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

**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 hyperionic stress. Several efflux systems were also highly up-regulated, as was the ATP synthase 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**

**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. Candidate genes 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 NaCl KCl stress (120 min) vs. 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 lysates, 220 unique proteins identified using the ProICAT software at > 95% 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 30%. Using the error as a cutoff, 64 proteins were evaluated to be significant changes.

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