

Paula Bernasconi

Development of a Genetically Engineered Seaweed That Can Detoxify TNT in Marine Waters

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2,4,6-Triitrotoluene (TNT) is one of the most recalcitrant and toxic of all military explosives. It occurs in coastal sites as a result of leakage from exploded and unexploded ordinance as well as runoff from firing ranges and munition dumps on land. Current methods for eliminating toxic compounds like TNT from contaminated waters and sediments involve dredging and disposal, and are extremely costly and potentially harmful to the environment. This project's goal is to develop a strain of seaweed capable of "phycoremediating" TNT in marine waters. Our model seaweed is a fast-growing, fast-reproducing strain of the red marine macroalga *Porphyra yezoensis*, which we genetically transform using a strain of *Agrobacterium tumefaciens* carrying the plasmid pNITRED3. This plasmid carries the bacterial nitroreductase gene, *nfsI*. Preliminary toxicity experiments determined that a TNT concentration of 5 mg/L could be used for isolating single lines of TNT-tolerant plants. Several lines have been produced which demonstrate a striking ability to take up, tolerate and detoxify TNT in seawater. One line, for example, can completely remove 10mg/L TNT from seawater in less than 3 days and still grow, whereas wild-type plants stop photosynthesizing and die. The presence of the *nfsI* transgene has been confirmed using PCR probes and has been shown to be both inheritable and stable (i.e. present through at least the T3 generation and inherited in the absence of selection). In addition, the products of TNT reduction by nitroreductase have been detected. This research is supported by a grant from the Office of Naval Research.

Sarah Strycharz

Development of a Genome-wide Screening Method to Identify Gene Candidates Involved in the Degradation of Halogenated Hydrocarbons Using Ion Chromatography

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As the use of phytoremediation for cleanup of halogenated hydrocarbons becomes increasingly widespread, it is imperative to determine the route of metabolism of such chemicals in plants to increase effectiveness and efficiency of degradation. In humans and other organisms, cytochrome P450s are involved in degradation of the common groundwater contaminant trichloroethylene (TCE). TCE and ethylene dibromide (EDB) are both metabolized by the same primary enzyme in humans, cytochrome P450 2E1. Other potential enzymes that may be involved in TCE degradation in plants include additional cytochrome P450s, peroxidases, dehalogenases, laccases, and reductases. In order to screen for multiple enzyme types, we have developed an assay in which bacterial cultures expressing a commercially purchased tobacco leaf (*Nicotiana tabacum*) cDNA library can be examined for the ability to degrade a halogenated hydrocarbon, EDB. EDB is being used instead of TCE as the bromide ion (Br⁻) is not prevalent in culture media whereas chloride ion (Cl⁻) can be found at high levels. Following 72 hours of growth to high density, cultures are concentrated and resuspended in a phosphate buffer containing glucose and 100 ppm EDB. Degradation of EDB is measured by detection of Br⁻ release using an ion chromatograph. We hope to narrow the number of possible candidate genes by comparing the ability of cDNA library cultures to degrade EDB, with the ability of a control culture expressing a gene known to degrade EDB.

Comparison of Native Southeastern Conifers to Hybrid Poplar for Suitability to Phytoremediate Trichloroethylene

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Phytoremediation of trichloroethylene (TCE) from contaminated groundwater has been extensively studied using the hybrid poplar tree (*Populus* spp.). Hybrid poplars are capable of rapid growth in which they take up large quantities of water and also possess the capacity to effectively metabolize TCE. Several metabolites of TCE have been identified in the tissue of poplar including trichloroethanol (TCEOH) and di- and trichloroacetic acids (DCAA, TCAA). This study follows a greenhouse-based comparison of four different native Southeastern conifers to a hybrid poplar species for the ability to take up and degrade TCE. Longleaf pine (*Pinus palustris*), Leyland cypress (x *Cupressocyparis leylandii*), two varieties of Loblolly pine (*Pinus taeda*), and hybrid poplar species OP-367 (*Populus deltoides* x *P. nigra*) were examined for the concentration of TCE and its metabolites in their tissue using gas chromatography following treatment with either a low dose of TCE (50 ppm) or a high dose of TCE (150 ppm) for two months. The amount of water taken up, height of the tree, TCE transpiration, and total fresh weight were also recorded. Our goal is to expand the effectiveness of phytoremediation in combination with land reclamation by supplying the option of creating a heterogeneous forest system for contaminated groundwater treatment.

Alison Watts

Uptake of Polycyclic Aromatic Hydrocarbons in *Spartina Alterniflora* - Evaluating Risk in Salt Marsh Vegetation

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Polycyclic Aromatic Hydrocarbons (PAHs) are present in many coastal and salt marsh sediments. Sources include oil spills, urban runoff, and coal gasification byproducts. Plants affect the movement of PAHs in several ways; they may inhibit erosion, enhance microbial degradation, or translocate compounds. Plant translocation can move a compound from the soil or water into the plant. The compound is then either stored in plant tissue, or degraded by microbial or metabolic processes, or excreted into the atmosphere. Contaminants stored in plant tissue may present a risk to the ecological community.

In this study, uptake of PAHs was measured in *Spartina alterniflora*, a common salt marsh plant. For three months *Spartina alterniflora* was grown outdoors in PAH-contaminated soil and in clean control soil. The PAH contaminated sediment was collected from an estuary near a former coal gasification plant, and contained an average of 200 ug/g total PAHs. Plant samples were also collected from a PAH-contaminated estuarine marsh and from an uncontaminated reference site. Plants grown in uncontaminated soil produced more flowers, and were taller, but plants grown in contaminated soil had more shoots, yielding a shorter, bushier morphology. The total above-ground biomass at the end of the growing season was similar in the controls and PAH-grown plants.

The harvested samples were separated into leaf and root material and analyzed for individual PAH compounds. Most of the samples were analyzed using a GC/MS/MS system with a chromatoprobe direct-sample-injection device. Small pieces of plant tissue can be analyzed directly by this method, allowing rapid evaluation of individual leaves or roots. However, the detection limit is higher than traditional extraction-injection methods due to the small sample size and co-elution of some plant-derived compounds.

PAHs were detected in both root and leaf tissue. Concentrations in root tissue were approximately an order of magnitude higher than leaf tissue, and plant concentration increased as soil concentration increased. PAH concentrations in plant tissue will be compared to ecological risk levels such as the NOAA ERM/ERLs, and potential ecological risks will be evaluated.