Nitrate stress response in *Desulfovibrio vulgaris* Hildenborough: Whole-Genome Transcriptomics and proteomics analyses

Qiang He[1], Zhili He[2], Wenqiong Chen[3], , Zamin Yang[4], Eric J. Alm[5], Katherine H. Huang[5], Huei-Che Yen[6], Dominque C. Joyner[7], Martin Keller[3,4], Adam P. Arkin[5], Terry C. Hazen[7], Judy D. Wall[6], and Jizhong Zhou[2]

[1]Department of Civil & Environmental Engineering, Temple University, Philadelphia, PA
[2]Institute for Environmental Genomics, Department of Botany and Microbiology, University of Oklahoma, OK
[3]Diversa Corporation, San Diego, CA
[4]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN
[5]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA
[6]Departments of Biochemistry and Molecular Microbiology & Immunology, University of Missouri-Columbia, Columbia, MO
[7]Earth Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA

Sulfate reducing bacteria (SRB) are of interest for bioremediation with their ability to reduce and immobilize heavy metals. Nitrate, a common co-contaminant in DOE sites, is suggested to inhibit SRB via nitrite. Previous results indicate that nitrite is indeed inhibitory to the growth of *Desulfovibrio vulgaris*. However, growth inhibition by nitrate alone was also observed. In this study, growth and expression responses to various concentrations of nitrate were investigated using the Omnilog phenotype arrays and whole-genome DNA microarrays. Changes in the proteome were examined with 3D-LC followed by MS-MS analysis.

Microarray analysis found 5, 50, 115, and 149 genes significantly up-regulated and 36, 113, 205, and 149 down-regulated at 30, 60, 120, and 240 min, respectively. Both transcriptomic and proteomic profiles shared little similarities with those of salt stress, indicating a specific inhibitory mechanism beyond osmotic stress. Many of the genes (~50% at certain time points) with altered expression level were of unknown functions; however, the increasing number of ribosomal protein genes down-regulated with time could provide a direct explanation to the growth inhibition effect of nitrate. Further, several lines of evidence suggested that the down-regulation of genes coding the ribosomal proteins could be the result of the changes in the energy flow upon nitrate exposure: 1) The down-regulation of genes for the ATPase subunits indicated reduced level of energy generation; 2) the up-regulation of phage shock protein genes (*pspA* and *pspC*) might indicate a reduced proton motive force; although damages to the cell envelope could also contribute to this outcome; 3) the gene for the hybrid cluster protein, a redox protein with roles in nitrogen metabolism, was highly up-regulated 120 and 240 min following nitrate stress at both transcriptomic and proteomic level, suggesting that nitrate was being actively reduced, shifting reducing equivalents away from normal energy production; 4) genes in the methionine biosynthesis pathway were among the most highly up-regulated genes throughout the experiment, potentially providing a convenient mechanism for the simultaneous disposal of excess sulfur (from sulfate reduction) and nitrogen (from nitrate reduction); 5) One gene cluster consistently among the most up-regulated genes consisted of genes encoding two TRAP dicarboxylate family transporters, a formate acetyltransferase, and a pyruvate formate-lyase activating enzyme, which might be regulated to provide an increased carbon flow to keep pace with demand from amino acids biosynthesis. These observations indicated that the growth inhibition effect of nitrate might be due to energy limitation.

Similar to the observations made during salt stress, the glycine/betaine transporter gene was among genes highly up-regulated, suggesting that $NaNO_3$ also constituted osmotic stress which was relieved by the mechanism of osmoprotectant accumulation. Osmoprotectant accumulation as the major resistance mechanism was further validated by the partial relief of growth inhibition by glycine betaine. It is also noted that, similar to nitrite stress, the ferric iron transporter genes were up-regulated during nitrate stress, suggesting an increased demand for iron. Unlike nitrite stress, however, no other genes in the Fur regulon were co-regulated during nitrate stress, pointing to a yet-to-known regulatory signal.

In conclusion, excess $NaNO_3$ resulted in both osmotic stress and nitrate stress. *D. vulgaris* shifted nitrogen metabolism and energy production in response to nitrate stress. Resistance to osmotic stress was achieved primarily by the transport of osmoprotectant.