Responses of *Desulfovibrio vulgaris* to Physiological Constraints Relevant to Bioremediation in the Field

- Field Occurrence
- *Desulfovibrio vulgaris* responses to field relevant conditions

Matthew W. Fields
Ecosystem
Identify key factors (i.e., stresses) that drive community structure and composition and impact the survival and efficacy of metal-reducers.

Community
How do communities respond to stress and how do the populations of interest interact in that community?

Populations
Determine the impact of stress on organisms (*Desulfovibrio vulgaris*).

Cell
Infer key stress response pathways and how gene networks interplay under different stress conditions to optimize biochemistry.
DOE Materials (Legacy Inventory)

http://web.em.doe.gov/takstock/fig11.html
Bioremediation of U(VI) Contaminated Sediments *in situ* at the FRC

**Outer loop**
- Injection: FW024
- Extraction: FW103

**Inner loop**
- Injection: FW104
- Extraction: FW026

Monitoring wells:
- FW101-2 (-40 ft)
- FW101-3 (-35 ft)
- FW102-2 (-40 ft)
- FW102-3 (-35 ft)

*Stanford/ORNL*
Principal Coordinate Analysis of Temporal and Spatial Population Changes

- Bioreduction wells at later times
- All wells at earlier times and outer wells at later times
- Injection and treatment wells during U(VI) reduction

Unc. *Desulfovibrionaceae* sp.
Unc. *δ*-proteobacterium sp.

Unc. *Burkholderia* sp.
Unc. *Comamonadaceae* sp.

Unc. *Burkholderia* sp.
Unc. *Actinobacteriaum* sp.
Unc. *Geobacter* sp.

Unc. *Desulfovibrionaceae* sp.
Unc. *δ*-proteobacterium sp.

Gallionella sp.

(Hwang et al., in prep)
Desulfovibrio are present at elevated numbers at the FRC during bio-stimulation.

Desulfovibrio spp. have also been observed as predominant populations at Hanford 100-D during bio-stimulation (Hazen et al.)

How do cellular responses to relevant field conditions impact cellular activities and survival?
Temporal Transcriptomics of Electron Donor Depletion

Cell Protein and Carbohydrate

Temporal Up- and Down-Expression

(Cell et al., 2006)
Lactate and Sulfate Permeases Displayed Different Trends of Expression

- Results suggested that different permeases were used with respect to changing nutrient levels
- An alternative explanation could be growth-rate dependent regulation
- Three presumptive LDH genes did not show significant changes

(Clark et al., 2006)
Major Changers as Electron Donor was Depleted

- Almost all phage-related genes were up-expressed into and during stationary-phase
- A possible $feo$ system was up-expressed and a ferritin was down-expressed

(Clark et al., 2006)
Cluster Analysis of Electron Donor Depletion

- Lactate
- Energy Replete
- Energy Deplete

Gene expression changes include:
- Carbon starvation protein (cstA) up
- Iron (II) transport (feoAB) up
- Phage genes up
- Super-oxide dismutase (sodB) up
- Carbohydrate-related genes up
- ATP synthase (atpG) down
- Ribosomal proteins down
- Lactate permease down
- Sulfate permease down

- Proteases up
- Lipoprotein up
-ngr, cydB up
- Phage genes up
- ATP synthase (atpG) down
- Ribosomal proteins down
- Lactate permease down
- Sulfate permease down

(Clark et al., 2006)
Some Conclusions from e- Donor Depletion

In addition to expected changes (e.g., energy conversion, protein turnover, translation, transcription, and DNA replication/repair)

Genes related to:
- phage
- carbohydrate flux
- outer envelop
- iron homeostasis

played a major role in the cellular response to nutrient deprivation under the tested growth conditions

*rpoS – universal stasis transcriptional factor?*

The results indicated that a subset of approximately 110 genes were uniquely up-expressed as the cells transitioned to stationary-phase (14 on the megaplasmid).*

*(Clark et al., 2006)*
Cr(VI) Responses in *D. vulgaris*

Cr is the third most common pollutant at hazardous waste sites and the second most common inorganic contaminant after Pb

*Cr(III)* can be detected on the cell surface and in the periplasm (Goulhen et al., 2005)

Energy production without growth in the presence of Cr(VI) (Chardin et al., 2002)

(acetate, sulfate, growth-??)
(re-establish $E_h$ ?)

U(VI) inhibited SO$_4$-reduction (Elias et al., 2004)

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*D. vulgaris* requires H$_2$S, hydrogenases and cytochrome c3 for the reduction of Cr(VI) (Chardin et al., 2002)
Growth Effects of Cr(VI) Exposure

(Klonowska et al., in prep)
Growth Effects of Cr(VI) Exposure

Cell Viability

Hydrogen Levels

Carb:Protein

(Klonowska et al., in prep)
Cr(VI) Responses

Up-Expression by COGs

Expression levels decline (30 min): Carbohydrate and amino acid metabolism; inorganic ions; envelope synthesis; energy

Expression levels increase (60 min): Coenzymes; translation; defense; protein turnover; energy

Expression levels increase (120 min): protein turnover; defense; repair; signaling; envelope synthesis

Temporal Up- and Down-Expression

(Fields et al., in prep)
Cr(VI) Responses

Response-Regulator and Protease

FTIR After Cr-Treatment

- Changes in the membrane lipids
- Changes in secondary protein structures
- Changes indicative of the PO$_2^-$ groups in nucleic acids
- Changes in the C-O-C and C-O-P groups in various oligo- and polysaccharides
Model for Cr(VI) Responses Based Upon Transcriptomics

- DNA repair
- Protein repair

FMN red.
NADPH$_2$ + FMN
NADP$^+$ + FMNH$_2$

NADP dehy

Metal ATPase
MerP
Cr(III)
AcrA
OM Efflux

Zur
Fur
PerR

SO$_4$ Influx

Cr(III)

Drug Resist.

ChrB

ChrA

Cr(III)

Cr(VI)

(Fields et al., in prep)
Ascorbate acts as a highly potent inducer of chromate mutagenesis via DSBs in epithelial cells (NAR 35:465-76. 2007).

Cr(VI) Exposure and Organo-Ligand Protection

- Role(s) for 1e⁻ and/or 2e⁻ transfers to Cr(VI)
- Cr(III) is considered less toxic (?)
- Carbon routing to produce specific ligand (?)
- Non-specific Cr(III) adducts (?)
**chrAB, Megaplasmid, and Cr(VI) Tolerance**

- Strain without the megaplasmid is more susceptible to Cr(VI) exposure

*(Fields et al., in prep)*
NO₂ Exposure

↓ ATPase synthase
↓ NO₃ reductase

↓ ribosomal proteins

↑ HP
↑ feoAB
↑ Fe(II) transport

↑ NO₂ reductase
↑ cytochrome

DVU0775 ATP synthase F1 beta subunit
DVU0776 ATP synthase F1 gamma subunit
DVU0777 ATP synthase F1 alpha subunit
DVU0778 ATP synthase F1 delta subunit
DVU0917 ATP synthase F0, C subunit
DVU0918 ATP synthase F0, A subunit
DVU1286 reductase, transmembrane subunit, putative
DVU1287 reductase, iron-sulfur binding subunit, putative
DVU1290 nitrate reductase, gamma subunit, putative
DVU0927 ribosomal protein L21
DVU1211 ribosomal protein L28
DVU1303 ribosomal protein L3
DVU1310 ribosomal protein L16
DVU1319 ribosomal protein L18
DVU1574 ribosomal protein L25
DVU2518 ribosomal protein L13
DVU2924 ribosomal protein L11
DVU2925 ribosomal protein L1
DVU2926 ribosomal protein L10
DVU0303 hypothetical protein
DVU0304 hypothetical protein
DVU2383 tonB dependent receptor domain protein
DVU2571 ferrous iron transport protein B
DVU2572 ferrous iron transport protein A, putative
DVU2573 hypothetical protein
DVU2574 ferrous iron transport protein
DVU2680 flavodoxin
DVU0624 NapC/NirT cytochrome c family protein
DVU0625 cyt c nitrite reductase, catalytic subunit NfrA
DVU1080 iron-sulfur cluster-binding protein
DVU1081 iron-sulfur cluster-binding protein
DVU2543 hybrid cluster protein
DVU2544 iron-sulfur cluster-binding protein

(He et al., 2006)
NO$_2$ Exposure

(He et al., 2006)
### Expression of Metabolic Differentiation Between Surface-Adhered and Planktonic Populations

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*(Fields et al., in prep)*
What can organismal biology and ecology do for mineral and contaminant biotransformation?

If one wants to understand and predict carbon and energy utilization (mass balance)---then we need to understand how cells respond to stressful conditions by altering carbon and energy flow.

At the cellular level and upward through the community.

What we want and what the bugs want may be two different things. **Bug wants:** grow efficiently - increase biomass – reproduce

Our wants: efficient activity of interest with minimal input

The more we know about how the cell (community) works as a system----the more we will be able to predict and control. Biological capacity f(t) and f(p)
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