Salt stress in *Desulfovibrio vulgaris* Hildenborough: An integrated genomics approach.

A. Mukhopadhyay[1], Z. He[2], E. Alm[1], A. Arkin[1,3], E. Baidoo[1], S. Borglin[1], W. Chen[4], T. Hazen[1], Q. He[2], H-Y. Holman[1], K. Huang[1], D. Joyner[1], M. Keller[4], P. Oeller[4], A. Redding[3], J. Sun[4], J. Wall[5], J. Wei[4], H-C. Yen[5], J. Zhou[2], J. Keasling[1,3]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA, [2]Oak Ridge National Laboratory, Oak Ridge, TN, [3]University of California, Berkeley, CA, [4]Diversa, Corp., San Diego, CA, [5]University of Missouri, Columbia, MO.

ESPP Environmental Stress Pathway Project

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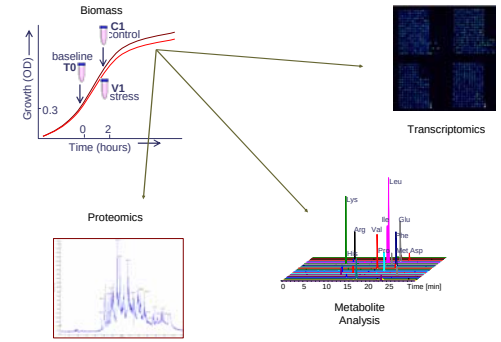
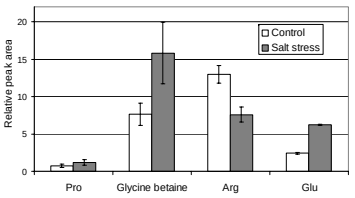
ABSTRACT

Recent interest in the ability of *Desulfovibrio vulgaris* Hildenborough to reduce, and therefore contain, toxic and radioactive metal waste, has made all factors that affect its physiology of great interest. Increased salinity constitutes an important and frequent fluctuation faced by *D. vulgaris* in its natural habitat. In liquid culture, exposure to excess salt resulted in a striking cell elongation in *D. vulgaris*. Using data from transcriptomics, proteomics, metabolite assays, phospholipid fatty acid profiling, and electron microscopy, we undertook a systems approach to explore the effects of excess NaCl on *D. vulgaris*. This study demonstrates that import of osmoprotectants such as glycine betaine and ectoine constitute the primary mechanism used by *D. vulgaris* to counter hyper-ionic stress. Several efflux systems were also highly up-regulated, as was the ATP synthesis pathway. Increase in both RNA and DNA helicases suggested that salt stress had affected the stability of nucleic acid base pairing. An overall increase in branched fatty acids indicated changes in cell wall fluidity. An immediate response to salt stress included up-regulation of chemotaxis genes though flagellar biosynthesis was down-regulated. Other down-regulated systems included lactate uptake permeases and ABC transport systems. The extensive NaCl stress analysis was compared with microarray data from KCl stress and unlike many other bacteria, *D. vulgaris* responded similarly to the two stresses. Integration of data from multiple methods has allowed us to present a conceptual model for salt stress response in *D. vulgaris* that can be compared to other microorganisms.

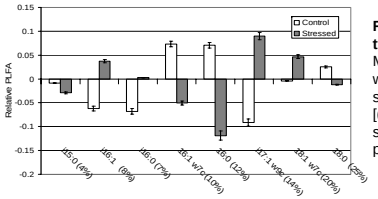
INTEGRATED GENOMICS



METABOLITE ANALYSIS

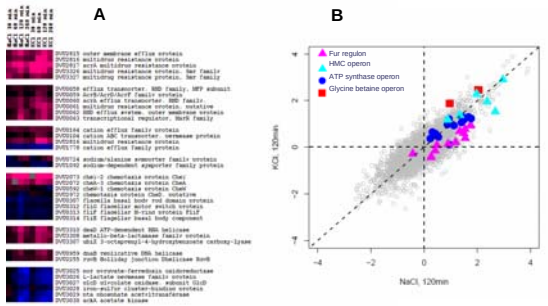


Quantities of selected metabolites estimated relative to the internal standard (set at 100%). Values are averages of three technical replicates. Metabolites were detected by comparison to standards using an Agilent CE-MSD



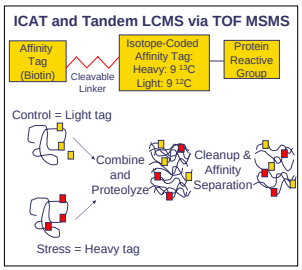
Relative change of eight major types of PLFA after NaCl stress: Mole fractions of individual PLFAs were measured in triplicate. Data shown are computed as [(V1/V0)/(C1/C0) -1] where V is salt stressed and C is control. Time point 1 = 120min, 0 = 0min.

Overview of Transcriptomics Data

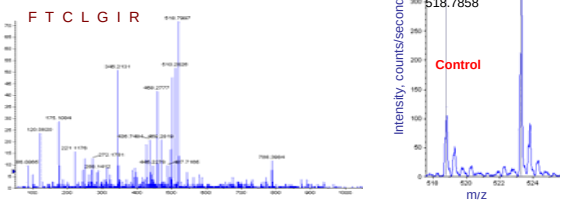


A) Selected hits from the NaCl and KCl microarrays. Color block figure shows changes in mRNA levels for selected *D. vulgaris* genes over time, in response to both NaCl and KCl stress. Pink, increase; Blue, decrease; Black, no change; Gray, data not available. Candidates are grouped by function or gene ID numbers and do not indicate clustering. **B) Comparison of NaCl and KCl stress response**. y-axis: Changes in mRNA levels in 250 mM KCl stress (120 min), x-axis: 250 mM NaCl stress (120 min) on x-axis. Figure illustrates the large overlap in the mRNA changes in response to KCl and NaCl. The plot represents the similarity between KCl and NaCl stress response. Points in the top right hand quadrant represent increases in both data sets. Values on both axes are log2 of the ratio of mRNA level under stressed conditions to mRNA from control genomic DNA. Since for both microarrays, genomic DNA was used as control, such a direct comparison can be made. Selected candidate operons and regulons have been highlighted and include the FUR and Hmc regulons, the ATP biosynthesis operon and Glycine betaine uptake operon.

ICAT and LC/MS/MS Proteomics

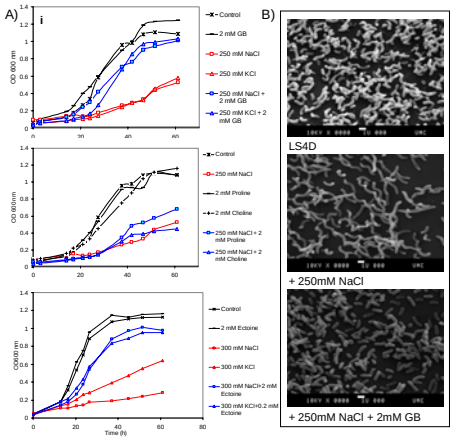


ICAT Proteomics Data: Using 300μg protein from Stressed and control Biomass lysate. 220 unique proteins identified using the ProICAT software at > 99% confidence. Of these high quality Stressed : Control ICAT ratios were obtained for 193 candidates. Using ratios from multiple peptides for proteins with high coverage, the error for ICAT ratios was established to be 30%. Using the error as a cutoff, 64 proteins were evaluated to be significant changers.



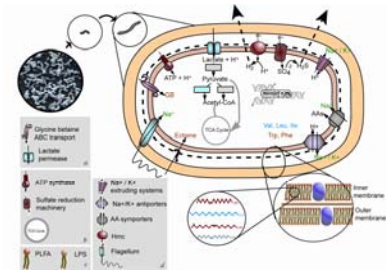
ICAT Proteomics data for the Glycine betaine permease OpuBB. The FTCLGIR sequence was obtained at 99% confidence from the MSMS of the parent ion at m/z = 523.29 (or mass =1046.59) and used to identify OpuBB (DVU2298). The +2 charged parent ion peaks are separated by an m/z = 4.5, The section of the TOF MS show the light (control) and heavy (NaCl stressed) ICAT ion pair for the peptide FTCLGIR and were used to obtain relative peak quantification using ProICAT. The stressed: control ratio of 3.45 is consistent with an upregulation in OpuBB. .

Salt stress and Osmoprotection in *D. vulgaris*



Effect of salt stress on growth and cell morphology of *D. vulgaris* and role of osmoprotectants A) (i) effect of 250 mM additional NaCl or KCl on growth of *D. vulgaris* in LS4D medium and also effect of the presence of 2 mM GB during salt stress, (ii) effect of proline and choline on NaCl stressed cells, (iii) effect of ectoine on NaCl or KCl stressed cells. Linear plots have been used for ease of visualizing the effect of the different growth conditions on the biomass. Log plots are available in supplementary information. **B)** Scanning electron microscopy images of *D. vulgaris* grown in different conditions as indicated (Electron Microscopy Core, University of Missouri, Columbia).

MODEL OF SALT STRESS RESPONSE



Molecular changes observed in *D. vulgaris* upon exposure to inhibitory NaCl concentrations. Symbols in red indicate an increase while those in blue indicate decrease. Gray indicates no change. (a) Data gathered from microarray, proteomics, metabolite and osmoprotection assays, (b) microarray and proteomics, (c) PLFA and FTIR analyses, and (d) microarray analysis only. Cell morphology data used electron microscopy.

ACKNOWLEDGEMENT

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