Elucidating the Molecular Basis and Regulation of Chromium(VI) Reduction by *Shewanella oneidensis* MR-1 and Resistance to Metal Toxicity Using Integrated Biochemical, Genomic, and Proteomic Approaches

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ABSTRACT

The mediation of metal reduction by microorganisms has been investigated intensively from physiological and biochemical perspectives; however, little is known about the genetic basis and regulatory mechanisms underlying the ability of certain bacteria to transform or immobilize a wide array of heavy metals contaminating DOE field sites. Chromium(VI), for example, is one of several risk-driving contaminants at DOE sites and has been targeted by the DOE for bioremediation research. The bacterium *Shewanella oneidensis* MR-1 can potentially be used to immobilize chromium, a toxic and mutagenic metal, by reducing soluble Cr(VI) to the insoluble and less bioavailable form of Cr(III), thus facilitating its removal from contained-storage and natural sites. The overall goal of this study is to integrate targeted biochemical and proteomic analyses with genome-wide gene expression profiling to examine the molecular basis and regulation of chromium(VI) reduction by *Shewanella oneidensis* MR-1. Towards this goal, we will (1) isolate and identify the terminal chromium(VI) reductase and the gene(s) encoding this activity using whole-genome sequence information for MR-1 and liquid chromatography-tandem mass spectrometry (LC-MS/MS) in conjunction with conventional protein purification and characterization techniques; (2) verify the function of the gene(s) encoding the terminal Cr(VI) reductase and compare whole transcriptome data with whole proteome data in order to understand the regulation of chromium reduction; and (3) investigate the molecular stress response and adaptation of *S. oneidensis* to toxic levels of soluble Cr(VI) and other heavy metals. This research will provide important information on the functional components and regulatory mechanisms of microbial metal reduction, which should prove valuable in developing effective assessment strategies for *in situ* bioremediation and genetically engineering desired bacteria for enhanced bioremediation.

RESEARCH GOAL 1: Isolate/identify the terminal Cr(VI) reductase and the gene(s) encoding this activity.

Cr(VI)-Reducing MR-1 Culture

↓ French Pressure Cell

Cell Disruption and Subcellular Membrane Fractionation

Cytoplasmic Membrane (CM) Fraction

Outer Membrane (OM) Fraction

↓

Solubilize Membrane Proteins

↓

Conventional Chromatography (e.g., phenyl sepharose)

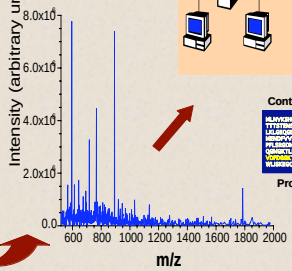
↓

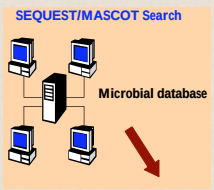
Monitor Purification using SDS-PAGE and Activity Assays

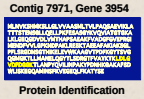
↓

LC-MS/MS Identification of Protein Constituents in Cr Reductase Complex

ÄKTA$_{FPLC}$ High Performance Purification System



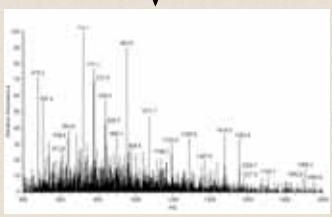




RESEARCH GOAL 2: Compare transcriptome and proteome data for *S. oneidensis* grown under Cr-reducing and Cr stress conditions.

Cells grown under Cr-reducing versus fumarate-reducing conditions

↓ Metabolic labeling with ^{15}N



Microarray Hybridization

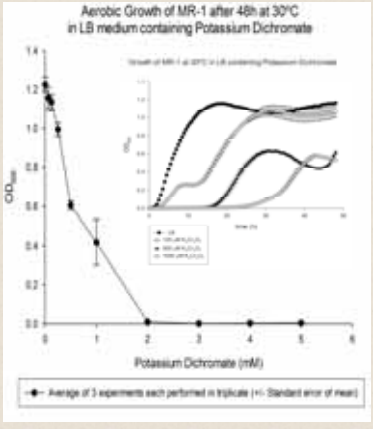
Shotgun LC-MS/MS analysis

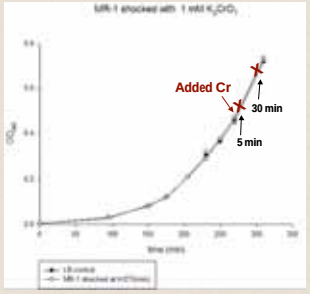
↓ Correlation? ↓

Transcriptome ⟷ Proteome

Recently, we have analyzed whole proteomes of aerobically grown wild type and *fur* (ferric uptake regulator) deletion strains of *S. oneidensis* by gel-less qualitative "shotgun" MS proteomics (submitted for publication). Replicate whole-proteome analysis of WT and FUR2 strains resulted in the identification of a total of 1104 proteins from both. Proteomic analysis clearly identified a number of transporters, binding proteins and receptors, related to iron uptake, that were up-regulated in response to a *fur* mutation. In general, the results showed that the microarray expression data correlated well with the proteomics data for genes showing large-scale differences at the transcript level.

RESEARCH GOAL 3: Molecular stress response and acclimation of *S. oneidensis* to Cr(VI) and comparison with other heavy metals.





Growth studies have shown that Cr(VI) is toxic to MR-1 at much lower concentrations than strontium [Sr(II)]. MR-1 was able to grow in LB medium with Sr concentrations as high as 180 mM, but showed substantial growth inhibition at levels above 180 mM (data not shown). The stress response of *S. oneidensis* to a shock addition of 180 mM Sr was examined after 5, 30, 60, and 90 minutes using microarrays. Siderophore biosynthesis and iron uptake genes were highly induced (up to 622 fold), and a siderophore biosynthetic mutant was more sensitive to strontium, suggesting that siderophore production plays an integral role in the ability of *S. oneidensis* to mediate strontium resistance. DNA microarray studies are under way to compare the molecular basis of resistance to the heavy metals Cr and Sr.

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