doty, S. L. 2002. Expression of functional mammalian P450 2E1 in hairy root cultures. Biotechnology and Bioengineering, 77(4):463-466.

Wang, X., M. P. Gordon, and S. E. Strand. Mechanism of Aerobic Transformation of Carbon Tetrachloride by Poplar Cells. (2002) Biodegradation, 13(5)297-305.

Matthews, D. W., J. Massmann, and S. E. Strand. Influence of Aquifer Properties on Phytoremediation Effectiveness.(2003) Ground Water, 41(1) 41.