

Genomic Approach to the Study of Microbial Reduction of Iron and Uranium in Subsurface Environments

Derek Lovley (dlovley@microbio.umass.edu), Barbara Methe, Maddalena Coppi, Kelly Nevin, Susan Childers, Jon Lloyd & Ching Leang

Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA

Recent studies have indicated that most, if not all, of the reduction of Fe(III) to Fe(II) and U(VI) to U(IV) in aquifers is the result of enzymatic reduction of these metals by micro-organisms. However, little is known about the micro-organisms involved in this process and their mechanisms for metal reduction. Therefore, studies were conducted to evaluate which micro-organisms were involved in metal reduction in the subsurface and to understand the physiology of these micro-organisms. For example, Fe(III) reduction can be an important process for the oxidation of aromatic hydrocarbon contaminants, such as toluene and benzene, in petroleum-contaminated aquifers. The composition of the microbial community in several aquifers contaminated with petroleum was evaluated by determining the distribution of ¹⁶S rDNA sequences in the sediments. Sediments from the zone in the aquifer in which Fe(III) reduction was most important were invariably enriched with sequences closely related to sequences of micro-organisms in the genera *Geobacter*. In a similar manner, when Fe(III) and U(VI) reduction in subsurface sediments were artificially enhanced with the addition of various organic substrates in order to promote the reductive precipitation of uranium from contaminated groundwater, the growth of *Geobacter* species was invariably stimulated. These results suggested that *Geobacter* species were the primary organisms responsible for the reduction of Fe(III) and U(VI) in these subsurface environments. This result was unexpected because a common finding in environmental microbiology is that the most environmentally significant micro-organisms can not readily be recovered in pure culture.

In order to take advantage of this unique opportunity to study an organism in pure culture that has been shown to have environmental relevance, the whole genome of one *Geobacter*, *G.*

sulfurreducens, is being sequenced. Preliminary analysis of the genomic data revealed the presence of genes for the production of flagella, suggesting for the first time that *Geobacter* may be motile under some conditions. It was found that *G. metallireducens* is in fact motile, but only when using insoluble Fe(III) oxides as an electron acceptor. This is consistent with companion studies that demonstrated that *G. metallireducens* does not release Fe(III) chelators, or soluble electron shuttling compounds to reduce Fe(III) oxides, indicating that *G. metallireducens* must search for and establish contact with Fe(III) oxides in order to reduce them. Analysis of the genomic data also revealed genes for the fixation of atmospheric nitrogen. Mutants defective in nitrogen fixation have been developed in order to determine the importance of this characteristic for survival in nutrient-poor subsurface environments. Biochemical studies suggested that the transfer of electrons from *Geobacter* to insoluble Fe(III) oxides proceeds through an electron transport chain that terminates with a c-type cytochrome that is exposed on the outer surface of the cell and donates electrons to the Fe(III) oxide. In contrast, U(VI), which is soluble, may be reduced by a shorter electron-transport chain that does not involve the cytochrome that protrudes from the outer membrane. The genes for many of the electron transport components believed to be involved in Fe(III) and U(VI) reduction have already been found in the preliminary genomic data and genetic studies to evaluate the role of each of these components are underway. These studies are expected to provide the first indication of how *Geobacter* species, the dominant Fe(III)- and U(VI)-reducing micro-organisms in a variety of subsurface environments, are able to transfer electrons to these metals. This information will significantly improve our models for iron and uranium geochemistry in subsurface environments.