Comparative Stress Response of *Desulfovibrio vulgaris* and *Shewanella oneidensis*

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INTRODUCTION

The transcriptional response of bacterial species to environmental stress has been the subject of considerable research, fueled in part by the widespread availability of gene expression microarray technology. Previous studies have established the similarity of gene expression networks across a wide range of organisms, yet in these studies different experiments were performed on different species preventing a direct comparison. We have compiled a core set of 'standard' stressors including salt, high/low pH, and temperature, and applied these stressors systematically to two metal-reducing bacteria.

We previously reported the genome-scale transcriptional response of the bacterium *Shewanella oneidensis* to a compendium of common environmental stressors. In this study, we report the transcriptional response of the bacterium *Desulfovibrio vulgaris* to a similar set of perturbations. We address two basic questions regarding the conservation of gene regulation across these distantly-relaed metal-reducing bacteria: (i) do genes respond to stressors in a way that is independent of their genetic background (*i.e.*, are orthologous genes both up/down regulated in response to the same stressors)?; and (ii) are there higher order 'modules' whose structure is conserved (regardless of whether their exact response is the same)?

We observe that while the overall network may be conserved (genes in the same pathways have high correlations over all conditions), the response of the network to the same perturbations can be very different in different species (pathways may respond to the same stressor in different ways). Differences between species can arise from differential behavior of the same regulons and because 'orthologous' regulons may comprise different sets of (non-orthologous) genes, both of which may lead to insights in the ecological factors that shape gene expression.

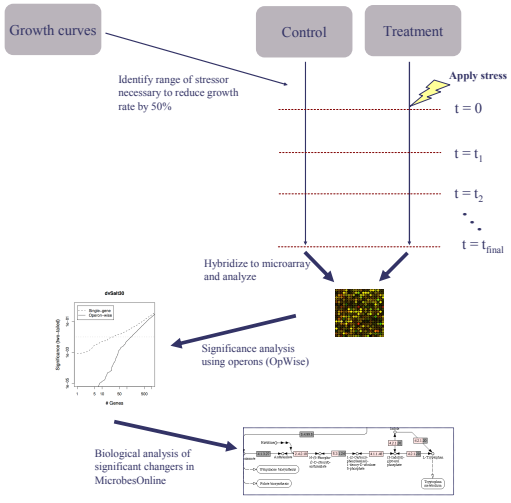
EXPERIMENTS

Stress response experiments were performed in both *S. oneidensis* and *D. vulgaris* cells grown in batch culture (in LS4D media) and harvested in log phase. *S. oneidensis* experiments were performed in the presence of atmospheric oxygen, and experiments in *D. vulgaris* were performed anaerobically. Experimental results are averaged over 3 biological X 2 technical replicates. Experimental design followed the flowchart shown below. Hybridizations were performed using either direct ratios (mRNA-mRNA) and genomic control (mRNA-gDNA), as noted in the table below.

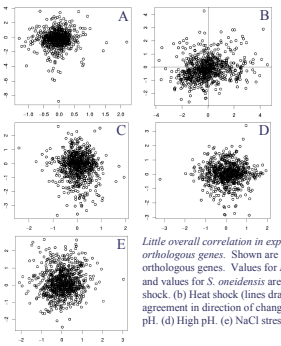
Organism	Stress	Treatment	Control	Hybridization
D. vulgaris	Heat	50C	37C	genomic control
D. vulgaris	Cold	8C	30C	genomic control
D. vulgaris	low pH	5.5	7	genomic control
D. vulgaris	high pH	10	7	genomic control
D. vulgaris	NaCl	250mM	0mM added	genomic control
S. oneidensis	Heat	42C	30C	direct ratio
S. oneidensis	Cold	8C	30C	direct ratio
S. oneidensis	low pH	4	7	direct ratio
S. oneidensis	high pH	10	7	direct ratio
S. oneidensis	NaCl	500mM	0mM added	direct ratio

Table of Stress Experiments

Experimental Flowchart



ORTHOLOGOUS STRESS RESPONSES

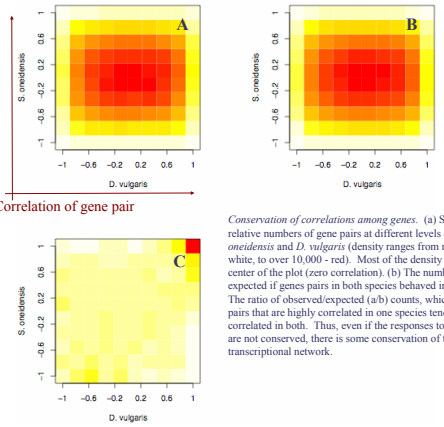


Little overall correlation in expression patterns observed for orthologous genes. Shown are $\log_2$ (fold-change) for 803 orthologous genes. Values for *D. vulgaris* are shown on the x-axis, and values for *S. oneidensis* are shown on the y-axis. (a) Cold shock. (b) Heat shock (lines drawn at x=0, y=0, to highlight general agreement in direction of change between the two species). (c) Low pH. (d) High pH. (e) NaCl stress.

Similar experiments performed in different species produced very different results. 803 orthologous genes were identified using the MicrobesOnline comparative genomics pipeline, and the expression of these genes were compared across all of the studied conditions (heat, cold, high/low pH, NaCl). Of these, only heat shock showed a modest similarity in transcriptional response.

LOOKING FOR 'MODULES'

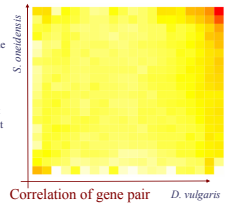
Although little correlation was observed between how these two organisms responded to the same stressors, we investigated whether there are higher order transcriptional modules, whose structure is conserved. To investigate whether some genes act together over our compendium of perturbations in both organisms, we looked at all (803 X 802) **pairs** of orthologous (non-self) genes, and surveyed the extent to which correlation (or lack of correlation) between two genes across all conditions was conserved.



Conservation of correlations among genes. (a) Shown are the relative numbers of gene pairs at different levels of correlation in *S. oneidensis* and *D. vulgaris* (density ranges from near zero counts - white, to over 10,000 - red). Most of the density is clustered in the center of the plot (zero correlation). (b) The numbers of genes expected if genes pairs in both species behaved independently. (c) The ratio of observed/expected (a/b) counts, which indicates that pairs that are highly correlated in one species tend to be highly correlated in both. Thus, even if the responses to given stressors are not conserved, there is some conservation of the structure of the transcriptional network.

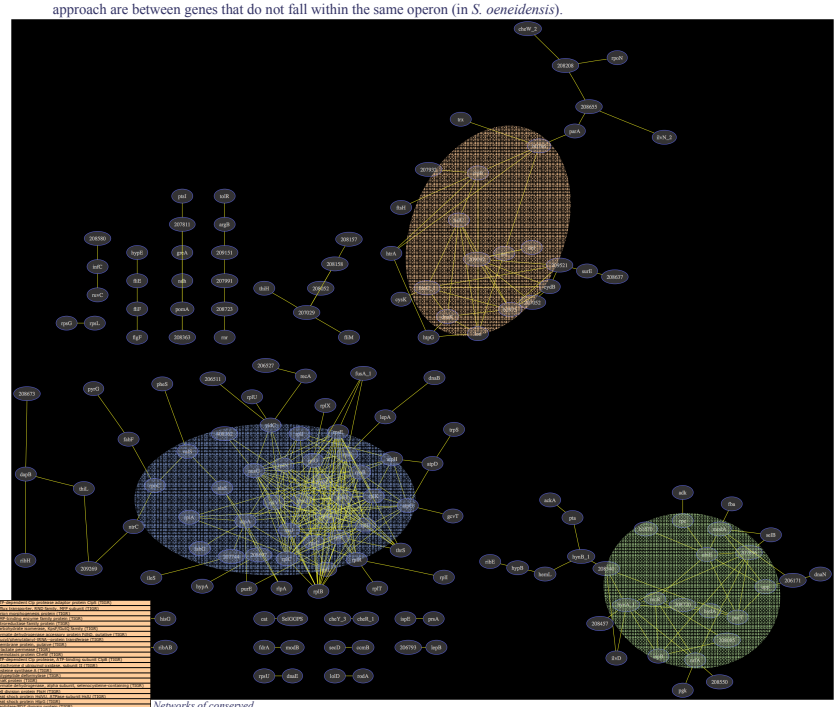
CONSERVATION AT MULTIPLE LEVELS

Conservation of sequence and expression. Genomes can differentiate *via* a number of mechanisms: (i) changes in sequence at orthologous loci; (ii) changes in gene content through horizontal transfer/deletion; and (iii) changes in the regulation of orthologous genes. We reasoned that genes with conserved transcriptional 'motifs', such as those in part (c) above, might also be highly conserved at the sequence level. To test this hypothesis, we plotted gene pairs on the same axes, but instead of density we looked at the sequence similarity of the genes in each pair. Shown at right is the average BLASTp score of orthologs (normalized by dividing the *S. oneidensis* self-BLASTp score) in a bin. For each pair, the minimum of the two genes scores is used, but similar results are observed using the average. Axes and colors are the same as above.

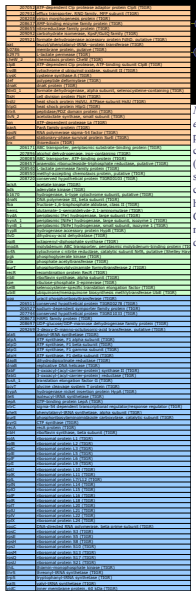


CONSERVED MODULES

We next looked at the links between gene pairs in these two species to see if there were higher order connections linking many genes into coexpressed networks. Shown is the expression network including all gene links conserved between these two species. There are three large connected components - one including the ribosome and ATP synthase genes, and a second including a number of less well-annotated genes that overall seems to be involved in energy production including several hydrogenases of key interest to our Genomics:GTL effort, and a third including several heat shock genes. Some smaller components include flagellar genes and biosynthetic pathways. Interestingly, over 60% of the gene links identified using this approach are between genes that do not fall within the same operon (in *S. oeneidensis*).



Networks of conserved expression. Above are pairs of genes that are closely co-expressed (Pearson r > 0.8) in both species. Major connected clusters of genes are highlighted in three colors. Genes within the three main components are detailed at left.



CONCLUSIONS

A similar set of stress response experiments were performed in both *S. oneidensis* and *D. vulgaris*. Little overall similarity was seen in the responses of orthologous loci, yet some transcriptional network structure was conserved at a level of order higher than operon structure. The genes that were found to be part of conserved expression networks were also highly conserved at the level of sequence identity, indicating that gene sequence and gene regulation at a given locus may be subject to similar evolutionary constraints. This conserved structure can provide insight into the function of a number of loci possibly involved in metal-reduction. Our ongoing work in *Geobacter metallireducens* should provide a better glimpse at this conserved network, especially since it is much more closely related to *D. vulgaris* than to *S. oneidensis*. The evolutionary distance at which the stress response of two organisms diverge remains an important open question in environmental microbiology.

ACKNOWLEDGEMENTS

This work was 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-AC03-76SF00098 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.