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ESPP 2 Environmental Stress Pathway Project

Investigation of Cr(VI) tolerant bacteria from Cr(VI)-contaminated 100H site at Hanford, WA

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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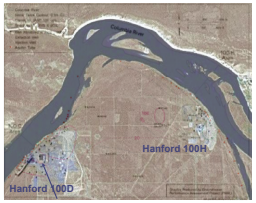
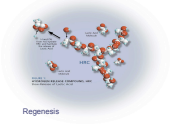
ABSTRACT

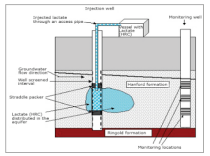
Hexavalent Chromium is a widespread contaminant found in soil, sediment, and groundwater. In order to stimulate microbially mediated reduction of Cr(VI), a poly-lactate compound HRC was injected into the chromium-contaminated aquifers at site 100H at Hanford, WA. Based on the results of the bacterial community composition using high-density DNA microarray analysis of 16S rRNA gene products, we investigated the diversity of the dominant anaerobic culturable microbial populations present at this site and their role in Cr(VI) reduction. Also, functional gene array (GeoChip) analysis of DNA extracted from monitoring well at the site indicated that genes involved in nitrate reduction, sulfate reduction, iron reduction, methanogenesis, as well as many chromium tolerance/reduction genes were highly abundant relative to the injection well. In addition, positive enrichments set up at 30°C using defined anaerobic media resulted in the isolation of an iron-reducing isolate strain HAF, a sulfate-reducing isolate strain HBLS and a nitrate-reducing isolate, strain HLN among several others. Preliminary 16S rDNA sequence analysis identified strain HAF as *Geobacter metallireducens*, strain HLN as *Pseudomonas stutzeri* and strain HBLS as *Desulfovibrio* species. Strain HAF utilized propionate, glycerol and pyruvate as alternative carbon sources, and reduced metals like Mn(IV) and Cr(VI). Growth was optimal at 37°C and pH of 6.5. Strain HLN utilized acetate, glycerol and pyruvate as alternative carbon sources, and reduced metals like Mn(IV) and Cr(VI). Optimal growth was observed at 37°C, at a pH of 7.5. Anaerobic washed cell suspension of strain HLN reduced almost 95µM Cr(VI) within 4 h relative to controls. Further, with 100µM Cr(VI) as sole electron acceptor, cells of strain HLN grew to cell numbers of 4.05 x 10^7/ml over a period of 24 h after an initial lag, demonstrating direct enzymatic Cr(VI) reduction by this species. These results demonstrate that Cr(VI) immobilization at the Hanford 100H site could be mediated by direct microbial metabolism apart from indirect chemical reduction of Cr(VI) by end products of microbial activity.

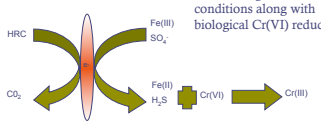


The DOE site at Hanford was established in 1943 to conduct research and development in nuclear energy technology.Chromium(VI) is a major contaminant at this site.



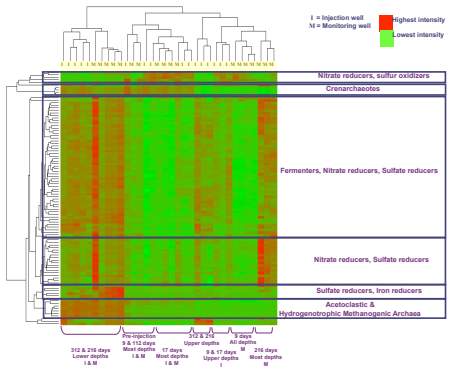
To stimulate bioremediation a poly-lactate compound HRC was injected into the chromium contaminated aquifers. It was expected that Cr(VI) would be immobilized by maintaining reduced conditions along with biological Cr(VI) reduction





Samples were monitored at regular intervals for microbial biomass and soluble chromium concentration. The water samples were also used to isolate indigenous chromium reducers from the site.

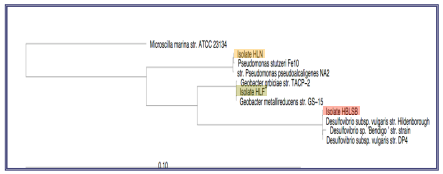
PHYLOGENETIC MICROARRAY



The biomass of significant organisms such as *Desulfovibrio spp.* and *Geobacter metallireducens* went up post -stimulation and continued to remain high.

Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation. PCA groups are indicated by brackets.

ISOLATION AND CHARACTERIZATION



Based on the phylochip data, anaerobic enrichments were initiated to study the dominant species in pure culture. Three organisms were isolated, an Iron-reducer, a sulfate reducer and a nitrate reducer named strain HAF, strain HBLS and strain HLN respectively. Phylogenetic analyses based on partial 16S rDNA sequencing revealed strain HAF was 100% similar to *G metallireducens*, strain HBLS was 99.9% similar to *D vulgaris Hildenborough* and strain HLN was 100% similar to *P stutzeri*.

Detailed genome analysis of strain HBLS revealed that it differs from *D vulgaris Hildenborough* by 100 genes.

Electron Donors

	HLN	HBLS	HAF
Butyrate	-	-	-
Proprionate	-	-	-
Ethanol	-	-	-
Pyruvate	+	+	+
Fumarate	+	-	+
Lactate	+	+	-
Acetate	+	-	+
Citrate	+	-	-
Glucose	+	-	-
Formate	+	-	-
Succinate	-	-	-
Benzoate	-	-	nd
Glycerol	+	-	+

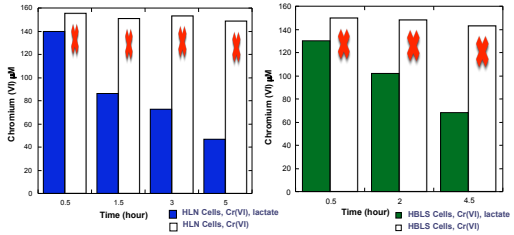
Electron Acceptors

	HLN	HBLS	HAF
Fumarate	+	-	N/d
Fe(III)-NTA	+	+	+
Chromium(VI)	+	+	+
Chlorate	-	-	-
Manganese(IV)	+	+	+
Sulfate	-	+	-
Nitrate	+	-	+
Oxygen	+	-	-
Sulfite	n/a	+	n/a
Thiosulfate	n/a	+	n/a

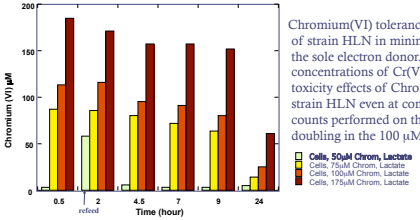
Both Strain HAF and strain HLN were able to utilize glycerol as carbon and energy source. Glycerol is a component of HRC. However strain HAF could not utilize lactate contrary to strain HBLS and strain HLN, which is also a major component of HRC

All strains were capable of Fe(III) reduction, Cr(VI) reduction as well as Mn(IV) reduction. Detailed experiments were set up to study direct biological enzymatic Cr(VI) reduction by the isolates

REDUCTION OF CHROMIUM (VI)

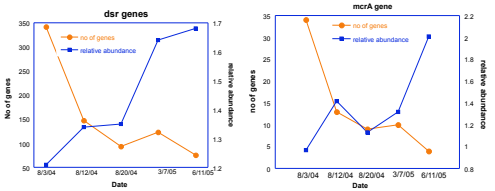


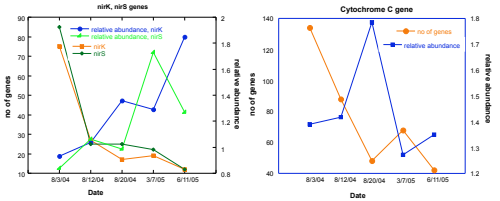
Cell suspension experiments were carried out with strain HBLS and HLN to determine their ability of Chromium(VI) reduction. with both strains, Chromium(VI) concentrations decreased with time when 10mM Lactate was supplied as the sole electron donor. When lactate was left out, little or no reduction of Chromium took place, demonstrating biological reduction of Cr(VI).



Chromium(VI) tolerance was tested on active cultures of strain HLN in minimal growth media. Lactate was the sole electron donor. Strain HLN reduced varying concentrations of Cr(VI) while oxidizing lactate. No toxicity effects of Chromium (VI) was observed on strain HLN even at concentrations of 175 µM. Cell counts performed on the incubations indicated a cell doubling in the 100 µM tube after 24 hours.

FUNCTIONAL GENE MICROARRAY





GeoChip functional gene microarray analysis demonstrated an enrichment of anaerobic electron accepting processes (nitrate reduction [nirK/nirS], iron reduction [Geobacter cytochrome C], sulfate reduction [dsrAB] and methanogenesis [mcrA] over time. These changes in functional gene density coincided with geochemical observations of sequential electron acceptor depletion.

In all cases an increase in relative intensity of functional genes coincided with a decrease in diversity of genes within that functional category demonstrating gradual dominance of each process by a few members of the population.

CONCLUSIONS

- Evidence suggests that the increased chromium immobilization coincides with the increase of the *Desulfovibrio, Geobacter, Pseudomonas* and *Dechloromonas* strains following HRC injection.
- Enrichments set up with water samples led to the isolation of a *Geobacter* species, a *Pseudomonas* species and a *Desulfovibrio* species.
- All the isolates grew best at 0.5% salinity in media and at circumneutral pH.
- The isolates were all able to reduce metals like Manganese(IV) and Chromium(VI) in cell suspension. No metal reduction occurred in the absence of electron donor.
- Strain HLN could sustain growth with lactate as the sole electron donor and Cr(VI) as the electron acceptor.
- Functional gene-chip (Geochip) analysis showed 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.
- Our study demonstrates that Cr(VI) immobilization at the Hanford 100H site could be mediated by direct microbial metabolism apart from indirect chemical reduction of Cr(VI) by end products of microbial activity.

ACKNOWLEDGEMENT

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