Global Transcriptional and Metabolite Analysis of *Desulfovibrio vulgaris* Hildenborough Responses to Long-Term Exposure to Elevated NaCl

ESPP2 Environmental Stress Pathway Project

Q-299

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ABSTRACT

The mechanisms of *Desulfovibrio vulgaris* Hildenborough responses to long -term NaCl exposure were studied by global transcriptional and metabolite analyses. The growth of *D. vulgaris* was inhibited by high salinity, and salt inhibition could be relieved by an addition of amino acids (e.g., glutamate, alanine) or yeast extract. Salt shock (sudden increase in salt concentration) and salt adaptation (inoculating cells in the medium containing high concentrations of salt) showed a significant difference in respective transcriptomes. Salt adaptation induced expression of genes involved in amino acid biosynthesis and transportation, electron transfer, hydrogen oxidation, and general stress responses (e.g., heat shock proteins, phage shock proteins, and oxidative stress response proteins). Genes involved in energy metabolism, cell motility, and phage structures were repressed. Genes involved in Na^+/H^+ transport, K^+ uptake and transportation, and proline biosynthesis and transportation were not significantly affected. Metabolite assays and external addition of amino acids into the growth medium of *D. vulgaris* suggest that amino acids, such as glutamate and alanine may accumulate as osmoprotectants in *D. vulgaris*. A conceptual model is proposed to link our observed results to currently available knowledge for further understanding the mechanisms of adaptation of *D. vulgaris* to sodium chloride.

MATERIALS AND METHODS

Cell culture and treatment: *D. vulgaris* cells were grown at the LS4D medium with or without yeast extract. To test the effects of amino acids on *D. vulgaris* growth, yeast extract was removed. NaCl was added into the LS4D medium to make desired concentrations when the LS4D medium was made.

***D. vulgaris* oligonucleotide array:** 70mer oligonucleotide arrays that containing all ORFs were constructed as described (He et al., 2006).

Target preparation, labeling and array hybridization: Total cellular RNA was isolated and purified using TRIzol™ Reagent, and then labeled with Cy5 dye. Genomic DNA was isolated and purified from *D. vulgaris* as described previously (Zhou et al., 1996), and then labeled with Cy3 dye. The labeled RNA and genomic DNA were co-hybridized to the array at 45°C with 50% formamide for 16 hrs in the dark. Image and data analysis were the same as described previously (Chhabra et al., 2006; Mukhopadhyay et al., 2006).

Metabolite determination: *D. vulgaris* cells were grown at the LS4D without added NaCl (the control), or with 250 mM additional NaCl (the treatment). A total of 150 ml of samples were collected for extraction of metabolites. Metabolite assays were conducted with capillary electrophoresis (CE) and mass spectrometric (MS) under optimal conditions.

RESULTS

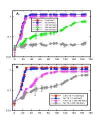


Fig. 1 Effects of yeast extract on the growth of *D. vulgaris* under NaCl stress

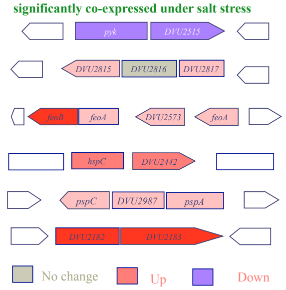
- **With yeast extract, *D. vulgaris* growth was inhibited ~15% by 250 mM NaCl (A); without yeast extract, its growth was inhibited ~50% by 250 mM NaCl (B).**
- **The results suggest that yeast extract significantly affected the growth of *D. vulgaris* in the presence of NaCl, which may be because certain substances in yeast extract help *D. vulgaris* cells adapt to high salinity environments.**

Transcriptomic analysis of *D. vulgaris* responses to long-term NaCl exposure

Table 1. Expression changes of representative genes of *D. vulgaris* involved in energy metabolism

Locus	Gene	Predicted function	$Log_2(R)$	Z-score
DVU0531	*hmcF*	HmcF, 52.7 kd protein in hmc operon	3.75	2.62
DVU0532	*hmcE*	HmcE, 25.3 kd protein in hmc operon	2.76	2.25
DVU0533	*hmcD*	HmcD, 5.8 kd protein in hmc operon	3.45	2.75
DVU0534	*hmcC*	HmcC, 43.2 kd protein in hmc operon	2.42	3.83
DVU0535	*hmcB*	HmcB, 40.1 kd protein in hmc operon	2.64	2.92
DVU0536	*hmcA*	HmcA, high-molecular-weight cytochrome c	3.00	2.75
DVU0587	*f dnG1*	Formate dehydrogenase, alpha subunit, selenocysteine-containing	1.81	2.42
DVU0588	*hybA*	Formate dehydrogenase, beta subunit, putative	0.55	0.52
DVU1769	*hydA*	Periplasmic [Fe] hydrogenase, large subunit	1.57	2.07
DVU1770	*hydB*	Periplasmic [Fe] hydrogenase, small subunit	1.62	2.19
DVU1919	NA	Hydrogenase expression/formation protein, putative	1.66	2.98
DVU2680	*f ld*	Flavodoxin, iron-repressed	1.90	3.31
DVU1568	*f tn*	Ferritin	-1.90	-3.19
DVU1569	*porA*	Pyruvate ferredoxin oxidoreductase, alpha subunit	-2.12	-1.90
DVU1570	*porB*	Pyruvate ferredoxin oxidoreductase, beta subunit	-1.96	-2.39
DVU1946	*oorB*	Pyruvate ferredoxin oxidoreductase, beta subunit, putative	-1.73	-2.08
DVU0596	*lytR*	DNA-binding response regulator LytR	-1.13	-1.54
DVU0597	*lytS*	Regulatory protein LytS	-1.23	-1.81
DVU0598	NA	Carbon starvation protein A, putative	-3.90	-4.99
DVU0599	NA	Carbon starvation protein A, putative	-3.46	-6.39
DVU3027	*glcD*	Glycolate oxidase, subunit GlcD	-2.10	-2.29
DVU3029	*pta*	Phosphate acetyltransferase	-2.47	-2.56
DVU3030	*ackA*	Acetate kinase	-1.89	-2.32
DVU3133	*glpF*	Glycerol uptake facilitator protein	-2.41	-2.40
DVU3134	*glpK*	Glycerol kinase	-1.44	-2.21

Fig. 2 Examples of operons whose genes were significantly co-expressed under salt stress



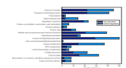


Fig. 3 Changes in gene expression profiling by category

- **Significantly changed genes were across different categories.**
- **Most significantly changed genes were involved in amino acid synthesis and transportation, energy metabolism, and regulatory processes.**

Fig. 4 Comparison of gene expression under short- and long-term NaCl exposure

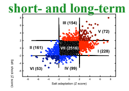


Table 2 Examples of up-regulated ORFs under long-term NaCl exposure but no significant changes under short-term NaCl exposure

	Short-term NaCl exposure		Long-term NaCl exposure		
Locus tag	Rati o	Zscore	Rati o	Zscore	Annotati on
DVU0375	0.696496	1.11554	2.70303	3.69125	Glu/Leu/Phe/Val dehydrogenase family protein
DVU0724	0.527134	1.0384	1.41683	2.6865	sodium/alanine symporter family protein
DVU2204	0.271192	0.467774	1.80918	3.21826	tryptophanase
DVU1068	0.418923	0.782345	2.70003	4.57373	branched-chain amino acid ABC transporter, permease protein
DVU2341	0.782525	1.17642	1.56143	2.75833	amino acid ABC transproter, His/Glu/Gln/Arg/opine family
DVU2740	0.609274	0.956004	3.18217	5.34415	high-affi nity branched-chain amino acid ABC transporter, ATP-binding protein
DVU2742	0.926925	1.75591	1.79111	2.0753	high-affi nity branched chain amino acid ABC transporter, permease protein
DVU1769	-0.691571	-1.30428	1.57488	2.07439	periplasmic [Fe] hydrogenase, large subunit
DVU1770	-0.0701596	-0.12201	1.61874	2.18869	periplasmic [Fe] hydrogenase, small subunit
DVU1919	0.0758716	0.131277	1.6601	2.97915	hydrogenase expression/formati on protein, putati ve
DVU2247	0.182349	0.309314	2.77459	4.76038	anti oxidant, AhpC/Tsa family
DVU2441	0.940294	1.6699	4.01167	7.41549	heat shock protein, Hsp20 family
DVU2442	0.721891	1.40396	3.63666	6.7162	heat shock protein, Hsp20 family
DVU2986	0.956861	1.86726	2.4673	4.47924	phage shock protein C
DVU0813	-0.0624729	-0.122593	1.34659	2.15932	heat-inducible transcripti on repressor HrcA
DVU2106	0.319206	0.495961	2.09413	3.60732	sigma-54 dependent transcripti onal regulator
DVU2129	-0.208266	-0.35661	2.88207	3.03662	sensory box histi dine kinase/response regulator
DVU2189	0.15792	0.284175	1.36288	2.1807	transcripti onal regulator dI, putati ve
DVU2423	0.89753	1.65938	2.40786	3.60319	transcripti onal regulator, putati ve
DVU2827	0.352753	0.612143	1.41227	2.06048	sigma-54 dependent transcripti onal regulator
DVU3095	0.688018	1.24951	2.06753	2.86857	transcripti onal regulator, Fur family
DVU3297	0.347412	0.648736	1.33884	2.47668	tryptophan-specifi c transport protein
DVU3313	0.0751262	0.135381	2.32176	4.06618	transcripti onal regulator, LysR family

Fig. 5 The correlation between microarray data and RT-PCR data for detecting gene expression changes under salt stress

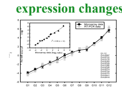


Table 3 Examples of down-regulated ORFs under long-term NaCl exposure but no significant changes under short-term NaCl exposure

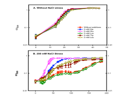
	Short-term NaCl exposure		Long-term NaCl exposure		
Locus tag	Ratio	Zscore	Ratio	Zscore	Annotation
DVU0189	-0.271622	-0.514763	-2.72175	-3.56399	phage/plasmid primase, P4 family
DVU0192	0.215571	0.424686	-1.69213	-2.88309	adenine specifi c DNA methyltransferase, putati ve
DVU0194	-0.342147	-0.589552	-2.46889	-3.82064	terminase, large subunit, putati ve
DVU0195	-0.109924	-0.206989	-1.43125	-2.36893	hypotheti cal protein
DVU0201	-1.02498	-1.84539	-2.8456	-3.57821	hypotheti cal protein
DVU0202	-1.07469	-1.95617	-2.70802	-3.28845	holin
DVU0214	-0.625818	-1.23073	-2.05834	-3.37688	tail/DNA circulati on protein, putati ve
DVU0216	-0.785014	-1.42542	-2.45481	-3.23594	phage baseplate assembly protein V, putati ve
DVU0217	-0.844964	-1.62192	-2.26391	-3.93748	tail protein, putati ve
DVU0218	-0.768425	-1.45918	-1.35436	-2.31798	tail protein, putati ve
DVU0992	-1.09603	-1.97737	-1.80829	-3.10264	chemotaxis protein CheV
DVU1593	0.734772	1.42466	-2.11874	-2.44204	chemotaxis protein CheY
DVU2585	-0.720215	-1.37516	-1.95132	-2.98183	methyl-accepti ng chemotaxis protein
DVU0305	-1.01269	-1.86858	-1.30296	-2.01242	ferredoxin II
DVU1570	-0.463957	-0.893221	-1.96091	-2.38622	pyruvate ferredoxin oxidoreductase, beta subunit
DVU1946	-0.354121	-0.685934	-1.72741	-2.07973	pyruvate ferredoxin oxidoreductase, beta subunit
DVU3349	-0.798089	-1.47416	-1.42986	-2.01549	pyruvate fl avodoxin/ferredoxin oxidoreductase
DVU2306	-1.021	-1.99179	-1.54907	-2.88716	phosphate transporter family protein
DVU2478	-0.798299	-1.41882	-1.97201	-2.65079	phosphate ABC transporter, permease protein
DVU1913	-0.389762	-0.766298	-2.36344	-2.7244	aspartate kinase, monofuncti onal class
DVU2983	1.00971	1.85838	-2.37213	-2.23063	3-isopropylmalate dehydratase, small subunit
DVU2984	0.975864	1.87158	-3.1365	-3.46474	conserved hypotheti cal protein
DVU2985	0.684439	1.34919	-1.5361	-2.54648	3-isopropylmalate dehydrogenase
DVU3133	-0.469317	-0.909555	-2.40934	-2.4024	glycerol uptake facilitator protein
DVU3134	-0.531008	-0.925009	-1.43954	-2.20885	glycerol kinase
DVU0258	-0.0169342	-0.0329078	-1.43068	-2.22621	sensory box histi dine kinase/response regulator
DVU0277	-0.171633	-0.331254	-1.39582	-2.29041	transcripti onal regulator, AraC family
DVU0653	-0.454123	-0.742163	-1.99882	-2.18976	sigma-54 dependent transcripti onal regulator
DVU0680	-0.107829	-0.191693	-2.04383	-3.92218	sensory box histi dine kinase
DVU0722	-0.686194	-1.07214	-3.17096	-5.43092	response regulator
DVU2677	-0.0194285	-0.0345315	-1.72208	-2.117	sensor histi dine kinase/response regulator
DVU3131	-0.232002	-0.446407	-1.94004	-3.12429	transcripti onal regulator, putati ve

Table 4 *D. vulgaris* cells grew at LS4D medium with or without 250 mM NaCl

Amino acid	Control (pMol/ml, mean±sd, n=5)	NaCl (pMol/ml, mean±sd, n=5)	NaCl/control
Glutamate	1144.41 ± 70.85	9248.83 ± 894.14	8.08
Histidine	8.31 ± 0.23	22.81 ± 4.24	2.74
Serine	145.25 ± 13.15	283.41 ± 28.55	1.95
Betaine	156.18 ± 9.35	299.44 ± 64.69	1.92
Lysine	17.137 ± 1.27	31.62 ± 6.19	1.85
Glutamine	404.88 ± 42.01	723.87 ± 56.45	1.79
Alanine	1104.17 ± 102.68	1958.00 ± 434.82	1.77
Leucine	56.07 ± 1.57	88.02 ± 6.05	1.57
Threonine	162.079 ± 9.40	247.04 ± 18.78	1.52
Proline	163.60 ± 20.24	226.95 ± 39.22	1.39
Aspartate	827.85 ± 53.81	1139.67 ± 126.50	1.38
Glycine	989.77 ± 190.33	1152.78 ± 352.48	1.16
Isoleucine	76.96 ± 4.35	80.64 ± 1.93	1.05
Methionine	5.60 ± 0.32	5.72 ± 0.93	1.02
Arginine	105.35 ± 3.56	99.08 ± 3.13	0.94
Valine	602.11 ± 159.71	479.96 ± 127.54	0.80
Tyrosine	23.92 ± 0.34	14.88 ± 0.34	0.62
Phenylalanine	1.47 ± 0.00	0.84 ± 0.00	0.57
Tryptophan	0.54 ± 0.00	0.30 ± 0.00	0.55
Asparagine	ND	209.01 ± 45.89	

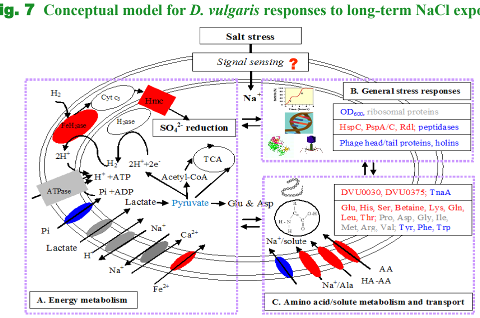
- **Glu and Ala significantly increased and intracellularly accumulated to ~9.2 and 2.0 µM, which may function as osmoprotectants.**
- **The results were consistent with microarray data and amino acid accumulation data (Table 3) in response to salt stress.**

Fig. 6 *D. vulgaris* cells grew at LS4D medium with or without 250 mM NaCl



- **Growth was not affected for *D. vulgaris* cells without NaCl addition.**
- **Glu, Ala, Leu and Trp significantly relieved the NaCl inhibition of *D. vulgaris* cells.**

Fig. 7 Conceptual model for *D. vulgaris* responses to long-term NaCl exposure



SUMMARY

- **Glu and Ala may be used as potential osmotic protection solutes in DvH under salt stress conditions.**
- **Electron transport flows were induced while carbon metabolism was repressed under salt stress conditions.**
- **Gene expression had a similar trend but also showed differences under short- and long-term exposure of *D. vulgaris* to NaCl.**
- **Many function-unknown genes were identified to be associated with salt tolerance, indicting salt-tolerance mechanisms are largely unknown.**

ACKNOWLEDGEMENT

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