Determination of the Structure of Metal- and Humics-Reducing Microbial Communities in Subsurface Environments Contaminated with Uranium and Other Metals

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The purpose of these studies is to determine the composition and activity of the metal- and humics-reducing community in subsurface environments and elucidate the physiological characteristics of subsurface metal and humics reducers. This will provide information that will aid in bioremediation of metal-contaminated subsurface environments. A combination of culturing, biochemical and molecular approaches is being employed in these studies.

In both laboratory and field studies, stimulation of Fe(III) reduction in diverse aquifers invariably resulted in a dramatic increase in the number of 16S rDNA sequences closely related to known *Geobacter* species. In laboratory studies, the addition of either various organic electron donors or electron shuttle compounds stimulated Fe(III) reduction and resulted in *Geobacter* sequences becoming important constituents of the *Bacteria* 16S rDNA sequences that could be detected with PCR amplification and denaturing gradient gel electrophoresis. Quantification of *Geobacteraceae* sequences with a most-probable-number technique indicated that the extent to which numbers of *Geobacter* increased was related to the degree of stimulation of Fe(III) reduction. *Geothrix* species were also enriched in some instances, but were orders of magnitude less numerous than *Geobacter* species. *Shewanella* species were not detected, even when organic compounds known to be electron donors for *Shewanella* species were used to stimulate Fe(III) reduction in the sediments. *Geobacter* species were also enriched in two field experiments in which Fe(III) reduction was stimulated with the addition of benzoate or aromatic hydrocarbons. The apparent growth of *Geobacter* species concurrent with increased Fe(III) reduction suggests that *Geobacter species* were responsible for much of the Fe(III) reduction in all of the stimulation approaches evaluated in three geographically distinct aquifers. Studies at the Shiprock UMTRA site also suggested that *Geobacter* species are likely to be the dominant metal-reducing microorganisms under Fe(III)-reducing conditions, even when the subsurface is contaminated with uranium. Therefore, strategies for subsurface remediation that involve enhancing the activity of indigenous Fe(III)-reducing populations in aquifers should consider the physiological properties of *Geobacter* species in their treatment design.

The finding that the *Geobacter* species that predominated in the various subsurface environments were closely related to *Geobacter* species already available in pure culture is surprising because the current dogma in environmental microbiology is that the most environmentally significant microorganisms can not be recovered in culture. Thus, we now have an apparently unique opportunity in which we can study the physiology of microorganisms in pure culture which are closely related to the microorganisms that we know are environmentally significant in the subsurface. This suggests that an in-depth characterization of the physiology and biochemistry of *Geobacter* species will provide important insights into the mechanisms for metal reduction in the subsurface.

As part of the investigation of the physiological characteristics of *Geobacter* species, the genome of *Geobacter sulfurreducens* is being sequenced in collaboration with TIGR. Sequence data available to date has already suggested novel metabolic characteristics of *G. sulfurreducens*, such as the ability to fix nitrogen, and has identified genes for proteins we have recently found to be involved in electron transport to Fe(III). The results of a more complete genomic analysis and preliminary comparisons of the *G. sulfurreducens* genome with other *Geobacter* genomes will be presented, as will a model for the current understanding of electron transport to extracellular Fe(III) oxides in cultures and subsurface environments.