Biomolecular Mechanisms for Microbe-Fe(III) Oxide Interactions in *Geobacter* species

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Previous studies have indicated that *Geobacteraceae* are the primary agents for metal reduction during in situ uranium bioremediation and that, even when uranium levels are high, electron transfer to Fe(III) reduction accounts for ca. 99% of the growth of the *Geobacteraceae*. Thus, it is important to understand how *Geobacteraceae* interact with the Fe(III) oxides.

Previous studies on the mechanisms for Fe(III) reduction in *Geobacter* species have focused on the role of outer-membrane *c*-type cytochromes, but this approach has ignored the fact that *Pelobacter* species, which are phylogenetically intertwined with *Geobacter* species, lack *c*-type cytochromes but still reduce Fe(III) oxides. Previous studies had demonstrated that pili were required for Fe(III) oxide reduction by *Geobacter sulfurreducens*, and *Pelobacter* species as well as other *Geobacter* species possess pili. Therefore, studies this year focused primarily on the role of pili in Fe(III) oxide reduction. Transmission electron micrographs of cultures grown with poorly crystalline Fe(III) oxide as the electron acceptor revealed that Fe(III) oxides were much smaller than the microorganisms and that the Fe(III) oxide particles were preferentially associated more with the pili of *G. sulfurreducens* than the outer cell surface. These results suggested that the pili might have a direct role in electron transfer to Fe(III) oxides. In order to evaluate this, the electrical conductivity through the pili was measured. Pili and other proteins released from the outer surface of *G. sulfurreducens* were immobilized on a graphite surface and analyzed with an atomic force microscope equipped with a conductive tip and electronics that permitted mapping of the local conductance from the tip to the substrate. Topographic analysis revealed pili as well as other, unidentified, more globular, proteins which were also sheared off the outer cell surface. When a voltage was applied to the tip there was a strong current response along the pilin filaments, which was positive when a positive voltage was applied and negative with a negative voltage.

In contrast, the non-pilin proteins had no detectable conductivity and in instances in which the non-pilin proteins covered the pili filaments, they insulated the pili from the conductive tip. When similar studies were carried out with pili from *Shewanella oneidensis*, no conductance was detected. This is consistent with the concept that this organism produces electron shuttles to transfer electrons onto Fe(III) oxides. Analysis of the sequence of *pilA*, which encodes for the pilin structural protein, suggested that the pilin subunits of *Geobacteraceae* are likely to have significantly different properties than the previously described pili of other microorganisms. The finding that the pili of *G. sulfurreducens* are highly conductive suggests that *G. sulfurreducens* requires pili in order to reduce Fe(III) oxides because pili are the electrical connection between the cell and the surface of the Fe(III) oxides. This contrasts with the nearly universal concept that outer-membrane cytochromes are the proteins that transfer electrons to Fe(III) oxide in Fe(III) reducers. In addition to serving as a conduit for electron transfer to Fe(III) oxides, pili could conceivably be involved in other electron transfer reactions.