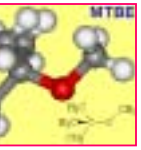
oundwater Remediation through Environmental Biotechnology: Transgenic Phytoremediation of Mt

I. Environmental Issue



The structure of MtBE (1).

- Methyl *tertiary* butyl ether (MtBE) (Figure 1) is used as a fuel oxygenate to reduce carbon monoxide and pre-smog emissions from automobiles.
- MtBE enters groundwater through leaking underground storage tanks and accidental spills.

groundwater, MtBE migrates faster and is more alcitrant than other gasoline constituents.

very low concentrations MtBE renders drinking water pplies unpalatable due to low taste and odor thresholds.

despread concerns regarding the occurrence of MtBE in oundwater prompted the US EPA to issue a drinking water visory of 20-40 ppb (2).

ny states are legislating the phase-out of MtBE from ned gasolines (Figure 8).

II. Background

aphium sp. (Figure 2), a filamentous fungus, grows on seous *n*-alkanes (3).

er exposure to *n*-alkanes, *Graphium* can degrade MtBE (Figure 3) and other persistent chemicals including orinated solvents, benzene, toluene, xylene, and yaromatic hydrocarbons (data not shown).

bsurface conditions and biological limitations may impede *aphium*'s ability to metabolize MtBE *in situ*. However, nsferring the metabolic capabilities of the fungus into a erent organism may result in new technologies for the nediation of polluted groundwater.

A typical synemmeta formed by the ete *Graphium* sp.

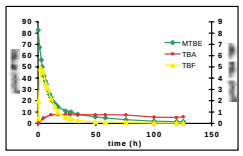


Figure 3. Propane-grown *Graphium* degrades MtBE and the MtBE metabolites TBF and TBA.

III. Scientific Approach

Figure 4. Northern blot of cultures grown on various substrates.

- *Graphium* cultures express a cytochrome P450 alkane monooxygenase (AMO) when grown on *n*-alkanes, alcohols, and acids, but not when grown on dextrose (Figure 4).
- Differential expression of the AMO facilitated the cloning of both *GRSPALK1*, the gene that encodes the *Graphium* AMO, and the *GRSPALK1* inducible promoter.
- Strategies to characterize the function of the AMO include:
  1) Knockout analysis in *Graphium* transformed with pCS10 (Figure 5);
  2) Heterologous expression of *GRSPALK1* in the fungus *Verticillium dahliae* (Figure 7) transformed with pCS15 (Figure 6).

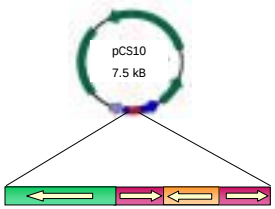


Figure 5. A map of the fungal transformation knockout vector, pCS10. Arrows indicate the direction of transcription. See legend at right.

- Green fluorescent protein (6) driven by the *ToxA* promoter.
- Hygromycin phosphotransferase, a selectable marker, driven by the *TrpC* promoter.
- *Graphium* P450 *GRSPALK1*.
- *Graphium* P450 *GRSPALK1* inducible promoter.

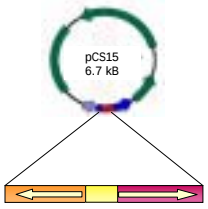


Figure 6. A map of the fungal expression vector, pCS15. Arrows indicate the direction of transcription. See legend at left.

Figure 7. Micrograph of conidia and mycelium of *V. dahliae*.

- Tobacco and/or poplar plants will be transformed with an *Agrobacterium tumefaciens* strain harboring a plasmid encoding *GRSPALK1*. *GRSPALK1* will be expressed *in planta* under the control of the 35S Cauliflower Mosaic Virus promoter.
- Transgenic plants expressing high levels of AMO will be used to test the efficacy of MtBE metabolism and to determine the fate of MtBE metabolites *in planta*.
- Rate limiting factors for MtBE uptake and metabolism in transgenic plants will also be determined.

IV. Environmental Impact

- Current MtBE remediation techniques are slow and expensive approaches.
- Many states have set regulatory limits on MtB concentrations (Figure 8). Inexpensive and reliab remediation techniques are urgently needed attenuate MtBE impacted soils to meet regulato limits.

Figure 8.

- This study will determine if transgenic phytoremedia will meet those needs.

V. Select References

1. Figure adapted from http://www.chm.bris.ac.uk/motm/leadtet/leadh.htm
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