Proteome Analyses of Subsurface Microbial Systems

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Objectives

This project is focused on the development and application of advanced proteomics techniques to understanding the microbes important to radionuclide fate and transport in the subsurface of the Hanford 300 area. Linking with the Biogeochemical Electron Transfer Reactions SFA Theme and the Microscale and Microbial Community Theme (L. Shi, E. Roden and M. Marshall), this project will provide mechanistic insights in to the electron transfer mechanisms of both model and Hanford relevant microorganisms, identify cell surface proteins that can interact with the environment and other microbes, characterize heme moieties that play important roles in metal reduction as well as develop new techniques for microbial extraction from soil.

Hanford Science Motivation:

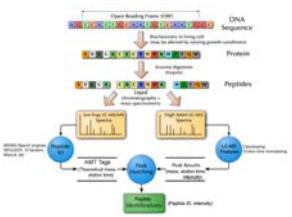
•Understanding the rates and molecular mechanisms used by Hanford relevant microorganisms for mediating extracellular redox transformation will require a fundamental understanding of the microbial electron transport mechanisms and composition of key cell surface-localized protein complexes.

•Microbes in the subsurface engage in a broad array of biochemical processes and thereby mediate biogeochemical transformations. These processes are governed by protein expression and activity, and understanding protein expression as a function of subsurface environmental conditions will be fundamental to the creation of conceptual and predictive models of the subsurface biogeochemical transformations.

Introduction

Proteomic characterization represents a powerful technology to elucidate the relevant biological processes when microbes and the communities they live in interact with metal contaminants in Hanford subsurface sediment and ground water. In-depth proteomic characterization is currently hampered by several factors including: a) the lack of genomic sequence from indigenous soil and ground water communities, b) the potential diversity and heterogeneity within communities at different depths of the Hanford strata, and c) the lack of relevant environmental isolates from the these materials. To overcome these barriers, we are focused on studies with defined systems to build a biological knowledge base to characterize the microbial function(s) important to biogeochemical processes at the site. We are also developing new incubation, sample preparation, and sample handling methods that will enable the characterization of the microbial communities from the soils and ground water.

AMT tag approach



AMT Tag Database Generation

- Complex samples fractionated, analyzed by LC-MS/MS, and spectra analyzed using SEQUEST
- Contains MS/MS peptide sequences, normalized elution times, and calculated masses.
- Serves as "look-up table" for subsequent high

LC-MS analyses

High-throughput Quantitative Proteomics

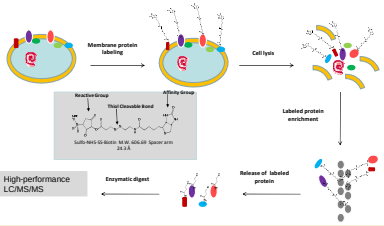
- Peptides identified from LC-MS peaks by matching both measured accurate masses and normalized elution times to those in AMT tag database within specified values
- Protein abundance can be determined

Internal SFA Collaborations:

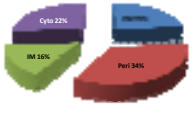
Proteomic analysis will be used for the characterization of environmental isolates cultured from the Hanford subsurface including the *Shewanella* strain isolated from the Hanford Reach of the Columbia River (HRCR) by Liang Shi and those from the project lead by M. Marshall. In the absence of metagenomic or genomic sequence, libraries of 16S rRNA gene sequences obtained from Hanford subsurface strata (A. Konopka et al.) will be used to determine the nearest phylogenetic neighbor that has sequenced genome , and that genome will be used. The AMT tag approach will also be employed for the characterization of single isolates described later in this poster and in the sub-surface models by M. Wilkins.

Selective Protein Enrichment for Environmental Samples

Cell Surface Protein Labeling and Characterization



Orbit-trap LC/MS/MS results in 408 proteins identification, 78% Of which are membrane localized



Internal SFA Collaborations:

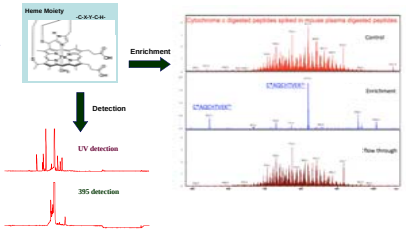
The cell surface labeling and identification of proteins will be employed by the Shi project entitled "**Functional Characterization of Microbial Proteins Involved in Biogeochemical Electron Transfer Reactions**" to identify the proteins that are on the surface of the cells of HRCR isolates and those that are interacting with the electron acceptors.

Heme Modification Characterization

•Heme moieties play a critical role in the reduction of metals in the environment.

• Proteomics is used to identify heme containing proteins and localization of the heme group.

•Selective enrichment of Heme containing peptides allows for better detection and identification.

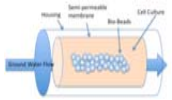


Internal SFA Collaborations:

The heme isolation and identification of proteins will be employed by the Shi project entitled "**Functional Characterization of Microbial Proteins Involved in Biogeochemical Electron Transfer Reactions**" for the characterizations of UndA-HRCR-6, a c-type cytochrome identified from a *Shewanella* strain isolated from the Hanford Reach of the Columbia River (HRCR).

Proposed: Proteome response to in situ subsurface conditions

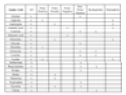
The characterization of microbial isolates in laboratory cultures can help elucidate the biological mechanisms present in an organism and under what conditions they are expressed. However, often the laboratory culture conditions do not accurately mimic the field conditions and biological conclusions can be biased by this disconnect. For this reason, we propose to develop and deploy an in situ culture system that will define protein expression patterns in model and Hanford-relevant microcrobes exposed to subsurface environmental conditions.



Conceptual design of the in situ microbial cultivation system. Cell culture (as represented by the brown space, would be exposed to both ground water in the well as well as BioSep beads.

This is similar to the approach taken by Wilkins et al in the " " project where *Geobacter sulfarreducens* will be allowed to grow anaerobically in a constrained pore size. This biomass will be enriched in this micromodel, and then extracted and analyzed using LC-MS/MS.

Methods Development. Ultimately, for the complete proteomic characterization of the microbial community, we believe that in-situ cell lysis will provide a broader and more representative view of the microbial community. Therefore , we have designed experiments aimed at the addition of chemicals to block potential protein adsorption sites on sediment prior to lysis of bacterial cells *in-situ*. Since the genomic sequence of the native microbial community was not available at the time, we used a solution of lysed *Escherichia coli* cells to represent the lysed proteins. In all experiments, the sediments were pretreated with the chemical mixture and then *E. coli* lysates was added. The metric used to assess protein adsorption was the number of peptides or proteins that were extracted from the sediment as compared to both the *E. coli* lysates that was not added to the sediment (upper control) and the *E. coli* added to the untreated sediment (lower control).



Treatment	Unique Peptides	Unique Proteins	Percent protein recovery from sediment	Improvement in protein recovery from no treatment
E. coli only	11731	1089	N/A	N/A
Sediment with no treatment	242	45	4%	N/A
All	2568	355	33%	8.2 fold
Polar Positive	3771	486	45%	11.5 fold
Polar Neutral	1246	210	19%	4.9 fold
Polar Negative	1199	185	17%	4.3 fold
Nonpolar Neutral	1172	204	19%	4.7 fold
Hydrophobic	2506	424	39%	9.9 fold
Hydrophilic	2612	291	27%	6.8 fold

Internal SFA Collaborations:

Proteome investigations will be coordinated with the project by Zhang et al. entitled "Investigating the impact of microenvironments on contaminant biogeochemical reactive transport" that will be using micro models to investigate *Geobacter* growth and metabolism grow in defined microenvironments. In addition, *Geobacter* colonizing BioSep beads or sediment will be introduced into the Hanford 300A subsurface in various locations within a natural redox gradient and allowed to incubate for 3-6 weeks. Comparative proteome analyses will then be conducted on these material as well as to laboratory batch cultivated cells.

Future Work

- *The AMT tag approach has been used to quantitatively characterize protein expression levels in many different environmental organisms. Working with both M. Marshall and E. Roden, this approach will be applied to Hanford isolates capable of Fe redox transformations such as G. bremensis and Acidovorax defluvii .*
- *Shebolinka and Roden have isolated Geobacter bremensis from the Hanford 300 area and Lin and Konopka have shown that Geobacter colonizes BioSep beads under in situ conditions (See Fredrickson et al. IFRC poster). As the organism is further characterized, it will serve as the focus for column experiments and cytochrome characterization.*
- *Interfacing with Shi et al. to understand electron transfer mechanisms in both model and Hanford relevant Fe redox transforming microorganisms, we will apply the cell surface labeling technologies and protein protein crosslinking approaches to define the structural relationships between c-type cytochromes and other key proteins to elucidate the mechanism for interfacial electron transport.*

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