Title
Genomewide Gene Expression Analysis of Response of Desulfovibrio vulgaris to High pH

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K-050 Genomewide Gene Expression Analysis of Response of *Desulfovibrio vulgaris* to High pH.

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ABSTRACT

As a part of the DOE Genomics:GTL initiative evaluating the use of microbial stress response to monitor the status of environmental systems, we studied the effect of exposure to high alkaline (pH10) media on exponentially growing *Desulfovibrio vulgaris* Hildenborough (*DvH*) cells. Any given Gram-negative bacterium under high pH stress is challenged by mainly three factors: intracellular alkalinized pH, diminished membrane potential and misfolded degrading proteins. To maintain viability in this stressful state, the cell could transport protons or acids into the cell, synthesize compounds that acidify the cytoplasm, activate systems for protein repairing, or increase degradation of denatured proteins. In our experiments, several genes reported to be upregulated in *E. coli* at high pH were also upregulated in *DvH*. These include three ATPase genes and a tryptophan synthase gene. As in *E. coli*, genes involved in flagella synthesis were downregulated during pH 10 stress. *DvH* also upregulated chaperone and protease genes such as dnaK and. an ATP-dependent Clp protease, an ATP-dependent protease La. Some energy production genes were consistently downregulated at high pH. These include pyruvate carboxylase, desulfoferredoxin, and ferredoxin II. Finally, the microarray data revealed a potential *DvH* specific pH homeostasis mechanism. In *DvH* but not other *Deltaproteobacteria* or *E. coli*, the Na^+/H^+ antiporter nhaC and a putative L-aspartate oxidase gene are adjacent in the genome and likely to be in the same operon. Both of these genes were upregulated in *D. vulgaris* during high pH stress. Thus, part of the *DvH* response to high pH stress appears to involve coupling pumping of protons into the cell with conversion of L-aspartate to oxaloacetate to acidity the cytoplasm.

EXPERIMENTAL APPROACH

D. vulgaris Microarray Manufacturing. 70 mer oligo probes were designed using a software ArrayOligoSelector (Zhu 2002). The whole genome microarray covered 3574 ORFs. Oligo probes were spotted onto UltraGAPSTM coated glass slides (Corning Life Science, NY) using a Microgrid II arrayer (Genomic Solution Inc., MI).

Cell Growth and High pH Upshift Conditions. *D. vulgaris* cells were grown in LS4D medium supplemented with lactate and sulfate. pH was adjusted to 10 by addition of KOH when culture reached middle exponential growth phase. Cells were harvested for RNA isolation after 30 and 60, 120 and 240 minutes.
To test growth rate of mutants at different pH, cells were grown in B3 medium (0.1g NaCl, 0.1g of $MgCl_2$•6H20, 0.1g $CaCl_2$•2H20, 0.5g NH_4Cl, 0.1g KCl, 1.4g of Na_2SO_4, 0.001g of Resazurine, 1ml of 1M K_2HPO4, 1ml of Trace Minerals, 1ml of Thauer's Vitamins, 1ml of 1M Cysteine and 1ml of 1M Na_2S in one liter) with lactate and sulfate using the following buffering agents: sodium bicarbonate for pH 7; and glycine for pH 8.0 and 9.0.

RNA Isolation and Microarray Hybridization. Total cellular RNA was isolated from cell cultures using the Trizol procedure (BRL), treated with RNase-free DNase I, and purified using RNeasy columns (Qiagen). cDNA was synthesized using total RNA (10 µg) as the template and fluorescently labeled with Cy5-dUTP in a reverse transcription reaction. Genomic DNA was labeled with Cy3-dUTP. Labeled cDNA and genomic DNA was then hybridized to the *D.vulgaris* whole-genome array at 50°C overnight in the presence of 50% formamide.

Microarray Analysis. Arrays were scanned using the scanning laser confocal fluorescence microscope of the ScanArray® Microarray Analysis System (GSI Lumonics), and hybridization signal intensities were quantitated using the software of ImaGene™ (Biodiscovery). Statistical analysis of the microarray data was performed using ArrayStat and cluster analysis was performed using TIGR MultiExperiment Viewer (MeV).

RESULTS

Figure1. K-means cluster analysis of gene expression pattern for all genes.

Table 1. Genes significantly upregulated in pH upshift stress (cluster 2)

Gene ID		Log2 ratio 30min	60 min	120min	240 min	Gene description
ORF04473	DVU3301	0.88	3.36	1.53	3.70	hypothetical protein
ORF04472	DVU3300	0.68	2.27	0.84	3.57	hypothetical protein
ORF00217	DVU0774	1.77	2.57	2.20	3.21	ATP synthase, F1 epsilon subunit (atpC)
ORF04773	DVU0086	1.18	0.84	1.88	3.00	hypothetical protein
ORF04091	DVU3081	0.27	2.38	1.35	2.64	integral membrane protein, putative
ORF04144	DVU3110	0.91	1.19	1.35	2.45	L-aspartate oxidase, putative
ORF04771	DVU0085	1.36	1.90	1.75	2.45	tryptophan synthase, beta subunit (trpB)
ORF02910	DVU2368	1.64	1.90	1.87	2.42	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase (fabZ)
ORF01110	DVU1314	ND	1.20	1.41	2.41	ribosomal protein L24 (rplX)
ORF01122	DVU1322	1.51	1.64	1.80	2.34	ribosomal protein L15 (rplO)
ORF02019	DVU1858	1.56	1.68	1.58	2.31	'Cold-shock' DNA-binding domain protein
ORF03992	DVU3028	1.57	2.07	1.92	2.30	glycolate oxidase iron-sulfur subunit,
ORF05070	DVU0258	0.96	0.95	1.24	2.28	sensory transduction histidine kinase-related
ORF00219	DVU0775	1.11	1.55	1.63	2.27	ATP synthase, F1 beta subunit (atpD)
ORF00220	DVU0776	0.87	1.60	1.13	2.26	ATP synthase, F1 gamma subunit (atpG)
ORF05195	DVU0331	1.36	1.18	1.83	2.23	putative histidine protein kinase
ORF03854	DVU2946	1.98	2.45	2.10	2.16	hypothetical protein
ORF04468	DVU3298	1.38	1.86	1.09	2.15	hypothetical protein
ORF04140	DVU3108	1.36	1.60	1.41	2.10	Na+/H+ antiporter NhaC (nhaC)
ORF00918	DVU1198	1.94	2.05	1.59	2.03	riboflavin synthase, beta subunit (ribH)
ORF00078	DVU0693	1.09	1.52	1.48	2.01	respiratory nitrate reductase, beta subunit (narH)
ORF03173	DVU2526	1.68	1.24	1.42	1.98	periplasmic (nife) hydrogenase large subunit precursor
ORF00079	DVU0694	2.17	1.91	1.01	1.97	molybdopterin oxidoreductase
ORF00354	DVU0855	1.64	1.76	1.60	1.96	coenzyme pqq synthesis protein, putative
ORF03647	DVU2816	1.04	1.80	1.87	1.95	efflux system protein
ORF04988	DVU0211	1.32	1.68	1.02	1.94	conserved hypothetical protein
ORF04508	DVU3325	1.27	2.13	2.12	1.94	hypothetical protein
ORF04586	DVU3371	0.54	2.18	1.55	1.87	mutE
ORF05148	DVU0303	1.58	1.87	2.05	1.82	hypothetical protein
ORF00660	DVU1035	1.09	2.03	2.11	1.82	glucokinase (glk)
ORF01117	DVU1319	1.66	1.64	1.34	1.80	ribosomal protein L18 (rplR)
ORF03251	DVU2572	1.25	1.36	1.80	1.76	ferrous iron transport protein A, putative
ORF00041	DVU0667	2.28	1.52	1.74	1.69	HD domain protein
ORF01094	DVU1304	1.53	1.67	1.46	1.66	ribosomal protein L4/L1 family
ORF02762	DVU2283	1.24	1.56	1.64	1.64	hypothetical protein
ORF02913	DVU2370	1.58	1.80	1.62	1.64	outer membrane protein OmpH, putative
ORF01324	DVU1446	2.98	2.30	2.56	1.63	Heptosyltransferase family
ORF05481	DVU0493	1.47	1.81	1.45	1.61	hypothetical protein
ORF05084	DVU0265	1.61	2.08	1.54	1.56	25.3 kd protein in hmc operon
ORF04004	DVU3035	1.41	1.74	1.31	1.56	methyl-accepting chemotaxis protein, putative
ORF01203	DVU1370	1.73	1.83	1.60	1.45	hypothetical protein

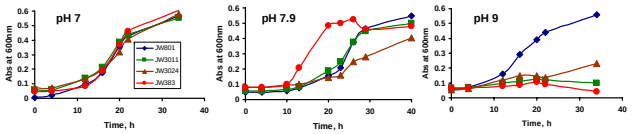
Figure 2. Gene organization of several chromosomal segments containing genes up regulated at high pH. Color of the contour lines indicates up regulation ; down regulation ; unchanged

Gene ID #	Protein assigned function	Cellular process
DVU3108	Na/H antiporter	Ion transport
DVU3109	Fe-S cluster binding	Electron transfer
DVU3110	L-aspartate oxidase	Amino acid metabolism
DVU3111	cAMP-binding proteins - catabolite gene activator	Gene regulation
DVU3112	Flp pilus assembly protein TadD, contains TPR repeats	Motility
DVU3113	carbamoyl-phosphate synthase, small subunit	Amino acid metabolism
DVU3114	3-deoxy-manno-octulosonate cytidylyltransferase	Lipopolysaccharide biosynthesis

Gene ID #	Protein assigned function	Cellular process
DVU2579	contains TPR repeats	Signal transduction
DVU2580	histidine kinase	Signal transduction
DVU2581	response regulator, CheY like reciever	Signal transduction
DVU2582	regulator, TetR family	Signal transduction
DVU2583	lipoprotein	Cell envelope biogenesis

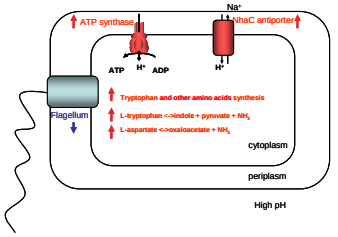
Gene ID #	Protein assigned function	Cellular process
DVU0330	response regulator	Signal transduction
DVU0331	histidine kinase	Signal transduction
DVU0332	hypothetical protein	
DVU0333	hypothetical protein	
DVU3334	D-alanine--D-alanine ligase	Cell envelope biogenesis
DVU0335	3-deoxy-D-manno-octulosonic-acid transferase, putative	Cell envelope biogenesis

Figure 3. Effect of pH on growth of *D. vulgaris* mutants



JW801 – wt; JW 3011- Tn insertion in DVU2580; JW 3024 - Tn insertion in DVU0331; JW 383 – deleted DVU 3108

Fugure 3. Conceptual model of *D. vulgaris* high pH stress response



CONCLUSIONS

• *D. vulgaris* response to high pH stress is somewhat similar to that in *E. coli* and other bacteria. ATPase, tryptophan synthase (DVU0085), tryptohanase TnaA (DVU 2204), one nhaC Na^+/H^+ antiporter (DVU3108) have been found to be upregulated showing commonality in pH stress response of DvH and *E.coli*. In addition to that, genes for some chaperones and proteases such as dnaK (DVU0811), ATP-dependent Clp protease (DVU1874), and DVU3303, coding for ATP-dependent protease La were also upregulated.

• Our data suggested that reactions involved in amino acid metabolism are part of the defense mechanism against high pH upshift. However, the exact mechanism of protection needs to be elucidated.

• Impaired growth of mutants JW3011, JW3024, JW3024 demonstrated that proton-sodium antiporter NhaC (DVU3108) and two regulators (DVU 311 and DVU 2580) are involved in high pH stress response of *D. vulgaris*

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