Title
Quantitative Proteomic Analysis of Nitrate Stress in Desulfovibrio vulgaris

Author
Keasling, J.D.

Publication Date
2006-05-22

Quantitative Proteomic Analysis of Nitrate Stress in *Desulfovibrio vulgaris*

A. Redding[1,2,3], A. Mukhopadhyay[2,3], D. Joyner[2,3], T. C. Hazen[2,3], J. D. Keasling[1,2,3]

[1]Virtual Institute for Microbial Stress and Survival (VIMSS), [2]University of California, Berkeley, Berkeley, CA, [3]Lawrence Berkeley National Laboratory, Berkeley, CA

ESPP Environmental Stress Pathway Project

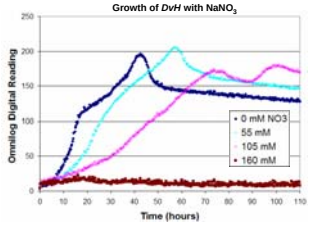
DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

VIMSS Virtual Institute for Microbial Stress and Survival | Miami University | University of Missouri | University of Washington | Diversa | Sandia National Laboratories | Oak Ridge National Laboratory | Berkeley University of California | Science 75 | http://vimss.lbl.gov/

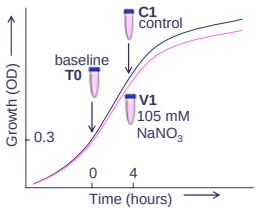
ABSTRACT:

Desulfovibrio vulgaris Hildenborough (*DvH*), a sulfate reducing bacterium, has historically been an environmentally important bacterium due its role in bio-corrosion of oil and gas pipelines and is one of the major sources of H_2S that cause bio-fouling of petroleum. Another reason for interest in *DvH* is due to its ability to reduce toxic and radioactive metals to their lower oxidation and insoluble forms, and therefore its potential use in bioremediation. While sulfate typically serves as the electron acceptor in *DvH*, alternate candidates for electron acceptors such as nitrate also exist. Exposure to excess nitrate occurs frequently since it is a common co-contaminant along with metals such as uranium in many waste sites. Therefore our knowledge of *DvH* response to nitrate will undoubtedly be critical in developing bioremediation strategies. This poster presents the results from a quantitative proteomic analysis evaluating the response of *DvH* to nitrate stress. Control proteome was compared with proteome from cells exposed to $NaNO_3$ levels that cause a 50% inhibition in growth. The ITRAQ peptide labeling strategy coupled with tandem liquid chromatography and mass spectrometry (triple-quad time of flight) was used. A total of 1166 unique proteins were identified, representing 34% of the total *DvH* proteome and spanning every functional category. Our results indicate that this was a mild stress, as confirmed by the lack of change observed in central metabolism or in the sulfate reduction pathway. Increases seen in transport systems for proline, glycine betaine and glutamate indicate that the $NaNO_3$ exposure led to both salt stress and nitrate stress. Up-regulation observed in a large number of ABC transport systems as well as in iron-sulfur cluster containing proteins, however, appear to be specific to the exposure to nitrate. Finally, a number of hypothetical proteins are among the most significant changers, indicating that there may be unknown mechanisms initiated upon nitrate stress. Our poster outlines both our results from the proteomic response to nitrate exposure as well as the methods used to optimize quantitative proteomics for *DvH*.

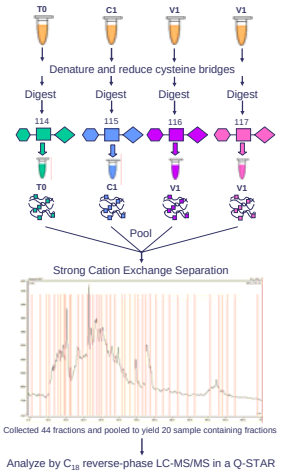
Experimental Design and Sample Prep:



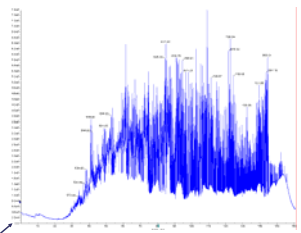
Determination of the minimum inhibitory concentration (MIC) of nitrate. MIC is defined as the concentration of stressor that causes the generation time of cells to double. The MIC was determined to be 105 mM $NaNO_3$.



Schematic of the $NaNO_3$ stress experiment conducted. Cells are grown to an optical density of 0.3, at which time the T0 sample is collected and 105 mM $NaNO_3$ is added. Samples C1 and V1 were collected at 4 hours (approximately one generation time).



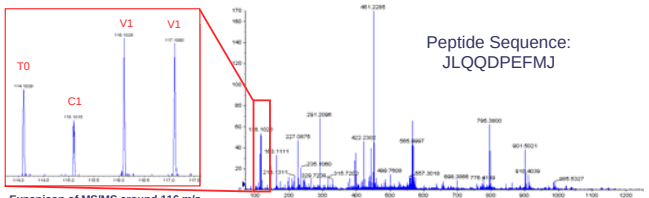
Harvested cells are lysed, and the resulting mixture is clarified to remove cellular debris. The BCA assay is used to quantify the resulting amount of protein. The same total amount of protein is taken for each labeling condition and labeled with iTRAQ tags. $NaNO_3$ sample (V1) was used in two tags, enabling determination of the internal error of the strategy.



Each fraction was split in half before being run. Each run generates a total ion chromatogram, as shown. Every fraction was run twice, and the results were combined.

Example iTRAQ Data:

Identified at 99% confidence via iTRAQ proteomics, the nitrite reductase gene, DVU0625, was seen to increase in stressed biomass. T0 represents baseline, C1 represents control, V1 represents 105 mM nitrate addition. The two V1 samples are technical replicates (i.e. the same sample labeled with two different tags). Peptide sequences are determined from the mass difference between peaks, which corresponds to the mass of each amino acid. The iTRAQ tags fragment at the low mass region, which is expanded, to show the ratio of the peptide.



Expansion of MS/MS around 116 m/z

MS/MS for peptide from DVU0625, NrfA, showing up-regulation

Overview of Proteomic Results

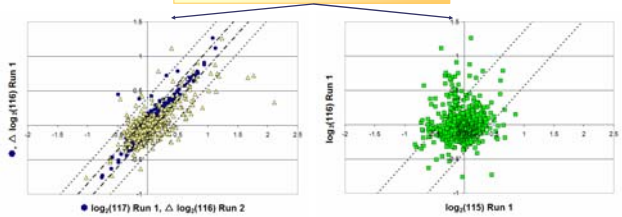
25,607 Total spectra collected

ProQuant **1,166** Proteins identified from 5,683 unique peptides

Mascot **1,221** Proteins identified

1047 Proteins identified by both programs

737 Proteins with 2 unique high confidence peptides

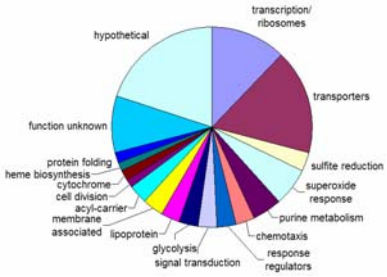


Log2(ratio) comparisons. (a) The log2 protein ratios between tag116 and tag117 for Run 1 are plotted (circles). The internal error for Run 1 was computed to be ±0.12 (— - —). As can be seen, the vast majority of proteins in Run 1 fall within this error. Similarly, the variation between tag116 in Run 1 and tag116 in Run 2 (technical replicates) is shown (triangles), along with error bars denoting the run to run variability which was computed to be ±0.4 (– – –). These error values establish the amount of variability that is associated with the method and cannot be distinguished from actual biological variation. In an ideal scenario, all of these points would fall on a single line. (b) The amount of sample variation between control (tag115) and stressed (tag116) samples in Run 1 (squares) is plotted along with the error bars from Fig. 1a. Because the error associated with the method was determined, the biological variation can be clearly seen. There are 65 of proteins whose ratio between stressed and control exceed the amount of run to run variability, thus they are considered to be the significant changers in the sample.

65 Proteins Significantly Changing

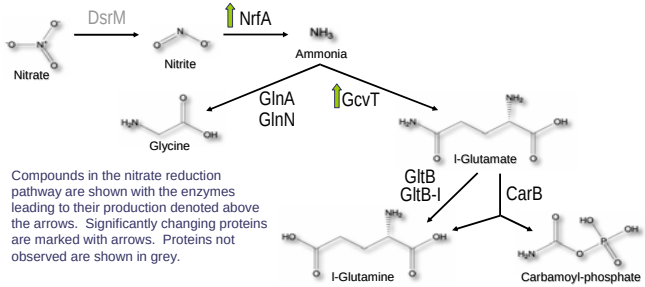
Biological Conclusions:

Distribution of Significant Changers



The functional breakdown of the 65 significantly changing proteins are shown. Hypothetical proteins represent 20% of all significant changers (13 proteins). All proteins in the function unknown category do have conserved folds, but belong to families that have many different functions.

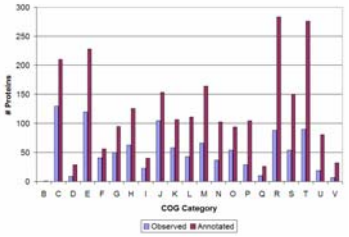
Nitrate Reduction Pathway



Compounds in the nitrate reduction pathway are shown with the enzymes leading to their production denoted above the arrows. Significantly changing proteins are marked with arrows. Proteins not observed are shown in grey.

Breakdown by Function: Clusters of Orthologous Groups

The COG distribution of all proteins observed. Every functional category was observed in the proteomic data set. There is little enrichment in any particular category.



A: RNA processing and modification
B: Chromatin structure and dynamics
C: Energy production and conversion
D: Cell division and chromosome partitioning
E: Amino acid transport and metabolism
F: Nucleotide transport and metabolism
G: Carbohydrate transport and metabolism
H: Coenzyme metabolism
I: Lipid metabolism
J: Translation, ribosomal structure and biogenesis
K: Transcription
L: DNA replication, recombination, and repair
M: Cell envelope biogenesis, outer membrane
N: Cell motility and secretion
O: Posttranslational modification, protein turnover, chaperones
P: Inorganic ion transport and metabolism
Q: Secondary metabolites biosynthesis, transport, and catabolism
R: General function prediction only
S: Function unknown
T: Signal transduction mechanisms
U: Intracellular trafficking and secretion
V: Defense mechanisms
W: Extracellular structures
Y: Nuclear structure
Z: Cytoskeleton

34% percent of the proteome was observed. 737 proteins passed all of our criteria, which is 22% of the proteome. Most of central metabolism remains unaffected, demonstrating that this was a mild stress. Salt stress was observed, which was expected as sodium nitrate was used as a stressor. Also, response in oxidative stress response proteins was observed, strengthening the hypothesis that they are involved in general stress response. Many ABC transport proteins increased, along with many hypothetical proteins, providing the foundation for more focused studies in the future.

ACKNOWLEDGEMENT

ESPP is part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.