Specific Aims

1,1,1 - Trichloroethane (TCE) has been widely used as an industrial solvent from the mid-1970's through the late 1990's. The use of TCE has been widely phased out due to its ozone depleting properties. Additionally, long term exposure above 200 ppb (the EPA maximum dosage) can cause damage to the liver, circulatory system, and nervous system [1]. Due to improper disposal and spills, TCE contaminates many sites throughout the United States. The soil biodegradation pathway is known that degrades TCE into non-toxic metabolites. In fact, there are two pathways: an anaerobic and an aerobic path. It is essential that the toxin is fully degraded because some of the secondary degradation products are more toxic than TCE itself. Both of these pathways require a consortium of bacteria to fully degrade TCE. The anaerobic pathway is the most obvious choice for manipulation. Although the dehalogenase enzymes responsible for reducing 1,1,1 – trichloroethane and 1,1 – dichloroethane to chloroethane have not been identified, several *Dehalobacter* species have been identified [2-3]. Additionally, chloroethane has been shown to be reduced to acetaldehyde by ammonia monooxygenase in *Nitrosamonas europea*.

The goal of this project is to generate a microbe for complete, efficient 1,1,1-trichloroethane degradation and produce a system to implement its degradation power at contamination sites.

Specific Aim 1: Perform directed evolution on the ammonia monooxygenase to increase catalytic activity on chloroethane. Chloroethane is not ammonia monooxygenase's natural substrate; therefore, the activity seen towards this substrate is a side reaction. This makes this enzyme an ideal candidate for directed evolution to increase catalytic activity.

Specific Aim 2: Integrate the evolved ammonia monooxygenase into the TCE degrading *Dehalobacter* species. Initially this would be inserted into the micbrobe via a constitutively active plasmid. Ideally, this gene and promoter would stably be incorporated into the host genome to minimize the chance of plasmid transfer between the laboratory strain and bacteria in the contamination site. Also, at this point it would be necessary to ensure that the addition of ammonia monooxygenase to the *Dehalobacter* is sufficient for complete TCE degradation.

Specific Aim 3: Generate a system that can control the location of the modified microbe by sequestering it and allowing for removal upon remediation of the contamination site. This could be done by cross-linking the microbe onto a filter. This filter could then be buried in soil or added to the contaminated water source. Upon removal of the contaminant, the filter could easily be removed from the water or soil without transferring the genetically modified organism to the soil. This is important concern due to GMO regulations.

References

1. Agency, T.E.P. *Basic Information about 1,1,1-Trichloroethane in Drinking Water*. 2010 3/5/2010 [cited 2010 5/17/2010]; Available from: http://www.epa.gov/safewater/contaminants/basicinformation/1-1-1-trichloroethane.html.
2. Grostern, A., et al., *Chloroform respiration to dichloromethane by a Dehalobacter population.* Environmental Microbiology, 2010. **12**(4): p. 1053-1060.
3. Sun, B.L., et al., *Microbial dehalorespiration with 1,1,1-trichloroethane.* Science, 2002. **298**(5595): p. 1023-1025.