Enhancing Phytoremediation and Plant Growth in Poplar and Willow

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Why do we need to enhance phytoremediation?
Removal of Pollutants by CYP2E1 Transgenic Poplar

Funding: Superfund Basic Research Program of NIEHS
P.I. Stuart Strand, UW
Co-P.I. Sharon Doty, UW
Trichloroethylene (TCE)

- Used for decades as metal degreaser, dry cleaning agent, solvent, and anesthetic
- Following use, it was dumped outside
- One of the most widespread contaminants in the environment (60% of SuperFund sites)
- Toxic to the liver, kidney, CNS, and likely carcinogenic
- Persistent in the environment
Mammalian Cytochrome P450 2E1 Catalyzes TCE Metabolism

Trichloroethylene $\xrightarrow{2E1}$ Chloral $\rightarrow$ Trichloroacetic Acid $\rightarrow$ Trichloroethanol
Transformation of Poplar (*P. tremula x alba* N717-1B4) using *A. tumefaciens*
Transgenic plants were assayed for increased metabolism of pollutants
CYP2E1 Transgenic Poplar Had Increased Metabolism of TCE

CYP2E1 transgenic poplar removed TCE at a faster rate

<table>
<thead>
<tr>
<th>Transgenic Plant</th>
<th>% Removal</th>
<th>Rate *</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plant control</td>
<td>0.8 +/- 1.1</td>
<td>0.1 +/- 0.1</td>
</tr>
<tr>
<td>Vector Control</td>
<td>2.6 +/- 0.3</td>
<td>0.4 +/- 2.8</td>
</tr>
<tr>
<td>CYP2E1 #78</td>
<td>86.9 +/- 11.4</td>
<td>20.3 +/- 4.6</td>
</tr>
</tbody>
</table>

Rate: ug TCE/day*gm fresh weight

Cytochrome P450 2E1 Has Multiple Substrates

- Chloroform
- Carbon Tetrachloride
- Vinyl Chloride
- Benzene
Increased Removal of Chloroform from Solution by CYP2E1 Transgenic Poplar

Doty, unpublished
Increased Removal of Carbon Tetrachloride from Solution By CYP2E1 Transgenic Poplar

![Graph showing the removal of carbon tetrachloride over time for different treatments.]

- Unplanted
- Vector Ctrl
- r2E1#78
- r2E1#20
CYP2E1 Transgenic Poplar Removed More Pollutants from Air

- TCE
- Benzene
- Vinyl Chloride
CYP2E1 Poplar Plants Removed TCE from Air At A Faster Rate
Identification of Plant Genes Involved in TCE Metabolism
Proposed Fate of TCE in Plants

Approaches to identify plant genes involved in TCE metabolism

- Poplar microarrays (Dr. Fred Farin, UW)
- Arabidopsis microarrays (Dr. Mary Schuler, UI)

Test candidate genes in yeast
Phases of detoxification in plants

(Benoit Van Aken, 2008 Trends in Biotechnology 26(5):225-227)
### Microarray Results: Vector Control vs CYP2E1
Poplar Genes Induced by TCE Exposure

<table>
<thead>
<tr>
<th>Function</th>
<th>Enzyme</th>
<th>KH200+TCE</th>
<th>#78+TCE</th>
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<tbody>
<tr>
<td><strong>Phase I</strong></td>
<td></td>
<td></td>
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<tr>
<td>Transformation</td>
<td>Cytochrome P450, Reductases, Dehalogenases</td>
<td>2</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
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<tr>
<td><strong>Phase II</strong></td>
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<tr>
<td>Conjugation</td>
<td>Glycosyltransferases (UGT), Glutathione-S transferases (GST), Acyltransferases</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
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<tr>
<td><strong>Phase III</strong></td>
<td></td>
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<tr>
<td>Compartamental</td>
<td>ATP binding cassette (ABC transporter)</td>
<td>1</td>
<td>7</td>
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</tr>
<tr>
<td></td>
<td>Peroxidase Oxidase Oxidoreductase</td>
<td>3</td>
<td>4</td>
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<td>6</td>
<td>7</td>
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<tr>
<td><strong>Unknown</strong></td>
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</table>

Log FC > 2

Kang, unpublished
Current Research

- Field testing of transgenic poplar (NIEHS funded)
- Analysis of plant genes involved in TCE metabolism (NIEHS funded)
- Developing transformation protocols for willow (CPBR funded)
**Summary of Transgenic Phytoremediation**

- Expression of mammalian P450 2E1 gene in transgenic poplar led to a strong increase in TCE metabolism and removal of TCE (53X), and a strong increase in removal of chloroform (9X), carbon tetrachloride, vinyl chloride (3X) and benzene (10X).

- Analysis of upregulated genes in response to TCE in poplar revealed many genes known to be involved in detoxification of pollutants. Analysis of these genes may lead to improved phytoremediation.
Enhancing Plant Growth using Nitrogen-Fixing Endophytes of Poplar and Willow
Contaminated areas often have poor soil

- Abandoned, polluted commercial properties
- Phytoremediation depends on good plant growth
Nitrogen fixation in root nodules

- Chemical fertilizers are petroleum-based; getting costlier
- Some microbes convert N2 gas to ammonia
- Nitrogen fixation occurs within root nodules of only some plant species
Endophytes

- Microorganisms that live within plants without causing disease
- Increased nutrient acquisition, pathogen resistance, stress tolerance
Nitrogen-fixing endophytes of poplar and willow

In wild settings, poplar and willow grow under low-nitrogen conditions.

Isolated endophytes; identified using 16S rRNA gene sequencing.
Some poplar and willow endophytes grow well in medium without ammonium or nitrate.

![Graph showing growth over time](image-url)
Plant Growth Promotion by Endophytes of Poplar
Grass growth promotion assay

Kentucky bluegrass

Sterile sand

NFM

Rope

Room temp, 16/8 hr light/darkness

After 50 days, cleaned and dried

Determined for N content using a CHN analyzer

Weighed for shoot and root respectively

Gang Xin, Genyun Zhang, and Sharon Doty (manuscript in preparation)
42% biomass increase with WPB inoculation

37% total N increase with WPB inoculation
WPB enhances the growth of rice under nitrogen limitation

Expt. Performed in Dr. Rodriguez’s lab
Summary of Endophyte Characterization

- Initially focused on Burkholderia strain, WPB.
- WPB shares high \textit{nifHDK} gene similarity with known \textit{B. vietnamiensis} strains.
- High nitrogenase activity was determined using both the $^{15}$N$_2$ incorporation assay and the Acetylene Reduction Assay.
- WPB can significantly promote growth of Kentucky bluegrass, crabgrass, and rice. Similar experiments with poplar and corn are underway.
Current Research

- Propagation of internally-sterile poplar
  - Growth effects
  - Microarrays
- Analysis of willow endophytes
Acknowledgements for Endophyte Research

Gang Xin: WPB studies

Gengyun (George) Zhang: Turfgrass studies

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Rice studies:

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