

**2006 ERSD Annual Report
Project #1010022**

Nanowires, Capacitors, and Other Novel Outer-Surface Components Involved in Electron Transfer to Fe(III) Oxides in Geobacter Species

Principal Investigator: Lovley, Derek R.

Organization: University of Massachusetts

Results To Date

In the first 8 months of this grant we initiated investigations on several of the new hypothesis in the proposal. Hypothesis 1-4 deal with the mechanisms of conductivity along the length of the pili of *Geobacter sulfurreducens*. The initial approach to evaluating these mechanisms was to attempt to measure end-to-end conductivity of the pili with lithographically-patterned electrodes in which conductive strips of graphite are placed on an insulating silicon dioxide surface. To our knowledge this is the first time that such conductivity measurements have been attempted with natural protein structures. Conductivity along the pili was measured with two methods. In the first method, the conductivity of the pili was measured by applying a voltage between the lithographic electrodes. In the second method, the voltage was applied between one lithographic electrode and the AFM tip. To date, we have not been able to consistently measure current of pili because of an inability to readily observe pili on the nanoelectrode system. Although we were able to make conductivity measurements in this manner on one attempt, we have not been able to consistently obtain appropriate preparations to consistently make such measurements. Therefore, we are evaluating strategies to modify this approach to make it more consistent.

Hypothesis 5 of the proposal was designed to evaluate the properties of pili in Geobacteraceae other than *Geobacter sulfurreducens*. The availability of new genome sequences within the family Geobacteraceae allowed us to identify putative genes encoding for pilin domain proteins. The following Geobacteraceae genomes for which the complete genome sequence is available were analyzed: *Geobacter metallireducens*, *Pelobacter propionicus*, *Pelobacter carbinolicus*, *Geobacter uraniumreducens* (Isolated from the DOE-site at Rifle, Colorado), *Geobacter* sp. FRC-32 (isolated from the DOE FRC site). Each genome contained several pilin domain candidates. Phylogenetic analyses of the amino acid sequences encoded by these genes identified only one gene per organism as homologous to the pilin gene (*pilA*) of *G. sulfurreducens*. All of them formed an independent line of descent with the pilin protein of *G. sulfurreducens* and distant from type-IV pilin proteins from other bacteria. We identified two subgroups among the "geopilins": (i) the short geopilins of *G. sulfurreducens*, *P. propionicus*, *G. metallireducens* and *Geobacter* sp. FRC-32; and (ii) the long geopilins of *G. uraniumreducens* and *P. carbinolicus*. The long geopilins contained a short (ca. 50 amino

acids) N-terminus domain conserved within the family Geobacteraceae and a large (ca. 200 amino acids), hyper-variable C-terminus domain showing no homology to any protein in the database. We studied the pili of *Pelobacter carbinolicus* because (i) its pilin subunit belongs to the long geopilins subgroup and (ii) the organism is representative of the *Pelobacter* species that are capable of using Fe(III) oxide as the sole electron acceptor yet do not contain the abundant outer-membrane c-type cytochromes found in other Geobacteraceae. Preliminary analyses of pili from *P. carbinolicus* with a conducting probe-atomic force microscope indicated that the pili were ca. 10-nm thick, larger than those of *G. sulfurreducens* as expected of pili composed of much larger subunits, and had conductive properties similar to those of *G. sulfurreducens*. These results further suggest the pili are important conduits for extracellular electron transfer in Geobacteraceae. Further studies of the properties of the pili of other Geobacteraceae, as well as heterologous expression of other geopilins in the pilin-deficient mutant of *G. sulfurreducens* background are underway. These experiments will provide new insights into the mechanisms by which the pili are conductive.

The goal of hypothesis 7 is to determine whether, like pili, some of the other electron transport proteins involved in electron transfer to Fe(III) oxide are localized on one side of the cell. OmcB, a polyheme c-type cytochrome, and OmpB, a multicopper protein, are required for the reduction of insoluble Fe(III) oxides. Antibodies to these proteins were produced and the localization of the proteins was then evaluated with immunogold labeling. Both proteins appeared to be distributed homogeneously within the outer membrane. These results indicate that neither of these proteins forms specific association with the conductive pili, suggesting that their role is not direct electron transfer to the pili. Further characterization of the protein-protein interactions between OmcB and OmpB and with other outer membrane proteins are expected to provide further understanding of the function of these outer surface-exposed proteins during Fe(III) oxide reduction.

Deliverables

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Mehta, T., M. V. Coppi, S. E. Childers, and D. R. Lovley. 2005. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* 71:8634-41.

Mehta, T., S. E. Childers, R. Glaven, T. Mester, and D. R. Lovley. 2006. A novel type II secretion system required for the secretion of a novel multi-copper protein specifically required for the reduction of insoluble, but not soluble Fe(III) in *Geobacter sulfurreducens*. *Microbiology* (in press).