Vinyl Chloride and 1,4-Dioxane Metabolism by *Pseudonocardia dioxanivorans* CB1190

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Background/Objectives: Vinyl chloride (VC) is a carcinogen and priority pollutant that serves as a continual threat to groundwater quality, and its presence in groundwater is mainly due to biological reduction of the widespread groundwater pollutant, trichloroethylene (TCE), which usually co-occurs with 1,4-dioxane (DX). However, the initiation and termination of TCE dehalogenation can be disturbed by the presence of oxygen, insufficient electron donor or carbon source, substrate inhibition, or competition with other microorganisms. These disturbances can lead to the accumulation of toxic intermediates such as cis-1,2-dichloroethene (cDCE) or VC. *Pseudonocardia dioxanivorans* CB1190 is able to degrade 1,4-dioxane aerobically, but its potential and molecular mechanisms for catabolizing VC were not previously reported.

Approach/Activities: Batch experiments were carried out using CB1190 culture previously grown on DX and exposed to various concentrations of VC with and without DX. VC and DX concentrations as well as abundance of biomarker genes were monitored over time. In order to track CB1190 metabolism during VC degradation, the cells were exposed to ^{13}C-labeled VC. The metabolites were measured using a liquid chromatograph coupled with a high-resolution mass spectrometer via electrospray ionization and analyzed via the Metabolomic Analysis and Visualization Engine.

Results/Lessons Learned: VC caused a dose-dependent inhibition of DX biodegradation by CB1190 whereas DX had little to no effect on VC degradation. DX mass removals generally decreased with increasing VC concentrations from 100 µg/L to 4,000 µg/L. After prior growth on DX, CB1190 was able to aerobically biodegrade VC, during which alkene monooxygenase genes were upregulated, whereas genes coding for dioxane monooxygenase (*dxmB*) and alkene dioxygenase were downregulated. VC incorporation into CB1190 cells was confirmed the appearance of ^{13}C in the intracellular metabolites engaged in lipid and protein synthesis, nucleotide biosynthesis, and energy production, verifying that CB1190 can use VC for its growth.

Biological treatment of mixed contaminations is often limited by the fact that certain microbes can only biodegrade a subset of compounds and are sensitive to prevailing geochemical conditions. The identification of CB1190's ability to aerobically metabolize VC, in addition to DX and cDCE, will increase our capacity to remove pollutant mixtures in varying redox zones across contaminated sites. Costs associated with injecting contaminant-degrading microorganisms, carbon substrates, and nutrient amendments are among the highest for in situ remediation methods. Our approach of using CB1190 could reduce the cost, energy, and substrates required as well as expand the number of sites where in situ bioremediation could be a viable remedy for co-occurring VC and DX as well as at sites previously treated with enhanced reductive dechlorination. The discovery of such microbial capabilities will be valuable for field applications where complex contaminant mixtures and biogeochemical conditions are present in the environment.