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An Evaluation of Microbial Community Structure and Function in Mercury Contaminated Stream Sediments

Anthony V. Palumbo, Steven D. Brown, Tatiana A. Vishnivetskaya, Meghan M. Drake, Marilyn K. Kerley, Scott Brooks, Lisa A. Fagan, Baohua Gu, Mircea Podar, Liyuan Liang, Miguel Rodriguez, Jr., Craig C. Brandt

Oak Ridge National Laboratory – ERSP Science Focus Area

ABSTRACT

Streambed microbial communities were examined in Oak Ridge, TN, across a mercury gradient using a functional gene array (FGA) and by phylogenetic characterization. The contaminated sites (e.g., mercury at 33.3 µg/g) exhibited elevated gene frequencies in general categories (sulfate reduction, denitrification, carbon utilization, and rubisco) and genes that could be associated with a response to contaminants (e.g., metal resistance and contaminant degradation) over the control site (e.g., mercury at 0.065 µg/g). The 16s clone libraries from the most highly contaminated sites had a higher proportion of cyanobacteria and lower diversity than the control. A synoptic snapshot of 6 sites (mercury range 0.071 µg/g to 39.1 µg/g) shows a poor correlation between mercury in stream sediments and in the water (r = 0.71). This observation is consistent with the complex relationship between stream sediment and stream water concentration that is likely influenced by geochemical factors and mercury speciation. The relationships among community structure, methyl mercury, total mercury, and other geochemical factors will be examined though further analysis (applying non-linear techniques such as artificial neural networks) of the data taken over a full year.

INTRODUCTION

- Discharges and spills of mercury associated with DOE facilities in Oak Ridge, TN, (e.g., Y-12) have contaminated groundwater and streams (e.g., East Fork Poplar Creek – EFK).
- The ratio of methyl Hg to total Hg in the streams is low.
- Total Hg in the streams has declined over time since the early 1980s but mercury in fish has not declined.
- As a step in identifying the geochemical controls on mercury methylation, we have collected samples in an experimental survey of mercury contaminated sites and a control site to obtain the geochemical conditions in which mercury methylation occurs.
- An initial survey of the functional capabilities of the communities in the streams was also conducted using the existing Functional Gene Array (FGA).but only a snapshot of that date is presented here.
- In this poster we present the results of the phylogenetic characterization of the bacterial communities in the sediments of the stream sites.

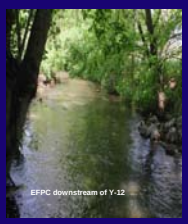
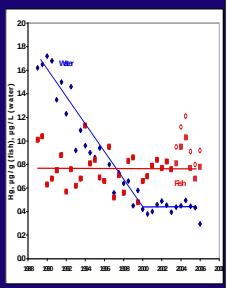


Figure 1. Mercury in stream and fish.



METHODS

Sampling Procedure for Stream Sediments

- Sediment Samples
  - Sediment samples were taken a) in the middle of the creek and b) adjacent to one of the stream banks.
  - The samples were collected by scooping sediment and bailing water in a large bucket and pouring into an autoclaved nalgene container.
  - 1-2 L of the water with fine sediments was centrifuged and ~0.5 g of the sediments was taken for DNA extraction.
  - The total community genomic DNA (cgDNA) was extracted from approximately 0.5-1 g of sediment using the PowerSoil™ DNA Isolation Kit (Mo Bio Labs, Inc., Carlsbad, CA).
- Water Samples
  - Water was back to the laboratory and split into several sets for different analyses geochemical analysis. Including SRP and DOC.
  - Uranium was measured using either a Kpa or an ICP-MS.
  - Centrifuged water samples were analyzed for major anions and cations using ICP and IC.
- Both Sediment and water were analyzed for total mercury content.

Sampling Locations

- Seven sets of samples (water and sediment) have been collected at a total of 6 sites (Figure 2) from October of 2007 to September of 2008.
- At each sampling time we either sampled
  - Two sites representing a highly contaminated (East Fork Poplar Creek 23.4) and a control site (Hinds Creek)
  - Six sites (including those above, 2 more sites in East Fork Poplar Creek identified as EFK, one in White Oak Creek and one in Bear Creek).

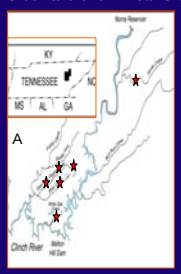


Figure 2. Sampling sites in creeks in the Oak Ridge Vicinity (★). A. Large scale view of 6 sites B. Detail of EFK sites indicated by star.

Phylogenetic Analysis

- The primary method of phylogenetic analysis was via PCR amplification and 454 Pyrosequencing
  - The hypervariable V4 region (~290 bp) of the 16S rRNA gene was amplified from the total community genomic DNA using the high fidelity AccuPrime Pfx DNA polymerase (Invitrogen, Carlsbad, CA) and specially designed primers.
  - The primers, in addition to the 16S rRNA priming region, contain sequences (adaptors) required for 454 FLX pyrosequencing, and the forward primer contains an additional short key (tag) sequence, so that 40 samples could be analyzed in one sequencing run.
  - Raw 454 data (~180,000 Mb from whole plate: region A and B) were initially processed through the RDP pyrosequencing pipeline. During this process the sequences were sorted by tag sequence, the 16S primers were trimmed off and low-quality sequences were filtered out. From ~8,000 to ~12,000 high quality sequences by size 200-220 bp were obtained for each sample.
- Four samples (March 2008 – Hinds Creek A and B, and EFK 23.4 A and B) were analyzed using a standard cloning and sequencing approach in addition to the 454 method
  - The total community genomic DNA was amplified using GoTaq Flexi DNA polymerase (Promega, Madison, WI) and Bacteria-specific primers targeted to Escherichia coli 16S rRNA positions 8-27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1510 to 1492 (5'-GGT TAC CTT TTA CGA CTT-3')
  - This was followed by standard cloning into E. Coli and sequencing with an Applied Biosystems 3730.

RESULTS:

Geochemical Range and Correlations

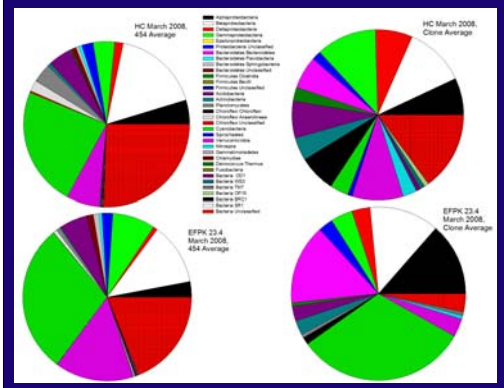
- There was a wide range in many geochemical parameters observed over the sampling sites (e.g., see Table 2 for range from March, May, July, September 2008 sampling dates).
- For the sampling dates with the most complete geochemical data (March to September of 2008) there were high correlations among water and sediment Hg (R=0.72), chloride and nitrate (r=0.976), uranium and both nitrate and chloride (r= 0.92, and 0.96).

	pH	Hgw	Hgs	Cl	NO3	SO4	DOC	U	SRP
		ng/L	ug/g	mg/L	mg/L	mg/L	mg/L	ug/L	ug/L
Max	8.87	107.65	46.80	2.67	18.32	4.31	59.69	267.03	767.09
Min	7.25	0.00	0.00	0.18	0.00	0.79	2.04	0.00	1.58

* Hgw = Mercury in Water; Hgs= Mercury in Sediment; SRP= Soluble Reactive Phosphorous

Table 2. Range of select geochemical parameters observed in the March to September time period at the six sites (average of replicate samples from each sampling time and location).

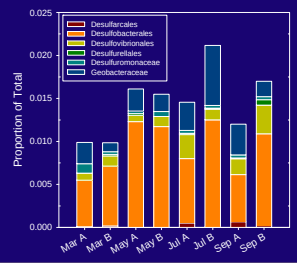
454 vs Cloning & Sequencing for Phylogenetic Characterization



- Clone data appear to over estimate differences between the samples
- Deltaproteobacteria make up a very small proportion of the total but are the only bacteria currently implicated in mercury methylation
- Cyanobacteria are a very prominent group
- A large proportion of the sequences are unclassified

Figure 3. Phylogenetic representation of control and contaminated site for March using the two methods.

RESULTS: Phylogeny by 454 Analysis



- Groups of deltaproteobacteria that include mercury methylators are Desulfovibrionales, Desulfobacteriales and Geobacteraceae
- Most major groups (Fig 4) are present in all samples (except for Desulfurellaceae)
- There appears to be within-site variability at EFK 23.4 in the presence of the Deltaproteobacteria that is as pronounced as the seasonal trend from March though September

Figure 4. Number of sequences indicating different groups of Delta proteobacteria present at EFK 23.4 from March to September 2008.

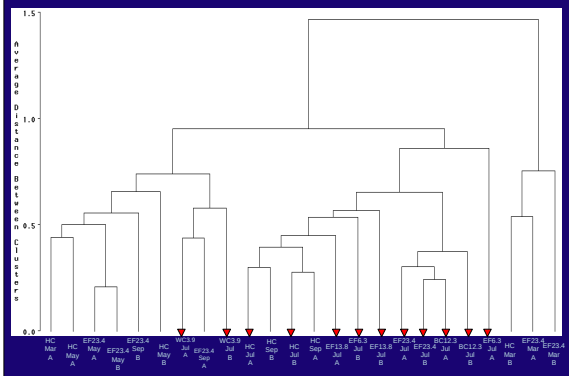


Figure 5. Cluster analysis of 454 sequence data for EFPK 23.4 from March to Sept and all sites for July. July samples are indicated by ▾.

454 Data Analysis

- Results from RDP Naïve Bayesian rRNA Classifier were loaded into SAS and phylogenetic assignment corresponding to 80% sequence similarity was retained
- The fraction of phylogenetic categories in each sample were calculated resulting in 1403 categories
- Only those categories that were present at 1% or higher in at least one sample were retained – 38 categories
- Thus many taxa identified were very rare
- Resulting fractions were analyzed by hierarchical cluster analysis using group average
- **The primary breakdown seems to be along seasonal trends with most of the July samples clustered**

RESULTS: Geochemical Relationships

- Mercury levels (Fig 8) are generally highest in the headwater of EFK (e.g., 23.4) nearest to the Y-12 plant (July data shown is typical)
- Nitrate and sulfate (Fig 8) dominate all the sites with nitrate highest in Bear Creek and White Oak Creek and sulfate highest in the rest
- U is highest in Black Oak Creek
- SRP is highest downstream in East Fork Poplar creek (likely due to a sewage treatment plant)
- MeHg typically increases downstream in EFK (Fig 9)
- The cluster analysis of the sites based on the November geochemical measurements broke out at the highest level according to the concentration of Hg in the sediments and water (Figure 10)

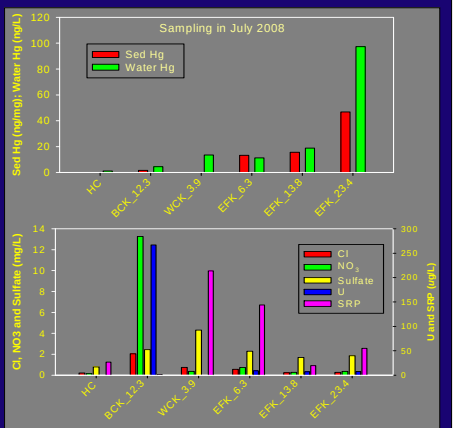


Figure 8. Geochemistry in July 2008.

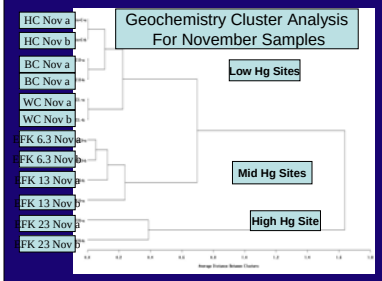


Figure 10. Cluster analysis of sampling sites based on geochemistry measurements for November snapshot sampling.

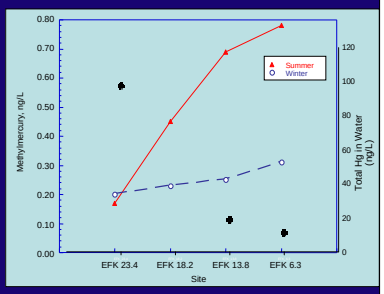


Figure 9. Lines and symbols indicate typical methylmercury concentrations in East Fork Poplar Creek (EFK). Black symbols indicate total Hg in water in July 2008 (right axis).

RESULTS: Functional Community Characteristics and Seasonal Effects

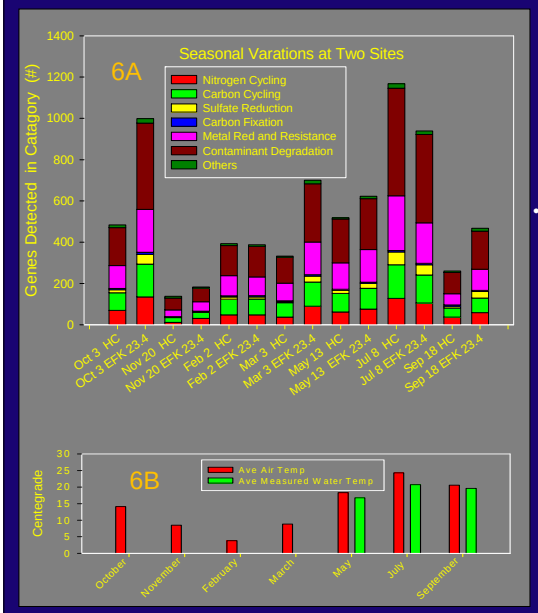


Figure 6. A. Gene frequencies observed for the contaminated (EFK 23.4) and control (Hinds Creek) sampled from November 2007 to September 2008 showing seasonal trends. B. Temperature trends over the same time period.

- There appears to be a strong seasonal trend (Fig 6) in the total numbers of genes detected by the microarray in all categories as illustrated by the trends at the uncontaminated (Hinds Creek) and highest mercury site (East Fork Poplar Creek station 23.4)
  - During most of the year, the uncontaminated site has lower numbers of genes detected.
  - At the time with the highest numbers and highest temperatures, the uncontaminated site has higher numbers of genes detected.

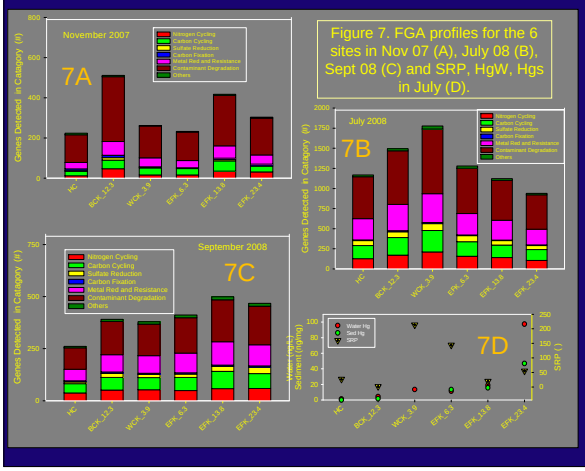


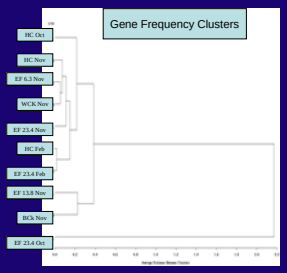
Figure 7. FGA profiles for the 6 sites in Nov 07 (A), July 08 (B), Sept 08 (C) and SRP, HgW, Hgs in July (D).

- There are different patterns in gene abundance (Fig 7) at the different sites at the 3 sampling times for which we have currently processed all six sites.
- In July (the warmest month sampled) the highest gene abundance was at the site with the highest SRP.
- In September there may have been a relationship to mercury and other contaminants that tends to follow the mercury pattern.
- More detailed multivariate analysis will be done.

Results: Community FGA Relationship to Geochemistry

- The results of simple linear correlations of gene frequencies with geochemical measurements for July and September samples of 2008 were different. In this analysis:
  - Sulfate was correlated (r of 0.45 or higher) with all gene categories
  - pH was positively correlated with gene frequency categories except for carbon fixation, metal activity, and contaminant degradation
  - Water mercury concentration was negatively correlated above an r value of 0.40 with the gene categories except for contaminant degradation and the total
  - U was correlated with the carbon fixation gene category at an r of 0.5
- Thus, the most robust relationships were with Sulfate but additional multivariate analysis needs to be carried out to account for other variables such as temperature.

Figure 11. Cluster analysis of sampling sites based on gene frequency from FGA measurements on the first 3 sampling dates.



- Cluster analysis (Figure 11) of the gene frequencies (by category) on the first three sampling dates indicated:
- The dominant feature was the clustering of the EFK 23.4 sample from October apart from everything else.
- The apparent seasonal influence in reduction in frequency in all gene categories across other sites may have suppressed differences in gene frequencies on other sampling dates.
- Additional analysis of the samples taken in warmer seasons may better elucidate the relationships between gene frequencies and geochemistry.

SUMMARY

- It appears that there are seasonal changes in functional community structure with reduced gene diversity in cold months and phylogenetic changes with season.
- The patterns of gene frequency among the sites also appear to vary with the most contaminated sites having higher gene frequencies except in July (the warmest month sampled).
- Additional analysis is underway to further understand the patterns observed and complete the phylogenetic analysis.

ACKNOWLEDGMENTS

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