Q-369/478. Response of *Desulfovibrio vulgaris* to acid medium

H.-C. Bill Yen[1], E. Alm[2], K. Huang[2], T.C. Hazen[3], A. Arkin[3], J. Zhou[4], and J. D. Wall[1]*

[1]University of Missouri-Columbia, Columbia, MO; [2]MIT, Cambridge, MA;

[3]Lawrence Berkeley National Laboratory, Berkeley, CA; and [4]University of Oklahoma, Norman, OK

ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

VIMSS Virtual Institute for Microbial Stress and Survival

http://vimss.lbl.gov/

ABSTRACT

The anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough is currently being explored for environmental bioremediation applications. Among the environmental constraints likely to be encountered in contaminated sites will be acid conditions. Therefore, the effect of low pH on the metabolic capacities of *D. vulgaris* is being explored. Transcriptional profiling was performed for cells exposed to low pH. According to pH 5.5 microarray data, many clusters of genes in *D. vulgaris* are up-regulated. Five of these gene clusters were chosen for physiological experiments to determine the relationship between the differentially expressed genes and acid resistance. Cells inoculated into acid pH media had a longer lag phase and lower final protein yields. When cells were pre-grown at pH 6 or 5, they did not show acid adaptation. The acid-grown cells showed greater mobility during microscopic observations and in a soft-agar plate assay, consistent with the increase in the transcription of flagellar genes. However, addition of thiamine, potassium, or individual amino acids showed no significant increase in acid resistance. Addition of casamino acids did have some protective effect. A mutant with a null mutation in *pspA*, a phage shock gene (JW704) showed an increased sensitivity to acid, having a much longer lag phase before growth in pH 5.5 medium. Finally, the effects of chemicals, such as polyamines which are products of the enzymes encoded in the norspermidine operon which was up-regulated, were investigated. Acid resistance and biofilm formation by adding spermidine, norspermidine, purescine, or onithine in growth medium will be reported.

RESULTS

Table 1. Growth of *D. vulgaris* on acid medium

Final protein yields and OD of cultures grown on acid media

Medium pH	Protein (μg/ml; % control) Exp. 1	Exp. 2	Exp. 3	Avg. %
7.0	97 (100%)	94 (100%)	93 (100%)	100%
6.5	84 (87%)	81 (86%)	83 (89%)	86%
6.0	55 (57%)	54 (57%)	48 (51%)	55%
5.5	50 (52%)	43 (46%)	47 (51%)	50%
5.25	39 (40%)	37 (39%)	32 (34%)	38%
5.0	29 (30%)	26 (28%)	28 (30%)	29%

Figure 1. Growth at different initial pHs

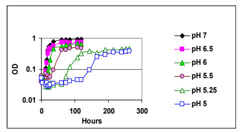
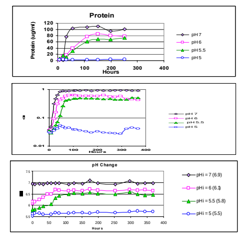


Figure 2. *D. vulgaris* growth and pH change on acid medium*



* Medium initially buffered with 30 mM PIPES

Figure 3. Acid pH shift at 0.4 OD 600 nm *D. vulgaris* growth and lactate consumption

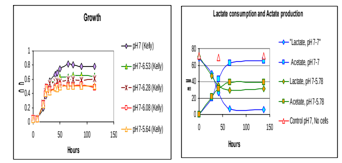
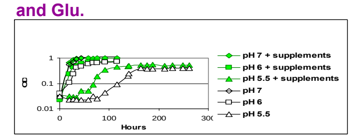


Table 2. Lactate consumption for pH adjustment?

Treatment	Initial pH	Final pH	Final Metabolite Conc: Protein μg/ml	Lactate* mM	Acetate mM	Lac + Ac Total
pH 7 to 7	6.94	6.96	120	0	53	53
pH 7 to 6.3	6.31	6.82	73	13	41	54
pH 7 to 6.02	6.08	6.64	66	23	35	58
pH 7 to 5.7	5.74	6.46	48	31	38	69

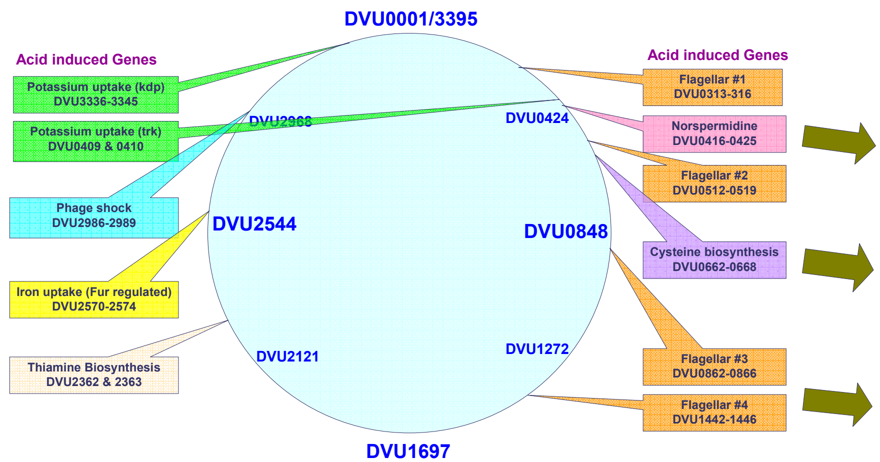
* Initial Lactate 60 mM

Figure 4. Effect of supplements on acid resistance: Thiamine, KCl, Arg, Lys, and Glu.



Supplements: 2 μM Thiamine, 8 mM KCl, 6 mM each Arg, Lys, and Glu.

MICROARRAY RESULTS



Microarray analysis of transcripts from pH 5.5-shifted *D. vulgaris*

Potassium metabolism (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU3336	*kdpD*	potassium channel histidine kinase doma	1.58	1.4		
		protein/universal stress protein				
DVU3337	*kdpC*	potassium-transporting ATPase, C subunit			1.58	1.4
DVU3338	*kdpB*	potassium-transporting ATPase, B subuni	0.98		3.43	2.92
DVU3339	*kdpA*	potassium-transporting ATPase, A subunit			2.57	2.42
DVU3342	NA	hypothetical protein		1.87	4.72	4.36
DVU3343	NA	hypothetical protein			0.75	0.72
DVU3344	NA	hypothetical protein			0.95	
DVU3345	NA	hypothetical protein		0.76	1.76	1.25
DVU0409	NA	(trk) hypothetical protein			1.26	0.97
DVU0410	NA	(trk) hypothetical protein	1.22	1.59	2.27	1.98

Phage shock proteins (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU2986	*pspC*	phage shock protein C			1.53	1.44
DVU2987	NA	hypothetical protein	0.68		1.96	2.19
DVU2988	*pspA*	phage shock protein A			2.65	2.72
DVU2989	*pspF*	response regulator				

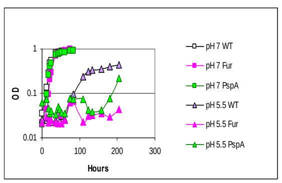
Fur-regulated *feo* operon (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU2570	*pleD*	GGDEF domain/HAMP domain protein				
DVU2571	*feoB*	ferrous iron transport protein B			0.92	1.08
DVU2572	*feoA*	ferrous iron transport protein A		0.75	1.56	1.71
DVU2573	NA	hypothetical protein			2.27	2.71
DVU2574	*feoA*	ferrous iron transporter component feoA			1.4	1.92

Thiamine biosynthesis (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU2363	*thiM*	hydroxyethylthiazole kinase	0.94	2.4	3.29	3.01
DVU2362	*thiE-2*	thiamine-phosphate pyrophosphorylase		1.88	2.37	2.17

Figure 5. Growth of WT, ΔFur, and PspA[-] mutants inoculated at acid pHs



Other up and down regulated genes (Z ≥ abs 2) from pH 5.5 microarray results

Gene Function	Gene Description	DVU	# genes
Up-regulated genes			
Energy	iron-sulfur cluster-binding protein	1080-1081, 2543-2544	4
	Formate dehydrogenase	0572-0573, 0576-0577, 0586-0588	7
Chaperon & Heat shock	*clpS, clpA, clpB, dnaJ, hspC, hsp20, lon*	1600-1603, 1874-1876, 2441-1442,3298-3305	17
Cell envelope & Wall	*fsxA, ripB*, kill protein, hydrolase, thioesterase, carbohydrate isomerase	1212-1213, 1579-1581, 1729-1730, 2974-2978, 0132-0135, 0159-0160	16
Down-regulated genes			
Pro-phages	Phage #2	2836-2837	2
	Phage #3	2709, 2725	2
	phage #4	1477-1480, 1509-1510	6
	Phage #5	2177-2183, 2189	8
	Phage #6	1110, 1144, 1150	3
Transposase	*orfA, orfB, ISDVU*	2004, 2006-7, 2010, 0555-0558, 0561-64	12
Megaplasmid	asparagine synthase, HP	A0025-A0027, A0066, A0073	5
Outer membrane & Lipoprotein	acetyltransferase, amidase, *omp85*, lipoprotein, peptidase, glycosyltransferase, ligase, methyltransferase, acyltransferase	2145-46, 2371-75, 2448, 2686, 0280-0281, 1849, 1860-1862	15
Regulatory	histidine kinase, sigma-54 dependent, *arsR*, transcriptional, chemotaxis, *tetR*	1760, 2827, 2956, 3080-3084, 5053, 0604-0606, 0629-0630	12
Others	AT-rich DNA binding, endoribonuclease, *metF*, *pyc* (pyruvate carboxylase)	0916, 0923, 0938, 0997, 1834	5
Unknown	hypothetical protein (HP) conserved hypothetical protein		24

Microarray analysis of transcripts from pH 5.5-shifted *D. vulgaris*

Norspermidine operon (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU0416	NA	GGDEF domain protein			0.71	0.69
DVU0417	*speA*	arginine decarboxylase		0.73	2.62	2.79
DVU0418	*lys1*	saccharopine dehydrogenase		0.99		0.8
DVU0419	*nspC*	carboxynorspermidine decarboxylase	2.6	3.52	4.63	4.69
DVU0420	NA	hypothetical protein		1	2.98	2.92
DVU0421	NA	agmatinase, putative			1.56	1.81
DVU0422	NA	sensory box/GGDEF domain/EAL domain protein			1.02	1.05
DVU0425	NA	hypothetical protein	0.75	1.52	2.7	2.73

Cysteine metabolism (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU0662	*cysE*	erine O-acetyltransferase			0.73	
DVU0663	*cysK*	cysteine synthase A		0.94	1.1	1.11
DVU0664	NA	cysteine desulfurase	1.93	2.27	1.9	1.97
DVU0665	NA	nitrogen fixation protein nifU	1.93	2.27	3.01	3.08
DVU0668	NA	methyl-accepting chemotaxis protein	0.72		1.67	1.38

Flagellar genes (Log2 Ratio of treated/control)

DVU	Gene	Description	60/0	120/0	240/0	240/240
DVU0862	NA	flagellar protein FliS/hypothetical protein, fusion			1.24	1
DVU0863	NA	flagellar hook-associated protein 2, putative			1.94	1.44
DVU0864	NA	glycoprotease family protein, putative			1	1.27
DVU0865	NA	membrane-associated zinc metalloprotease, putative			1.92	2.04
DVU0866	*dxr (isp)*	1-deoxy-D-xylulose 5-phosphate reducto	1.38	1.43	3.27	3.34
DVU1442	NA	flagellin FlaG, putative		0.91	1.62	1.59
DVU1444	*flgD*	basal-body rod modification protein FlgD		0.97	1.37	1.04
DVU1445	NA	flagellar hook-length control domain protein		1.32	2.48	1.86
DVU1446	NA	heptosyltransferase family protein			1.38	
DVU0313	*fliF*	flagellar M-ring protein FliF			0.94	
DVU0314	*fliE*	flagellar basal body component FliE			1.54	1.11
DVU0315	*flgC*	flagellar basal-body rod protein FlgC		1.12	2	1.75
DVU0316	*flgB*	flagellar basal-body rod protein FlgB	0.96	1.66	2.53	1.91
DVU0512	*flgF*	flagellar basal-body rod protein, putative		1.46	2.02	1.88
DVU0513	*flgG*	flagellar basal-body rod protein FlgG		0.94	1.18	0.91
DVU0515	*flgH*	flagellar L-ring protein FlgH	1.01	1.86	2.69	2.39
DVU0516	*flgI*	flagellar P-ring protein FlgI			1.21	0.9
DVU0517	NA	peptidase, M23/M37 family			0.98	0.96
DVU0519	NA	flagellar hook-associated protein FlgK, putative			1.31	1.21

RESULTS & CONCLUSIONS

1. Cells inoculated into acid media had longer lag phases and lower final protein yields. Lactate appeared not to be completely oxidized at acid pH.
2. When cultures were pH shifted at OD 0.4, no cell growth occurred below pH 6.0, although medium pH increased ~ 0.6 pH unit.
3. Addition of potassium, thiamin, and amino acids in pH 5.5 medium increased the acid resistance slightly.
4. Both ΔFur and PspA[-] mutants were more acid sensitive than the wild type.
5. Although many flagellar genes were increased in transcription at pH 5.5, an increase in motility could not be demonstrated (Data not shown).
6. Biofilm formation was increased in acid medium.

Additional observations:

I. Acid adaptation was not apparent, when cells were pre-grown at pH 6 or 5 before subjecting to a larger pH shift.
II. Stationary cell acid adaptation could not be demonstrated.
III. No obvious amino acid decarboxylase systems for acid resistance (e.g. glutamate and lysine) could be recognized in *D. vulgaris* DNA.
IV. F_0F_1 ATPase is not regulated by acid pH as observed in some Gram-positive bacteria.

ACKNOWLEDGEMENT

ESPP is part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.