

Evaluation of stress responses in sulfate-reducing bacteria through genome analysis: identification of universal responses

J.D. Wall¹, H.-C. Yen¹, E.C. Drury¹, A. Mukhopadhyay², S. Chhabra³, Q. He^{4,7}, M.W. Fields⁴, A. Singh³, J. Zhou^{4,7}, T.C. Hazen², and A.P. Arkin⁶





Abstract

The model bacterium, *Desulfovibrio vulgaris* Hildenborough, for which the genome sequence has been fully determined, is being examined for its responses to a variety of stresses that may be expected to be encountered in natural/contaminated settings. We have examined the preliminary transcriptional data from ten treatments to learn whether there are general responses or common themes for responses to stresses by D. vulgaris. This anaerobe apparently does not have an ortholog encoding RpoS implicated in the universal stress response in y-Proteobacteria. Interestingly genes predicted to be controlled by the global regulator Fur appear to be among the most frequently responsive in the genome. The transcriptional responses to increased concentrations of sodium and potassium overlapped strongly, as would be predicted. Curiously, it was not predicted that these salt responses would be shared by the response to reduced temperature. Also counter to our prediction, the response to nitrate was not a simple sum of the responses to sodium and intrite. Further insights into general patterns of transcription during stresses wild be discussed.

Stresses

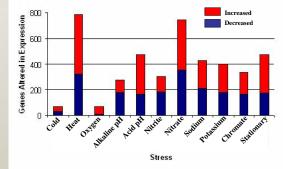
 Table 1. Stressors examined for transcriptional responses

 in D. vulgaris Hildenborough

Stressor	Concentration or Condition	Time (min)	Comparison culture
Cold	8 C	240	30 C, 240 min
Heat	50 C	15	37 C, 15 min
Oxygen	0.1 %	240	No O ₂ , 240 min
Alkaline pH	pH 10	120	pH 7, 0 min
Acid pH	pH 5.5	240	pH 7, 0 min
Nitrite	2.5 mM	60	No NO ₂ ⁻ , 60 min
Nitrate	105 mM	240	No NO ₃ ⁻ , 240 min
Sodium	250 mM	120	No added Na+, 120 min
Potassium	250 mM	120	No added K+, 120 min
Chromate	0.55 mM	120	No CrO ₄ ⁼ , 0 min

Results

Figure 1. Numbers of differentially expressed genes (abs $(Z) \ge 2$) for each stress



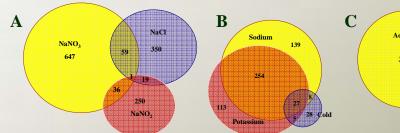
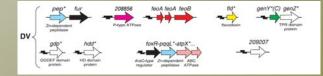


Table 2. *D. vulgaris* Hildenborough transcriptional responses of representative genes in energy metabolism

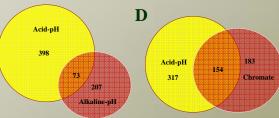
SO4 ⁼ Reduction			Stress										
ORF No. Gene Description		Cold	Heat	0,		pH5.5	ess NO ₂	NOv	NaCl	KCI	CrO4		
				Heat	02	pH10		NO ₂	NO ₃	NaCi	KCI	CrO ₄	
DVU0053	NA°	Sulphate permease, putative	1.38				-1.15						
DVU0279	NA	Sulphate permease family protein			1.09			-1.60		2.28	1.85		
DVU1295	sat	Sulphate adenylyltransferase							1.10				
DVU1566	cysD	PAPS reductase, putative		1.99			1.54		2.98				
DVU1597	sir	Sulphite reductase, assimilatory-type							1.61				
DVU0847	apsA	Adenylyl-sulphate reductase, alpha subunit		-1.71					1.73				
DVU0846	apsB	Adenylyl-sulphate reductase, beta subunit		-2.11					1.21				
DVU0402	dsrA	Dissimilatory sulphite reductase A, alpha		-3.28									
DVU0403	dvsB	Dissimilatory sulphite reductase B, beta		-2.59									
DVU0404	dsrD	Dissimilatory sulphite reductase D		-2.96			1.78					1.81	
DVU2776	dsrC	Dissimilatory sulphite reductase C, gamma											
DVU1286	dsrP	Integral membrane protein						-1.64					
DVU1287	dsr0	Periplasmic (Tat), binds 2[4Fe-4S]						-2.40					
DVU1288	dsrJ	Periplasmic (Sec), trihaem cytochrome c						-2.37					
DVU1289	dsrK	Cytoplasmic, binds 2[4Fe-4S]		-1.54				-2.20					
DVU1290	dsrM	Inner membrane protein binds 2 haem b						-2.65					
DVU1636	ppaC	Inorganic pyrophosphatase, Mn-dependent			-1.14				2.14				
		Hmc Complex											
DVU0529	rrf2	Rrf2 protein		2.84						2.77	2.29		
DVU0530	rrf1	Rrf1 protein		2.09						2.44	2.34		
DVU0531	hmcF	HmcF, 52.7 kD protein		2.19						2.37	1.96		
DVU0532	hmcE	HmcE, 25.3 kD protein		1.86			1.63			1.34	1.38		
DVU0533	hmcD	HmcD, 5.8 kD protein		2.39						1.97	2.29		
DVU0534	hmcC	HmcC, 43.2 kD protein		1.49	-1.07				1.86				

Fur Regulon		Stresses										
ORF No.	Gene	Description	Cold	Heat	02	pH 10	pH5.5	NO ₂	NO ₃	NaCl	KCI	CrO ₄ =
DVU0303	NA	Hypothetical protein		2.86	1.49	2.02	1.35	2.79				2.19
DVU0304	NA	Hypothetical protein						2.77				2.34
DVU0763	gdp	GGDEF domain protein		1.50		1.10				1.74		1.97
DVU2378	foxR	Transcriptional regulator, AraC family		1.38				1.66	1.61			
DVU2571	feoB	Fe ²⁺ transport protein B		3.41		1.74		2.95		1.54	1.15	1.50
DVU2572	feoA	Fe ²⁺ transport protein A		3.28		1.86	1.56	3.25		1.49		2.95
DVU2573	NA	Hypothetical protein		3.77			2.27	2.49		1.25		2.60
DVU2574	feoA	Fe2+ transporter component, feoA		3.54			1.40	2.49		1.18		1.97
DVU2680	fid	Flavodoxin, iron-repressed		1.27	1.22				-1.59			1.74
DVU3330	NA	Fe-regulated P-type ATPase, hypothetical		1.93			1.12	2.09				



D.A. Rodionov, I. Dubchak, A. Arkin, E. Alm, M.S. Gelfand. Reconstruction of regulatory and metabolic pathways in metalreducing delta-proteobacteria. *GenomeBiology 2004. 5:R90*

Figure 2. Illustration of the overlap in differentially expressed genes in various stresses



Observations

- A) *D. vulgaris* Hildenborough does not encode nitrate reductase. The transcriptional response to nitrate is unexplained and not a simple combination of the response to sodium and that to nitrite.
- B) The responses to high concentrations of NaCl or KCl have many transcript changes in common. A remarkable number of the genes altered in expression by mildly cold temperature overlapped the salt responses.
- C) Few transcriptional responses are shared by opposite pH stresses.
- D) An unexpected number of the same genes were differentially expressed in acid and chromate treatments.
- E) Genes encoding enzymes for sulfate reduction are not generally upregulated during stress treatments, with the exception of nitrate.
- F) Genes of the predicted *fur* regulon respond to most stress treatments. A classical "general stress response" has not been identified.
- G) In the absence of an apparent *rpoS*, a general stress sigma factor, responses to stresses in *D. vulgaris* occur through unidentified signaling pathways.

Acknowledgements

This work was part of the Virtual Institute for Microbial Stress and Survival (http://VIMSS.lbl.gov) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, and Genomics Program:GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy