Project 1022485

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

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RESULTS TO DATE: 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. Specifically, we explore the physiological requirements of available Anaeromyxobacter isolates, design molecular biology tools to detect and quantify Anaeromyxobacter in pure cultures, consortia, and environmental samples, assess their diversity, distribution, and abundance in the environment, including DOE sites, and attempt the isolation of additional Anaeromyxobacter species from the Oak Ridge Field Research Center (FRC). The performers on this project include Frank Loeffler (PI), Robert Sanford (Co-PI), Qingzhong Wu (postdoc), Sara Henry (graduate student with fellowship, no charges to NABIR project), Ivy Thomson (graduate student, no charges to NABIR project), and Ryan Wagner ("Special Topics" bioinformatics undergraduate student, no charges to NABIR project). Exploratory MALDI-TOF MS experiments for the specific detection of Anaeromyxobacter species were performed by Kerry Preston (graduate student, no charges to NABIR project). The 2nd year's efforts used the tools designed during the first year of NABIR support and focused on characterizing U(VI) reduction in Anaeromyxobacter dehalogenans strain 2CP-C. First, in collaboration with Dr. Martial Taillefert, a sensitive, accurate, and reproducible laser-excited spectrofluorescence assay was designed for quantifying U(VI) reduction in bacterial cultures. Secondly, an Anaeromyxobacter 16S rRNA gene-specific PCR assay was developed and validated. The assay specifically amplifies Anaeromyxobacter 16S rRNA genes, and was applied to detect Anaeromyxobacter species in environmental samples. Thirdly, a quantitative real-time PCR (qRTm) PCR approach was designed to enumerate Anaeromyxobacter cells in cultures and in environmental samples. Our work also yielded the first Geobacter isolate, designated strain SZ that uses tetrachloroethene as metabolic electron acceptor. Similar to other well-characterized Geobacter species, strain SZ reduces Fe(III) and U(VI). A qRTm PCR approach was designed for strain SZ and allows us to quantitatively monitor growth of Geobacter species. We demonstrated previously that Anaeromyxobacter dehalogenans strain 2CP-C reduces U(VI) to U(IV). Using the new analytical tools, we quantified U(VI) reduction and showed that strain 2CP-C required hydrogen as an electron donor for U(VI) reduction. Anaeromyxobacter isolates use acetate and hydrogen for the reduction of other electron acceptors, such as Fe(III), nitrate, fumarate, etc., but apparently require hydrogen for U(VI) reduction. The analysis of 16S rRNA gene copy numbers using qRTm PCR indicated that U(VI) reduction in cultures of strain 2CP-C is not a fortuitous reaction. Rather strain 2CP-C uses oxidized U(VI) as metabolic electron acceptor and grows on the expense of U(VI) reduction. No increase in cell numbers was observed in cultures that did not receive U(VI) as an electron acceptor. The geochemistry at contaminated DOE sites is complex, and other energetically favorable electron acceptors such as nitrate, Fe(III), or chloro-organic compounds can interfere with efficient U(VI) reduction. The addition of nitrate to U(VI)-reducing cultures of strain 2CP-C inhibited U(VI) reduction, and an increase in U(VI) concentrations was observed, apparently caused by the re-oxidation of reduced U(IV). Nitrate was reduced to ammonium with the intermediate formation of nitrite. The increase in U(VI) concentration coincided with the transient formation of nitrite, suggesting that nitrite, rather than nitrate, was responsible for U(IV) re-oxidation. Following the reduction of nitrate and nitrite to ammonium, U(VI) reduction resumed. To explore the effects of soluble and insoluble forms of Fe(III) on U(VI) reduction, Fe(III) citrate and amorphous Fe(III) (FeOOH) were added to U(VI)-reducing cultures of strain 2CP-C. U(VI) reduction in cultures that received Fe(III) citrate was completely inhibited but Fe(III) reduction occurred without a lag time. The addition of citrate alone also caused cessation of U(VI) reduction. The formation of stable, binuclear ((UO2)2 citrate2)2- and Fe-U-citrate complexes has been described, and may explain the observed citrate inhibition. U(VI) reduction continued to completion in cultures that received amorphous Fe(III) (FeOOH) although at 3-fold lower rates compared with control cultures. Fumarate and 2-chlorophenol had no inhibitory effects on U(VI) reduction and both electron acceptors were consumed concomitantly with U(VI). In order to enrich, identify, and isolate Anaeromyxobacter species responsible for metal reduction

at the FRC site, microcosms were established with materials collected from FW113 and FB089. A variety of treatments including different electron donor, electron acceptor, and pH conditions were established. The microcosms are being analyzed for activity (e.g., electron acceptor reduction) and the increase in Anaeromyxobacter 16S rRNA gene copy numbers using the qRTm PCR approach. The Anaeromyxobacter 16S rRNA gene-targeted approach was employed to analyze a large number of samples including materials collected from the FRC, microcosms established with FRC materials, DNA from microcosms established with FRC materials (kindly provided by J. Kostka), DNA extracted from FRC groundwater (kindly provided by T. Gentry and J. Zhou), and DNA extracted from agricultural soils (kindly provided J. Chee-Sanford). These analyses suggested that Anaeromyxobacter species are present at the FRC though not ubiquitously distributed at the site. Anaeromyxobacter were detected in many agricultural soil samples suggesting a widespread distribution in those environments. Most interestingly, Anaeromyxobacter 16S rRNA gene sequences were detected at two locations at the FRC (Areas 1 and 3) following biostimulation with ethanol but not in nearby locations that did not receive this enhanced treatment. These findings strongly suggest that Anaeromyxobacter-species respond to biostimulation, and thus, enhanced rates of contaminant removal can be expected during enhanced treatment. The draft genome sequence of strain 2CP-C has been made available by JGI, and we are using this information to learn more about the organism's features and capabilities. For instance, the genome analysis suggested that strain 2CP-C possesses a large number of c-type cytochromes, including many with multiple heme-binding sites. One cytochrome contains 33 CXXCH sites, one CXXXCH site, and six CXXXXCH sites. c-type cytochromes with such unusually large numbers of heme binding sites are implicated in metal reduction. To elucidate the function of individual c-type cytochromes, we are exploring if a genetic system developed for Myxococcus xanthus can be adapted for Anaeromyxobacter spp. Our preliminary studies showed that a number of M. xanthus genetic systems work for A. dehalogenans strain 2CP-C. One of the methods utilizes the pBJ114 suicide vector, a conditionally-replicating plasmid constructed for gene disruption in M. xanthus via homologous recombination. Alternatively, the pMycoMar plasmid is a donor of the mariner transposable elements magellan-3 and magellan-4. The pMycoMar plasmid was shown to be effective for transposon mutagenesis in M. xanthus as well as in another members of the order Myxococcales. The Loeffler group is collaborating with Dr. J. Kirby, a faculty member in Georgia Tech's School of Biology, to develop a genetic system for Anaeromyxobacter, and the progress achieved in the past year gives us confidence that we will be able to adopt a Myxococcus genetic system to work in Anaeromyxobacter. Once developed, the genetic system can be used to investigate the mechanisms of metal-reduction mediated by Anaeromyxobacter. Further, questions relating to how Anaeromyxobacter is similar or different from Geobacter and Shewanella can then be addressed.

DELIVERABLES: Peer-reviewed publications: Wu, Q., R. A. Sanford, and F. E. Loeffler. 2005. Characterization of U(VI) reduction in Anaeromyxobacter dehalogenans strain 2CP-C. To be submitted in 2005. Sung, Y., K. M. Ritalahti, R. A. Sanford, and F. E. Loeffler. 2005. Characterization and description of Geobacter lovleyi strain SZ sp. nov., a novel metal-reducing and tetrachloroethene (PCE)-dechlorinating bacterium. Appl. Environ. Microbiol. Submitted. Rademacher, L., C. Lundstrom, T. Johnson, R. Sanford, J. Zhao, and Z. Zhang. 2005. Experimentally determined uranium isotope fractionation during biotic and abiotic reduction. Geochimica et Cosmochimica Acta. Submitted. Poster presentations: Preston, K. E., J. R. Barr, H. Moura, A. Woolfitt, B. Amos, Y. Sung, S. Henry, and F. E. Loeffler. 2005. MALDI-TOF MS profiling of metal-reducing bacteria including Anaeromyxobacter, Geobacter, and Desulfuromonas species. The Joint International Symposia for Subsurface Microbiology and Environmental Biogeochemistry, Jackson Hole, WY. Wu, Q., F. E. Loeffler, R. A. Sanford, and S. Henry. 2005. Uranium reduction by Anaeromyxobacter species. Annual DOE-NABIR investigator meeting. Wu, Q., R. A. Sanford, F. E. Loeffler. 2005. Uranium reduction by Anaeromyxobacter species, abstr. Q-038. In Abstracts of the 104th General Meeting of the American Society for Microbiology, Atlanta, GA. Thomson, I. N., S. Henry, R. Krajmalnik-Brown, K. M. Ritalahti, F. E. Loeffler. 2005. Cloning and expression analysis of Anaeromyxobacter reductive dehalogenase genes, abstr. Q-029. In Abstracts of the 104th General Meeting of the American Society for Microbiology, Atlanta, GA. Henry, S., F. E. Loeffler, R. A. Sanford, J. Kirby. 2005. Pilus-based motility and energy taxis in the subsurface: Anaeromyxobacter dehalogenans as a model organism for bioremediation applications, abstr. I-080. In Abstracts of the 104th General Meeting of the American Society for Microbiology, Atlanta, GA. Preston, K. E, F. E. Loeffler, H. Moura, A. Woolfitt, B. Amos, Y. Sung, S. Henry, J. R. Barr. 2005. MALDI-TOF MS profiling of environmentally relevant bacteria including Anaeromyxobacter, Geobacter, and

Desulfuromonas species, abstr. Q-262. In Abstracts of the 104th General Meeting of the American Society for Microbiology, Atlanta, GA. Preston, K. E., F. E. Loeffler, H. Moura, A. Woolfitt, S. Henry, Y. Sung, B. Amos, J. R. Barr. 2005. Detection of Anaeromyxobacter dehalogenans biomarkers by MALDI-TOF MS analysis of whole cells. Association of Biomolecular Resource Facilities, Savannah, GA. Sanford, R. A., Q. He, and F. E. Loeffler. 2004. Variation in hematite reduction activity among strains of Anaeromyxobacter dehalogenans. In Microbial Planet - Sub-Surface to Space, p. 370. ISME-10, Cancun, Mexico. Oral presentations: Dr. Loeffler gave several invited talks where he presented the work performed under this NABIR grant. Project URL: Dr. Loeffler's web site is currently being updated and improved. The new web site will be operational by September 2005, and will include a link to the ongoing NABIR project.