Transcriptome and Proteome Dynamics of the Cellular Response of Shewanella oneidensis to Chromium Stress

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ABSTRACT

The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. The transcriptomic analysis was conducted using DNA microarrays, with 1808 genes selected for analysis. The proteomic analysis was conducted using high mass resolution (≥ 1.6 Da/amu) data-dependent LC-MS/MS analysis. The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. The transcriptomic analysis was conducted using DNA microarrays, with 1808 genes selected for analysis. The proteomic analysis was conducted using high mass resolution (≥ 1.6 Da/amu) data-dependent LC-MS/MS analysis.

METHODS

Bacterial Growth Conditions and Total RNA Isolation:

For time-course microarray experiments, batch cultures of S. oneidensis MR-1 were grown in mid-exponential phase (OD₆₀₀ 0.6-0.8) at 30°C in LB media, followed by the addition of 25 mM K₂CrO₄ to a final concentration of 1 mM. Untreated control cultures were grown in parallel with treated cultures. Control and treated cells were harvested for total cellular RNA extraction at 5, 30, 60, and 90 min post-exposure. Total RNA was isolated from untreated control cultures and harvested for total cellular RNA extraction at 30, 60, and 90 min post-exposure addition using the TRIzol reagent.

Microarray Data Analysis:

Array hybridization signals were quantified using Affymetrix version 5.1. Data transformation and statistical determination of Gene death significance (p<0.05) changes in expression were further analyzed using the program Hierarchical Clustering Explorer version 2.0.

Chromatin Redox Assay and Metal Analysis:

Residual chromate was measured using the 1,5-diphenylcarbazide method (Park et al., 2000) at 540 nm. The effect of the growth medium (LB) on potassium chromate was examined using a Varian (Cary-1E) UV-Visible Spectrophotometer. UV-Visible spectra were collected for K₂CrO₄ in LB at 5, 10, 30, 60, 90 min, and 24 h with reference to K₂CrO₄ in HB water. The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. The transcriptomic analysis was conducted using DNA microarrays, with 1808 genes selected for analysis. The proteomic analysis was conducted using high mass resolution (≥ 1.6 Da/amu) data-dependent LC-MS/MS analysis. The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. The transcriptomic analysis was conducted using DNA microarrays, with 1808 genes selected for analysis. The proteomic analysis was conducted using high mass resolution (≥ 1.6 Da/amu) data-dependent LC-MS/MS analysis.

RESULTS

The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. Control and treated cells were harvested for total cellular RNA extraction at 5, 30, 60, and 90 min post-exposure addition using the TRIzol reagent.

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^1/2 Cr₂O₇²⁻ + 2H⁺ + e⁻ → Cr₃O₄ + H₂O

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CONCLUSIONS & FUTURE RESEARCH

Global temporal alterations in the transcriptome and proteome of S. oneidensis MR-1 exposed to potassium chromate exposure were determined in order to understand the cellular responses to acute chromate stress. Genes and their corresponding protein products involved in iron and carbohydrate transport, cellular detoxification, and DNA repair were upregulated in response to acute Cr(VI) exposure. Transcriptomic profiles generated from cells exposed to 0.5 and 1.0 mM K₂CrO₄ for 24 h differed markedly from those exposed to untreated cells exposed to acute Cr(VI) stress without adaptation. The new linear-tape array allows for a much greater detailed analysis of the proteome with ~2-3 times the proteome coverage allowing for comparisons of low abundance proteins not identified by conventional quadrupole time mass analysis. Reconstitute proteomic profiles with the linear tape are ongoing.

Many of the genes found to be up-regulated at the transcript level were found to have reproducible dramatic difference in % signal to average coverage of > 40 unique peptides, and special cases (e.g. unique inducible protein abundance). The new linear-tape array allows for a much greater detailed analysis of the proteome with ~2-3 times the proteome coverage allowing for comparisons of low abundance proteins not identified by conventional quadrupole time mass analysis. Reconstitute proteomic profiles with the linear tape are ongoing.

Chemical analysis indicated that Cr remains predominantly in the +6 oxidation state in LB medium (no cells) over the time course examined.

MR-1 orthologs of known members of the LexA regulon (Sträff et al., 2006) of S. oneidensis are present in MR-1 and are currently being characterized. This work has revealed other key gene targets for antimicrobials.

We are in the process of investigating the possibility that SO3585, SO3586, and SO3587 form a protein complex anchored in the periplasmic region of S. oneidensis. The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response in Shewanella oneidensis strain MR-1. Temporal genomic profiling and mass spectrometry-based proteomic analysis were employed to characterize the dynamic molecular response of S. oneidensis MR-1 to both acute and chronic Cr(VI) exposure. The acute stress response of aerobic, mid-exponential phase cells shocked to a final concentration of 1 mM potassium chromate (K₂CrO₄) was examined at post-exposure time intervals of 5, 30, 60, and 90 min relative to untreated cells. The transcriptomic and proteomic analyses were conducted in parallel with treated cultures. Control and treated cells were harvested for total cellular RNA extraction at 5, 30, 60, and 90 min post-exposure addition using the TRIzol reagent.

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