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

Melissa R. Thompson1,2, Nathan C. VerBerkmoes2, Karuna Chourey2, Steven D. Brown3, Robert L. Hettich2, and Dorothea K. Thompson4

1Graduate School of Genome Science and Technology, UT-ORNL, Knoxville, TN
2Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN
3Department of Biological Sciences, Purdue University, West Lafayette, IN

OVERVIEW

Shewanella oneidensis MR-1 is a gram-negative, facultatively anaerobic bacterium originally isolated from a freshwater lake. (Science 240, 1988, 1319-1320)

S. oneidensis MR-1 has the ability to reduce toxic metal ions [e.g., Cr(VI)] found in industrial and governmental waste sites.

Cells were grown and exposed to three different metal concentrations in order to probe the dosage response of S. oneidensis MR-1 to Cr(VI) in the form of chromate.

Protein fractions were digested with trypsin and analyzed with a multidimensional HPLC-NanoESI-MS/MS protocol.

The goal of this work is to identify protein components of pathways/mechanisms responsible for both detoxification and reduction of chromate.

EXPERIMENTAL

S. oneidensis MR-1 dosage response cells were grown under aerobic conditions with the addition of 0.3, 0.5, or 1.0 mM K2CrO4 when cells reached mid-exponential phase. These cells were then allowed to grow for an additional 30 minutes in the presence of chromate.

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

Analysis was carried out by 24 hour multidimensional HPLC-MS/MS protocol. (See Figure 3.)

Separation was accomplished by online 2-D chromatography using strong cation exchange as the first dimension and C18 reverse phase as the second dimension of separation.

Peptide identification was completed by the search engine SEQUEST with a two unique peptide cut-off (X-corr values 1.8(V1), 2.5(V2), and 3.5(V3)).

Semi-quantitation: Proteins were considered differentially expressed (up- or down-regulated) with a difference in at least two of the following categories: 3 or more peptides, a difference of 40% sequence coverage, or 20X more spectra identified between treated and control samples.

RESULTS

Global Results

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 third spectrum, which contains the sequence of a peptide from SO3308 (previously identified as SO3308p) was identified up-regulated at the 0.5 mM and 1.0 mM chromate levels.

Functional Category Assignments

Table 1: Differential expression of proteins under at least one of the four growth conditions.

Table 2: A total of 2,445 proteins identified under at least one of the four growth conditions.

CONCLUSIONS

After exposure to three different concentrations of K2CrO4, we found:

• A total of 2,445 proteins identified under at least one of the four growth conditions.

• 128 proteins differentially expressed with respect to the control after the addition of 0.3 mM chromate (42 up- and 86 down-regulated).

• 96 proteins were differentially expressed after 0.5 mM chromate (44 up- and 52 down-regulated).

• A total of 92 proteins were differentially expressed after exposure to 1.0 mM chromate (66 up- and 26 down-regulated).

• A total of 29 proteins were differentially expressed over all three chromate concentrations (See up- and down-regulated protein tables).

The 30-minute time point for the 1.0 mM chromate sample analyzed here is similar to our 45-minute 1.0 mM chromate shocked sample (Brown et al, MCP, in press).

• 69% of proteins identified as differentially expressed after 45 minutes were also at the 30-minute time point.

A putative azoreductase (SO3585), a glyoxalase family protein (SO3586), and a hypothetical protein (SO3587) are identified only under chromate conditions (See Figure 2), and may be involved in a detoxification mechanism for chromate. Studies are underway to determine whether these proteins function in a complex and whether the putative azoreductase can reduce Cr(VI).

• SO3585 and SO3586 were found to be up-regulated at both the mRNA and protein level after 45 and 90 minutes of exposure to K2CrO4.

• In this dosage response study, a concentration of 0.5 mM chromate, SO3585 (3 peptides) and SO3586 (3 peptides) were identified at the protein level, but were not detected at the mRNA level.

• At the 0.5 mM chromate level, SO3585 was identified as up-regulated, however SO3586 and SO3587 were identified with 3 peptides each but not up-regulated.

• At a concentration of 1.0 mM chromate only SO3585 is up-regulated with identification of SO3586 and 20% by 4 peptides each.

ACKNOWLEDGMENTS

Research support was provided by the U.S. Department of Energy Office of Science, Biological and Environmental Research program.

M. Thompson acknowledges 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, and UT-Battelle, LLC, under contract DE-AC05-00OR22725 with the U.S. Department of Energy.