Aerobic Biodegradation of 1,2-Dichloroethane at the Botany Industrial Park

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Background/Objectives. 1,2-Dichloroethane (DCA) is a problematic groundwater pollutant at the Botany Industrial Park in Sydney, Australia. Our lab has been studying the potential for aerobic biodegradation of DCA at this site, using culture-based methods and molecular tools. Our aims have been to determine the distribution and diversity of aerobic DCA-degrading bacteria, and to isolate and characterize bacteria potentially useful for bioaugmentation.

Approach/Activities. Enrichment and isolation experiments were conducted using site materials, including groundwater and sediments from the subsurface, sludge samples from a pilot-scale membrane bioreactor (MBR), and activated carbon samples from an on-site groundwater treatment plant (GTP). Bacterial community analysis based on 16S rDNA sequencing, and quantitative PCR (qPCR) targeted at the DCA dehalogenase gene *dhlA* were used in parallel on these materials. The genetic basis of DCA biodegradation in isolates was investigated by complete sequencing of catabolic plasmids found in these bacteria.

Results/Lessons Learned.

1. Membrane bioreactor study. A pilot-scale MBR was found to be an effective bioremediation method for DCA-contaminated groundwater. Biodegradation of DCA in the MBR began after a 26-day adaptation period, and was sustained for approximately 100 days, with effluent DCA concentrations below regulatory discharge limits. A qPCR assay for *dhlA* was developed to monitor DCA-degrading bacteria in the MBR, and a positive correlation was seen between *dhlA* gene abundance and the amount of DCA degraded. Genera previously associated with aerobic DCA biodegradation (*Xanthobacter, Ancylobacter, Azoarcus*) were present in the MBR, and the abundance of *Azoarcus* correlated with *dhlA* gene abundance. Pure cultures of *Xanthobacter*, *Starkeya*, and *Leifsonia* capable of growth on DCA as sole carbon source were isolated.
2. Genetic studies of DCA-degrading isolates. Three bacterial isolates from the MBR or GTP (*Starkeya* EL1, *Xanthobacter* EL4, and *Xanthobacter* EL8) were found to contain a *dhlA* gene identical to that in the well-studied *Xanthobacter* strain GJ10. However, unlike GJ10, the Botany isolates carried the *dhlA* gene on a 37-kb plasmid (pDCA), related to pTAR family catabolic plasmids, which enable growth on tartrate, dimethylformamide, and aromatics. Two chloroacetate dehalogenases (*dhlB*) were detected in the Botany isolates; a chromosomal *dhlB1* gene similar to *dhlB* of GJ10, and a plasmid-borne *dhlB2* gene, which was not closely related to previously-studied dehalogenases. Expression of *dhlB2* in *E.coli* confirmed that this gene encoded an enzyme capable of chloroacetate dechlorination.
3. Survey of DCA degraders in groundwater via qPCR. The dehalogenase gene *dhlA* was detected in all groundwater samples tested from the Botany site (n=15). The abundance of *dhlA* genes was positively correlated to DCA concentration, and was unexpectedly highest in samples with low oxygen conditions. Based on this finding, we tested *Xanthobacter* EL8 for anaerobic DCA degradation under denitrifying conditions, and this ability was confirmed.

Conclusions. Aerobic bacteria capable of growth on DCA are widespread at the Botany site, indicating that natural attenuation or biostimulation could be valid approaches for site cleanup. The *dhlA* gene is a unique biomarker for these bacteria, and the *dhlA* qPCR is thus an excellent tool for detecting and monitoring these bacteria. We have shown that some ‘aerobic’ DCA degraders can also use nitrate as electron acceptor, and these should therefore be described as ‘hydrolytic’ DCA degraders, to differentiate them from reductive dehalogenators.