Sponsor:	U.S. Department of Energy
Principal Investigator:	Dorothea K. Thompson, 765-496-8301
Project Title:	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
Award NBR:	DE-FG02-06ER64163

FINAL TECHNICAL REPORT

Coordination with other DOE-ERSP Projects. This DOE-ERSP project is a collaborative endeavor between the project PI, Dorothea Thompson (now at Purdue University, formerly at Oak Ridge National Laboratory) and co-PI Robert Hettich in the Chemical Sciences Division at ORNL.

Research Objectives. The overarching objective of this project was to characterize the molecular basis and regulation of the cellular stress response and detoxification/reduction of chromate by *S. oneidensis* MR-1. We have identified and characterized (in the case of a DNA-binding response regulator [SO2426] and a putative azoreductase [SO3585]) the genes and gene products involved in the molecular response of MR-1 to Cr(VI) stress using whole-genome sequence information for MR-1 and recently developed proteomic technology (liquid chromatography-mass spectrometry [LC-MS]). The proteome datasets were integrated with temporal gene expression data obtained from whole-genome microarrays for *S. oneidensis* MR-1. The genes and their encoded products identified in this study are of value in understanding metal reduction and bacterial resistance to metal toxicity, which are necessary for developing effective metal immobilization strategies.

Summary of Project Results. This final report summarizes the status of the work achieved as of the end of year 3 of this project. Major research results are highlighted in the following numbered points:

1) Temporal genomic profiling and whole-cell proteomic analyses were performed to characterize the dynamic molecular response of the metal-reducing bacterium *Shewanella oneidensis* MR-1 to an acute chromate shock. Differential proteomics based on multidimensional high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used to complement the transcriptome data, resulting in comparable induction and repression patterns for a subset of corresponding proteins. In total, expression of 2,370 proteins were confidently verified, with 624 (26%) of these annotated as hypothetical or conserved hypothetical proteins. The initial response of *S. oneidensis* to chromate shock appears to require a combination of different regulatory networks that involve genes with annotated functions in oxidative stress protection, detoxification, protein stress protection, iron and sulfur acquisition, and SOS-controlled DNA repair mechanisms. These data provide key insights into how MR-1 responds to acute chromate exposures, which would be necessary to anticipate how this organism might respond when added to a Cr(VI)-contaminated site.

2) The biological impact of 24-hr "chronic" chromate exposure on *Shewanella oneidensis* MR-1 was assessed by analyzing cellular morphology as well as genome-wide differential gene and protein expression profiles. Transcriptome profiling and mass spectrometry (MS)-based proteomic characterization revealed that the principal molecular response to 24-h Cr(VI) exposure was the induction of prophage-related genes and their encoded products as well as a number of functionally undefined hypothetical genes that were located within the integrated phage regions of the MR-1 genome. In addition, genes with annotated functions in DNA metabolism, cell division, biosynthesis and degradation of the murein (peptidoglycan) sacculus, membrane response and general environmental stress protection were upregulated 24 hr after chromate addition, while genes encoding chemotaxis, motility, and transport/binding proteins were largely repressed under these experimental conditions. The data suggest that prophage activation may be the primary factor leading to cell lysis under long-term chromate exposure. The accompanying physiological response to chronic Cr(VI) exposure included marked changes in cellular morphology as revealed by scanning confocal microscopy and atomic force microscopy (AFM). Transcriptional induction of cell wall biosynthesis and cell division genes (i.e., the putative *mur* and *fts* operon), as well as *rodA*, *mreD*, and *era*, appeared to correlate with the extreme filamentous (elongated) morphology observed using confocal microscopy and AFM topographic imaging.

3) Large-scale proteome measurements were conducted to study the dosage-dependent chromate shock growth effects in *Shewanella oneidensis*. Proteome alterations in this metal-reducing bacterium in response to different acute dose challenges (0.3, 0.5, or 1 mM) of the toxic metal chromate [Cr(VI)] were characterized with multidimensional HPLC-MS/MS on a linear trapping quadrupole MS. A total of 2,406 functionally diverse proteins were identified, with a subset demonstrating dosage-dependent up- and down-regulated expression, such as proteins involved in detoxification and iron binding and transport.

4) Global studies suggested that a predicted DNA-binding response regulator (SO2426), part of a two-component signal transduction system, plays a potentially important regulatory role in the MR-1 response to chromate. Gene SO2426 was highly up-regulated at the transcription level in response to chromate shock and in MR-1 cells actively reducing Cr(VI). The protein product for this gene was expressed and detected only in chromate-treated samples using HPLC-MS/MS. To further define the functional role of SO2426 in the stress response to chromate, an in-frame deletion of the *so2426* locus in MR-1 was created using a *cre-lox*-based recombination system and the phenotype of the resulting mutant was characterized in terms of growth in the presence of varying concentrations of chromate, copper, cobalt, strontium, ferric citrate, manganese dioxide, and hydrogen peroxide under aerobic respiratory conditions. Growth studies indicated that the *so2426* deletion mutant was hypersensitive to Cr, Cu, Co, and Sr compared to the wild-type reference, and chromate reduction (as measured by the 1,5-diphenylcarbazide method) by the mutant was impaired significantly. For example, 100% of 0.3 mM chromate was transformed in the presence of WT MR-1 cells after approximately 24 hs, whereas only 50% Cr(VI) was transformed by the *so2426* deletion mutant at the same time point. Complementation of the *so2426* deletion mutant with a plasmid expressing the full-length SO2426 restored the growth phenotype and Cr(VI) reduction ability of the complemented strain to near wild-type levels. These findings were

recently used as the basis for the submission of an NSF proposal by Dr. Dorothea Thompson to study signal transduction systems mediating metal stress responses in *S. oneidensis* MR-1.

5) We have completed an extensive time-series microarray study to characterize temporal changes in the transcriptome of the *so2426* deletion mutant relative to that of the wild-type MR-1 strain in response to 1 mM chromate exposure. Temporal changes in the transcriptome of the *so2426* deletion mutant were determined at 5, 30, 60, 90, 180 min and 24 hs post-chromate addition. Because SO2426 is annotated as a transcriptional regulator, the purpose of this study was to define the regulon of this potentially important heavy metal response regulator. Based on global transcriptional profiling, the most notable differences between the mutant and wild-type strains was the down-regulation of genes encoding TonB-dependent heme receptors (SO1580, SO4743), a siderophore biosynthesis operon (*alcA-so3031-32*), a bicyclomycin resistance protein (SO2280), a cation efflux family protein (SO2045), a sodium:alanine symporter family protein (SO3063), and ferritin (*ftn*) in a Δ*so2426* genetic background in response to chromate challenge. These same genes are normally up-regulated in wild-type cells in response to Cr(VI), thus suggesting that SO2426 may directly or indirectly regulate the expression of these genes under chromate stress conditions in a positive fashion. Most notable was the down-regulation of a two-component signal transduction system (*cpxA*/*cpxR*) known to be involved in envelope stress responses in other bacterial systems and the strong repression of a conserved hypothetical operon (*so1188-89-90*) encoding proteins predicted to be membrane associated.

6) Experimental protocols have been developed and tested to reduce the amount of cellular biomass required for deep characterization of bacterial proteomes by multidimensional LC-MS/MS measurements. By devising a method to lyse bacterial cells and conduct proteolytic digestions in a single microcentrifuge tube, it was possible to greatly reduce sample handling and losses, thereby enabling the examination of low milligram biomass samples. In particular, a large-scale proteomic measurement of *S. oneidensis* (i.e., greater than 2000 proteins identified) was obtained from a starting amount of 1 mg of wet cell paste. Detailed examination of the proteome datasets revealed no significant discrimination of protein types relative to the larger scale more conventional sample preparation methods.

Papers and Other Products Delivered. In total, we have presented results from this ERSP project at 17 national and international scientific conferences over the three-year funding period, with 8 abstracts/presentations given in 2006 alone. Most recently, the following poster presentations were given at the Gordon Research Conference on Microbial Stress Response (July 9-14, 2006) and the 11th International Symposium on Microbial Ecology (August 20-25, 2006) held in Vienna, Austria.

- Thompson, D. K., K. Chourey, M. R. Thompson, S. D. Brown, N. C. VerBerkmoes, and R. L. Hettich. 2006. Functional genomics of the *Shewanella oneidensis* response to chromium stress. Poster presentation at the Gordon Conference on Microbial Stress Response, July 9-14, South Hadley, MA.

- Thompson, D. K., K. Chourey, M. R. Thompson, S. D. Brown, N. C. VerBerkmoes, and R. L. Hettich. 2006. Functional genomics of the *Shewanella oneidensis* response to

chromium stress and involvement of a DNA-binding response regulator. Poster presentation at the 11th International Symposium on Microbial Ecology (ISME), August 20-25, Vienna, Austria.

A list of the publications derived from this ERSP project is given below:

- Thompson, M. R., N. C. VerBerkmoes, K. Chourey, M. Shah, D. K. Thompson, and R. L. Hettich. 2007. Dosage-dependent proteome response of *Shewanella oneidensis* MR-1 to acute chromate challenge. *Journal of Proteome Research*, in press.
- Brown, S. D., M. R. Thompson, N. C. VerBerkmoes, K. Chourey, M. Shah, J. Zhou, R. L. Hettich, and D. K. Thompson. 2006. Molecular dynamics of the *Shewanella oneidensis* response to chromate stress. *Molecular & Cellular Proteomics* 5:1054-1071.
- Chourey, K., M. Thompson, J. Morrell-Falvey, N. C. VerBerkmoes, S. D. Brown, M. Shah, J. Zhou, M. Doktycz, R. L. Hettich, and D. K. Thompson. 2006. Global molecular and morphological effects of 24-h chromium(VI) exposure on *Shewanella oneidensis* MR-1. *Applied and Environmental Microbiology* 72:6331-6344.

The following manuscripts describing work from this project are currently in preparation and are planned for submission in 2007.

- Thompson, M. R.**,** Froelich, J. M., Erickson, B., VerBerkmoes, N. C., and Hettich, R. L. 2007. Experimental approach for large-scale proteome measurements from small amounts (low mg) of microbial cultures.
- Chourey, K., W. Wei, X.-F. Wan, and D. K. Thompson. 2007. Identification and transcriptional characterization of a DNA-binding response regulator involved in metal stress responses.

In addition to data dissemination through published papers and scientific meetings, we have developed a project website at the following URL: http://compbio.ornl.gov/shewanella_metal_stress/. This website is freely accessible to the public domain and provides a description of the major research objectives of our ERSP project including complete microarray and proteome datasets for the acute stress, chronic, and chromate dose-response studies.

Continuing Work. In Dorothea Thompson's lab at Purdue University, we are continuing to investigate the functional role and regulatory mechanism mediated by the orphan reponse regulator SO2426 in regards to metal stress response pathways. NSF funding is being sought for this work. Current effort in Robert Hettich's group at ORNL is directed at finishing bioinformatics work to extract protein post-translational information from the proteome datasets to ascertain whether protein modification is important in the response of *S. oneidensis* to chromate shock. A second collaborative proposal was submitted to the DOE-ERSP and was selected for funding. Based on the initial proteome and transcriptome measurements with single isolates in lab cultures, we plan to extend our experimental approach to the examination of target organisms in soil microcosms.