ABSTRACT: The mechanisms for Fe(III) oxide reduction in Geobacter species are of interest because of its important role in global carbon cycling. This is also of specific interest to the Subsurface Biogeochemical Research Program because Geobacter species, growing primarily via Fe(III) oxide reduction, have been shown to be important agents in the bioremediation of uranium-contaminated groundwater. There has been substantial debate about the mechanisms for Fe(III) oxide reduction in Geobacter species. One model is that electrons are transported outside the cell along proteinaceous pili that have metallic-like conductivity and that the multi-heme c-type cytochrome OmcS, which is specifically localized along the pili, facilitates electron transfer from the pili to the Fe(III) oxide. The alternative model is that outer-surface c-type cytochromes not associated with pili are the primary electrical contacts with Fe(III) oxides. Conclusively differentiating between these two hypotheses has been difficult, in part because of the non-specific reduction of Fe(III) by many different cytochromes in vitro. Furthermore, genetic approaches have often yielded strains in which the deletion of a single gene influenced the production and/or localization of multiple proteins.

In order to investigate Fe(III) reduction mechanisms further, a strain of G. sulfurreducens, designated Aro-5, was constructed which produced pili with diminished conductivity. This was accomplished by modifying the amino acid sequence of PilA, the structural pilin protein. An alanine was substituted for each of the five aromatic amino acids in the carboxyl terminus of PilA, the region in which G. sulfurreducens PilA differs most significantly from the PilA of microorganisms incapable of long-range extracellular electron transport. Strain Aro-5 produced pili that were properly decorated with OmcS. Other abundant outer-surface c-type cytochromes, such as OmcZ, were properly expressed and localized. However, the Aro-5 pili had greatly diminished conductivity and Aro-5 cultures were severely limited in their capacity to reduce Fe(III) compared to the control strain. The capacity of the Aro-5 strain to produce electrical current with a graphite anode serving as the electron acceptor was less than 10% of the control strain and the conductivity of the Aro-5 biofilms was 10-fold lower than the control strain. These results further demonstrated that loss of pili conductivity reduced the capacity for long-range electron transport. Thus, the expression of outer-surface c-type cytochromes is insufficient for Fe(III) reduction in G. sulfurreducens. The pili of G. sulfurreducens must be conductive in order for the cells to be effective in extracellular long-range electron transport.

Previous evidence for the alternative hypothesis, that G. sulfurreducens reduces Fe(III) with outer-surface c-type cytochromes not associated with pili, was the finding that deletion of gene GSU1501, which controls production of exopolysaccharide, inhibited Fe(III) oxide reduction. However, further examination of the GSU1501-deletion mutant revealed that this strain does not properly localize OmcS on the pili. OmcS is produced and is abundant in the extracellular matrix. Thus, the phenotype of the GSU1501-deletion mutant is also consistent with the model in which long-range electron transport is along the pili, which delivers electrons to OmcS for the final electron transfer to Fe(III) oxide.

These studies rule out the possibility that outer-surface c-type cytochromes can effectively facilitate Fe(III) oxide in the absence of pili conductivity and support the concept that long-range electron transport along pili is the primary mechanism for Fe(III) oxide reduction by G. sulfurreducens.