Title
Integrated Ecogenomics Study for Bioremediation of Cr(VI) at Hanford 100H Area

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Integrated ecogenomics study for bioremediation of Cr(VI) at Hanford 100H area

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Chromium(VI) contamination at Hanford

- Cr(VI) highly soluble, toxic compound
- Chemical manufacturing, waste streams from electroplating, leather tanning, textile industries, dyes and pigments industry, reactors, coal ash.
- Causes ulcer, convulsions, lung cancer, asthma, organ damage
➢ 450 billion gallons of waste from nuclear reactors at Hanford released that made its way to groundwater.

➢ Treatability tests employed by pumping HRC an injection well
In-situ bioremediation at Hanford 100H area

- Stimulate indigenous microbial populations
- Immobilize Cr(VI) through maintaining reduced conditions
NO$_3$ Concentration after HRC Injection

Injection & Pumping

Pumping

Pumping

Concentration (mg/l)

Injection Well

Monitoring Well

Aug-04
Feb-05
Aug-05
Jan-06
Aug-06
Jan-07
Jul-07
SO$_4$ Concentration after HRC Injection
Average Cr(VI) Concentration after HRC Injection

- Upgradient monitoring well
- Downgradient monitoring well
- Injection well
- Detection limit
- MCL
Genomic approach

- **Phylochip**: 16S rRNA based microarray
- **Clone libraries**: by MDA
- **Geochip**: Functional gene based microarray
- **Isolation, 16S-phylogenetic analysis and physiology**
PhyloChip – 500,000 probes (300k target 16S)

16S rRNA gene used as biomarker due to large database and availability of "universal" primers.

16S rRNA gene is amplified from genomic extract or 16S rRNA molecules are used directly.

Amplicon pool fragmented, biotin labeled.

PhyloChip is scanned, fluorescence data analyzed and probe sets with >90% probes positive are considered present.

PhyloChip stained and washed using automatic fluidics station.
Data mining – Bidirectional clustering

I = Injection well
M = Monitoring well

Highest intensity
Lowest intensity

Nitrate reducers, sulfur oxidizers
Crenarchaeotes

Fermenters, Nitrate reducers, Sulfate reducers

Nitrate reducers, Sulfate reducers

Sulfate reducers, Iron reducers

Acetoclastic & Hydrogenotrophic Methanogenic Archaea

312 & 216 days
Lower depths
I & M

Pre-injection
9 & 112 days
I & M

17 days
Upper depths
I & M

9 & 17 days
Upper depths
I

9 days
All depths
M

216 days
Most depths
M
Phylochip results of significant bacterial groups:

- Desulfovibrio halophilus
- Geobacter metallireducens
- Dechloromonas agitatus
- Pseudomonas putida

The graph shows the corrected hybridization intensity over days since HRC injection.
Functional groups – Iron reduction

Fe(II) can abiotically reduce Cr(VI) to Cr(III)

Deltaproteobacteria (Geobacteraceae)
Functional groups – Sulfate reduction

H₂S can abiotically reduce Cr(VI) to Cr(III)

Days post HRC injection

Deltaproteobacteria
(Desulfovibrionaceae)

Injection well

Monitoring well

Hybridization intensity

Days post HRC injection

H₂S can abiotically reduce Cr(VI) to Cr(III)
Functional groups – Methanogenesis

Presence of methanogens indicates strongly reducing conditions.
Clone libraries

- 16S clone libraries generated by MDA
- Analysis in progress.
- Initial results implicate *Caulobacter crescentus*, *Pseudomonas*, *Stenotrophomonas* and *Desulfovibrio* spp.
Geochip

- Approx 25000 oligonucleotide (50 mer) probes
- covering >10000 genes in >150 functional groups
- Genes for nitrogen, carbon, sulfur and phosphorus cycling, metal reduction and resistance, and organic contaminant degradation.
Geochip microarray results:

- Injection well
- Monitoring well

**Graph:**

- Mean signal to noise ratio

**Legend:**
- Chromium
- Nitrate
- Cytochrome
- Sulphate
- Methanogenesis
- Methane oxidation

- Many chromium tolerance/reduction genes.
Sulfite reductase

Methyl coenzyme-M reductase
Isolating microorganisms:

- Sediment collected
- Enrichments set up
- Lactate
- Electron acceptor
- Periodic transfers and microscopic counts
- Isolated colonies developed by agar shake tubes method
- Pure culture of bacterium
16S-rDNA based Phylogenetic tree
Characterization using the OMNILOG phenotypic microarray

Cells grown to mid log phase

Centrifuged at 6000rpm for 10min

Cell pellet resuspended to obtain density of $10^7$ cells/ml

100µl of cell suspension inoculated each well

Growth results obtained and data analysis

Plates sealed and incubated anaerobically for 96 hours
## Electron Donors and Carbon source

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<th>RCH1</th>
<th>RCH2</th>
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<td>Glycerol</td>
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</table>
Chromium reduction by active cells

Pseudomonas strain HLN

Chromium (VI) mM

Time (hour)

0.5 2 4.5 7 9 24

Cells, 50uM Chrom, Lactate
Cells, 75uM Chrom, Lactate
Cells, 100uM Chrom, Lactate
Cells, 175uM Chrom, Lactate

3.83 \times 10^7
6.33 \times 10^7
**Pseudomonas stutzeri**
strain HLN

**Desulfovibrio vulgaris**
strain RCH1

- **Cr(VI) exposed**
- **Control**
- **Std**

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**Heme staining**

**RCH1, Cr(VI) 250µM, Lactate**

**RCH1, Cr(VI) 250µM**

**Time (hour)**

0 0.5 2 4.5

**Chromium µM**

0 20 40 60 80 100 120 140 160
To Conclude:

- Phylochip suggests that increased Cr(VI) immobilization coincides with the increase of the *Desulfovibrio*, *Geobacter*, *Pseudomonas* and *Dechloromonas* strains following HRC injection.

- Clone library analysis indicated Psedumonas, Desulfovibrio spp along with others.

- Geochip reveals that following HRC injection, the richness of gene diversity corresponding to the dominant metabolisms decreased, however the relative abundance of these genes increased over time. This implies gradual dominance of each process by a few members of the population.
Iron reducer, nitrate reducer, sulfate reducer isolated from the Hanford 100H site capable of Iron(III) reduction and Chromium(VI) reduction.

Organisms mediate Chromium(VI) removal by direct enzymatic as well as abiotic interactions.

Environmental *Desulfovibrio* isolate currently being sequenced by JGI.
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