Analysis of the Heat Shock Response in *Desulfovibrio vulgaris* through Global Proteomics and Transcriptomics Studies.

Swapnil Chhabra[1], Qiang He[2], Zhili He[2], Sara Gaucher[1], E. Alm[3], M. Hadi[1], A. Arkin[3], T. Hazen[3], J. Zhou[4], A. K. Singh[1];
[1]Sandia National Labs, Livermore, CA, [2]Oak Ridge National Labs, Oak Ridge, TN, [3]Lawrence Berkeley National Labs, Berkeley, CA, [4]Oakridge National Labs, Oak Ridge, TN.

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Abstract

Sulfate reducing bacteria (SRB) have the unique capability of using sulfate as the final electron acceptor in respiration. Among Eubacterial SRBs, members of the genus *Desulfovibrio* have been extensively studied. *Desulfovibio* spp. have been shown to reduce toxic metals (such as uranium) to water insoluble species making them excellent model systems for the study of bioremediation of contaminated ground waters or soils. An understanding of regulatory networks in the SRB will thus be important in utilizing these organisms for various bioremediation applications. As a part of studying stress response behaviour in *Desulfovibrio vulgaris* - a model SRB with a fully sequenced genome, we investigated the heat shock response in this organism. *D. vulgaris* cell cultures grown on LS media through mid-log phase (60ml) were heat shocked at 50°C for up to 2h and cell samples were collected at the following time intervals (min): 0,15,30,60,90 and 120. A control culture remained unperturbed for the same time period. Three biological replicates each for the control and variable samples were generated and the resulting cell mass was processed to obtain total RNA or total protein for subsequent analysis. The total protein for the 60 min time point was analyzed through Differential-In-Gel-Electrophoresis (DIGE) while the transcriptome was analyzed for all time points through whole-genome micro-arrays. The proteomics analysis revealed overproduction of major heat shock proteins including HSP70, HSP60 and HSP10 as well as other chaperones. Data analyses correlating gene expression data from whole genome-microarrays to protein production data from DIGE is presented here.
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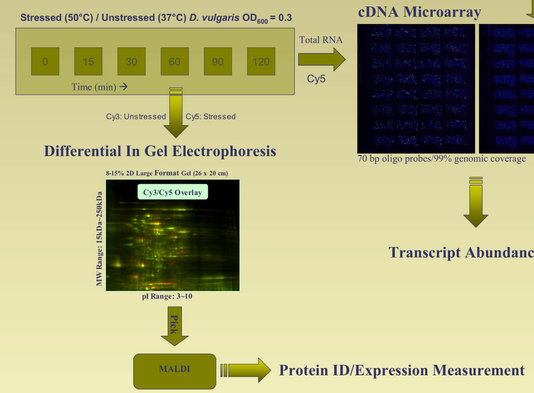
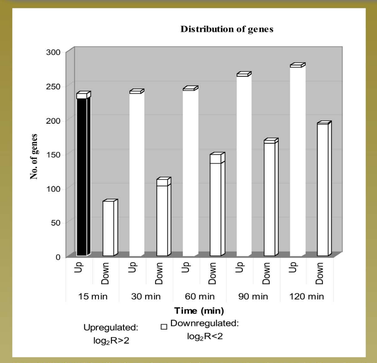
The Heat Shock Experiment



D. vulgaris cell cultures (V) (60ml) were heat shocked at 50°C for 60 min. A time dependent study was performed for measurement of the transcript abundance. A control culture (C) remained unperturbed at each time point. Transcriptional response corresponding to a Z score of 1.5 or greater varied from 1158 genes at 15 min (*t1*) through 1490 genes at 120 min (*t5*). This included 21 megaplasmid genes at *t1* and 11 megaplasmid genes at *t5*. A time dependent plot of total number of genes with Z≥1.5 and -2≥$\log_2$ R≥2 is plotted in Fig. 1. Three biological replicates each for the control and variable samples were used in this analysis. For the proteomics experiment biological replicates at *t3* were pooled together. Three technical replicates (Gel17, Gel35 and Gel46) of this biological pool were analyzed through DIGE. Cy-dye analysis of the resulting 2-D gels indicated a roughly 1500 spots for each gel. Based on Cy3/Cy5 ratio estimates for individual gels different number of spots were picked from all three gels. MALDI-MS data was manually acquired for 270 spots originating from Gel17; 106 and 173 spots from Gels 35 and 46 respectively. MASCOT analysis of the resulting spectra resulted in ~32 spots with significant scores of 60 or above and are listed in Table 2. A few proteins including DnaK (DVU0811), GroES (DVU1976) and GroEL (DVU1977) were observed in number of closely clustered spots suggesting the presence of post translational modifications.

Transcript Abundance Measurements



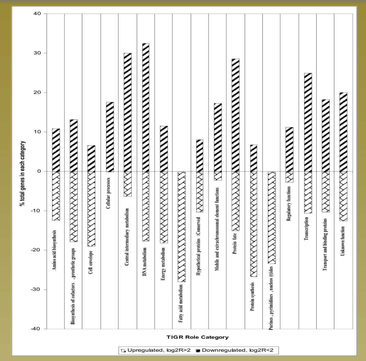
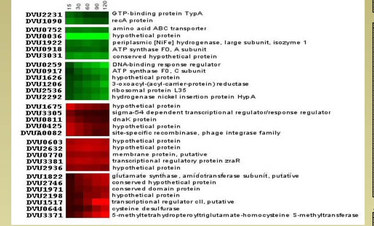


Fig. 1 (Left): Distribution of up- and down-regulated genes in *D. vulgaris* Hildenborough as a function of time upon a temperature up-shift of 13°C. Only those genes with Z>1.5 and -2>$\log_2$R>2 were included in the plot. Grey region at the top of each data bar corresponds to genes from the megaplasmid.
Fig. 2 (Right): Distribution of up- and down-regulated genes in *D. vulgaris* Hildenborough at t=15 min upon a temperature up-shift of 13°C based on TIGR Role category. Numbers of genes up- or down-regulated were plotted as a percentage of the total number of genes from the genome assigned to that particular category. Only those genes with Z>1.5 were included in the plot.



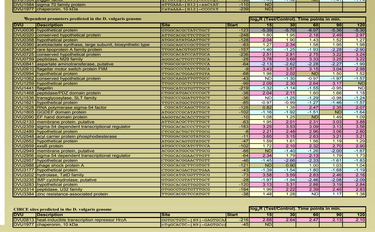


Fig. 3 (Left): Hierarchical clustering of up- and down-regulated genes in *D. vulgaris* Hildenborough from t=15 min to t=120 min upon a temperature up-shift of 13°C. Only those genes with Z>1.5 at all time points and at least one time point meeting the criteria -2>$\log_2$R>2 were included in the clustering analysis. Sample clusters displaying similar time-dependent or independent patterns are shown in greater detail with the corresponding gene annotations – on the right.
Table 1 (Right): Computational predictions of σ–32 (Ref), σ–54 promoters and CIRCE sites in the *D. vulgaris* genome. Log_2R values for available time points are indicated for the corresponding genes. Data shown only for Z>1.5. ND indicates data not available.

Protein Expression vs. Transcript Abundance

DVU	Description	Expression Ratio Protein	Expression Ratio *m*RNA
DVU0160	carbohydrate isomerase, KpsF/GutQ family	4.16	2.33
DVU0811	dnaK protein	6.57	1.63
DVU0847	adenylyl-sulphate reductase, alpha subunit	-2.92	-1.79
DVU0910	flagellar motor switch protein FliM	3.08	3.15
DVU1468	peptidase/PDZ domain protein	3.92	1.60
DVU1636	inorganic pyrophosphatase, manganese-dependent	-4.62	-1.69
DVU1772	pyridine nucleotide-disulfide oxidoreductase	-4.41	-1.18
DVU2247	alkyl hydroperoxide reductase C	6.64	1.83
DVU2548	acyl carrier protein phosphodiesterase	4.28	2.63
DVU2643	heat shock protein HtpG	2.75	1.33
DVU2744	high-affinity branched-chain amino acid ABC transporter	5.00	2.55
DVU0095	polyamine ABC transporter, periplasmic polyamine-binding protein	5.94	-1.62
DVU0177	molybdenum ABC transporter, periplasmic molybdenum-binding protein	3.38	-2.33
DVU0712	amino acid ABC transporter, periplasmic-binding protein	2.73	-1.31
DVU0745	ABC transporter, periplasmic substrate-binding protein	3.97	-2.24
DVU0966	amino acid ABC transporter, periplasmic amino acid-binding protein	2.88	-2.70
DVU1932	adenylate kinase	2.62	-3.87
DVU2667	phosphate ABC transporter, periplasmic phosphate-binding protein	3.53	-1.72
DVU3245	transcription elongation factor GreA	-3.49	2.11
DVU0322	enolase	3.20	ND
DVU0386	amino acid ABC transporter, periplasmic amino acid-binding protein	3.87	ND
DVU0415	cytosol aminopeptidase	18.05	ND
DVU0750	methyl-accepting chemotaxis protein	3.87	ND
DVU0851	hypothetical protein	-2.78	ND
DVU0978	ABC transporter, periplasmic substrate-binding protein, putative	3.20	ND
DVU1976	chaperonin, 60 kDa	4.90	ND
DVU1977	chaperonin, 10 kDa	4.03	ND
DVU2138	conserved hypothetical protein	2.85	ND
DVU2427	hypothetical protein	2.52	ND
DVU2649	hypothetical protein	2.65	ND
DVU3061	sensory box histidine kinase	4.40	ND
DVU3150	ribosomal protein S1	3.00	ND

Table 2: Comparison of *m*RNA transcript abundance with corresponding protein levels. Data compared for *D. vulgaris* cell cultures – heat shocked vs control samples (60 min). Protein levels were determined from Differential In-Gel Electrophoresis of total soluble protein while transcript abundance was determined from cDNA Microarray analysis of *D. vulgaris* cell cultures under stressed and non-stressed conditions. Proteins identified by MASCOT search analysis with MOWSE score ≥ 60. Transcript abundance ratios reported for z>1.5. ND indicates data not available

Heat Shock Response in *D. vulgaris* vs. other Gram +/– Bacteria

COG	Function	*D. vulgaris* Hildenborough		*B. subtilis* MO945		*E. coli* K12		*S. oneidensis* MR-1	
COG463	Glycosyltransferases involved in cell wall biogenesis, *cshB*								
		DVU3013	-2.08	Bsu1975	-0.99	b2254	-2.65	SO3180	ND
		DVU1892	-0.96	Bsu3552	1.17	b2351	-1.89	SO4868	ND
				Bsu0859	2.79				
COG28	Thiamine pyrophosphate requiring enzymes, *ydaP*								
		DVU0360	1.27	Bsu2827	-1.96	b3671	1.85	SO2279	ND
				Bsu3599	3.7			SO3262	ND
								SO4347	ND
COG39	Malate/lactate dehydrogenases, *ldh*								
		DVU0600	-2.46	Bsu0306	2.98	b3236	ND	SO0770	-0.96
COG63	Predicted sugar kinase, *yokO*								
		DVU1910	-0.99	Bsu3868	3.45	b4167	ND	SO0598	ND
COG280	Phosphotransacetylase, *yqiS*								
		DVU3029	-3.77	Bsu3763	-1.11	b0297	ND	SO2916	1.01
						b2458	ND		
COG394	Protein-tyrosine-phosphatase, *ydLI*								
		DVU1646	1.26	Bsu0788	2.62	b0982	ND	SO0533	ND
						b2061	ND	SO2208	ND
COG526	Thiol-disulfide isomerase and thioredoxins, *trxA*								
		DVU1586	-1.03	Bsu2846	1.32	b2195	ND	SO0476	0.9
				Bsu1425	1.03	b2582	ND	SO0406	1.94
						b3781	ND	SO0462	3.51
COG557	Exoribonuclease R, *vvsJ*								
		DVU2467	1.92	Bsu3358	1.13	b4179	ND	SO3935	ND
COG589	Universal stress protein UspA and related nucleotide-binding proteins, *yhaX*								
		DVU1030	1.03	Bsu0969	4.42	b0607	ND	SO2355	-0.99
COG693	Putative intracellular protease/amidase, *yfkM*								
		DVU1933	-1.22	Bsu2698	-1.53	b1967	2.46	SO1378	ND
								SO1887	ND
COG753	Catalase, *katB*								
		DVUA0091	-1.11	Bsu3859	4.1	b1732	ND	SO1070	ND
				Bsu3901	4.44				
COG1012	NAD-dependent aldehyde dehydrogenases, *aldY*								
		DVU3294	0.93	Bsu1931	0.82	b0312	ND	SO4480	1.01
				Bsu3982	0.91	b1300	ND	SO3683	1.87
				Bsu3879	5.61	b1385	ND	SO1678	-2.14
				Bsu0392	2.73	b1387	ND		

Table 3.1

COG	Function	*D. vulgaris* Hildenborough		*B. subtilis* MO945		*E. coli* K12		*S. oneidensis* MR-1	
COG443	Molecular chaperone, *dnaK*								
		DVU0811	2.94	Bsu2543	1.48	b0014	2.35	SO1126	2.4
COG484	DnaJ-class molecular chaperone with C-terminal Zn finger domain, *dnaJ*								
		DVU1876	0.83	Bsu2542	0.83	b0015	2.91	SO1127	1.96
		DVU3243	1.57						
COG576	Molecular chaperone GroES (heat shock protein), *grpE*								
		DVU0812	1.83	Bsu2544	1.35	b2614	2.04	SO1524	2.63
COG1420	Transcriptional regulator of heat shock genes, *hrcA*								
		DVU0813	2.68	Bsu2545	2.23	***NH***		***NH***	
COG2264	Ribosomal protein L11 methylase, *yqe7*								
		DVU2329	-1.30	Bsu2541	0.8	b3259	ND	SO0395	ND

Table 3.2

COG	Function	*D. vulgaris* Hildenborough		*B. subtilis* MO945		*E. coli* K12		*S. oneidensis* MR-1	
COG542	ATPases with chaperone activity, ATP-binding subunit, *clpC*								
		DVU1602	2.8	Bsu0086	3.04	b0882	2.73	SO3577	2.86
		DVU1874	4.24						

Table 3.4

COG	Function	*D. vulgaris* Hildenborough		*B. subtilis* MO945		*E. coli* K12		*S. oneidensis* MR-1	
COG57	Glyceraldehyde 3-phosphate dehydrogenase, *gap*								
		DVU0665	-1.62	b1416	ND	Bsu3391	-1.17	SO2347	-2.64
				b1779	ND			SO0538	1.07
				b2927	ND			SO2345	2.15
COG71	Molecular chaperone (small heat shock protein), *ibpA/B*								
		DVU2442	3.66	b3686	5.13	Bsu1798	1.59	SO2277	3.14
				b3687	5.1				
COG265	Trypsin-like serine proteases, typically periplasmic, *degQ, htrA*								
		DVU1468	2.04	b0161	ND	Bsu4033	2.36	SO3942	2.89
				b3234	ND			SO3943	1.48
				b3235	ND				
COG293	23S rRNA methylase, *rlmJ*								
		DVU2260	-1.65	b3179	1.92	***NH***		SO1196	2.5
COG326	Molecular chaperone, HSP90 family, *htpG*								
		DVU2643	2.63	b0473	ND	Bsu3978	1.59	SO2016	2.64
COG465	ATP-dependent Zn proteases, *hflB*								
		DVU1278	2.53	b3178	1.13	Bsu1243	-1.48	SO1197	1.91
COG466	ATP-dependent Lon protease, bacterial type, *lon*								
		DVU1191	2.02	b0439	1.86	Bsu2816	ND	SO1796	2.11
		DVU1337	1.77						
COG501	Zn-dependent protease with chaperone function, *htpX*								
		DVU2494	3.44	b1829	1.87	Bsu1029	ND	SO0110	-1.43
						Bsu1351	ND		
						Bsu3874	ND		
COG568	DNA-directed RNA polymerase, sigma subunit (sigma70/sigma32), *rpoHD2*								
		DVU1788	1.76	b3067	1.66	Bsu2517	ND	SO4583	1.46
				b3461	1.72			SO1284	1.3
COG1220	ATP-dependent protease HslVU (ClpYQ), ATPase subunit, *hslU*								
		DVU1467	1.92	b3931	1.82	Bsu1617	ND	SO4163	2.76
COG5405	ATP-dependent protease HslVU (ClpYQ), peptidase subunit, *hslV*								
		DVU1577	1.84	b3932	2.36	Bsu1616	ND	SO4162	2.96
COG1028	Dehydrogenases with different specificities, *ycdF*								
		DVU1206	-1.44	Bsu0284	6.01	b0596	ND	SO0772	1.34
				Bsu0419	4.55	b1093	ND	SO1683	-1.7
				Bsu0945	3.86	b1271	ND	SO1873	1.02
				Bsu1043	3.96	b1606	ND	SO2776	-1.48
				Bsu1592	-0.89	b1619	ND		
				Bsu1688	1.14	b2137	ND		
				Bsu2214	-0.85	b2426	ND		
				Bsu2938	0.93	b2541	ND		
				Bsu3316	1.12	b2705	ND		
				Bsu3358	3.3	b2774	ND		
				Bsu3893	1.01	b2842	ND		
				Bsu3980	4.26	b2902	ND		
COG1309	Transcriptional regulator, *ydgC*								
		DVU1690	-1.67	Bsu2851	-1.07	b0313	ND	SO3277	1.12
		DVU1760	0.83	Bsu0558	1.23	b0464	ND	SO3494	1.03
				Bsu2713	2.82	b0796	ND	SO3684	2.51
						b1013	ND	SO4326	0.8
						b1618	ND	SO4468	1.7
						b3264	ND	SO1578	0.92
COG1366	Anti-anti-sigma regulatory factor, *yshA*								
		DVU1449	-1.23	Bsu2474	2.84	***NH***		*ND*	
COG1442	UDP-D-glucose (galactose/glucose) saccharide glucosyltransferase), *gspA*								
		DVU2971	0.95	Bsu3839	4.19	b3626/27	ND	*NH*	
COG1544	Ribosome-associated protein Y (PSrp-1), *yvyD*								
		DVU1629	1.9	Bsu3528	1.26	b2597	ND	SO3403	2.42
						b3203	ND	SO3422	2.44
COG1670	Acetyltransferases, including N-acetyltases of ribosomal proteins, *yobA*								
		DVU2486	2.18	Bsu2156	-0.99	b1427	ND	*ND*	
				Bsu0421	0.93	b1584	ND		
				Bsu1853	2.07				
COG1734	DnaK suppressor protein, *yocK*								
		DVU0878	1.03	Bsu1923	3.53	b0145	ND	*ND*	
COG1825	Ribosomal protein L25 (general stress protein Ctc), *ctc*								
		DVU1574	-1.91	Bsu0052	2.94	b2185	ND	*ND*	
COG2217	Cation transport ATPase, *yvgW*								
		DVU2324	4.55	Bsu3346	2.97	b0484	ND	*ND*	
		DVU3332	2.3			b3469	ND		
COG2814	Arabinose efflux permease, *bmr*								
		DVUA0096	2.62	Bsu3393	0.82	b1981	-1.83	SO2389	1.34
				Bsu0740	0.98	b1528	-2.04		
				Bsu3754	1.17	b2587	-2.3		
				Bsu3506	1.16				
				Bsu3143	1.17				
				Bsu1854	1.54				
				Bsu0854	1.56				
				Bsu4081	1.63				
				Bsu3977	3.93				
				Bsu3581	4.05				

Table 3.3

Table 3: Comparison of transcriptional response ratios ($\log_2$R) during early heat shock conditions – *D. vulgaris* (15 min.), *E. coli* (5 min.), *B. subtilis* (3 min.) and *S. oneidensis* (5 min.). Genes chosen for comparisons belong to the heat shock regulon in *E. coli* (Table 3.1) and Class I ~ IV (Table 3.2~3.4) heat shock genes from *B. subtilis*. Data shown only for Z>1.5. ND indicates data not available. ***NH*** indicates no homolog in that organism corresponding to the specific COG.

Summary

We present in this poster, a global overview of the heat shock response in *D. vulgaris*. The recently published genome sequence of *D. vulgaris* reveals the presence of positive regulatory features found in in the gram negative bacterium *E.coli* and negative regulatory features found in the gram positive bacterium *B. subtilis* during heat shock. Whereas the negative regulatory feature involving the *hrcA* operon, responds as expected, responses of operons/regulons promoted by Sig32 seemed unclear. Although, *D. vulgaris* shares a large number of genes that respond similarly in both *E. coli* and *B. subtilis* during heat shock, it seems to possess unique control mechanisms for up- or down- regulation of these genes. An interesting observation includes genes promoted by Sig54 that displayed both up- and down- regulation. The protein expression data correlated well with the transcript abundance ratios for the commonly observed chaperones/proteases during heat shock conditions. These include the chaperones, DnaK (DVU0811). HtpG (DVU2643), and the peptidase, DVU1468. Most of the proteins for which expression data didn't correlate with the corresponding gene up/down regulation data included periplasmic ABC transporter type proteins. In addition proteomics revealed expression patterns for genes for which statistically significant data for the corresponding transcript did not exist. Notable among these were the chaperones GroES (DVU1976) and GroEL (DVU1977) for which no transcript data was available.

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