

## OVERVIEW

*Shewanella oneidensis* MR-1 (Figure 1) is a gram-negative, facultatively anaerobic bacterium that has the ability to reduce toxic metal ions [e.g., Cr(VI) and U(VI)] found in industrial and governmental waste sites.

Wildtype MR-1 cells were grown under aerobic conditions and exposed to a dosage of 0, 0.3, 0.5, or 1.0 mM  $K_2CrO_7$ .

Two dimensional HPLC-MS/MS allows for identification of proteins from the predicted proteome with an extensive depth of coverage.

Putative proteins differentially expressed that may be involved in a dosage response to chromate are investigated.



Figure 1: Atomic force microscopic image of *S. oneidensis* MR-1 grown under physiologically optimal conditions. The flagellum and unknown appendages that are located on the outside of the cell are clearly visible in the image. (Micrograph courtesy of K. Chourey)

## INTRODUCTION

*Shewanella oneidensis* MR-1 (Figure 1) is a gram-negative, facultatively anaerobic bacterium originally isolated from a freshwater lake<sup>1</sup>.

*S. oneidensis* has the ability to reduce toxic metal ions [e.g., Cr(VI) and U(VI)] found in industrial and governmental waste sites.

Chromium is found in the environment as two abundant stable species (Cr(III) and Cr(VI)).

Cr(VI) is found as either chromate ( $CrO_4^{2-}$ ) or dichromate ( $Cr_2O_7^{2-}$ ) and is considerably more soluble than Cr(III), which contributes to its bioavailability. Cr(VI) as well can lead to the formation of reactive oxygen species (ROS) within the cell due to long-term exposure.

Immobilizing Cr(VI) to Cr(III) chemically is very costly, therefore utilizing bacteria as bioremediation agents for Cr(VI) immobilization is more cost effective.

Two previous proteome studies of Cr(VI) exposure to *S. oneidensis* MR-1 yielded distinct responses. The first study analyzed proteome samples from an acute exposure of 45 and 90 min to 1.0 mM  $K_2CrO_7$  and identified putative proteins differentially expressed involved in an initial response to chromate<sup>2</sup>.

The second study analyzed proteome samples from a chronic exposure of 24 h to 0.3 mM  $K_2CrO_7$  and differentially expressed proteins were identified as well<sup>3</sup>. However, the identified gene products were unique with 25% of the proteins found up-regulated mapped to an area of the MR-1 genome containing a phage genome and emphasize the toxicity of chromate to living organisms.

# Dosage-Dependent Proteome Response of *Shewanella oneidensis* MR-1 to Chromate Insult

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## EXPERIMENTAL

*S. oneidensis* MR-1 were grown under aerobic conditions to exponential phase and subsequently exposed to a dosage of 0, 0.3, 0.5, or 1.0 mM  $K_2CrO_7$ . The cells were then allowed to grow for an additional 30 minutes in the absence or presence of chromate.

Cells were lysed using sonication and protein fractions were separated into crude and membrane associated fractions by centrifuging the samples at 100,000 x g for 60 minutes. Following lysis, a trypsin digestion using a standard protocol was employed.

Protein identification was carried out by a 24 hour multi-dimensional HPLC-MS/MS protocol. (See Figure 2)

Separation was accomplished by online 2-D chromatography using strong cation exchange as the first dimension and  $C_{18}$  reverse phase as the second dimension of separation.

A LTQ linear trapping quadrupole (Thermo Electron) or a LCQ Deca XP three dimensional ion trap (Thermo Electron) were coupled to an Ultimate HPLC (LC Packings) operated in the data-dependent mode for the dosage response samples.

Peptide identification was completed by the search engine SEQUEST with a two unique peptide cut-off (X-corr values 1.8(+1), 2.5(+2) and 3.5(+3)).

Semi-quantitation: Proteins were considered differentially expressed (up- or down-regulated) with a difference in at least two of the following categories:

For the LTQ Dataset: 5 or more peptides, a difference of 40% sequence coverage, or 2X more spectra identified between treated and control samples.

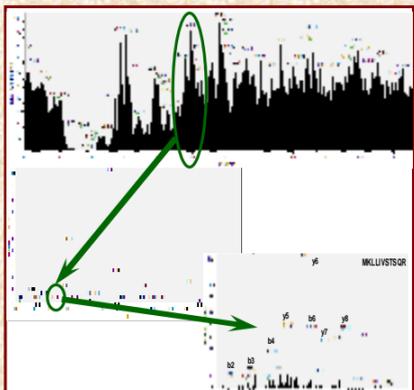


Figure 2: The ion chromatogram from the 0.3 mM chromate sample illustrates a full MS scan that contains a peak at m/z 638. This peak was subsequently isolated and fragmented giving the MS/MS spectrum, which contains the sequence of a peptide from SO3585 (see Figure 5) that was identified up-regulated at the 0.5 mM and 1.0 mM chromate levels.

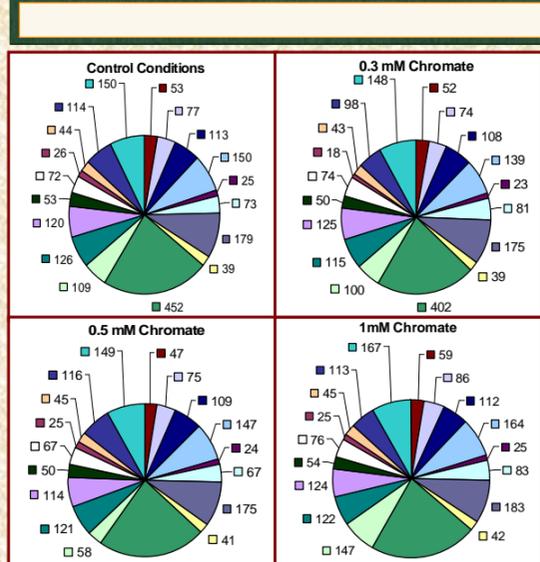


Table 1: Proteome Results of Chromate Dosage on *S. oneidensis*.

Condition	Instrument	Proteins Identified <sup>1</sup>	Proteins Identified <sup>2</sup>	Average Coverage <sup>3</sup>
Control	LCQ	1263	809	26.00%
	LTQ	2593	1975	35.60%
0.3 mM $K_2CrO_7$	LCQ	1267	835	30.00%
	LTQ	2419	1864	32.80%
0.5 mM $K_2CrO_7$	LCQ	1221	780	31.10%
	LTQ	2425	1807	37.20%
1.0 mM $K_2CrO_7$	LCQ	1307	879	29.10%
	LTQ	2630	2085	37.50%

Total Non-Redundant: 2455

LCQ: Thermo Electron NanoESI quadrupole ion trap

LTQ: Thermo Electron NanoESI-linear trapping quadrupole

<sup>1</sup>Identified with at least 1 peptide per protein

<sup>2</sup>Identified with at least 2 peptides per protein

<sup>3</sup>Average sequence coverage with at least 2 peptides per protein

Table 2: Select Proteins Up-Regulated in 0.3 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	0.3mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description
SO0026	0.0%	0	9	conserved hypothetical protein
SO0996	34.7%	5	18	glyoxalase family protein
SO1471	17.9%	5	0	site-specific recombinase, phage integrase family
SO4281	31.5%	7	15	DNA-binding response regulator
SO2827	30.1%	5	9	conserved hypothetical protein
SO0317	10.8%	6	13	5-nucleotidase e. putative
SO3675	21.7%	4	17	hemin ABC transporter, ATP-binding protein
SO4003	18.5%	5	15	response regulator
SO4218	25.7%	7	0	UDP-N-acetylmuramyl-L-alanine ligase (murC)
SO4564	11.3%	1	17	TonB2 protein, putative

Select Proteins Down-Regulated in 0.3 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	0.3mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description
SO0026	0.0%	0	14	transcriptional regulator, ArsR family
SO0102	0.0%	0	7	transcriptional regulator, nitrate-reducible, iron-sulfur subunit (tdrH)
SO0297	0.0%	0	13	lipopeptide, putative
SO0423	0.0%	0	14	pyruvate dehydrogenase complex repressor (pdrR)
SO0560	0.0%	0	5	formate-ferredoxinase (fhd)
SO0847	0.0%	0	11	iron-sulfur cluster-binding protein NapG (napG)
SO1265	0.0%	0	14	transcriptional regulator, putative
SO1287	0.0%	0	9	conserved hypothetical protein
SO1339	0.0%	0	8	conserved hypothetical protein
SO1429	0.0%	0	11	anaerobic dimethyl sulfoxide reductase, A subunit (dmsA-1)
SO1935	0.0%	0	14	regulator of nucleoside diphosphate kinase (nck)
SO1952	0.0%	0	2	gamma-galactosyltransferase (ggp-2)
SO2525	0.0%	0	17	ABC transporter, ATP-binding protein
SO2622	0.0%	0	9	conserved hypothetical protein
SO3036	0.0%	0	9	conserved hypothetical protein
SO3096	0.0%	0	16	RNA polymerase sigma-70 factor, ECF subfamily
SO3243	0.0%	0	4	flagellar long protein FlgH (flgH)
SO3538	0.0%	0	14	transcriptional regulator HU (huU)
SO3656	0.0%	0	11	hypothetical protein
SO3890	0.0%	0	6	serine protease, subtilase family
SO3949	0.0%	0	18	boW/hta family protein
SO4561	0.0%	0	9	conserved hypothetical protein
SO4598	0.0%	0	7	heavy-metal efflux pump, CzcA family
SO4745	0.0%	0	20	UDP-N-acetylglucosamine pyrophosphorylase (gmU)
SO49153	0.0%	0	5	heavy-metal efflux pump, CzcA family

0.3 mM  $K_2CrO_7$  dosage response differentially expressed proteins

- A total of 42 proteins were identified as up-regulated after a 30 min exposure to chromate.
  - Four of the functional categories (Numbers 2, 8, 10, and 16; see Figure 3) were not represented in the list of up-regulated proteins.
  - 36% of the proteins up-regulated were annotated in the transport and binding proteins (17) category (Table 2).
  - 84 proteins were found down-regulated after the 30 min exposure.
    - Two of the functional categories (Numbers 10 and 15; see Figure 3) did not contain any members that were identified as down-regulated.
    - Hypothetical Proteins (9) and Cellular Processes (4) had the highest representation of down-regulated proteins with 18 and 10 members identified, respectively (Table 2).

## DOSAGE RESPONSE RESULTS

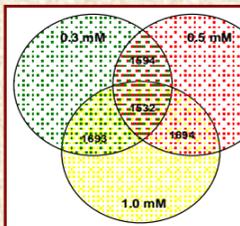


Figure 4: Venn Diagram of Dosage Response Proteome Samples. The Blue circle is the 0.3 mM  $K_2CrO_7$  sample, the Pink is the 0.5 mM, and the Orange is the 1.0 mM. The numbers are the total number of proteins shared between the respective samples. For instance, 1532 proteins were identified in all three dosage conditions, while 1693 proteins were shared between the 0.3 mM and 1.0 mM samples.

Table 3: Select Proteins Up-Regulated in 0.5 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	0.5mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description
SO1189	56.7%	6	15	conserved hypothetical protein
SO2426	22.4%	5	18	DNA-binding response regulator
SO3871	16.0%	5	8	TonB system transport protein ExbB1 (exbB1)
SO3875	40.1%	8	12	hemin ABC transporter, ATP-binding protein (hmuV)
SO4218	36.3%	11	19	UDP-N-acetylmuramyl-L-alanine ligase (murC)
SO4478	31.0%	5	9	sphingolipid protein y precursor, putative
SO4493	43.2%	12	65	sulfate ABC transporter, periplasmic sulfate-binding protein (sbp)

Select Proteins Down-Regulated in 0.5 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	0.5mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description	
SO0560	0.0%	0	16,4%	7	formate-ferredoxinase (fhd)
SO1744	0.0%	0	17,1%	6	AMP-binding protein
SO3521	0.0%	0	23,9%	13	SSB4, transposase
SO3538	0.0%	0	39,8%	5	transcriptional regulator HU (huU)
SO4758	0.0%	0	24,2%	6	sphingolipid acetylase (mexB)

Table 4: Select Proteins Up-Regulated in 1.0 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	1.0mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description
SO1035	15.3%	5	7	nicotinic-acetylcholine-dimethylbenzimidazole-phosphoribosyltransferase (cobT)
SO1275	17.7%	6	7	succinate-semialdehyde dehydrogenase (gabD)
SO2426	32.3%	8	13	DNA-binding response regulator
SO3655	42.7%	10	32	acetate kinase, putative
SO3871	20.6%	6	24	TonB system transport protein ExbB1 (exbB1)
SO3875	64.6%	11	27	hemin ABC transporter, ATP-binding protein (hmuV)
SO3728	32.8%	8	10	uroporphyrin-III C-methyltransferase (cobA)
SO4003	25.0%	6	8	response regulator
SO4349	24.8%	6	6	ketol-acid reductoisomerase (hnc)
SO4480	12.1%	5	8	acetylcholinesterase (aceA)
SO4652	49.7%	19	35	sulfate ABC transporter, periplasmic sulfate-binding protein (sbp)

Select Proteins Down-Regulated in 1.0 mM  $K_2CrO_7$  Exposed *S. oneidensis*

Locus	1.0mM average % coverage peptide spectra	Control average % coverage peptide spectra	Functional Category #	Description	
SO0102	6.3%	1	10	formate dehydrogenase, nitrate-reducible, iron-sulfur subunit (fhdH)	
SO0311	4.9%	3	3	conserved hypothetical protein	
SO0398	6.1%	2	28,5%	flavoprotein subunit (hda)	
SO0696	6.8%	3	4	thiol disulfide interchange protein (dtd) (dtdD)	
SO0847	0.0%	0	47,4%	8	iron-sulfur cluster-binding protein NapG (napG)
SO1429	3.4%	2	20,4%	11	anaerobic dimethyl sulfoxide reductase, A subunit (dmsA-1)
SO1776	9.0%	4	6	solar membrane protein precursor M56 (m56)	
SO1805	3.8%	2	23,4%	8	hemin ABC transporter, periplasmic peptide-binding protein (sbpA)
SO3797	3.0%	2	15,8%	10	peptidase, U2 family
SO4093	9.3%	6	20	methyl-accepting chemotaxis protein	
SO4093	2.2%	2	12,2%	11	conserved hypothetical protein
SO40141	2.3%	1	51,4%	24	hypothetical protein

0.5 mM  $K_2CrO_7$  dosage response differentially expressed proteins

- 39 proteins were identified as up-regulated following exposure to 0.5 mM chromate.
  - Ten functional categories (1, 3, 5, 6, 7, 9, 11, 15, 17, and 18) Figure 3) comprised up-regulated members.
  - As with the 0.3 mM dose; the category of Transport and Binding Proteins represented 44% of the proteins up-regulated (Table 3).
  - A total of 39 proteins as well were found to be down-regulated after exposure to chromate.
    - The highest represented category, Energy Metabolism (7), corresponded to 8 of the proteins identified as down-regulated under this dose.
    - Five proteins were identified only under control conditions (Table 3); where at least 5 peptides were identified confidently.

1.0 mM  $K_2CrO_7$  dosage response differentially expressed proteins

- A total of 66 proteins were up-regulated after exposure to chromate.
  - In a comparison to two other time-points for this dosage from a previous study with the highest represented category of Transport and Binding Proteins (45 and 90 min exposure to chromate); only 1 protein (SO1072; a putative chitin-binding protein) was unique to the 30 min time-point (this study).
  - Three of the proteins identified up-regulated in this study under the category of Transport and Binding Proteins were shared with the 90 min time-point, but not the 45 min: SO3599; CysP, SO3671 (Table 4); ExbB1, and SO4743; a putative TonB dependent receptor.
  - 26 proteins were identified as down-regulated after exposure to chromate.
    - The most highly represented functional category down-regulated was Energy Metabolism (7) with 9 members. This category was also represented well in a previous study with two other time-points (45 and 90 min); where the 30 min time-point in this study is more comparable to the 90 min time-point than the 45 min.
    - Four members of Energy metabolism are found in Table 4; where their expression is detected at a higher level under control conditions versus after exposure to 1.0 mM chromate.

Differentially expressed proteins found under all three dosage conditions are highlighted in light green in the tables.

## CONCLUSION

After exposure to three different doses of chromate and control conditions, we identified:

- A total of 2,429 proteins under at least one of the four growth conditions and two instruments.
- After exposure to 0.3 mM chromate for 30 min; 126 proteins were identified as differentially expressed.
- For 0.5 mM chromate; 78 proteins were differentially expressed.
- After 1.0 mM chromate; 92 proteins were differentially expressed.

At a concentration of 1.0 mM  $K_2CrO_7$ ; we now have proteomics data on three time-points (30, 45, and 90 min).

41% of the proteins identified up-regulated after 1.0 mM chromate exposure were also identified after the 90 min exposure study.

- Out of this group; almost 60% of the proteins were shared with the 45 min time-point.<sup>2</sup>

An unique putative protein complex that was identified only under exposure to chromate was SO3585 (a putative azoreductase), SO3586 (a glyoxalase family protein), and SO3587 (a hypothetical protein) (See Figure 5). This putative complex may be involved in a mechanism of chromate detoxification.

According to our proteome results, we hypothesize that this putative complex may be associated with the membrane.

- This is due to our observation that the hypothetical protein is only identified in the membrane associated fraction and contains a putative transmembrane domain.<sup>2</sup>

SO3585 (Figure 2) and SO3586 were identified at the 2-peptide level for all three doses and up-regulated at the 0.5 and 1.0 mM chromate doses.

SO3587 was identified in the 0.5 and 1.0 mM chromate samples, but failed the criteria set out in the Experimental section to be differentially expressed.

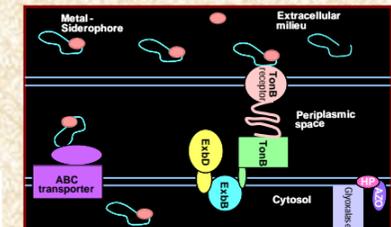


Figure 5: Schematic view of membrane region in Gram-negative bacterium. Key transport molecules are a metal-siderophore complex, TonB receptor, TonB complex (TonB, ExbB, & ExbD), and ABC transporter. These proteins are annotated under transport and binding proteins (17) in *S. oneidensis* MR-1 and are found up-regulated under the dosage response conditions presented here (See table of up-regulated proteins). Also shown is a putative azoreductase (AZO, HP, and Glyoxalase) complex of the inner membrane.

## REFERENCES

- Myers and Nealson. 1988. *Science* 240, 1319-1320.
- Brown et al. 2006. *Molecular & Cellular Proteomics*, In press.
- Chourey et al. 2006. *Applied and Environmental Microbiology*, submitted.

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