Stress Response in *Desulfovibrio vulgaris* Hildenborough: A Proteomics Approach

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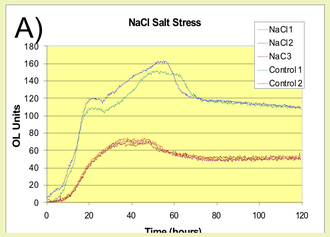
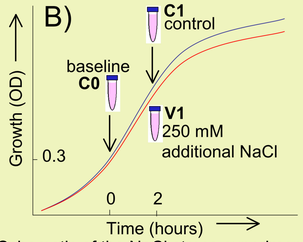
Abstract:

Desulfovibrio vulgaris is classified as an anaerobic, sulfate reducing δ-proteobacterium, which has the ability to reduce several heavy metals including chromium and uranium. Reduction of heavy metals places organisms under stress as the bacterium must adapt to new environmental conditions and different energy sources. In an attempt to gain an understanding of the physiology of the anaerobe *D. vulgaris*, and evaluate its potential application in bioremediation, we are studying this bacterium under environmentally applicable stress conditions. To date we have analyzed oxygen, sodium chloride, and nitrate stress. Bacterial stress response is reflected in changes in gene expression levels, protein levels, and enzymatic activity. We are utilizing proteomics to study the changes in whole cell stress response by monitoring the changes in the level of protein expression. Both the isotope-coded affinity tag (ICAT), and ITRAQ labeling strategies are utilized to differentially label peptides from a stressed sample and unstressed (normally grown) sample. These techniques allow peptide samples to be pooled after labeling and analyzed together. Liquid chromatography coupled with dual mass spectrometry (LC-MS/MS) is used to analyze and sequence the peptides. Peptide identification, reconstruction, and quantification is performed using ProICAT and ProQuant software from Applied Biosystems. These studies have confirmed the activity of several stress response mechanisms present in *D. vulgaris*, including the rubrythrin/rubredoxin pathway under oxygen stress, and the glycine betain transport system under salt stress. The results from these three proteomic studies are presented.

Experimental Scheme:

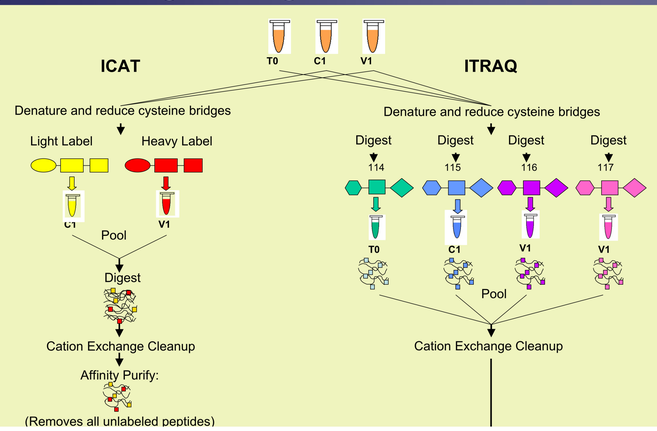


(A) The MIC for salt stress. Similar growth curves were produced for each stress considered. Stress was defined as the concentration of stressor that caused the generation time of cells to double. The time points used for proteomics analysis in each stress condition were determined via microarray analysis.



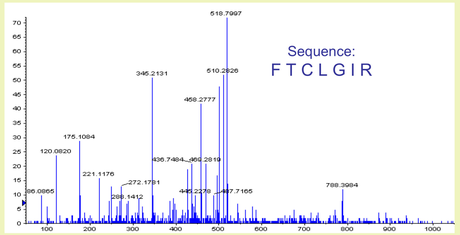
(B) Schematic of the NaCl stress experiment conducted during biomass collection. Cells are grown to an optical density of 0.3, at which time the C0 sample is collected and the stressor is added to one of the cultures. Samples C1 and V1 were collected at 2 hours (approximately one generation time).

Sample Labeling Strategies:

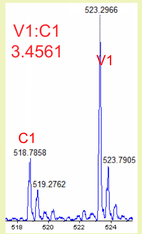


Sample ICAT Data:

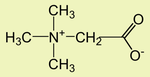
Identified at 99% confidence via ICAT proteomics using LCMS, DVU2298, the glycine betaine ABC transporter/permease protein was shown to be highly up-regulated in the salt stressed biomass using. C1 represents control, V1 represents 250 mM NaCl.



MSMS for peptide sequence of parent ion at m/z = 518.79



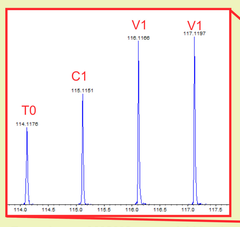
MS for relative peak quantification of parent ions



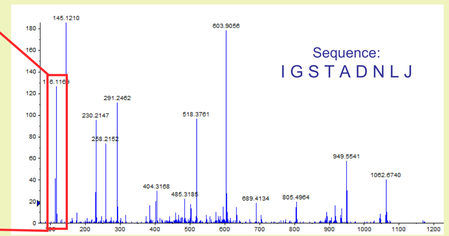
Accumulation of small neutral molecules such as glycine betaine (left) appears to be one of the predominant protective mechanisms during salt stress in *D.vulgaris*.

Sample ITRAQ Data:

Identified at 99% confidence via ITRAQ proteomics using LCMS, DVU2218, a putative rubrythrin candidate was shown to be highly up-regulated in the oxygen stressed biomass. T0 represents baseline, C1 represents control, V1 represents 0.1 ppm oxygen addition. The two V1 samples are technical replicates (i.e. the same sample labeled with two different tags).



Expanison of MSMS around 116 m/z



MSMS for peptide from DVU2318, rbr2, showing up-regulation

Experimental Summary:

Stressor	Amount	Labeling Method	# Peptides*	# Proteins
Salt	250 mM additional	ICAT	358	220
Oxygen	0.1 ppm continuous	ITRAQ	1063	331
Nitrate	6500 ppm added	ITRAQ	3903	275

*Indicates the total number of spectra which were able to be assigned to any peptide. For example, during oxygen stress 795 unique peptides were observed.

Observations From Oxygen Stress:

VIMSS ID	Name	Description	Log_2(V1:C1)
307732	AhpC	alkyl hydroperoxide reductase C	0.6102
208611	Rbr2	rubrerythrin	0.3974
208610	Rdl	rubredoxin -like protein	0.3576
207805	Rbr2	rubrerythrin, putative	0.3241
208912	ZraP	zinc resistance -associated protein	0.2906
206977		decarboxylase family protein	0.2538
209119		conserved hypothetical protein	0.1918
207907		conserved hypothetical protein	0.1899
207257	RpsU	ribosomal protein S21	-0.2128
206739	RpsL	ribosomal protein S12	-0.17
206696		RNA -binding protein	-0.1522

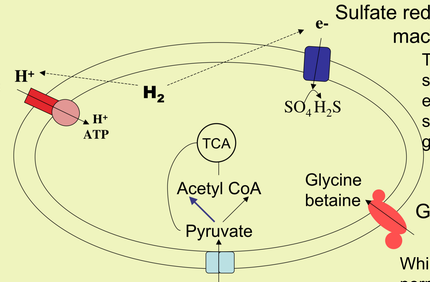
This is a partial list of proteins that changed significantly under oxygen stress. Up-regulated proteins are in green, down-regulated are in red. Rubrerythrin and rubredoxin proteins among the most upregulated proteins present.

Observations From Salt Stress:

During salt stress, many different significantly changing proteins were identified. The list of significant changers was investigated to provide a functional understanding of the changes observed. Many of the significant changes are also supported by microarray data (courtesy of Joe Zhou's lab). Based on these observations a picture of what is occurring inside the cell could be developed.

↑ ATP synthesis

The operon encoding the F1 subunit of the ATP synthase is highly up-regulated.



Sulfate reduction machinery ↑

This pathway is showing up-regulation, especially in the adenyl-sulphate reductase gene.

Glycine betaine transport ↑

While most ABC permease genes show no significant change or decrease after salt stress, the ABC transporter opuBB for glycine/betaine/L-proline transport is highly up-regulated.

↓ Cellular metabolism

The lactate permease was found to be unchanging in proteomics data, but was found to be highly down-regulated in microarray data. However, one of the conversion steps to acetyl CoA (glycolate oxidase) was found to be significantly down-regulated.

Other observations:

The ATP dependent RNA helicase, *deaD* is highly up-regulated.

Desulfoferrodoxin is highly down-regulated.

Conclusions:

The data from the oxygen stress experiment are very encouraging, as they show an increase in the rubrerythrin/ rubredoxin pathway, which is known in other organisms to counter free oxygen radicals. Also, a hydroperoxide reductase was strongly up-regulated. Further investigation may reveal the importance of the up-regulation in the zinc resistance associated protein, and may help elucidate the function of the up-regulated conserved hypothetical proteins.

Additional studies based upon the salt stress results confirmed that addition of glycine betaine to the media protected cells from the effects of salt stress. Other studies currently under way include creating gene deletions of the genes that changed significantly, such as the RNA helicase *deaD*. With additional information we hope to confirm and expand on the current gene regulatory network developed for *D. vulgaris*.

The data from the nitrate stress experiment revealed very few significantly changing proteins. This experiment is being repeated with biomass collected after approximately 4 doubling times, hopefully revealing a more complete picture of what is occurring inside the cell upon nitrate stress.

Many scientists involved with the VIMSS project are pooling information from a variety of techniques to try to gain a more complete understanding of these stress responses. ICAT and ITRAQ labeling techniques have proven very useful in finding a number of cases where microarray data shows large down-regulation in gene levels which is not supported by actual protein content. In the future we will begin studying the effects of heavy metals. By constructing gene regulatory networks based upon stress response, we hope to be able to predict what will happen to *D. vulgaris* upon bioremediatory stimulation under various environmental conditions.

Acknowledgements:

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