Adaptation to salt stress in experimental evolution of *Desulfovibrio vugaris* Hildenborough

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ESPP Environmental Stress Pathway Project

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ABSTRACT

One of the greatest challenges in biology is to understand the interaction between genotype and environment to determine the fitness of an organism. With the recent advances in genome sequencing and high-throughput genomic technologies, it is possible now to link sub-cellular molecular/metabolic processes with the population-level processes, functions and evolution. Sulfate reducing bacteria *Desulfovibrio vulgaris* Hildenborough is an ideal model environmental organism to address such fundamental questions. In this study, a long-term evolution experiment was carried out under controlled laboratory conditions for *D. vulgaris*. The salt tolerance was tested periodically by monitoring the growth of cell lines on LS4D + 250 mM NaCl. The results demonstrated that the adaptation to salt stress was a dynamic process. Enhanced salt tolerance to higher salt (250 mM NaCl) was observed at 300 generations and it became more obvious with the increase of generations. De-adaptation of cell lines by removal of salt stress at 500, 1000 and 1200 generation cell lines did not affect the increased salt tolerance, indicating that the observed phenotype changes was due to genetic changes instead of physiological adaptation. Furthermore, results from the de-adaptation experiment suggest the dynamic trend of genetic adaptation and the genetic mutation may become stable at 1000 generations, which was also confirmed by the microarry data from 500 and 1000 generations samples. *hmcF-E-D-C-B-A, rrf2-rrf1, lysA-2-lysX* and *DVU3290-3291-3292* (glutamate synthase) were examples of significantly up-regulated polycistronic operons in long-term stressed lines. Whole genome sequencing of selected colonies is underway to identify the beneficial genetic mutations.

MATERIALS AND METHODS

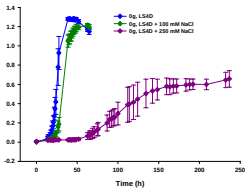
Bacteria strain: Single colony-based liquid culture was obtained from the original *D. vulgaris* Hildenborough stock. Six lines each were used for control and treatment respectively.

Medium and culture condition: LS4D was used as standard medium for the control. Medium for salt stress treatment was LS4D +100 mM NaCl. Cells were kept at 37°C and transferred every 48 hrs.

Handling of the samples: At some selected points, for example, every 100 generations, the glycerol stocks were archived and a variety of genetic, molecular, physiological, and genomic analyses were conducted to determine their evolution/adaptation to environmental stresses.

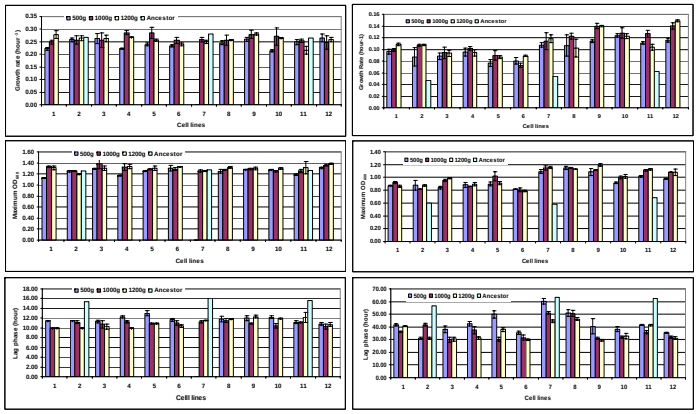
Microarray analysis: 70mer oligonucleotide arrays for *D. vulgaris* Hildenborough that containing all ORFs (He et al., 2006) were used in this study. Total cellular RNA was isolated using TRIzol (Invitrogen) and RNeasy mini column and labeled with Cy5 dye. Genomic DNA was isolated from *D. vulgaris* Hildenborough as described previously (Zhou et al., 1996) and labeled with Cy3 dye. The labeled cDNA and genomic DNA were co-hybridized to the array. Microarray data were processed as described before (Chhabra et al., 2006; Mukhopadhyay et al., 2006).

RESULTS



Elevated salt in the medium arrest the growth of the *D. vulgaris* strain

With 100 mM of NaCl in the medium, the growth of DvH was delayed for a few hours without obvious perturbation on growth rate and final biomass; however, with 250 mM of NaCl in the medium, significantly decreased growth rate and final biomass yield were observed.



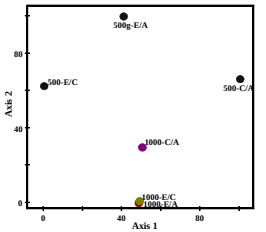
The enhanced salt tolerance of the long-term evolved cell lines is due to genetic changes instead of physiological adaptation

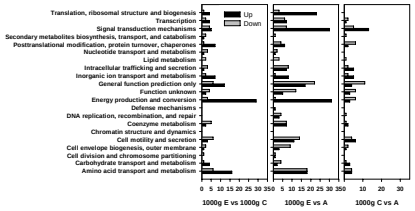
Long-term transferred cells lines with mild salt stress archived at 500, 1000 and 1200 generations were revived and cultured on standard medium LS4D for about 50 generations before the phenotype test on medium with elevated salt (LS4D +250 mM NaCl).

Cell lines #1~6 are control lines long-term transferred on LS4D; #9~12 are de-adaptive lines.

Left panel shows the phenotype with standard medium LS4D. Not much difference were seen except all the long-term transferred lines have shorter lag phase compared to the ancestor;

Right panel shows the phenotype with LS4D + 250 mM NaCl. Compared to ancestor, all the long-term transferred lines have faster growth rate, higher biomass yield and shorter lag phase. The trend is more obvious for the long-term transferred lines under mild salt stress.





The transcriptional response of the long-term transferred *D. vulgaris* cells lines

Left panel: The transcription profiling change became stable at 1000g DCA analysis of the all the gene expression data was shown.

Right panel: The transcription profiling change of 1000 generation cell lines according to the COG roles. Energy production and conversion, signal transduction mechanisms are among the gene categories with most number of genes up-regulated.

E: long-term transferred lines under salt stress; C: long-term transferred lines without stress A: ancestor

Selected polycistronic operons with increased gene expression levels in 1000 generation cells

		E vs A		C vs A		E vs C	
DVU	Description	Log2R	Z	Log2R	Z	Log2R	Z
DVU0774	ATP synthase, F1 epsilon subunit (atpC)	1.0	1.9	0.5	0.8	0.5	0.9
DVU0775	ATP synthase, F1 beta subunit (atpD)	0.9	1.7	0.2	0.3	0.7	1.2
DVU0777	ATP synthase, F1 alpha subunit (atpA)	0.9	1.8	0.0	0.0	1.0	1.6
DVU0778	ATP synthase, F1 delta subunit (atpH)	1.0	1.7	0.0	-0.1	1.0	1.5
DVU0779	ATP synthase F0, B subunit, putative	0.7	1.3	-0.1	-0.1	0.8	1.1
DVU0780	ATP synthase F0, B subunit, putative	0.2	0.4	-0.1	-0.1	0.3	0.5
DVU0846	adenylylsulphate reductase, beta subunit (apsB)	0.5	0.9	-0.2	-0.2	0.6	0.7
DVU0847	adenylyl-sulphate reductase, alpha subunit (apsA)	0.8	1.5	-0.1	-0.1	0.9	1.3
DVU0848	Quinone-interacting membrane-bound oxidoreductase(QmoA) (Shelley Haveman)	0.7	1.1	0.0	0.0	0.7	0.9
DVU0849	Quinone-interacting membrane-bound oxidoreductase(QmoB) (Shelley Haveman)	1.1	2.2	0.0	0.0	1.1	1.9
DVU0850	Quinone-interacting membrane-bound oxidoreductase(QmoC) (Shelley Haveman)	1.0	1.4	-0.2	-0.3	1.2	1.8
DVU0851	hypothetical protein	0.6	1.1	-0.2	-0.3	0.8	1.2
DVU2019	conserved hypothetical protein	0.7	1.2	-0.5	-0.8	1.3	2.0
DVU2020	conserved hypothetical protein	0.9	1.6	-0.1	-0.2	1.1	1.7
DVU2379	peptidase, M16 family, putative	0.6	0.7	-0.8	-0.9	1.2	1.3
DVU2380	ABC transporter, ATP-binding protein	0.9	0.5	0.1	0.1	0.9	0.5
DVU2381	conserved hypothetical protein	2.1	1.2	1.1	0.9	1.1	0.6
DVU2382	conserved domain protein	0.6	0.5	-0.2	-0.3	0.8	0.7
DVU2383	tonB dependent receptor domain protein	1.7	0.8	1.0	1.0	0.9	0.4
DVU2384	ABC transporter, periplasmic substrate-binding protein	0.6	0.7	-0.6	-0.7	1.2	1.0
DVU2385	ABC transporter, permease protein	0.8	1.4	-0.4	-0.4	1.2	1.5
DVU2386	ABC transporter, permease protein	0.4	0.6	-0.7	-0.8	1.0	1.1
DVU2387	ABC transporter, ATP-binding protein	0.6	0.8	-0.7	-0.7	1.2	1.2
DVU2388	tolQ protein (tolQ-1)	0.6	0.6	-0.1	-0.1	0.7	0.8
DVU2389	biopolymer transport protein, ExbD/TolR family	1.0	1.0	-0.3	-0.4	1.2	1.3
DVU2390	TonB domain protein	0.2	0.2	-0.2	-0.4	0.5	0.5
DVU2566	diaminopimelate decarboxylase (lysA-2)	1.7	2.4	-0.3	-0.4	2.0	2.5
DVU2567	predicted transcriptional regulator for lysine biosynthesis and transport	0.9	1.0	0.0	0.0	0.8	1.1
DVU2571	ferrous iron transport protein B (feoB)	1.9	1.3	1.4	1.4	0.5	0.3
DVU2572	ferrous iron transport protein A, putative	1.6	1.0	1.7	1.5	-0.1	-0.1
DVU3290	conserved domain protein, glutamate synthase	0.9	1.1	-0.2	-0.2	1.0	1.0
DVU3291	glutamate synthase, iron-sulfur cluster-binding subunit, putative	2.5	2.3	0.1	0.1	2.4	2.6
DVU3292	pyridine nucleotide-disulfide oxidoreductase	1.4	2.1	-0.1	-0.1	1.4	2.1
DVU0529	transcriptional regulator, rrf2 protein, putative	1.3	1.7	-0.1	-0.2	1.5	1.9
DVU0530	response regulator, rrf1 protein	1.8	2.0	0.1	0.1	1.7	2.3
DVU0531	hmc operon protein 6	2.0	2.2	0.2	0.2	1.8	2.4
DVU0532	hmc operon protein 5	2.4	3.9	0.7	0.9	1.8	2.9
DVU0533	hmc operon protein 4	2.2	3.8	-0.2	-0.3	2.4	2.9
DVU0534	hmc operon protein 3	1.2	2.3	0.1	0.3	1.1	1.9
DVU0535	hmc operon protein 2	0.3	0.3	-0.3	-0.4	0.5	0.7
DVU0536	high-molecular-weight cytochrome C (hmcA)	1.0	1.2	-0.6	-0.6	1.6	1.8

CONCLUSIONS

- The enhanced salt tolerance was obtained in the long-term evolved lab culture and it became more obvious with the increase of generations;
- Evolved cell lines under mild salt stress were more tolerant to the increased salt concentration (250 mM NaCl);
- De-adaptation experiment suggests that there might be genetic bases behind the phenotype;
- Microarray data showed that there were extensive gene expression change due to long-term evolution in the lab.

Future work

- Whole genome sequencing to discover the possible beneficial mutations;
- Verification of the possible beneficial mutations;
- Gene complementation to confirm gene functions;
- Phenotypic array to find out the possible phenotypic changes.

ACKNOWLEDGEMENT

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