Distribution and Activity of Dissimilatory Metal-Reducing Microorganisms in Contaminated Subsurface Environments

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Investigations into the diversity and ecology of dissimilatory metal-reducing microorganisms in uranium-contaminated subsurface environments were conducted with a combination of culturing, molecular ecology, and genomic techniques. Culturing studies yielded several novel isolates capable of dissimilatory metal reduction that have unique physiological properties that could be useful for engineered bioremediation of metal-contaminated environments. Molecular analyses of microbial communities associated with in situ metal remediation were carried out at the FRC and UMTRA sites.

For example, as part of a field experiment evaluating the potential for in situ microbial U(VI) reduction to remove U(VI) from contaminated groundwater, the microbial community associated with U(VI) removal was evaluated via analysis of 16S rDNA sequences. When dissimilatory metal reduction was stimulated with the addition of acetate, microorganisms in the family *Geobacteraceae* became the predominant organisms, accounting for well over half the microbial community when the rates of Fe(III) and U(VI) reduction were the highest. These results are in accordance with a number of studies that have demonstrated that *Geobacteraceae* are the predominant dissimilatory metal-reducing microorganisms in a wide variety of environments in which dissimilatory metal reduction is an important process.

Given the predominance of *Geobacteraceae* in subsurface environments in which dissimilatory metal reduction is important, studies on the genetic potential of *Geobacteraceae* living in these sediments were initiated. Genomic DNA extracted from uranium-contaminated subsurface sediments was used to make a large-insert BAC library, and the library was screened to identify genomic DNA from *Geobacteraceae*. One of the most interesting findings was that an "as-yet-uncultured" *Geobacter* species living in sediments from the Old Rifle field experiment site was found to contain an important gene duplication event that has recently been studied in *Geobacter sulfurreducens*. This gene duplication includes a gene for an 89 kDa *c*-type cytochrome shown to be essential for Fe(III) reduction in *G. sulfurreducens*. This and other results from sequencing environmental genomic DNA suggest that the *Geobacteraceae* living in uranium-contaminated subsurface sediments have important genetic similarities with *Geobacteraceae* currently being intensively studied in pure culture.

In order to assess the activity and metabolic state of the *Geobacteraceae* predominating in these environments, techniques to extract mRNA from subsurface sediments were optimized, and analysis of gene expression in *Geobacter* species under various growth conditions helped identify key genes for monitoring metabolism of *Geobacteraceae* in the subsurface. Results of ongoing sediment studies using the mRNA approach will be presented.