

OVERVIEW

- Shewanella oneidensis* is a gram-negative facultatively anaerobic bacterium that utilizes metal ions as terminal electron acceptors during cellular metabolic processes.
- S. oneidensis* cells were grown under three different metal-exposure conditions:
 - a short chromium (Cr) shock exposure followed by growth for either 45 or 90 minutes at mid exponential phase
 - continual exposure to chromium for 24 hours for adaptation evaluation at stationary phase
 - exposure to various concentrations of Cr(VI): 0.3 mM, 0.5 mM, and 1 mM followed by growth
- Protein fractions were digested with trypsin and analyzed with a multidimensional HPLC-NanoESI-MS/MS protocol.
- The goal is to identify the metabolic machinery involved in the metabolism and cellular response to chromium.

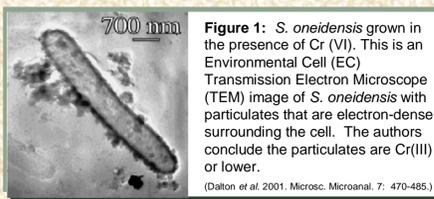


Figure 1: *S. oneidensis* grown in the presence of Cr (VI). This is an Environmental Cell (EC) Transmission Electron Microscope (TEM) image of *S. oneidensis* with particulates that are electron-dense surrounding the cell. The authors conclude the particulates are Cr(III) or lower.

(Dallon et al. 2001. Microsc. Microanal. 7: 470-485.)

INTRODUCTION

- Shewanella oneidensis* utilizes metal ions such as manganese, uranium, and chromium as terminal electron acceptors during cellular processes.
- Our goal is to understand the metabolic processes of *S. oneidensis* that utilize chromium as a terminal electron acceptor for the purpose of using this microbe for bioremediation as well as understanding the molecular response to toxic Cr levels.
- The exposure to chromium should cause a change in the proteins that are expressed, with those involved in metabolism or stress response to chromium being expressed at a much higher level than proteins found in the control cells.
- We exposed *S. oneidensis* cells to 45 and 90 minute Cr(VI) shock periods, a 24 hour growth adaptation period with continual exposure to Cr(VI), and to varying concentrations of Cr(VI). The proteome of Cr-exposed cells was compared to that of control (unexposed) cells.

EXPERIMENTAL

- S. oneidensis* chromium shock cells were grown under aerobic conditions with the addition of 1 mM K_2CrO_4 [Cr(VI)] when cells reached mid-exponential phase. The cells were then allowed to grow for 45 and 90 minutes in the presence of Cr.
- S. oneidensis* chromium adaptation cells were grown under aerobic conditions with a pre-adaptation concentration of 0.3 mM K_2CrO_4 [Cr(VI)] followed by the addition of 1 mM K_2CrO_4 [Cr(VI)] for 24 hours until cells reached stationary phase.
- Cells were lysed using sonication and protein fractions separated into a crude and membrane fraction by centrifuging the samples at 100,000g for 60 minutes. Following lysis, a trypsin digestion using a standard protocol was employed.
- Analysis was carried out by a 24 hour multidimensional HPLC-MS/MS protocol.
 - separation accomplished by 2-D chromatography using strong cation exchange as the first dimension and C18 reverse phase as the second dimension of separation
 - an LQC Deca XP Plus 3-D ion trap (Thermo Finnigan) and Ultimate HPLC pump (LC Packings) were operated in the data dependent mode for all the samples
 - an LTQ linear trapping quadrupole (Thermo Finnigan) and Surveyor pump (Thermo Finnigan) were operated in the data dependent mode for the Cr-shocked 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 of 4 or more peptides and/or a difference of 30% sequence coverage between states.

GLOBAL RESULTS

Table 1

Condition	Instrument	Proteome Analysis Cr Shock Samples		
		No. proteins identified	No. proteins identified	Average Sequence coverage
Control 1	LQC	1318	894	28.03%
45 min. shock	LQC	1238	816	31.47%
Control 2	LQC	1368	959	30.95%
90 min. shock	LQC	1267	856	31.69%
Control 1	LTQ	2552	1793	35.36%
45 min. shock	LTQ	2644	1959	37.32%
Control 2	LTQ	2571	1873	36.61%
90 min. shock	LTQ	2664	1992	36.78%
Total		3291	2358	

* identified with at least 1 peptide per protein
identified with at least 2 peptides per protein
\$ average sequence coverage of proteins (2-peptide level)

Table 2

Condition	Instrument	Proteome Analysis Cr Adapt Samples		
		No. proteins identified	No. proteins identified	Average Sequence coverage
Control Run 1	LQC	1119	802	32.47%
Control Run 2	LQC	1098	784	32.50%
Control Run 3	LQC	1336	949	29.66%
Cr adapt Run 1	LQC	1039	699	27.14%
Cr adapt Run 2	LQC	1053	686	28.19%
Cr adapt Run 3	LQC	1043	672	24.79%
Total		1800	1199	

* identified with at least 1 peptide per protein
identified with at least 2 peptides per protein
\$ average sequence coverage of proteins (2-peptide level)

Table 1

- Summary of the 45 and 90 minute Cr shock samples
- LQC and the LTQ datasets analyzed in duplicate
- 2358 proteins identified out of >4900 proteins (2-peptide level)

Table 2

- Summary of the Cr adaptation experiments
- LQC dataset consisted of triplicate runs
- 1199 proteins identified out of >4900 proteins (2-peptide level)

Proteome Characterization of Chromium-shocked and Chromium-adapted *Shewanella oneidensis*

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CHROMIUM SHOCK RESULTS

Figure 2

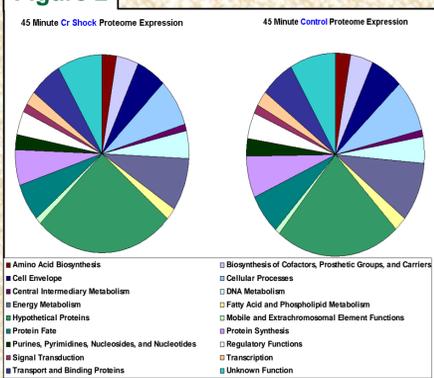


Figure 3

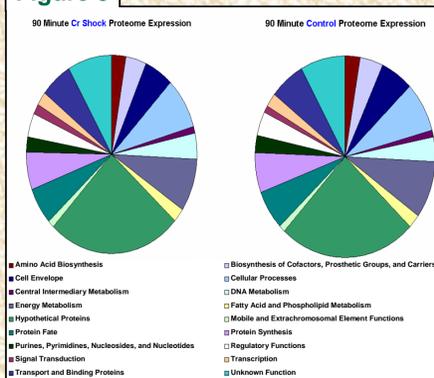


Figure 4

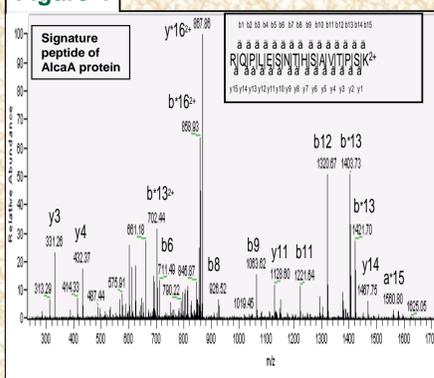


Table 3

Up-Regulated Proteins From 45 Minute Cr Shock Sample On The LTQ											
Locus	control_run1	control_run2	chrom_run1	chrom_run2	Total	Description					
SO3442	22.9	7.9	66.0	49.3	80.7	thioredoxin 2 (trx2)					
SO3394	12.2	64.7	37.8	76.3	96.6	conserved hypothetical protein					
SO1482	32.2	34.4	82.7	80.5	82.7	TonB-dependent receptor, putative					
SO3030	10.9	0.0	62.4	66.1	69.3	siderophore biosynthesis protein (AlcA)					
SO3033	12.0	11.6	57.6	57.3	51.1	heme-alkaligen siderophore receptor					
SO3599	12.8	14.0	68.7	64.8	68.7	sulfate ABC transporter, periplasmic sulfate-binding protein (cysP)					
SO3667	5.4	0.0	91.9	96.9	97.3	conserved hypothetical protein					
SO3669	9.3	5.5	71.6	77.9	79.0	heme transport protein (hgmB)					
SO3673	0.0	0.0	63.1	61.2	63.1	heme ABC transporter, periplasmic heme-binding protein (hmuT)					
SO3675	0.0	0.0	72.9	69.0	72.9	heme ABC transporter, ATP-binding protein (hmuV)					
SO3737	30.8	20.0	62.1	73.3	75.3	sulfate reductase (NADPH) hemoprotein beta-component (cysR)					
SO3967	0.0	0.0	15.0	48.1	60.0	conserved hypothetical protein					
SO3914	18.5	31.4	68.1	67.8	69.9	TonB-dependent receptor, putative					
SO3652	0.0	0.0	46.6	62.0	62.0	sulfate ABC transporter, periplasmic sulfate-binding protein (cysB)					
SO4655	0.0	0.0	54.5	55.1	59.0	sulfate ABC transporter, ATP-binding protein (cysA2)					

Table 4

Up-Regulated Proteins From 90 Minute Cr Shock Sample On The LTQ											
Locus	control_run1	control_run2	chrom_run1	chrom_run2	Total	Description					
SO3442	22.9	7.9	66.0	49.3	80.7	thioredoxin 2 (trx2)					
SO3394	12.2	64.7	37.8	76.3	96.6	conserved hypothetical protein					
SO1482	32.2	34.4	82.7	80.5	82.7	TonB-dependent receptor, putative					
SO3030	10.9	0.0	62.4	66.1	69.3	siderophore biosynthesis protein (AlcA)					
SO3033	12.0	11.6	57.6	57.3	51.1	heme-alkaligen siderophore receptor					
SO3599	12.8	14.0	68.7	64.8	68.7	sulfate ABC transporter, periplasmic sulfate-binding protein (cysP)					
SO3667	5.4	0.0	91.9	96.9	97.3	conserved hypothetical protein					
SO3669	9.3	5.5	71.6	77.9	79.0	heme transport protein (hgmB)					
SO3673	0.0	0.0	63.1	61.2	63.1	heme ABC transporter, periplasmic heme-binding protein (hmuT)					
SO3675	0.0	0.0	72.9	69.0	72.9	heme ABC transporter, ATP-binding protein (hmuV)					
SO3737	30.8	20.0	62.1	73.3	75.3	sulfate reductase (NADPH) hemoprotein beta-component (cysR)					
SO3967	0.0	0.0	15.0	48.1	60.0	conserved hypothetical protein					
SO3914	18.5	31.4	68.1	67.8	69.9	TonB-dependent receptor, putative					
SO3652	0.0	0.0	46.6	62.0	62.0	sulfate ABC transporter, periplasmic sulfate-binding protein (cysB)					
SO4655	0.0	0.0	54.5	55.1	59.0	sulfate ABC transporter, ATP-binding protein (cysA2)					

Down-Regulated Proteins From 90 Minute Cr Shock Sample On The LTQ											
Locus	control_run1	control_run2	chrom_run1	chrom_run2	Total	Description					
SO3847	45.9	43.9	6.1	0.0	51.6	iron-sulfur cluster binding protein NapS (napS)					
SO3002	49.8	43.6	26.6	18.4	62.3	alpha-ubiquitin oxidoreductase, Nta translocating, alpha subunit (ntrA)					
SO3870	59.9	63.6	34.7	34.9	67.1	kumate reductase flavoprotein subunit precursor					
SO1111	75.6	80.9	35.7	42.7	65.4	beta-defensin subunit 2 (bds)					
SO1405	40.1	31.4	11.1	5.6	66.0	transglutaminase family protein					
SO1420	42.8	30.5	0.0	0.0	49.4	aminoacyl ornithine sulfide reductase, A subunit (dnaA1)					
SO1420	31.2	40.2	0.0	0.0	45.1	aminoacyl ornithine sulfide reductase, B subunit (dnaA1)					
SO1480	67.3	70.2	27.2	39.9	71.5	alcohol dehydrogenase (adhB)					
SO1518	80.4	69.1	48.1	52.4	85.7	conserved hypothetical protein					
SO4651	37.7	47.4	11.4	9.7	47.4	conserved hypothetical protein					

45 Minute Cr Shock Results

- Figure 2
 - Cr 45-min shock and control samples
 - Proteins organized within seventeen functional categories assigned by TIGR (www.tigr.org)
 - Exception is the hypothetical proteins category
 - Over 100 more hypothetical proteins identified in Cr 45-min shock vs. Control
- Table 3
 - Top 15 out of 29 proteins up-regulated
 - 3 of the top 15 proteins have no peptides identified in Control sample
 - 2 proteins identified as down-regulated

90 Minute Cr Shock Results

- Figure 3
 - Cr 90-min shock and control samples
 - Proteins organized within functional categories
 - No significant differences between shock and control samples
 - Hypothetical proteins category not changed here
- Table 4
 - Top 15 out of 44 proteins up-regulated
 - 4 of the top 15 proteins have no peptides identified in Control sample
 - Top 10 out of 12 proteins identified as down-regulated

AlcA Siderophore Biosynthesis Protein

- Figure 4
 - MS/MS spectrum of peptide RQPLESNTHSAYTPSK of siderophore biosynthesis protein AlcA (SO3303)
 - Highly expressed in the 45-min and 90-min Cr shock conditions relative to the respective Control sample
 - Down-regulated under Cr-adaptation conditions
- Table 5
 - List of peptides identified for AlcA in Cr 90-min shock and Control samples
 - Total of 23 unique peptides from Cr 90-min shock condition
 - 69.3% sequence coverage
 - 3 peptides identified under Control conditions

CHROMIUM ADAPTATION RESULTS

Figure 5

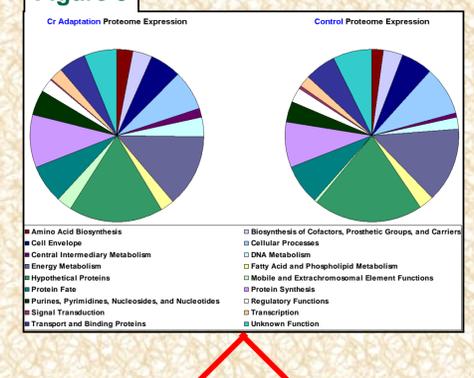


Table 6

Up-Regulated Proteins From Cr Adapt Sample On The LQC											
Locus	control_run1	control_run2	chrom_run1	chrom_run2	Total	Description					
SO3030	10.9	0.0	62.4	66.1	69.3	siderophore biosynthesis protein (AlcA)					
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Table 7

Down-Regulated Proteins From Cr Adapt Sample On The LQC											
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SO1480	67.3	70.2	27.2	39.9	71.5	alcohol dehydrogenase (adhB)					
SO1518	80.4	69.1	48.1	52.4	85.7	conserved hypothetical protein					
SO4651	37.7	47.4	11.4	9.7	47.4	conserved hypothetical protein					

Figure 5

- Cr adaptation and Control proteins categorized by functional category
 - Hypothetical proteins category reduced by 70 proteins under Cr-adaptation conditions (down-regulated)
 - Mobile and extra-chromosomal elements category increased from 6 to 23 proteins under Cr-adaptation conditions
- Table 6
 - Differentially expressed proteins from Cr adaptation and Control conditions
 - Top 15 out of 19 proteins considered up-regulated under Cr-adaptation conditions
 - Top 16 out of 63 proteins down-regulated under Cr-adaptation conditions
- Table 7
 - Peptides identified from HK97 family prophage lambdaSO major capsid protein
 - Highly expressed under Cr-adaptation conditions
 - Demonstrates the bacterial stress of Cr-adaptation conditions

DISCUSSION

- S. oneidensis* has the ability to utilize metal ions, like Cr, as a terminal electron acceptor. This ability creates the opportunity to use this bacterium for bioremediation and to understand the process of metal ion uptake and metabolism.
- With the LTQ, we were able to identify approximately twice as many proteins relative to the LQC. Identified 1793 proteins (2-peptide level) with 45-min control sample and 1959 with 45-min Cr shock sample. 90 minute control sample contained 1873 proteins and 90 minute Cr shock sample consisted of 1992 proteins.
- Under Cr adaptation conditions, many proteins are down-regulated relative to the Control condition. Most of the proteins found up-regulated are located in a region of the genome that contains a large number of phage genes and hypothetical proteins that may be from the phage sequence.
- Proteins identified as up-regulated after 45 minutes of Cr shock include AlcA (see Fig4 and Table5), azoreductase, heme ABC transporter members (HmuT and HmuV), and 4 hypothetical proteins (SO3667, SO4079, SO4651, & SOA0080). Only two proteins were down-regulated: a hypothetical protein (SO2929) and a transcriptional regulator (HlyU).
- After 90 minutes of Cr shock; AlcA, azoreductase, HmuT, HmuV, and two of the hypothetical proteins (SO3667 & SO4079) under 45 minutes of Cr shock were still up-regulated. Other proteins include a ferric alcaligen siderophore receptor and a TonB-dependent receptor. Fumarate reductase flavoprotein subunit precursor, Bfr2 (bacterioferritin subunit 2), and AhdB (alcohol dehydrogenase II) were identified as down-regulated after 90 minutes of Cr shock.
- Preliminary results of the Cr concentration experiments reveal many of the same trends observed with the Cr shock and adaptation experiments. AlcA, HmuT, HmuV, and SO3914 (a TonB-dependent receptor) were found up-regulated.

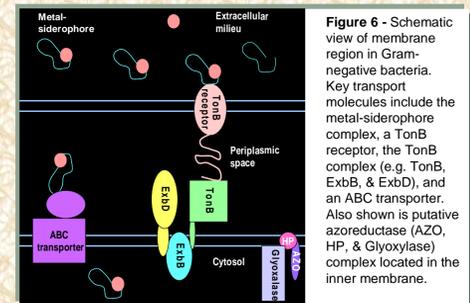


Figure 6 - Schematic view of membrane region in Gram-negative bacteria. Key transport molecules include the metal-siderophore complex, a TonB receptor, the TonB complex (e.g. TonB, ExbB, & ExbD), and an ABC transporter. Also shown is putative azoreductase (AZO, HP, & Glyoxylase) complex located in the inner membrane.

ACKNOWLEDGMENTS

- Research support was provided by the U.S. Department of Energy Office of Biological and Environmental Research, Natural and Accelerated Bioremediation Research (NABIR) Program.
- M. Thompson acknowledges financial support from the ORNL-UTK Genome Science and Technology Graduate School.
- Oak Ridge National Laboratory is managed and operated by the University of Tennessee-Battelle, L.L.C., under contract DE-AC05-00OR22725 with the U.S. Department of Energy.