34. Identification of Iron Reductases Using Top-down Proteomics and Heterologous Expression

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Project Goals: The goal of this project is to identify and analyze novel iron reductases in three microorganisms, *Geobacter sulfurreducens PCA, Desulfotomaculum reducens MI-1* and *Anaeromyxobacter dehalogenans 2CP-1,* using a combination of non-denaturing separation, proteomics-based discovery and heterologous expression to confirm and further characterize enzyme function.

Identification and analysis of enzymes involved in heavy metal reduction by microorganisms can provide better mechanistic understanding as well as improve bioremediation techniques for heavy metal contamination. In this project, novel iron reductases in three microorganisms were identified by using a combination of non-denaturing protein separation, functional screens, proteomics-based discovery and heterologous expression to confirm and further characterize enzyme function. *Geobacter sulfurreducens PCA, Desulfotomaculum reducens MI-1* and *Anaeromyxobacter dehalogenans 2CP-1* were each grown anaerobically and the cells were collected and lysed. The soluble and membrane-bound protein fractions were separated for individual analysis. The proteins were separated using strong anion exchange (SAX) chromatography, size exclusion (SEC) chromatography, and native gel electrophoresis coupled with solution-phase and in-gel iron reduction assays. Protein bands displaying iron- reduction activity in the in-gel activity assay were excised for protein digestion and peptide identification by mass spectrometry. Proteins identified were over-expressed in *E.coli* and purified by metal-affinity chromatography for characterization studies, including *in vitro* iron reduction activity. Thus far, a total of six iron reducing proteins/protein complexes have been confirmed in these three organisms. These proteins/complexes were NADPH-dependent enal/enone/nitroreductase from *G. sulfurreducens,* Oxidoreductase FAD/NAD(P)-binding subunit/Dihydroorotate dehydrogenase 1B complex and NADH: flavin oxidoreductase from *D. reducens* as well as Pyruvate flavodoxin/ferredoxin oxidoreductase homologs in each microorganism.

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