Improved Understanding of Microbial Iron and Sulfate Reduction Through a Combination of Bottom-up and Top-down Functional Proteomics Assays

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Project goals: Our overall project objectives are to improve our understanding of the systems biology of the dissimilatory iron reducing bacteria (DIRB) and sulfate reducing bacteria (SRB) and to use this knowledge to aid in site-monitoring and management via informative biomarkers. The work will employ a wide range of proteomic and biochemical assays. These include both "bottom-up" and "top-down" approaches. In the bottom up approaches, gene targets are selected for heterologous expression based upon proteomic expression patterns and are then put through a range of *in silico*, proteomic and biochemical tests. In the top-down approach, we will develop a high-throughput functional protein assay that screens for function first before then identifying the responsible enzymes– thereby focusing mass spectrometry resources on the identification of functionally-relevant oxidoreductase enzymes and their interactors.

Abstract: The advent of the genomics era has advanced our understanding not only of the genetic makeup of a wide range of microorganisms (and microbial communities) it has also enabled remarkably high throughput monitoring of gene expression for thousands of genes simultaneously. With current *in silico* bioinformatic tools, inferences can often be made as to the function of some of these gene targets simply from gene sequences or (in the case of "hypothetical" proteins) from expression patterns under different conditions. However, inferences are only the start with respect to learning the true function of poorly annotated gene targets. To turn these observations and inferences into an improved understanding of the systems biology of organisms of interest, functional annotation techniques and approaches must be improved.

We have created an experimental plan to tackle this functional annotation bottleneck in two groups of microorganisms of particular interest for heavy metals and radionuclide bioremediation: the dissimilatory iron reducing bacteria (DIRB) and the sulfate reducing bacteria (SRB). We anticipate that our approaches will both generate a better fundamental understanding of the systems biology of the DIRB and SRB, and lead to improved diagnostic tools for application at contaminated field sites – namely peptide biomarkers that can be used to document and enhance *in situ* activity. To this end we are particularly interested in improved functional characterization of oxidoreductase enzymes as they are key at the biotic/abiotic interfaces in aquifers.

The work is taking take place in three complementary phases. In Phase 1 ("bottom-up"), a combination of *in silico*, proteomic, and biochemical approaches are being applied to phylogenetically-diverse DIRB and SRB cultures (grown under different operating conditions of type of electron acceptor and rate of respiration). This phase will first identify the core proteomes specific to iron and sulfate reduction, then will focus on improved functional characterization of this core proteins via: follow-up i*n silico* analysis of sequences, follow-up proteomics to identify

protein-protein interaction partners, and biochemical assays focused on documentation of redox capabilities. In Phase 2 ("top-down"), we are developping a high-throughput functional redox protein assay consisting of three steps: non-denaturing separation of a (meta)proteome, parallel assaying of wells for desired redox activity, and tandem mass spectrometry identification of proteins in positive wells. In Phase 3, proteotypic peptides will be elucidated for a suite of functionally-informative enzymes (already known and newly discovered in Phases 1 and 2) and Mass Western assays will be developed for the simultaneous quantification of dozens of such peptide biomarkers in a single nanoLC-MS/MS injection. In Phases 2 and 3, assays will first be tested on lab culture proteomes and then used to quantify biomarkers in biomass samples from sites undergoing in situ bioremediation (e.g. the Rifle site). In our poster presentation we will highlight work performed in the first six months of the project.

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