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Towards a More Complete Picture: Dissimilatory Metal Reduction by Anaeromyxobacter Species

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RESULTS TO DATE: Sanford and Loeffler: Towards a More Complete Picture: Dissimilatory Metal Reduction by Anaeromyxobacter Species

The overarching goal of this 3-year project is to explore uranium reduction in Anaeromyxobacter species. We investigate the physiological requirements of available Anaeromyxobacter isolates, and assess their distribution and abundance in the environment, including DOE sites. The performers on this project include Frank Loeffler (PI), Robert Sanford (Co-PI), Qingzhong Wu (postdoc), Sara Henry (graduate student) and Cornell Gayle (undergraduate student). Year-1 efforts focused on method and tool development to address the research objectives. First, we compared different analytical assays (based on fluorescent light emission and calorimetric methods) to quantify U(VI) in cultures of Anaeromyxobacter dehalogenans strain 2CP-C. The assays were optimized to reflect specific culture conditions, and we found that a laser-excited spectrofluorescence assay provided reproducible and accurate information on the amount of U(VI) reduced in bacterial cultures. To demonstrate the ability of Anaeromyxobacter dehalogenans strain 2CP-C to reduce U(VI), washed suspensions of fumarate-grown cells were prepared. These experiments confirmed that the rapid reduction of U(VI) to U(IV) depended on the presence of live cells, and no U(VI) reduction occurred in cell-free controls. Additional experiments explored the ability of three different Anaeromyxobacter strains to grow with the mineral hematite, an insoluble form of ferric iron, as electron acceptor. All strain grew equally well with soluble ferric iron (provided as ferric citrate) but distinct differences were observed between strains when grown with hematite. All strains tested shared a 16S rRNA gene similarity of >99.5%, suggesting that closely related strains may differ in their ability to access insoluble forms of ferric iron. These experiments have been expanded, and the available strains are being tested for differences in their ability to reduce U(VI) and Mn(IV). Cultures of Geobacter metalireducens and Geobacter sulfurreducens served as positive or negative controls for the experiments. In addition, we initiated experiments with colleagues in the Geology department at the University of Illinois to explore uranium isotope fractionation during biotic and abiotic reduction. We compared isotope fractionation between U(VI)-reducing bacterial cultures and systems dominated by abiotic, chemical reduction of U(VI). A manuscript detailing these results is in preparation. Further, our work on chlorinated solvent reductive dechlorination yielded the first isolate of a Geobacter species, designated strain SZ, that uses tetrachloroethene as metabolic electron acceptor. Strain SZ was included in some of the experiments performed with Anaeromyxobacter, and we could demonstrate that strain SZ readily reduced ferric iron and U(VI). Hence, Geobacter sp. strain SZ is the first member of the Geobactereacea with the ability to reduce chlorinated ethenes and radionuclides (e.g., uranium). The Year-1 molecular work focused on four themes: First, we initiated efforts to develop genetic systems for Anaeromyxobacter to generate mutants and allow the heterologous expression of foreign genes. The springboard to develop a genetic system for A. dehalogenans is based on genetic systems utilized in the closely related species Geobacter sulfurreducens and Myxococcus xanthus. Ten Anaeromyxobacter genes have been selected for initial mutational analysis. We solicited the expertise of Dr. John Kirby, an Assistant Professor in the School of Biology and a Myxococcus expert. The second goal is to design an Anaeromyxobacter 16S rRNA gene-targeted approach for identifying this bacterial group in environmental samples. Anaeromyxobacter 16S rRNA gene sequences, closely related environmental clone sequences deposited in public databases (e.g., GenBank), as well as sequences of closely related organisms were aligned. Several regions suitable for primer design were identified. A total of 14 primers were designed, and multiple primer combinations were tested with genomic DNA from Anaeromyxobacter strains and plasmid DNA containing a single 16S rRNA gene of strain 2CP-C. Following optimization of the PCR conditions several primer pairs specific to the Anaeromyxobacter group, as well as strain-specific (i.e., strain 2CP-C) primers, were obtained. These primers have been tested to detect Anaeromyxobacter

populations in pure culture, consortia and in environmental samples. Sequencing the resulting amplicons confirmed primer specificity, and only Anaeromyxobacter 16S rRNA genes amplified. These primers form the basis of the third objective, which is to design a real-time (RTm) PCR approach for quantifying A. dehalogenans 16S rRNA gene copies in cultures and environmental samples. Initial experiments using the SYBR green reporter system were promising, and we anticipate that a quantitative approach will become available in Year 2. Another goal is to obtain key information from the A. dehalogenans strain 2CP-C genome. A draft genome sequence of strain 2CP-C became available to us in August of 2004. We are using the available sequence information to identify and examine genes that are relevant for bioremediation (i.e., reductive dechlorination, and radionuclide reduction).

DELIVERABLES: Peer-reviewed publications: Rademacher, L., C. Lundstrom, T. Johnson, R. Sanford, J. Zhao, and Z. Zhang. 2004. Experimentally determined uranium isotope fractionation during biotic and abiotic reduction. Geochimica et Cosmochimica Acta. To be submitted in 2004.

Sung, Y, R. A. Sanford, and F. E. Loeffler. 2004. Characterization and description of Geobacter sp. strain SZ sp. nov., a tetrachloroethene (PCE)-, metal- and radionuclide-respiring bacterium. Appl. Environ. Microbiol. To be submitted in 2004.

Oral presentations: Sanford, R. A., Q. He, and F. E. Loeffler. 8/2004. Variation in Hematite Reduction Activity Among Strains of Anaeromyxobacter dehalogenans. In Microbial Planet - Sub-Surface to Space, p. 370. ISME-10, Cancun, Mexico.

Poster presentations: Henry, S., F. E. Loeffler, and J. Kirby. 7/2004. Characterization of Anaeromyxobacter dehalogenans. The 31st International Meeting on the Biology of the Myxobacteria, July 3-7, Elsinore, Denmark.

Sanford, R. A, Qingzhong Wu, Cornell Gayle, and F. E. Loeffler. 3/2004. Dissimilatory metal reduction by Anaeromyxobacter species. DOR-NABIR Workshop, March 15-17, Warrenton, VA.