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

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Shewanella oneidensis strain MR-1 is a metal-reducing bacterium that can respire a wide array of organic and inorganic substrates, including Cr(VI), U(VI), and Tc(VII). *S. oneidensis* can potentially be used to immobilize metals/radionuclides by reducing soluble and more mobile forms to sparingly soluble, less bioavailable forms. However, the prediction and assessment of bioremediation performance is compounded by the lack of fundamental knowledge on microbe-metal interactions and on a microorganism's ability to survive and function in relevant contaminated environments.

The overall goal of this DOE NABIR project is to characterize the molecular basis and regulation of hexavalent chromium [Cr(VI)] stress response and reduction by *S. oneidensis* MR-1. Here we describe transcriptome and proteome analyses of the molecular stress response and adaptation of MR-1 to toxic levels of Cr(VI). The acute stress response of aerobic, mid-exponential phase cultures shocked to a final concentration of 1 mM K_2CrO_7 was examined after 5, 30, 60, and 90 min and compared to untreated cultures. The transcriptome of mid-exponential cultures was also examined 30 min after shock doses of 0.3 mM, 0.5 mM, or 1 mM K_2CrO_7. The *tonB1-exbB1-exbD1* genes comprising the TonB1 iron transport system were some of the most highly induced genes after 90 min (up to ~240 fold), followed by other genes involved in iron acquisition and sulfate transport and metabolism. Transcripts predicted to encode hydrogenases (e.g., HydA, HydB), iron-sulfur cluster binding proteins (e.g., SO4404), and cytochromes (e.g., OmcA, OmcB) were some of the most highly repressed genes after 90 min. Transcripts for DNA repair and detoxification genes such as *recA*, *kat2*, and a putative azoreductase-encoding gene were also more abundant in cells exposed to chromium. Transcriptome profiles generated from MR-1 cells adapted to 0.3 mM Cr(VI) differed significantly from those characterizing cells exposed to acute Cr(VI) stress without adaptation.

Parallel proteomic characterization of soluble protein and membrane protein fractions extracted from Cr-shocked and Cr-adapted MR-1 cells was performed using multidimensional HPLC-ESI-MS/MS. For a 45 min shock sample, ~720 proteins were confidently identified, which compared favorably with the ~800 identifications from a control cell culture (i.e., cells grown in the absence of Cr). A total of 12 proteins were found to be up-regulated and 7 proteins were found to be down-regulated in the Cr-shocked *S. oneidensis* samples, relative to the untreated control. The up-regulated proteins included a putative azoreductase and proteins involved in iron binding and transport. The 90-min shock sample revealed 14 proteins that were up-regulated, including two subunits of sulfate adenylyltransferase (CysN and CysD) and a sulfate ABC transporter (CysA-2) that were not found to be up-regulated in the 45 min shock sample. These studies provide important insights into cellular chromium tolerance. Future research will focus on the structural and regulatory genes implicated in Cr(VI) reduction and detoxification.