2006 ERSD Annual Report

DOE-BER Environmental Remediation Sciences Project # 1022403

Identification of Metal Reductases using Proteomic Analysis

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Research Objectives:

Central to the NABIR goal to develop the scientific basis for *in situ* remediation of radioactive contaminants is the fundamental understanding of microorganisms with dissmilatory metal reducing activity. In order to effectively exploit these bacteria, it is necessary to know which enzymes and pathways are involved. Additionally, it would be advantageous to understand the similarities and differences of these pathways across different bacteria for effective deployment in bioremediation, as well as to identify new microbes capable of such activities. Most approaches to identify these enzymes or enzyme complexes rely on biochemical purification to homogeneity with subsequent N-terminal sequencing of digested peptides. However, loss of activity before achieving purity often necessitates repetition of the entire process. Newly developed proteomics capabilities at PNNL allow for the identification of many proteins from a single sample through mass spectrometry analysis. Thus, the need for absolute sample purity is eliminated, and potential enzymatic targets for metal reduction are reduced to a small subset of proteins whose metal reduction activity can be related by genetic manipulation. We are utilizing a combination of high throughput proteomics and biochemical purification techniques to identify the proteins responsible for metal reduction activity across 5 organisms: *Shewanella oneidensis* MR-1, *Geobacter sulfurreducens, Desulfovibrio desulfuricans* G20, and *Deinococcus radiodurans* allowing inferences to be made as to the similarities and differences of activities throughout the organisms.

Research Progress:

This report summarizes the work in three years of a three-year project. The follow on work is captured in a new proposal that has been submitted and currently under review. This project has focused on the characterization of proteins that play roles in the reduction of metals in organisms of interest to the Department of Energy (DOE).

Heavy metal and radionuclide contamination at (DOE) sites nationwide constitute a major environmental problem. Of particular interest are U and Tc, as well as Fe and Mn due to their potential direct and indirect effects on contaminant biogeochemical behavior. For the past decade bacteria that utilize metals as terminal electron acceptors have been isolated and identified. These bacteria include members of three major anaerobic groups; the denitrifying, sulfate- and Fe(III)- reducing bacteria. The electron transfer pathways within these bacteria are still not well understood. Moreover, this lack of information substantially impedes efforts to increase *in situ* bioremediation efficiency. Hence, identification of metal reductases, and determination of their similarity between these bacterial groups is essential for understanding these mechanisms and assessing bioremediative potential at DOE sites.

Historically, the identification of these proteins associated with metal reduction activity has been challenging due to the need to purify proteins to homogeneity. In the past, the procedures for protein identification have necessitated this purification, however, during the purification process the metal reduction activity is often lost due to inactivation of the protein or dissociation of complexes that are necessary for metal reduction. However, new advances in by mass spectrometry allowed for the identification of multiple proteins from global lysates and mixtures.

In this project, we have used cell fractionation techniques to resolve sub-cellular protein fractions and quantify the purity of proteins within each enriched fraction. The identification of metal reducing proteins in cell cultures has been relatively straight forward, and additionally, we have developed methods for the characterization of cytochrome containing peptides from metal reducing proteins. We have illustrated this advance by the direct characterization of cytochrome proteins isolated from *Shewanella* to determine the site of modification and build a mass tag database for these peptides for use in the field samples.

For the identification of the enzymes that manifest metal reduction activity, we applied cell lysates to orthogonal protein purification techniques such as strong cation exchange, strong anion exchange, weak cation exchange, weak anion exchange, metal affinity, and hydrophobic chromatography. Each of the eluting fractions were assayed for metal reduction activity and the fractions that displayed activity were processed and analyzed by tandem mass spectrometry (LC-MS/MS). The data from the LC-MS/MS experiments was processed by SEQUEST, and analysis tool that will compare the out put of the peptide fragmentation pattern to what would be predicted from a sequenced genome and provide a peptide identification. From this information, the proteins manifesting metal reduction activity were determined. This process has been applied to Shewanella oneidensis, Geobacter species and Desulfovibrio desulfuricans. While facing challenges of maintaining anaerobic conditions throughout the enrichment procedure, will have been able to identify and validate a small number of proteins responsible for metal reduction.

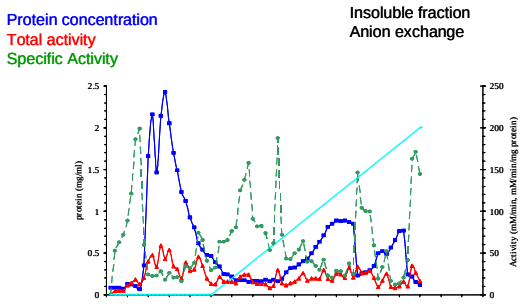

Figure 1: *Illustration of anion exhange standard separation of the insoluble fraction Shewanella oneidensis*

The procedure starts with an enrichment of the metal reduction proteins and an assay of the metal reduction activity. The output of such an experiment is shown in Figure 1. Each of the fractions are analyzed and the proteins contained therein are identified. Of the organisms identified, a number of candidates were identified as playing roles in metal reduction. Figure 2 shows a breakdown of the number of proteins that were identified by organism.

Organism	Number of Candidate Proteins
S. oneidensis MR-1	30 (6)
Geobacter metallireducens GS-15	60 (0)
Geobacter sulfurreducens PCA	75 (12)
Desulfovibrio desulfuricans G20	57 (1)

Figure 2: Number of candidate proteins from each of the 4 microbes that were examined in this study. The number of candidate proteins indicates those proteins that were observed in orthologous separation experiments. The numbers in the parentheses are the number of candidates already identified in the literature.

The results of the identifications compared across all 4 organisms revealed a subset of proteins that were conserved in each organism and the list is shown in figure 3. While it is clear that these proteins are not the only candidates that play a role in metal reduction, the identification of these proteins across all 4 candidate organisms could point to a conserved pathway for the reduction of metals in all microbes.

NCBI alias	*G. sulf.* ORF	*G. sulf.* Protein Name	*Dsv. des.* ORF	*Dsv. Des.* Protein Name	*S. oneid.* ORF	*S. oneidensis* MR-1 Protein Name
Gmet0328	GSU3334	cytochrome c family protein	Dde0561	cytochrome c family protein	SO2363	cytochrome c oxidase, cbb3
Gmet0100	GSU0274	cytochrome c family protein	Dde0580	cytochrome c family protein	SO1778	decaheme cytochrome c MtrC
Gmet2356	GSU2267	outer membrane protein	Dde1371	outer membrane protein OmpH		
Gmet2902	GSU0612	cytochrome c3 (ppcA)	Dde3710	acidic cytochrome c3	SO1779	decaheme cytochrome c OmcA
Gmet2930	GSU0592	cytochrome c family protein	Dde0653	high-molecular-weight cyt C	SO1782	decaheme cytochrome c MtrD
Gmet0909	GSU2732	cytochrome c family protein	Dde0300	hypothetical	SO1427	decaheme cytochrome c
Gmet0909	GSU2738	cytochrome c family protein	Dde0300	hypothetical	SO1777	decaheme cytochrome c MtrA

Figure 4: The list of 7 proteins from figure 3 was compared to the list of orthologous from MR-1 and resulted in 6 candidate proteins that were common to all 4 organisms.

Planned activities:

This project has transitioned into a new project that is aimed at the characterization of the proteomic complement of microbes isolated from the Hanford 100H site and the UMTRA field site at Rifle Colorado, which will be led by Dr,Mary Lipton of Pacific Northwest National Laboratory This specific work has transitioned into a companion proposal led by Dr. Dwayne Elias of the University of Missouri, Columbia.

We will apply the global proteomics techniques described in numerous manuscripts as well as the directed approach described here to the characterization. It is expected that we will receive the first samples in early summer and the initial analysis and evaluation will be completed by the start of FY08.

Information Access:

List of publications:

Elias, D.A., Yang, F., Mottaz, H.M., Beliaev, A.S., Lipton, M.S. "Enrichment of Functional Redox Reactive Proteins and Identification by Mass Spectrometry Results in Several Terminal Fe(III)-reducing Candidate Proteins in Shewanella oneidensis MR-1" Journal of Microbiological Methods in press

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Shen, Y., Tolic, N., Masselon, C., Pasa-Tolic, L., Camp, D. G., 2nd, Lipton, M. S., Anderson, G. A. and Smith, R. D., *Nanoscale proteomics.* **(2004),** Anal Bioanal Chem 378, 1037-1045.

Smith, R. D., Anderson, G. A., Lipton, M. S., Masselon, C., Pasa-Tolic, L., Udseth, H., Belov, M., Shen, Y. and Veenstra, T. D., *High-performance separations and mass spectrometric methods for high-throughput proteomics using accurate mass tags.* **(2003),** Adv Protein Chem 65, 85-131.