DOE-ERSP Annual Progress Report
October 30, 2006

Project Title: *Elucidating the Molecular Basis and Regulation of Chromium (VI) Reduction by Shewanella oneidensis MR-1 Using Biochemical, Genomic, and Proteomic Approaches*

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Coordination with other DOE-ERSP projects: This ORNL research is a subproject correlated with Prof. Dorothea Thompson's (Purdue University) primary ERSP project of the same title.

Research Objective: Although microbial metal reduction has been investigated intensively from physiological and biochemical perspectives, little is known about the genetic basis and regulatory mechanisms underlying the ability of certain bacteria to transform, detoxify, or immobilize a wide array of heavy metals contaminating DOE-relevant environments. The major goal of this work is to elucidate the molecular components comprising the chromium(VI) response pathway, with an emphasis on components involved in Cr(VI) detoxification and the enzyme complex catalyzing the terminal step in Cr(VI) reduction by *Shewanella oneidensis* MR-1. We have identified and characterized (in the case of DNA-binding response regulator [SO2426] and a putative azoreductase [SO3585]) the genes and gene products involved in the molecular response of MR-1 to chromium(VI) stress using whole-genome sequence information for MR-1 and recently developed proteomic technology, in particular liquid chromatography-mass spectrometry (LC-MS), in conjunction with conventional protein purification and characterization techniques. The proteome datasets were integrated with information from whole-genome expression arrays for *S. oneidensis* MR-1 (as illustrated in Figure 1). The genes and their encoded products identified in this study are of value in understanding metal reduction and bacterial resistance to metal toxicity and in developing effective metal immobilization strategies.

We anticipate that this fundamental information would help guide the experimental design of i*n situ* bioremediation efforts focused on exploiting the intrinsic respiratory processes of dissimilatory metal ion-reducing bacteria. By employing an integrative approach focused on both large-scale gene (transcriptomics) and protein (proteomics) expression, we hope to provide the key information necessary to understand how *S. oneidensis* responds to acute and chronic metal insult (particularly for chromate).

Research Progress: This report summarizes the status of the work as of the end of year 3 of this project. Major research results are summarized into the following bullet points:

- 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. This data provides key insight into how *S. oneidensis* responds to acute chromate shock, which would be necessary to anticipate how this organism would respond when added to a contaminated site.

- The biological impact of 24-hr "chronic" chromium(VI) [Cr(VI) or 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. This data provides key insight into how *S. oneidensis* responds to chronic chromate exposure, which would be necessary to evaluate the longer-term effect of how this organism might adjust to a contaminated site.

- 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, as illustrated in Table 1 and Figure 2. (Manuscript "*Dosage-Dependent Proteome Response of Shewanella oneidensis MR-1 to Acute Chromate Challenge*" under review for the *Journal of Proteome Research*, September, 2006). This data provides key insight into how *S. oneidensis* responds to changing concentration gradients of chromate shock, which would reflect a likely real-world scenario.

- 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. This work would provide the basis for real-world measurements in which the amount of biomass is severely limited.

Planned Activities: Current effort 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. 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.

Information Access:

"Global molecular and morphological effects of 24-hour chromium(VI) exposure on *Shewanella oneidensis* MR-1," Chourey K, Thompson MR, Morrell-Falvey J, VerBerkmoes NC, Brown SD, Shah M, Zhou JZ, Doktycz M, Hettich RL, Thompson DK *Applied and Environmental Microbiology* 72 (9): 6331-6344 SEP 2006

"Molecular dynamics of the *Shewanella oneidensis* response to chromate stress," Brown SD, Thompson MR, VerBerkmoes NC, Chourey K, Shah M, Zhou JZ, Hettich RL, Thompson DK *Molecular & Cellular Proteomics* 5 (6): 1054-1071 JUN 2006

All proteome datasets and supplemental information are freely accessible at the web-site: (http://compbio.ornl.gov/shewanella_metal_stress/dosage/ms_analysis/)

Figure 1.

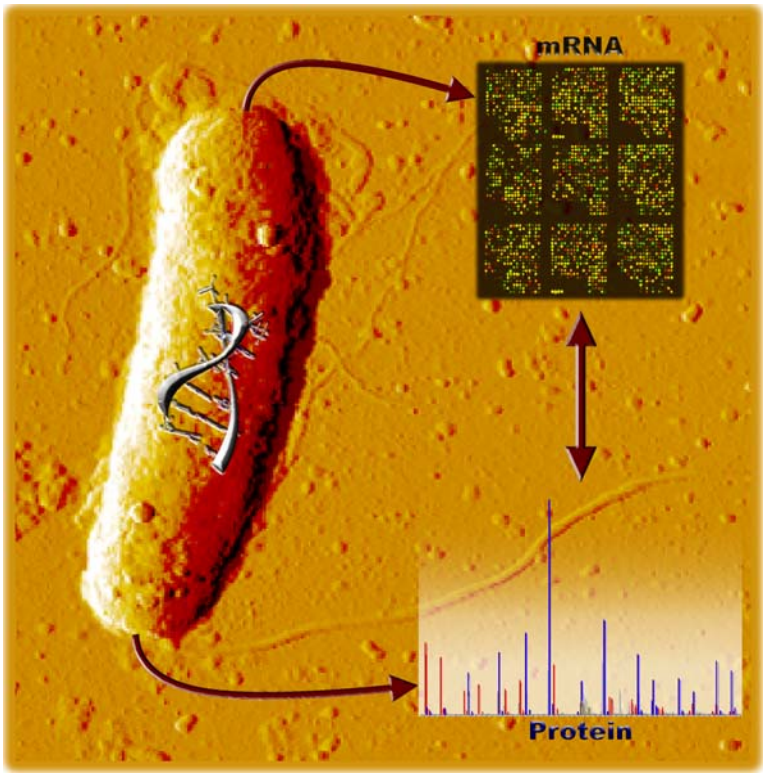

Table 1

Up-regulated Proteins under all dosage conditions														
Locus	Control			0.3mM Chromate			0.5mM Chromate			1.0mM Chromate			Functional	Description
	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra	seq cov.	peptide	spectra	Category #	
SO0344	11.6%	3	3.5	31.5%	12	19	42.4%	20	30	48.2%	24.5	34	7	methylcitrate synthase (prpC)
SO1190	48.0%	11	19	55.3%	24	74	85.1%	32	102	70.2%	28	88	9	conserved hypothetical protein
SO1482	52.2%	26	35	76.40%	75	266	74.8%	81	328	70.5%	60	160	17	TonB-dependent receptor, putative
SO2426	0.0%	0	0	31.5%	7	10	22.4%	4	14	32.3%	8	13	15	DNA-binding response regulator
SO3030	4.0%	1	2	49.1%	24	37	47.7%	20	40	48.4%	22	39	17	siderophore biosynthesis protein (alcA)
SO3032	0.066	3.5	3.5	0.19	8	19	22.3%	10	22.5	0.28	15.5	25.5	17	siderophore biosynthesis protein, putative
SO3033	27.5%	15	23	60.40%	47	148	59.9%	45	172	57.0%	46	158	17	ferric alcaligin siderophore receptor
SO3420	54.4%	9	30	69.80%	16	58	75.8%	23	99	69.8%	20	66	7	cytochrome c
SO3462	5.8%	2	2	15.7%	7	10	27.5%	12	16	39.9%	15	23	6	DNA repair protein RecN (recN)
SO3667	25.2%	4	4	78.2%	21	78	91.4%	32	266	92.2%	34	241	9	conserved hypothetical protein
SO3669	11.2%	4	4	47.4%	30	205	74.8%	57	425	72.4%	54	199	17	heme transport protein (hugA)
SO3673	13.9%	3	3	40.9%	11	18	69.6%	22	74	69.1%	26	66	17	hemin ABC transporter, periplasmic hemin-binding protein (hmuT)
SO3675	0	0	0	0.271	5	10	40.9%	7.5	13	0.6455	10.5	27	17	hemin ABC transporter, ATP-binding protein (hmuV)
SO3738	6.6%	2	3	23.7%	8	18	20.4%	9	21	22.0%	8	19	5	sulfite reductase (NADPH) flavoprotein alpha-component (cysJ)
SO3914	0.445	29.5	49	0.668	66	236	65.5%	62	225.5	0.565	52.5	217	17	TonB-dependent receptor, putative
SO4743	67.1%	46	100	74.10%	70	307	75.0%	73	406	69.6%	61	266	17	TonB-dependent receptor, putative
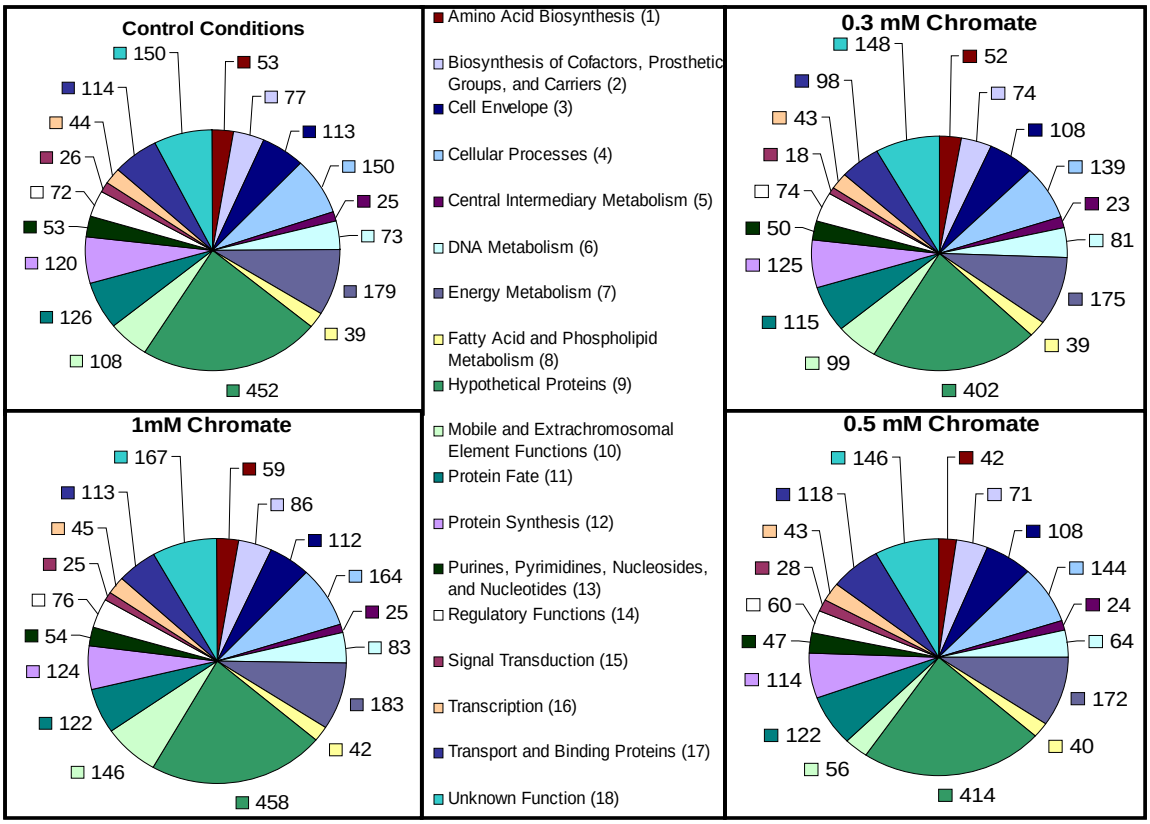
Figure 2.