

Proteome Characterization of Chromium-shocked and Chromium-adapted *Shewanella oneidensis* MR-1

M. Thompson¹, N. VerBerkmoes¹, K. Chourey², S. Brown², D. Thompson², R. Hettich¹

¹Chemical Sciences Division and ²Environmental Sciences Division; Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.

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 two different metal-exposure conditions:
 - a short chromium (Cr) shock exposure followed by growth for either 45 or 90 minutes
 - continual exposure to chromium for a 24 hour growth for adaptation evaluation
- Protein fractions were digested with trypsin and analyzed with a multidimensional HPLC-NanoESI-MS/MS protocol.
- The search engine SEQUEST was used for peptide identifications.

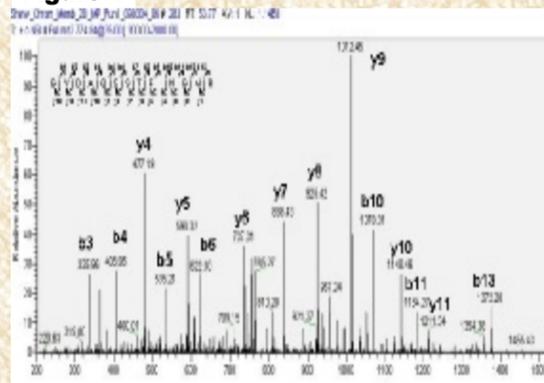
INTRODUCTION

- 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 purposes 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 chromium metabolism being expressed at a much higher level than proteins found in control cells.
- We exposed *S. oneidensis* cells to 45 and 90 minute Cr(VI) shock periods. The proteome of Cr-exposed cells was compared to that of control (unexposed) cells.

EXPERIMENTAL

- S. oneidensis* cells were grown under aerobic conditions with the addition of 1 mM K₂CrO₇ [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.
- Cells were lysed using sonication and protein fractions separated into a crude and membrane fraction by centrifuging the samples at 100,000g for 60 min.
- A trypsin digestion was accomplished by first reducing the crude or membrane fraction in 6M Guanidine and 10mM DTT. Trypsin was added at 1:100 (wt:wt) and digested overnight at 37 °C with gentle shaking. The following morning a second trypsin aliquot was added with a final reduction with 20mM DTT.
- Analysis was carried out by a 24 hour multidimensional HPLC-MS/MS protocol. Briefly, separation occurred by 2-D separation using strong cation exchange as the first dimension and C18 reverse phase as the second dimension of separation. An LCQ Deca XP Plus 3-D ion trap was operated in the data dependent mode where a full scan is acquired followed by four tandem mass spectra.
- Peptide identification was completed by the search engine SEQUEST with a protein considered a true "hit" if two unique peptides are identified.

Figure 1



MS/MS spectrum of the +2 peptide GYDAQSSTFINGMR from a TonB receptor protein (SO3914) in *S. oneidensis* 45 min shock membrane fraction.

RESULTS

Table 1: Proteins up-regulated in Cr shock

Seq. coverage 45 min Cr	Seq. coverage 45 min Control	Seq. coverage 90 min Cr	Seq. Coverage 90 min Control	Description
97.5	63.1	97.5	63.9	ribosomal protein L7/L12 (pIL)
53.8	36	47.9	37.3	methylcrotonate lyase (ppB)
76	42.7	76	66.7	chaperonin GroES (groES)
55.1	22.1	57.4	12.5	conserved hypothetical protein
46.7	24	71.2	0	TonB-dependent receptor, putative
71.6	58.9	80.8	69.2	heat shock protein HspG (hspG)
59.8	22.9	64.6	37	NifU family protein
88.4	34	78.9	50.3	16 kDa heat shock protein A (hpaA)
93.2	67.7	83.5	78.6	cysteine synthase A (cysK)
21.5	0	50.2	0	siderophore biosynthesis protein (alcA)
27.4	20.7	43.6	6.9	ferric alcaligin siderophore receptor
45.9	11.6	36.6	30.7	cipB protein (cipB)
22.1	0	27.5	0	azoreductase, putative
83.2	10.3	69.7	0	conserved hypothetical protein
67.7	3.4	71	0	heme transport protein (hugA)
57.1	0	62.8	0	hemin ABC transporter, periplasmic hemin-binding protein (hmuT)
40.2	0	53	0	hemin ABC transporter, ATP-binding protein (hmuV)
25.3	0	37.1	0	sulfate adenylyltransferase, subunit 1 (cysN)
36.4	21.2	58.6	0	sulfate adenylyltransferase, subunit 2 (cysD)
35.2	18.1	51.6	0	TonB-dependent receptor, putative (SO3914)
35.3	40	57.3	18.1	iron-regulated outer membrane virulence protein
21.2	0	45.8	0	sulfate ABC transporter, ATP-binding protein
48.4	38	57.5	28.6	TonB-dependent receptor, putative

Figure 2

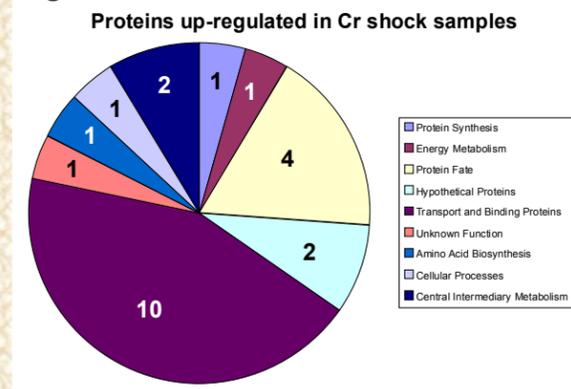
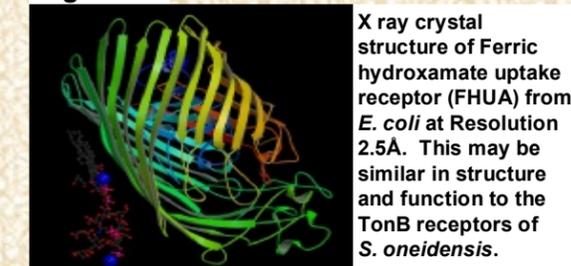


Figure 3

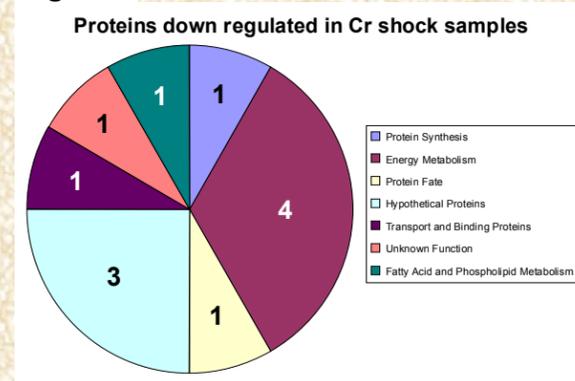


[Ferguson, A. D., et. al.: Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. *Science* 282 pp. 2215 (1998)]

Table 2: Proteins down-regulated in Cr shock

Seq. coverage 45 min Cr	Seq. coverage 45 min Control	Seq. coverage 90 min Cr	Seq. Coverage 90 min Control	Description
23.2	35.7	0	50.4	periplasmic nitrate reductase (napA)
20.3	23.3	18.3	34.2	lumarate reductase flavoprotein subunit precursor
21.2	21.2	17.9	32.8	conserved hypothetical protein TIGR0092
9	17.3	8.5	20.5	aminopeptidase P, putative
18.1	40.3	11.8	62.8	alcohol dehydrogenase II (adhB)
30.4	33.8	14.8	62.9	acyl-CoA dehydrogenase family protein
15.1	25.2	16.3	37.3	decaheme cytochrome c (omcA)
0	32.2	0	36.3	extragenic suppressor protein SuhB (suhB)
10.4	27.6	11.8	52.1	hypothetical protein
14.4	26.7	31.9	48.4	poly(r)-RNA synthetase (proS)
23.3	17.9	0	57.6	molybdenum ABC transporter, periplasmic molybdenum-binding protein, putative
81	69	38.3	81	conserved hypothetical protein

Figure 4



- A total of ~800 proteins were identified at the 2 peptide level in the 45 minute Cr shock sample and ~900 proteins were identified in the control sample.

Out of the 45 minute shock and control samples, four proteins (AlcA, azoreductase, HmuT, and HmuV) were absent in at least one of the control runs (Table 1). SuhB was absent in one of the Cr runs, indicating a decreased expression level (Table 2).

- ~850 proteins were identified in the 90 minute shock sample at the 2 peptide level and ~950 proteins were found in the corresponding control sample.

In the 90 minute Cr shock samples, a total of eleven proteins were missing in at least one of the control sample runs, including two TonB receptors, AlcA, a conserved hypothetical protein, HugA, three different ABC transporter proteins, and two subunits of sulfate adenylyltransferase (Table 1). Proteins that were missing in at least one of the Cr shock runs, include a hypothetical protein, an alcohol dehydrogenase, a nitrate reductase, and a molybdenum ABC transporter.

CONCLUSION

S. oneidensis is able to utilize different metals as alternative electron acceptors. Here we find some potential candidate proteins that may be involved in Cr metabolism and/or the cellular response to Cr stress in *S. oneidensis*.

Figures 2 and 4 are a graphical representations of Tables 1 and 2, respectively, with the results organized by their functional categories. In the Cr shock data, ten proteins under the category of transport and binding proteins (Figure 2) were more prevalent as up-regulated when compared to all 23 proteins identified as up-regulated. However, energy metabolism is down-regulated in the Cr shock samples (Figure 4).

Also shown in Table 1, the gene labeled TonB-dependent receptor (SO3914) is found to be expressed at a higher level in the 90 minute shock sample than in the 45 minute shock sample relative to the control sample. The TonB receptor peptide illustrated in Figure 1, which is a MS/MS spectrum of GYDAQSSTFINGMR is indicative of the presence of TonB in the Cr shock sample.

Figure 3 is the X-Ray crystal of an *E. coli* ferric uptake receptor, which may be similar in structure to TonB receptors of *S. oneidensis*. This data helps demonstrate proteins and protein complexes that may be involved in cellular response to Cr toxicity.

Improvements in definitive quantification methods available and improvements in the dynamic range of mass spectrometry are needed to verify the list of proteins presented here.

Cells grown in the presence of Cr for a 24 hour growth adaptation period are currently being analyzed. This data should demonstrate proteins and protein complexes involved in maintaining cell survival under conditions of chronic Cr exposure.

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