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Composition, Reactivity, and Regulations of Extracellular Metal-Reducing Structures (Bacterial Nanowires) Produced by Dissimilatory Metal Reducing Bacteria

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Research Objective:

This research proposal seeks to describe the composition and function of electrically conductive appendages known as bacterial nanowires. This project targets bacterial nanowires produced by dissimilatory metal reducing bacteria Shewanella and Geobacter. Specifically, this project will investigate the role of these structures in the reductive transformation of iron oxides as solid phase electron acceptors, as well as uranium as a dissolved electron acceptor that forms nanocrystalline particles of uraninite upon reduction.

Research Progress and Implications:

This report summarizes research accomplishments collected during the first 2 years of a 3 year project. In 2004, the US Department of Energy initiated funding on a proposal entitled "Composition, Reactivity, and Regulation of Extracellular Metal-Reducing Structures (Bacterial Nanowires) Produced by Dissimilatory Metal Reducing Bacteria", (CoPIs Yuri Gorby and Terry Beveridge). During two years of research, the PI's demonstrated that bacterial nanowires are produced by metal reducing bacteria, such as *Geobacter* and *Shewanella*, under conditions that limit the availability of electron acceptors. Using a combination of Scanning Electron Microscopy (SEM) and Scanning Tunneling Microscopy (STM), research confirmed that the wild type strain of MR-1 produced electrically conductive nanowires (Figure 1) that connected cells together into an electrically integrated cellular network. Conductivity of nanowires produced by *Shewanella oneidensis* strain MR-1 requires the presence of electron transport proteins known as cytochromes, which were previously shown to enzymatically reduce solid phase iron and manganese oxides as a form of microbial respiration. Mutants lacking these cytochromes produced appendages that resembled nanowires but were non-conductive. Notably, these mutants did not reduce solid phase iron oxides, did not produce electricity in a microbial fuel, and did not form thick biofilms. These results are fully described in a manuscript recently published in the Proceedings of the National Academy of Science (Gorby et al., 2006).

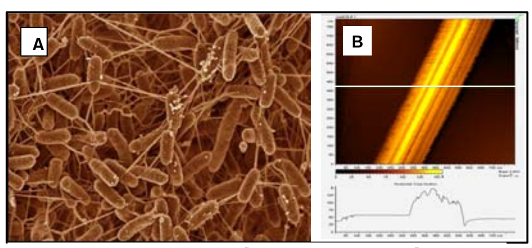

Figure 1. Scanning electron micrograph (A) and scanning tunneling micrograph (B), revealing the morphological and electrical properties of bacterial nanowires from Shewanella oneidensis strain MR-1.

Recent results demonstrated that nanowires are able to reach out and access iron that is bound in pore spaces that are too small for bacteria to access directly. Figure 2A is an SEM image of porous silica beads with pore spaces measuring about 100 nanometers in diameter. Surfaces throughout the bead matrix were coated with a thin layer of iron oxide that served as the sole terminal electron acceptor. When inoculated with Shewanella, biofilms developed on the surface of the beads and nanowires were observed to enter the porous matrix (Figs. 2B, C, and D). With time *Shewanella* reductively dissolved all iron within the porous matrix. Mutant strains lacking electrically conductive nanowires did not reduce iron within the porous silica matrix.

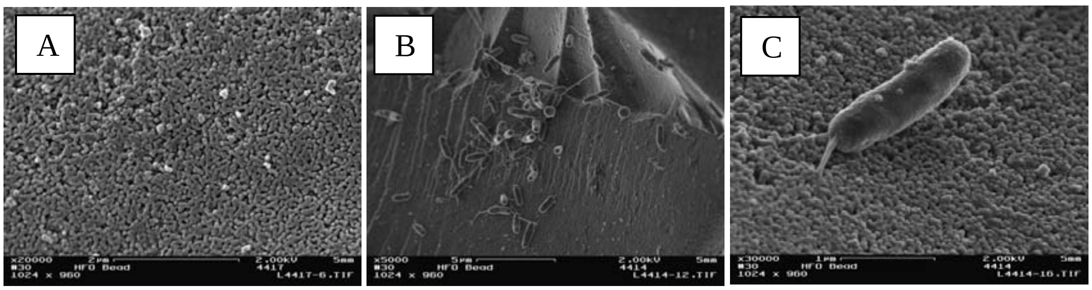

Figure 2. SEM images of Shewanella on iron-bearing porous silica beads. (A) Uninoculated control. (B&C). Beads inoculated with S. oneidensis strain MR-1 showing nanowires extending into the porous matrix.

The results presented above significantly alters our current understanding of the mechanisms by which dissimilatory metal reducing bacteria deliver electrons to solid phase electron acceptors that dominate most anaerobic subsurface sediments, including those contaminated with heavy metals and radionuclides. As scientists and engineers we must reassess our preexisting concepts regarding the influence of these bacteria on influencing the fate and transport of multivalent contaminants in groundwater, especially when considering those processes that are previously considered to be principally controlled by diffusion.

Although not part of the original proposal, investigations conducted in our lab have revealed that nanowires are not exclusive to dissimilatory metal reducing bacteria. Indeed, *bacteria ranging from oxygenic, photosynthetic cyanobacteria to thermophilic methanogenic cocultures produce electrically conductive nanowires*. These findings support a hypothesis that electrically conductive nanowires represent a common bacterial strategy for efficient electron transfer and energy distribution. These results present immediate implications for redox processes in subsurface sediments controlled by organisms other than dissimilatory metal reducing bacteria.

Planned Activities:

The final year will be devoted toward completing the objectives outlined in the original proposal. Specifically, antibodies generated against proteins suspected to be involved in electron transport will be used to investigate the distribution of these proteins along nanowires. Antibodies have been generated against the decaheme cytochromes MtrC and OmcA that have been implicated in electron transport to solid phase iron oxides by S. oneidensis MR-1. Preliminary data collected by Dr. Terry Beveridge at the University of

Guelph suggests that these cytochromes, which were previously believed to be associated with the outer membrane, are located along the length of the nanowires. Antibodies produced against the mulitheme cytochromes OmcE and OmcS in Geobacter sulfurreducens have been produced and are being assessed for specificity and avidity. These antibodies will be used to investigate the distribution of cytochromes in cells that are producing nanowires in response to electron acceptor limitation. Additionally, collaborators at Lawrence Berkeley National Lab will conduct labeling experiments using tagged constructs generated by Dr. Jim Fredrickson and associates involved in the Shewanella Federation research collaboration. Techniques being applied by Dr. Manfred Auer and associates at LBNL are expected to yield high resolution transmission electron micrographs that reveal the distribution of electron transport components associated with nanowires. Such studies are essential for conclusively demonstrating that nanowires produced by Geobacter and Shewanella are 'functionalized' by electron transport proteins (cytochromes).

Information Access:

Gorby, Y.A., A. Dohnalkova, K. M. Rosso, S. Yanina, M. J. Marshall, A. S. Beliaev, D. Moyles, A. Korenevski, T. J. Beveridge, J. S. McLean, D. E. Culley, S. B. Reed, M. F. Romine, D. A. Saffarini, I. S. Chang, B. H. Kim, K. S. Kim, L. Shi, D. A. Elias, D. W. Kennedy, G. Pinchuk, E. A. Hill, J. M. Zachara, K. H. Nealson, and J. K. Fredrickson. 2006 "Electrically Conductive Bacterial Nanowires Produced by *Shewanella oneidensis* strain MR-1 and Other Microorganisms" (2006) PNAS: 130:11358-63

Hu JZ, Wind RA, McLean J, Gorby YA, Resch CT, Fredrickson JK. 2004. High-resolution H-1 NMR spectroscopy of metabolically active microorganisms using non-destructive magic angle spinning. Specroscopy 19 (12): 98-103

Gorby, Y.A. Bacterial nanowires: novel structures for extracellular electron transfer and mineral transformation. Gordon Research Conference on Environmental Bioinorganic Chemistry. June 18-23, 2006. Proctor Academy, Andover, NH. (Invited).

Gorby, Y.A. Biofilms as the Mediators of Electron Flow in Microbial Fuel Cells. Southern California American Society for Microbiology annual meeting. Nov. 3-5, 2006, San Diego, CA (Invited).

Gorby, Y.A. Bacterial Nanowires and Their Role in Extracellular Electron Transfer. Joint Programme on Science and Technology for Sustainability. Gyangju Institute for Science and Technology. October 31 to November 2, 2006. Damyang, Korea (invited).

Clarification:

This project was awarded to Dr. Yuri Gorby. He recently left PNNL and Dr. Johannes Scholten has assumed the PI role for this project. Furthermore, Dr. Yuri Gorby will have an advisory role and continued interest in successful completion of the stated objectives.