# Applied Environmental Microbiology Core:
# Rapid Deduction of Stress Response Pathways in Metal/Radionuclide Reducing Bacteria

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ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival — Massachusetts Institute of Technology — University of Missouri — University of Washington — Montana State University — Southern Illinois University Carbondale — Sandia National Laboratories — Oak Ridge National Laboratory — Berkeley University of California — Science — http://vimss.lbl.gov/

## INTRODUCTION

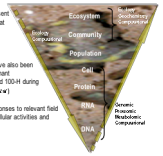
**AEMC of the ESPP project is the source of environmental data and samples that determine the stressors that will be studied, provides the environments for growing the organisms to be tested, simulates stressed environments, and verifies the conceptual models to determine how these stress regulatory pathways control the biogeochemistry of contaminated sites**

## Environmental Characterizations

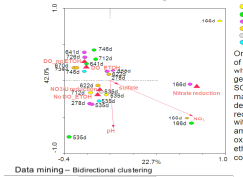
*Desulfovibrio* are present at elevated numbers at the FRC during biostimulation

*Desulfovibrio* spp. have also been observed as predominant populations at Hanford 100-H during biostimulation (Hazen et al.)

How do cellular responses to relevant field conditions impact cellular activities and survival?

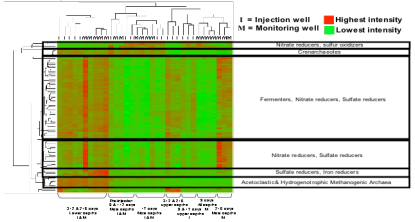



Phylogenetic relationship of cloned SSU rRNA genes classified in the Δ-Proteobacteria from bio-stimulated wells at S-3 waste ponds compared with reference sequences (in bold) from GENBANK (accession numbers in parenthesis). The numbers on the trees refer to bootstrap values on 500 replicates. *Methanosarcina mazei* (EF452664) was used as the outgroup.




Ordination diagram from CCA of SSU rRNA community data where groundwater geochemistry ($U^{6+}$, $NO_3$ and $SO_4$) and phase of subsurface manipulation (residual denitrification, initial uranium reduction, ethanol amendment with no DO control, ethanol amendment with dissolved oxygen control, and no ethanol amendment with DO control) were variables.

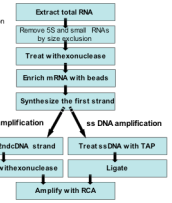
Data mining – Bidirectional clustering

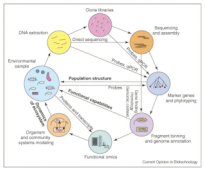


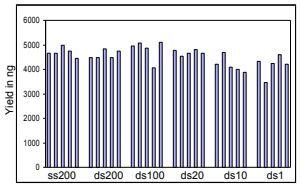
The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7.2H_2O$)

## Technique Development for Environmental DNA and mRNA analysis

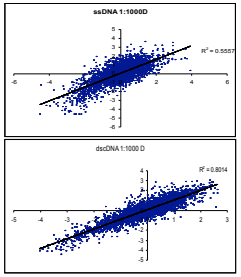
Diagrametic representation of the experimental workflow for mRNA enrichment and cDNA amplification, using single or double stranded amplifications.





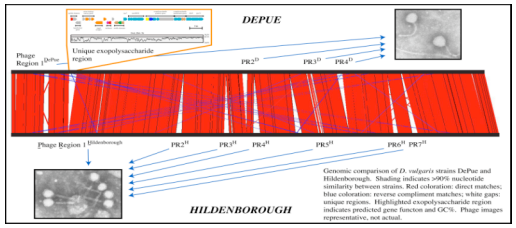

Amplification yelds of 5 replicate single stranded (SS) and double stranded (DS) reactions beginning from various amounts of starting total RNA material (in ng)




Normalized log expression ratios comparing single stranded (top) and double stranded amplifications (bottom) to unamplified enriched cDNAs from log phase DvH cells. Many fewer transcripts are detected, with higher variability using the single stranded approach

- Using single stranded or double stranded templates phi29 can efficiently amplify cDNAs in the 200 to 1KB range over 10000 fold in 4 hr reactions
- Double stranded amplifications tend to result in more uniform and unbiased amplifications than single stranded when compared to unamplified controls via microarray hybridizations.
- Solexa sequencing comparisons with the developed methods are currently ongoing

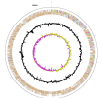
## Genome Sequence




*Anaeromyxobacter* sp. Fw109

- 5.3 Mb sequenced genome
- 73.5% G+C content
- 4386 ORFs (4336 protein coding)
- 1 SSU rRNA gene
- 29.5% Unknown Function
- 27.8% CHP 1.7% HP

The fraction of the genes annotated for Signal Transduction Pathways is greater than the fraction observed for 95% of completed genomes.



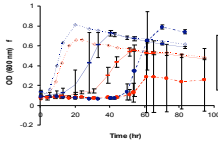
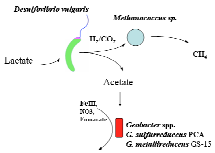
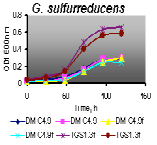



**Figure 1:** Growth of DvH and DP4 exposed to 50 μM $CrO_4^{2-}$. Two different inoculums were tested, values shown (0.10, 0.20) represent calculated initial $OD_{600}$.

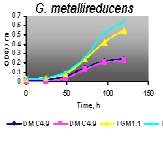
## Artificial Communities




We are using organisms having fully sequenced genomes (*Desulfovibrio vulgaris, Geobacter sulfurreducens* PCA, *Geobacter metallireducens* GS-15 and *Methanococcus maripaludis*) to construct different tri-cultures and develop tools to monitor community composition. *G. sulfurreducens* and *G. metallireducens* consume acetate and use alternative electron acceptors, including nitrate, fumarate and iron.

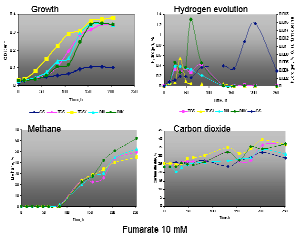



Lactate 30 mM, Fumarate 50 mM



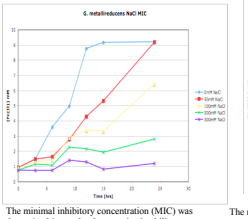

Lactate 30 mM, Nitrate 10 mM

DM – *D. vulagaris* and *M. maripaludis* coculture;
TGS - *D. vulagaris, G. sulfurreducens* PCA and *M. maripaludis* triculture;
TGM - *D. vulagaris* , *G. metallireducens* GS-15 and *M. maripaludis* triculture;
f - presence of fumarate

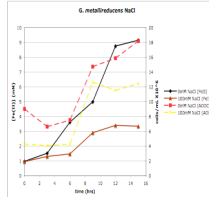



Methanogenic tri culture of *D. vulgaris, M. maripaludis* and *G. sulfurreducens* reduces fumarate, produces methane and evolves hydrogen.
DM – *D. vulagaris* and *M. maripaludis* coculture;
TGS - *D. vulagaris*, *G. sulfurreducens* PCA and *M. maripaludis* triculture;
GS - *G. metallireducens* GS-15monoculture;
f - presence of fumarate

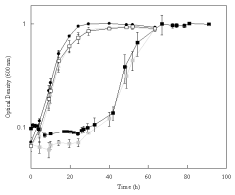
## Stress Experiments



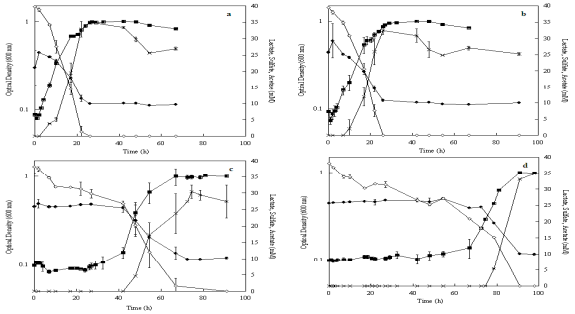

The minimal inhibitory concentration (MIC) was determined in part by the organism's ability to biotically reduce Fe(III) to Fe(II) with the amendments of five different NaCl concentrations.




The minimal inhibitory concentration (MIC) of NaCl was determined through the combination of Acridine Orange Direct cell Counts (AODCs) (dashed lines) and the organism's capacity for biotic Fe(III) reduction (solid lines).

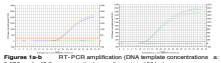



Growth of Cr(VI) exposed *D. vulgaris* cells in the presence of ascorbate. Washed cells were inoculated into LS4D alone (●), with 0.05mM Cr(VI) (■), 0.05 mM ascorbate (□), 0.05 mM Cr(VI) plus 0.05 mM ascorbate 3 h post Cr(VI)-exposure (■), and 0.05 mM Cr(VI) plus 0.05 mM ascorbate added concurrently (▲). Growth was monitored via $OD_{600}$.




Substrate analysis of washed *D. vulgaris* cells exposed to Cr(VI). Washed cells were inoculated to an $OD_{600}$ (■) of approximately 0.08 and lactate (◊), sulfate (●), and acetate (x) levels were monitored over time for control (**a**), 0.02 mM Cr(VI) (**b**), 0.05 mM Cr(VI) (**c**), and 0.1 mM Cr(VI) (**d**).

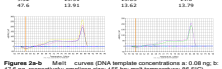
## High Throughput Biomass Production



**Figures 1a-b** RT-PCR amplification (DNA template concentrations a: 0.076 ng; b: 47.6 ng, respectively; amplicon size: 155 bp)

**Table 1** Threshold cycle data for three parallel amplifications

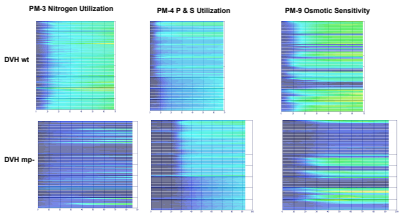
| DNA [ng] | Ct values for 1 | Ct values for 2 | Ct values for 3 |
|---|---|---|---|
| 0.076 | 21.63 | 21.99 | 21.6 |
| 0.381 | 19.39 | 19.45 | 19.62 |
| 1.904 | 17.24 | 17.39 | 17.49 |
| 9.52 | 15.51 | 15.65 | 14.6 |
| 47.6 | 13.91 | 13.62 | 13.79 |



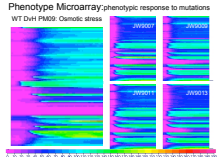
**Figures 2a-b** Melt curves (DNA template concentrations a: 0.08 ng; b: 47.6 ng, respectively; amplicon size: 155 bp; melt temperature: 86.5°C)

Real time PCR provides a highly reproducible and relatively inexpensive way of high-throughput analysis of culture purity in a continuously running bioreactor. A combination of universal and DvH-specific primers targeted less than 200-bp fragments of the highly conserved 16S rRNA-gene, respectively. Using SYBR® GreenER™ (Invitrogen) for amplicon detection, genomic DNA was amplified in a wide dynamic range with excellent reproducibility (Figures 1a-b). Template concentration, as expected, only influenced threshold cycle ($C_t$) but not the melt temperature (Table 1). The latter is only influenced by the length and sequence of the amplicon (Figures 2a-b).

## Phenotypic Responses




Phenotype Microarray: phenotypic response to mutations




Data processing interface:
http://vimss.lbl.gov/~pjacobsen/GTL/VIMSS/HazenLab/OmniogrPM/home.html

These results show the profile of osmotic stress comparing DvH WT with 4 mutants of DvH. Here, WT is more sensitive than the mutant strains to increasing concentrations of sodium acetate, KCl, NaCl and $NaNO_3$.

## Select Publications FY07

Hubbard, S. S., K. H. Williams, M. E. Conrad, B. Faybishenko, J. Peterson, J. Chen, P. E. Long, and T. C. Hazen. In Press. Geophysics for monitoring of hydrological and biogeochemical transformations associated with contaminant remediation. ES&T.
Wu, L., X. Liu, M. W. Fields, D. K. Thompson, C. T. Hughes, J. Tiedje, T. C. Hazen, and J. Zhou. In Press. Microarray-based whole-genome hybridization as a tool for determining soil bacteria relatedness. ISME.
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Tang, Y., F. Chakraborty, A. Mukhopadhyay, R. Phan, T. C. Hazen, J. D. Keasling. 2007. Pathway confirmation and flux analysis using $^{13}C$ isotopic labeling of metabolites in *Desulfovibrio vulgaris* Hildenborough via FT-ICR Mass Spectrometry. J. Bacterial. 1 89:940-949. LBNL-61051.
Lin, L.-H., P.-L. Wang, D. Rumble, J. Lippmann-Pipke, B. Sherwood Lollar, E. Boice, L. Pratt, E. Brodie, T. C. Hazen, G. L. Anderson, T. DeSantis, D. P. Moser, D. Kershaw, and T. C. Onstott. 2006. Long term biosustainability in a high energy, low diversi ty crustal biosphere. Science 314: 479-482. LBNL-60468
Brodie, E. L., T. Z. DeSantis, D. C. Joyner, S. M. Baek, J. T. Larsen, G. L. Anderson, T. C. Hazen, P. M. Richardson, D. J. Herman, T. K. Tokunaga, J. M. Wan, and M. K. Firestone. 2006. Bacterial population dynamics during uranium reduction and re-oxida tion: Application of a novel high density oligonucleotide microarray approach. Appl. Environ. Microbiol. 72:6288-6298. LBNL-59761.
Clark, M. E., Q. He, Z. He, K. H. Huang, E. J. Alm, X. Wan, T. C. Hazen, A. P. Arkin, J. D. Wall, J. Zhou, and M. W. Fields. 2006. Temporal transcriptomic analysis of *Desulfovibrio vulgaris* Hildenborough transition into stationary phase growth during el ectron donor depletion. Appl. Environ. Microbiol. 72:5578-5588. LBNL-60466.
Redding, A. M., A. Mukhopadhyay, D. Joyner, T. C. Hazen, and J. D. Keasling. 2006. Study of Nitrate Stress in *Desulfovibrio vulgaris* Hildenborough Using iTRAQ Proteomics. Briefings in Functional Genomics and Proteomics 5: 133-143. LBNL-59867.
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He, Q., K. H. Huang, Z. He, E. J. Alm, M. W. Fields, T. C. Hazen, A. P. Arkin, J. D. Wall, and J. Zhou. 2006. Energetic consequences of nitrite stress in *Desulfovibrio vulgaris* Hildenborough inferred from global transcriptional analysis. Appl. Environ. Microbiol. 72:4378-4385. LBNL-60422.
Mukhopadhyay, A., Z. He, E. J. Alm, A. P. Arkin, E. E. Baidoo, S. C. Borglin, W. Chen, T. C. Hazen, Q. He, H.-Y. Holman, K. Huang, R. Huang, D. C. Joyner, N. Katz, M. Keller, P. Oeller, A. Redding, J. Sun, Z. Yang, J. D. Wall, J. Wei, H.-C. Yen, J. Zhou, and J. D. Keasling. 2006. Salt stress in *Desulfovibrio vulgaris* Hildenborough: An integrated genomics approach. J. Bacterial. 188:4068-4078. LBNL-59862.
Fields, M. W., C. E. Bagwell, S. L. Carroll, T. Yan, X. Liu, P. M. Jardine, C. S. Criddle, T. C. Hazen, and J. Zhou. 2006. Phylogenetic and functional biomarkers as indicators of bacterial community responses to mixed-waste contamination. Environ. Sci. Technol. 40:2601-2607. LBNL-60460

## ACKNOWLEDGEMENT

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