37. Investigations into the Proteome of Gram-positive Metal-reducing Bacterium *Desulfotomaculum reducens* MI-1

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Project Goals: The major goal of this project is to improve characterization of iron and sulfate respiration in *Desulfotomaculum reducens* MI-1. Towards this goal, other aims of the project included creating and optimizing a top-down proteomic workflow involving nondenaturing separations in order to screen for iron reduction active proteins and then heterologously expressing and characterizing identified proteins. Additional aims included selecting proteins of interest for roles in sulfate or iron respiration in *D. reducens* based on comparative proteomic analyses across different growth conditions.

Microbial respirations based on iron and sulfate occur readily in subsurface environments and have important impacts on heavy metal and radionuclide contaminants. *Desulfotomaculum reducens* MI-1 provides a unique study system as a Gram-positive sulfate reducing bacteria (SRB) that is also capable of respiring a variety of metals. Predictions of sulfate reduction pathways in *D. reducens* currently rely on homology to model Gram-negative SRB. Furthermore, no metal reductases have been described in this organism, and no orthologs to characterized metal reductases from other organisms exist in the *D. reducens* genome. In this project, we utilized proteomic-based techniques in order to investigate iron and sulfate reduction in *D. reducens*. We optimized a top-down proteomic approach based on a workflow of multidimensional protein complex separation followed by iron reduction activity assays and implemented it in order to identify proteins capable of iron reduction from the proteome of *D. reducens*. These proteins have been heterologously expressed and validated as iron reductases in purified form. In addition, we have performed comparative proteomic analyses on the proteome of *D. reducens* across different growth conditions. This bottom-up proteomic technique has highlighted proteins of interest based on differential abundance and has led to predictions of proteins involved in the respiration of iron and sulfate in *D. reducens*. Targets for heterologous expression and characterization have also been selected based on these comparative proteomic analyses.

Funding for this project was provided by the Department of Energy's Genomic Sciences Program within the Office of Biological and Environmental Research