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

Terry C. Hazen*[1], C. Abulencia[3], G. L. Anderson[1], S. E. Borglin[1], E. L. Brodie[1], S. van Dien[8], M. Fields[6], J. Fortney[1], Jil Geller[1], E. Hendrickson[5], Hoi-Ying Holman[1], J. Leigh[5], T. Lie[5], Richard Phan[1], Janet Jacobsen[1], Dominique Joyner[1], Romy Chakraborty[1], Martin Keller[2], Aindrila Mukhopadhyay[1], Chritopher Schadt[2], David Stahl[5], Sergey Stolyar[5], Chris Walker[5], Judy Wall[4], Eleanor Wozei[1], Zamin Yang[2], Huei-che Yen[4], Grant Zane[4], Jizhong Zhou[7]

1. E. O. Lawrence Berkeley National Laboratory, 2. Oak Ridge National Laboratory, 3. Diversa, Inc., 4. University of Missouri, Columbia, 5. University of Washington, 6. Montana State University, 7. University of Oklahoma, 8. Genomatica

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 — University of Oklahoma — Sandia National Laboratories — Oak Ridge National Laboratory — Berkeley — 75 Years of World Class Science

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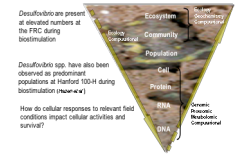
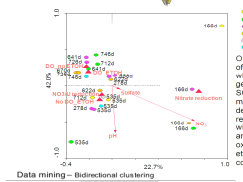
INTRODUCTION

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

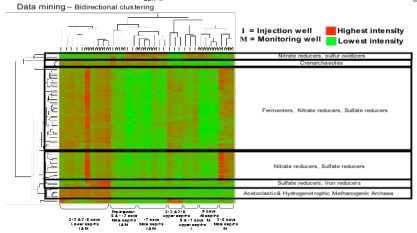
Environmental Characterizations



Phylogenetic relationship of cloned SSU rRNA genes classified in the ■-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* (EF462664) was used as the outgroup.

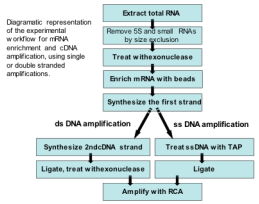


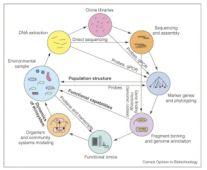
Ordination diagram from CCA of SSU rRNA community data where groundwater geochemistry (U^{6+}, NO_3 and SO_4) and phase of subsurface manipulation (residual denitrification, initial uranium reduction, ethanol amendment with no DO control, ethanol amendment with dissolved oxygen control, and no ethanol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

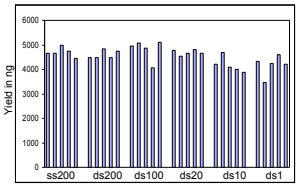


The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$)

Technique Development for Environmental DNA and mRNA analysis





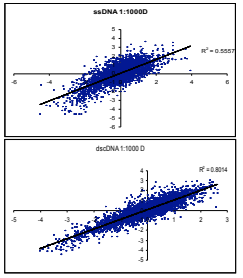


Amplification yields of 5 replicate single stranded (SS) and double stranded (DS) reactions beginning from various amounts of starting total RNA material (in ng)

•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

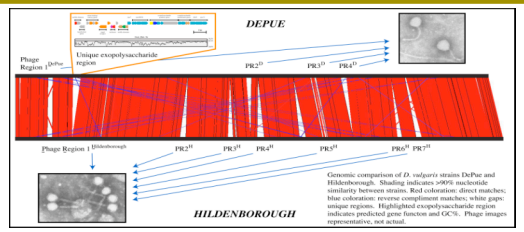
• Double stranded amplifications tend to result in more uniform and unbiased amplifications than single stranded when compared to unamplified controls via microarray hybridizations.

•Solexa sequencing comparisons with the developed methods are currently ongoing



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

Genome Sequence



Genomic comparison of *D. vulgaris* strains DePue and Hildenborough. Shading indicates >90% nucleotide similarity between strains. Red coloration: direct matches; blue coloration: reverse compliment matches; white gaps: unique regions. Highlighted exopolysaccharide region indicates predicted gene functon and GC%. Phage images representative, not actual.

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.

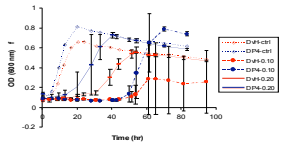
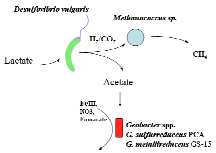
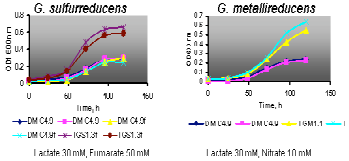


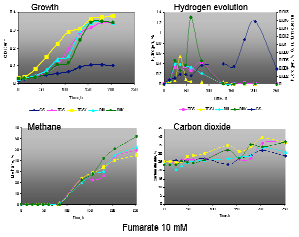
Figure 1: Growth of DvH and DP4 exposed to 50 μM $CrO_4^{=}$. Two different inoculums were tested, values shown (0.10, 0.20) represent calculated initial OD_{600}.

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.

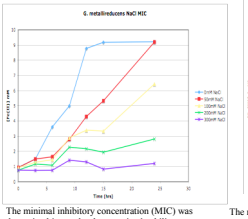




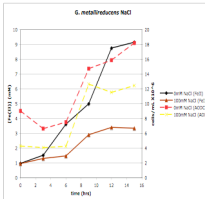
DM – *D. vulgaris* and *M. maripaludis* coculture;
TGS - *D. vulgaris*, *G. sulfurreducens* PCA and *M. maripaludis* triculture;
TGM - *D. vulgaris* , *G. metallireducens* GS-15 and *M. maripaludis* triculture;
f - presence of fumarate

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

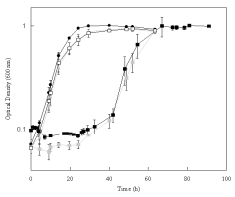
Stress Experiments



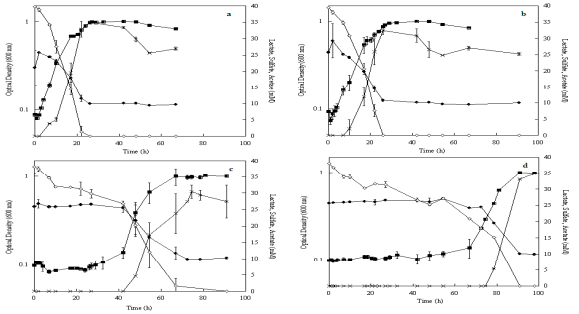
The minimal inhibitory concentration (MIC) was determined in part by the organism's ability to biotically reduce Fe(III) to Fe(II) with the amendments of five different NaCl concentrations.



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).

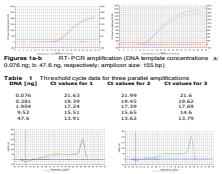


Growth of Cr(VI) exposed *D. vulgaris* cells in the presence of ascorbate. Washed cells were inoculated into LS4D alone (●), with 0.05mM Cr(VI) (■), 0.05 mM ascorbate (□), 0.05 mM Cr(VI) plus 0.05 mM ascorbate 3 h post Cr(VI)-exposure (■), and 0.05 mM Cr(VI) plus 0.05 mM ascorbate added concurrently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

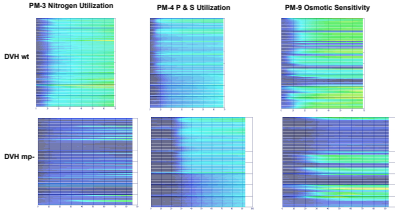
Table 1 Threshold cycle data for three parallel amplifications

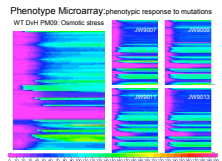
DNA (ng)	Ct values for 1	Ct values for 2	Ct values for 3
0.076	21.63	21.99	21.6
0.381	19.39	19.45	19.62
1.904	17.24	17.39	17.69
9.52	15.51	15.65	14.4
47.6	13.91	13.62	13.79

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





Data processing interface:
http://vimss.lbl.gov/~rjacobsen/GTL/VIMSS/HazenLab/OmnilogPM/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.

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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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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. Bacteriol. 188:4068-4078. LBNL-59862.

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