Isolation and Characterization of Bioaugmentation Strains for 1,4-Dioxane Bioremediation

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Background/Objectives. There have been extremely few reports regarding successful in situ bioremediation of 1,4-dioxane, which may require bioaugmentation at sites exhibiting insufficient catabolic capacity. One potential limitation to bioaugmentation is the limited availability of suitable strains. While both metabolic and co-metabolic biodegradation has been demonstrated in numerous bacteria, the vast majority of these are highly related actinomycetes. However, recent studies have suggested a broader diversity of 1,4-dioxane degraders exists. This diversity is important as a main cause of bioaugmentation failures is a lack of survival of the introduced strain or consortium, making strain selection an extremely critical factor for success. Thus, the isolation of novel dioxane-degraders from ecologically disparate environments may enhance our ability to utilize bioaugmentation as a remediation strategy. Additionally, knowledge of the regulation of gene expression in isolated strains will facilitate the identification of novel inducers for use in biostimulation or to enhance secondary substrate utilization.

Approach/Activities. A broad variety of environments (e.g., soil, sludge, seawater, groundwater) have been or will be sampled to obtain dioxane-degrading species. Aerobic and microaerobic enrichment cultures will be prepared using both both complex and defined media at normal and diluted concentrations. Cultures demonstrating dioxane biodegradation will be subcultured three times prior to attempting isolation on agar or gelatin plates. Diffusion chamber-like isolation chips will also be utilized to enhance the isolation of “uncultivable” degraders. To facilitate the identification of novel inducing substrates, select isolates will be engineered to express a fluorescent reporter (e.g., GFP) upon induction of the genes involved in dioxane degradation.

Results/Lessons Learned. Results to date indicate that dioxane biodegradation potential exists in a wide variety of environments, including those with no known prior exposure to dioxane. 16S rRNA community analyses using enrichments prepared from soil has revealed the presence of several bacterial genera previously associated with dioxane degradation (*Mycobacterium, Ralstonia,* and *Afipia*), but are primarily dominated by *Ferruginibacter*. *Bosea, Cupriavidus,* and *Acidovorax* are also highly abundant. Interestingly, time-series analyses have indicated an increase in oligotrophic species at lower dioxane concentrations. Future efforts will be focused on isolating strains capable of metabolic degradation, and preparing enrichment cultures from a broader diversity of environmental samples.