Genetically Enhanced Rhizoremediation

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Phytoremediation Mechanisms

- Stabilization
- Uptake
  - Degradation
  - Volatilization
  - Storage
- Rhizosphere degradation
(Respiration) $\text{O}_2$, $\text{CO}_2$

$\text{H}_2\text{O}$ (transpiration)

$\text{O}_2$, $\text{CO}_2$ (Photosynthesis)

Phloem

Xylem

Respiration $\text{O}_2$, $\text{CO}_2$

Organics, exudates

$\text{H}_2\text{O}$, Nutrients
Technological Approach

• Stabilization
  – Utilizing high transpiration rates to hydraulically isolate contaminated site

• Genetically Enhanced Rhizoremediation
  – Utilize rhizosphere as a selective environment.
  – Promote established symbiotic plant-microbe relationships to promote biodegradation.
  – Select ideal plants for contaminated site.
Plant-Microbe Symbiosis

Genetically engineered microbes (GEMs) languish in uncontrolled environment, flourish in selective lab

Rhizosphere is a selective environment

- Organic substrate: Unique and plentiful
- pH properties: Can vary > 1 unit from bulk soil
- Nutrient availability: Enhanced by disturbance and hydraulic gradient
- Attachment sites: High surface area roots
Engineering The Rhizosphere

Find Prevalent Rhizobacteria

Incorporate selected genes into native strains

Isolate viable recombinants

Inoculate roots

Test viability
State of Technology

Proof of concept at bench scale with numerous contaminants

Little on optimizing for application

No known work on Dioxins and Furans
Completed work: TCE

Toluene $o$-monooxygenase incorporated into wheat and poplar colonizing bacteria, working with Tom Wood (U. Connecticut)

✓ Viability
✓ Growth rates
✓ Selectivity
✓ Degradation

Survival Plate Counts

![Graph showing survival plate counts over time]

- Y-axis: 
  - # cells/in. root
  - # cells/g soil

- X-axis: 
  - Day 14
  - Day 28
  - Day 49

The graph illustrates the survival plate counts over different days, with symbols indicating the number of cells at each time point.
Transport Experiment

Water table dropped every 60 days.

Root transport only as plants were watered from below by hydrostatic equilibrium.
Transport Experiment; 180 days
Recent: Visual Detection

Photograph of poplar root from Transfer experiment under an epifluorescent microscope. Visualized using specific GFP filters.
PCR Results

Lane 1 1 kb Ladder
Lane 2 Pb5gfp2-2 (+Control)
Lane 3 Pb5TOM (- Control)
Lane 4 P. f. 2-79 (- Control)
Lane 5 Pb5gfp2-2   Day 14
Lane 6 Pb5gfp2-2   Day 14
Lane 7 Pb5gfp2-2   Day 14
Lane 8 Transfer isolate (150 d)
PCR Results

Lane 1 1 kb Ladder
Lane 2 Pb5gfp2-2 (+ control)
Lane 3 Pb5TOM (- Control)
Lane 4 Pb5 #2/R upper
Lane 5 Pb5 #3/S lower
Lane 6 Pb5 #2/S lower (not gfp)
Lane 7 Rhiz #1/R upper
Lane 8 Rhiz #3/S lower
Findings: GEMs/Rhizosphere

- Survival for up to 180 days
- Increased degradation
- Selective to plant type (wheat and poplar colonizers did not cross inoculate)
Limitations

- Survival populations were not extremely competitive
- Degradation was minor in overall fate, still primarily taken up and volatilized.
- Poplar roots do not have a high specific surface area.
Ongoing efforts

Targeting hydrophobic contaminants

- Higher surface area, more fibrous root systems
- Hearty plants, shallow rooting
- Optimize microbial recipient
Selecting Better Bacteria

Replicate plating of bacteria from PCB contaminated root zone

- LB media (culturable)
- Macerated root biomass
- Sterile root exudate
- Biphenyl
Initial Plating

Rhizosphere sample

- LB media
- Master Plate

Macerated root biomass
- Sterile root exudate
- Biphenyl
Replica Plating

- Rhizosphere sample
- LB media
- Master Plate
- Macerated root biomass
- Sterile root exudate
- Biphenyl
Replica Plating

- Rhizosphere sample
- LB media
- Master Plate
- Macerated root biomass
- Sterile root exudate
- Biphenyl
Replica Plating

Rhizosphere sample → LB media
   → Master Plate

- Macerated root biomass
- Sterile root exudate
- Biphenyl
Engineer selected colonizers

Sequence and Select cultures to represent the diversity present in Rhizosphere
Testing Degradation

- Cultures – track individual congeners (not all expected now)
- Soil microcosm – Planted/Not Sterile/Not
- Greenhouse Column/Lysimeter – investigate degradation patterns and rooting patterns
- Enhanced survivability
## Current progress

<table>
<thead>
<tr>
<th>Target</th>
<th>Plant/ Microbe</th>
<th>Enzyme/ gene</th>
<th>Engineering</th>
<th>Survival/ thriving</th>
<th>Degradation (planted)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TCE</strong></td>
<td>Poplar/Pb &amp; Rhiz.</td>
<td><em>tom</em></td>
<td>Yes</td>
<td>&gt;180 days no</td>
<td>Yes</td>
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<tr>
<td><strong>GFP Marker</strong></td>
<td>Poplar/Pb &amp; Rhiz.</td>
<td><em>gfp</em></td>
<td>Yes</td>
<td>&gt;120 days</td>
<td>Visual Marker</td>
</tr>
<tr>
<td><strong>PCBs</strong></td>
<td>Alfalfa/S. <em>Mel.</em> Fescue/?</td>
<td><em>bph</em></td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Dioxins &amp; Furans</strong></td>
<td>Fescue/?</td>
<td><em>dfd</em></td>
<td>TBA</td>
<td>TBA</td>
<td>TBA</td>
</tr>
</tbody>
</table>
Limitations

- Bioavailability
- Potential replanting and reinoculation
- Slow: must think years not weeks – months.
- Depth, rooting depth of grasses/vadose zone
- \textit{bph} attacks limited PCB congeners *

Benefits

• Site may still be useful, limited restrictions and disturbance

• Remediation and restoration at the same time. Leaving site in useful state

• Stabilization, hydraulic containment and remediation – concurrently (poplar – willow)
Hydraulic Control:
July 2001 Aberdeen Proving Ground
Conclusion & hypothesis

Engineered systems can alter this fate and enhance degradation mechanisms, and the selective nature of the rhizosphere can foster GEM survival and transport.

Proper selection of plant and native bacteria can increase survival and thereby degradation of hydrophobic contaminants in-situ.
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