Title

GeoChip Analysis of Subsurface Microbial Communities Impacted by Heavy Metal and Nitrate Contamination

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GeoChip analysis of subsurface microbial communities impacted by heavy metal and nitrate contamination

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ESPP2 Environmental Stress Pathway Project

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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ABSTRACT

The objective of this study is to examine the bacterial community structure in wells of varying heavy metal and acid contamination to determine which contaminants have the greatest effect. Five monitoring wells and an uncontaminated background well from the Field Research Center (FRC) site of the U.S. DOE ERSP (Environmental Remediation Science Program) at Oak Ridge, Tennessee, were sampled to provide a gradient of groundwater nitrate, pH and uranium concentrations. DNA from these samples was analyzed with a comprehensive functional gene array containing 24,243 probes for >10,000 genes involved in carbon, sulfur, nitrogen, and metal cycling. Genes with the highest signal intensities from each sample were correlated with the groundwater geochemistry of that well. Wells with similar geochemical profiles had greater gene overlap than dissimilar wells. A higher percentage of nitrogen fixation genes were detected in groundwater with lower nitrate concentrations, while the percentage of nitrate reduction genes generally decreased with decreasing nitrate. Wells with elevated sulfate concentrations had a greater percentage of genes dedicated to sulfate reduction, and higher signal intensities for *dsrAB* genes than the background, indicating a greater abundance of those genes. Contaminated wells did not have a higher percentage of metal reduction and resistance genes than the background, but the total signal intensity of those genes was 1.4- to 2.3-fold greater than the background, indicating that metal-related genes were more prevalent in the contaminated wells. Uranium, nitrate and sulfate were identified by CCA as important factors in determining community structure. This study provides an overall view of the functional genes present in a highly contaminated environment, and shows the differences in functional populations between wells with varying contamination. As indicated by this work, contaminant level has significant effects on bacterial community structure, the knowledge of which may be important in planning and implementing successful bioremediation strategies in the future.

METHODS

- Six groundwater monitoring wells at the FRC were selected for sampling, to provide a gradient of contaminants, including one uncontaminated background well. Groundwater was analyzed for metal and ion concentrations.
- High molecular weight DNA was extracted from groundwater by filtration and a freeze-thaw grinding and phenol/chloroform extraction procedure.
- A quantity of DNA equal to the amount extracted from 1 L of groundwater was amplified by whole community rolling circle amplification using a TempliPhi kit (GE Healthcare, Piscataway, NJ). DNA was then labeled with a Cy5 fluorescent dye.
- Labeled DNA was purified and then hybridized to the GeoChip functional gene array in a Tecan hybridization station (Durham, NC) at 42ºC 10 h with agitation.
- Arrays were washed, dried and digitally scanned. The signal intensity for each probe was determined using Imagene software (Biodiscovery Inc., Los Angeles, CA).
- Probes were considered positive if the SNR > 1.5 (Signal to Noise Ratio). Genes were considered positive if at least 33% of the probes for a single gene were positive.
- Cluster analysis and Mantel tests were performed with microarray signal intensity data and geochemical data using PC-ORD.
- Canonical Correlation Analysis (CCA) was performed using CANOCO (Biometris).
- The Vegan package in R (v. 2.6.0) was used to perform the BioEnv analysis to select the geochemical variable that explains the greatest amount of variation observed in the gene diversity.

Gene Category	Probe #
Nitrogen Mineralization	1432
Denitrification	2306
Nitrification	347
Nitrogen fixation	1225
Metal reduction and resistance	4546
Org. contam. degradation	8028
Methane oxidation	336
Methane generation	437
Sulfate reduction	1615
Carbon fixation	1018
Carbon degradation	2808
Phosphorus utilization	145
Total	**24,243**

Table 2. Numbers of 50-mer oligo probes for each functional gene category printed on the GeoChip.

RESULTS

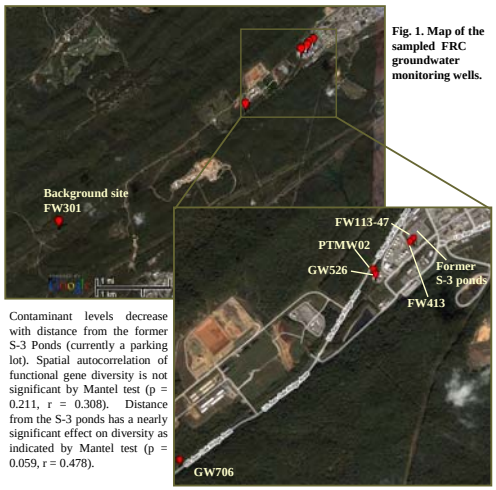


Fig. 1. Map of the sampled FRC groundwater monitoring wells.

Contaminant levels decrease with distance from the former S-3 Ponds (currently a parking lot). Spatial autocorrelation of functional gene diversity is not significant by Mantel test ($p = 0.211$, $r = 0.308$). Distance from the S-3 ponds has a nearly significant effect on diversity as indicated by Mantel test ($p = 0.059$, $r = 0.478$).

Table 2. Geochemical analysis of groundwater from selected FRC wells. Contaminant levels decrease with distance from the former S-3 ponds.

	Distance from ponds (m)	Al (mg/l)	Ca (mg/l)	Cl (mg/l)	Mg (mg/l)	Mn (mg/l)	Ni (mg/l)	Nitrate (mg/l)	Sulfate (mg/l)	U (mg/l)	pH
FW301	7021	0.37	30.51	1.03	2.40	0.09	--	0.47	4.92	--	5.46
GW706	2269	0.35	108.14	42.3	24.43	--	6.65	79.8	23.9	0.06	7.29
GW526	438	0.7	181.89	3.6	76.18	--	--	741	2.36	--	8.00
PTMW02	407	20.4	1846.9	142	193.35	70.1	1.31	7536	114	1.84	4.64
FW413	125	80.74	106.14	73.3	23.7	28.37	4.03	706	1193	21.45	3.75
FW113-47	103	486.07	786.57	456	112	50.22	14.56	5811	2660	66.96	3.44

Fig. 2. Distribution of gene categories in selected wells. All genes detected were divided into functional gene categories. Each gene category is represented in each well, but the percentage of total genes varies by well.

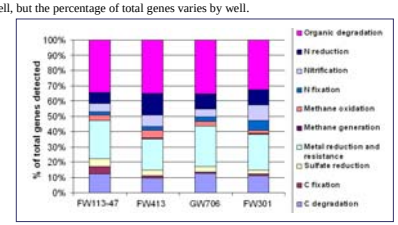


Fig. 3. Cluster analysis of microarray signal intensity of all genes detected by GeoChip. Wells with similar geochemical profiles clustered more closely than wells with less similar water chemistry. Clustering of wells based on signal intensities from GeoChip hybridizations using DNA amplified from the quantity of DNA present in 1 liter of groundwater as template in selected wells.

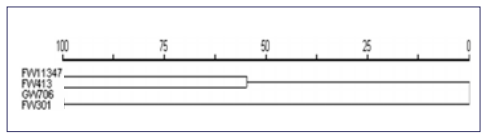


Fig. 4. Relationship between sulfate concentrations and the presence and abundance of sulfate reduction genes (*dsrAB*) in selected wells. In the 170 genes detected with the greatest signal intensity, the percentage of genes detected that are devoted to sulfate reduction appear to decrease with decreasing sulfate concentrations. The total signal intensities also drop off as sulfate concentrations decrease.

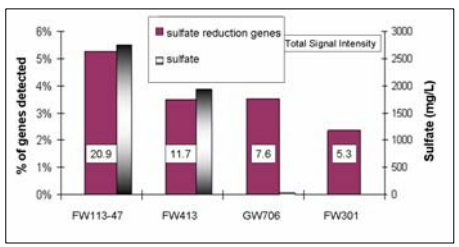


Fig. 5. Relationship between nitrate concentrations and the presence and abundance of nitrogen cycling genes in selected wells. In the 170 genes detected with the greatest signal intensity, the percent of genes devoted to nitrogen fixation appears to increase as nitrate concentrations decreases, though the signal intensities vary. The percent and total signal intensity of nitrate reduction genes appear to be elevated in the middle-nitrate wells, but drops off in the well with the highest nitrate concentration.

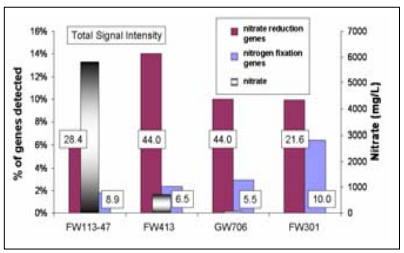


Fig. 6. Relationship between metal concentrations and the presence and abundance of metal-related genes in selected wells. In the 170 genes with the greatest signal intensity, there is little relationship between metal concentrations and the percent of genes detected that are involved with metal reduction and resistance, but the total signal intensity is higher in wells with elevated metal concentrations, indicating a greater abundance of these genes.

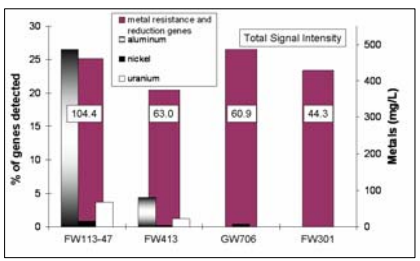
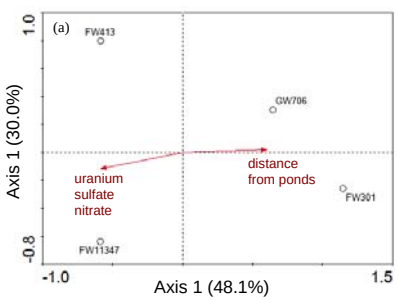
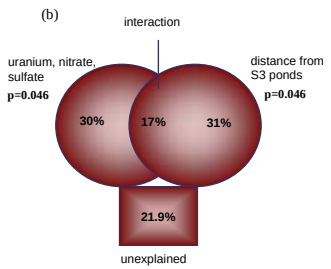


Fig. 7. (a) Canonical Correlation Analysis (CCA) and (b) Variance Partitioning Analysis (VPA) of functional gene diversity with distance from former S3 ponds and geochemical variables (uranium, sulfate and nitrate). (a) Concentrations of uranium, sulfate and nitrate are important factors in the determining the community structure of the contaminated wells. Distance from the ponds is important in the community diversity observed in the low-contamination and background well. These variables explain approximately 70% of the variation observed, at a nearly significant level ($p = 0.083$).
(b) Contaminant levels (uranium, nitrate and sulfate) and distance from the S3 ponds each explain approximately one third of the variation observed in the genetic diversity detected by GeoChip. The interaction between these two variables explains 17% of the variation observed, with 21.9% unexplained by the tested variables.





SUMMARY

- Bacterial communities from similar contaminant levels have more similar functional gene communities and cluster together based on these similarities.
- Functional gene diversity and abundance often correlate with the concentrations of relevant contaminants in these wells.
- CCA and VPA indicate that contaminant levels, specifically sulfate, nitrate, and uranium, and distance from the former S3 ponds are significant factors in determining bacterial community structure.

ACKNOWLEDGEMENT

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