Summary of a 2005 NABIR PI Meeting Breakout Session

Monday, April 18, 2005

How distinct are microbial communities at different field sites?

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Fredrick Brockman – *Pacific Northwest National Laboratory*

Invited Presenters: Heath Mills – *Florida State University*

Jack Istok – *Oak Ridge National Laboratory*

Chris Schadt – *Oak Ridge National Laboratory*

Matthew Fields – *Miami University*

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Abstract

ERSD sponsors research at field sites as part of both the NABIR and EMSP programs, and in much of this research microbial communities at contaminated and uncontaminated control sites are being examined. This Breakout Session focuses on the properties of the microbial community at NABIR and EMSP field sites prior to (active) bioremediation, and the methods used to characterize these properties. The objective of this Breakout Session is to identify gaps in our understanding of microbial communities that, if we could attain that knowledge prior to accelerated bioremediation, would most advance our ability to manage bioremediation at field sites.

Key issues to be discussed include:

- What is the utility of characterizing the distinctiveness of communities from a phylogenetic (e.g., 16S rRNA) versus functional (activity or gene characterization) vs. individual genomes/proteomes versus metagenomic/metaproteomic standpoints?
- What is our ability to characterize the distinctiveness of communities from a phylogenetic (e.g., 16S rRNA) vs. functional (activity or gene characterization) vs. individual genomes vs. metagenomic standpoints? How will the merits of each of these approaches change over the next 10 to 20 years?
- What level of detail (or level of aggregation) is most useful for characterizing the distinctiveness and diversity of microbial communities at bioremediation field sites? For example, at what taxonomic level (strains, species, genus, family, or above) is phylogeny most useful?

- At what scale(s) do communities vary within a field site, and at field sites?
- Is the distinctiveness/similarity of communities tied to broad hydrogeochemical conditions or to more localized effects? For example, are vadose zone communities hundreds of miles apart more similar than a vadose zone and a saturated zone community a few tens of meters apart?
- To what extent can climatic, geochemical, and geophysical properties of sites/strata be used as proxies for estimating the bioremediation-related properties and capabilities of a microbial community?

Some subsidiary questions to the above issues include:

- What are the implications and reasons for the different views of the community given by molecular methods and culture methods?
- What is the value of heroic efforts to cultivate "uncultured" bacteria?
- Are methods that indicate the metabolic status and/or activity of microbes at the site necessary for a more complete understanding of the community?

How distinct are microbial communities at different field sites?

Introduction and Workshop Format

Fred Brockman gave a short introduction to the breakout section that emphasized the questions and key issues that the session was designed to address, and that were described in detail in the abstract.

Following the introduction, a series of speakers presented very short (2-5) PowerPoint slide presentations using data from the field sites to provoke discussion. Rather than holding all discussion to the end of the series of speakers, the participants in the workshop chose to comment and question during and after each presentation. The presentations and discussions are summarized below.

Major ideas points and concerns brought up during the discussion were capture on a flip chart by Fred Brockman and were used in preparing this summary.

Following the presentations, the input captured on the flip charts was used to establish a list of topics on microbial communities at field sites that need to be addressed. These topics were listed on a new flip chart and the participants were given the opportunity during the break to participate in a weighted voting exercise to establish priorities among the topics for future research emphasis (e.g., funding). Each participant could vote for three priorities giving a weight of 3 to their first priority, 2 to their 2nd and 1 to their 3rd priority. The results were tabulated and are presented below.

Following the break there was a presentation by Jizhong Zhou on his views of the research needs.

There was also a short final discussion on the results of the priority setting exercise and the overall workshop.

Invited Presentation Summary

Heath Mills from Florida State University spoke about 16s clone data from the FRC site. He reported on research lead by Joel Kostka using samples taken from a vertical profile of a borehole at 2-6 m bgs. The samples had a pH from 3.7-6.7. Heath showed significant differences in the community profile dependent on if DNA or RNA was used to assess the community (Figure 1). Over this limited pH range there was no obvious tie to the community structure.

The discussion following the presentation centered on the significance of the differences between results from DNA and RNA. The central question was whether the focus of

community studies should focus on the total population (DNA) or the active population (RNA). This appeared to be one of the critical questions of the session.
Terry Gentry of Oak Ridge National Laboratory spoke on results from a functional gene array approach to characterizing microbial communities at the FRC with Jizhong Zhou. Terry reported very large difference in genes detected between wells that are separated by only ~ 1m at the FRC site. He also showed results at a fairly high level of gene aggregation that still indicated large differences among groups (Figure 2).

There was extensive discussion on the technical aspects of the application of the functional microarrays. These included questions on the ability of the functional microarrays to identify genes from specific bacteria such as Geobacter cytochromes. Questions also related to normalization of the samples, e.g., did they all have the same quantity of DNA. What degree of replication is appropriate given the relatively high cost? Is the cost "high" considering the quantity of information obtained? Can we prioritize probes?

There were also questions related to the results from the array. These included questions such as "Is variability spatial and/or temporal?" At what point can we identify a set of dominate players in a community? This brought the discussion to more general issues such what is the best method. Again there was a consensus that there is no best method but multiple methods are needed to determine the active fraction of the bacterial community.

Chris Schadt of Oak Ridge National Laboratory spoke on application of DNA amplification for functional gene arrays to communities at the FRC site which he is working on with Jizhong Zhou. When dealing with samples from sites where no biostimulation has taken place, Chris identified the fundamental problem of low biomass in the samples. Chris discussed a whole community genome amplification method being worked on at ORNL with Jizhong Zhou as a promising approach to the low biomass problem. He presented some results from the application of the functional genome microarray to the FRC samples amplified using the method (e.g. Figure 3). Out of 5 wells, Chris reported both unique genes and some overlapping genes. It was obvious from the data that there were large differences in communities in samples at just this one site.

Discussion following the presentation centered on the quality of data from PCR-based approaches and array-based approaches. Questions arose on the potential bias of using PCR methods and the potential for the whole community genome amplification to be less biased. In addition, the discussion centered on the following questions: Do geochemical factors affect certain set of genes? Are dead cultures considered unculturable? If so what are the implications and is this a cause of bias. A consensus was expressed that there is a need to assay using more than one method and that several molecular techniques that are complimentary need to be applied. There were opinions that no one Abest method≅ exists. Also, experiments should be tried such as spiking with dead cells.

Matthew Fields of Miami University spoke about microbial diversity in groundwater at the FRC site and focused on interpretation of cloning and sequencing results of different

gene categories. He indicated that at the FRC site microbial diversity from cloning and sequencing of specific genes (DNA) is typically low when there were either high or low levels of contamination and that diversity was highest in intermediate contamination zones. Also, bacteria present in highly contaminated regions of the FRC are very difficult to culture. He indicated that there were difficulties in interpreting data. The diversity of the methods is vast and the application of many of the methods developed for standard ecology to microbial data is largely untested. Even similar clustering approaches can yield differences (Figure 4). More attention to and validation of the methods developed for ecology is warranted to increase progress in microbial ecology.

Some of the discussion provoked by his presentation on groundwater communities addressed the unknown relationship between what is present in the groundwater communities and what is present in the sediments. It was pointed out that this question has not been adequately addressed especially in low cell density environments such as the sediments at the FRC. In addition there was discussion on how long a cell was metabolically active after it was not able to replicate.

Jizhong Zhou of Oak Ridge National Laboratory summarized some of the ideas from the workshop in the final presentation. He addressed the differences between microbial communities and macroscopic communities that make addressing microbial communities particularly difficult. These differences include the extremely high diversity and very unequal abundance often present in microbial communities. This makes some analysis methods (e.g., species diversity indices) difficult to interpret. He addressed the question of whether we can use genomics and sequencing to understand whole community diversity. He pointed out the difficulties in addressing activity using mRNA as it is difficult to isolate and amplify. He also addressed the question: Is microbial ecology qualitative and/or quantitative?

Discussion following his presentation included consideration of the difficulties in sampling. Terry Hazen spoke about the problems of sampling and how microbial communities can be seriously altered by heat and vibration associated with drilling. Also, the representativness of the samples to the aquifer and sediments must be considered. Additional considerations for near surface sediments are the influence of rain events or a rainy season on the community.

How does the rainy season affect the community? Bioremediation?
Can we use culturing to study the behavior of a small consortium (without removing cells from the community and focusing on single cell isolation)?

Discussion Summary

Key issues identified before the workshop were extensively addressed during the discussions. These included:

- Importance of phylogenetic vs functional characterization

- Ability to characterize communities - phylogenetic and functional
- Level of detail or aggregation
- Scale of variation
- Localization vs. broad hydrogeochemical conditions

Items identified as secondary issues prior to the meeting were also discussed but some emerged as actually being key or primary issues that need to be addressed in future research.

- Are methods to indicate metabolic status or activity critical – This issue became a very important discussion topic
    - Live vs. dead?
    - RNA vs. DNA
    - stable isotope techniques
- Culturing
    - can be worth the effort but not necessary for all studies
    - the more culturing that is done the better the molecular methods can be interpreted.

An objective of the workshop was to address the question - How distinct are field sites?

- The consensus was that the answer depended on the level of aggragation (e.g., how similar on clone, species, genus, or higher level) used to answer the question.
    - Communities can be very distinct at clone level
    - More similar at higher levels
    - What level is an appropriate description? The answer may depend on the specific reason behind the question.

Additional Issues discussed

- Importance of Quantitative vs Qualitative measures
- Ecological Issues - Methods to compare communities
    - Value of diversity indices
    - How to compare communities
        - Adapt ecological methods
        - PCA
        - Cluster analysis
    - Dimensionality of data vs number of samples
- Importance of Technique development
    - Need to cross validate methods to gain confidence
        - e.g., Validate RNA libraries/arrays with FISH
- Difficulty in addressing temporal and spatial variability with limited samples
- The lack of information on the scale of spatial variations

Prioritization Exercise

After the presentations and discussions, the following potential set of research priorities arising from the discussion were presented to the group and the results of the voting on priorities is given on the far right.

- Technique development 18
    - E.g., extraction and analysis
- Temporal (7.5) and Spatial resolution (10.5) 18
- Spatial resolution 18
- Relationship between Bacteria in GW & sediment 1
- DNA (presence) vs. RNA (activity) 18
- Ecological issues 47
    - Understanding relationships in different environmental contexts between:
        - Functional diversity
        - Functional redundancy
        - Structural stability/instability
- Training grants 3

Conclusions

By far the highest priority or need identified by the workshop participants was addressing ecological issues. Following that was a tie for 2nd among technique development, spatial and temporal resolution taken together, and the importance of DNA vs RNA in assessment of communities.

The workshop provoked serious and lengthy discussions among the participants and there was a surprising level of consensus reached on the priorities and needs for future research. Clearly, the highest priority was for targeting ecological issues related to community structure such as functional diversity, functional redundancy and structural stability. These may all be important factors in planning, monitoring, and measuring the success of bioremediation activates targeted at bioimmobilization of metals.

Figure 1

High level aggregation of clone library results from FRC samples

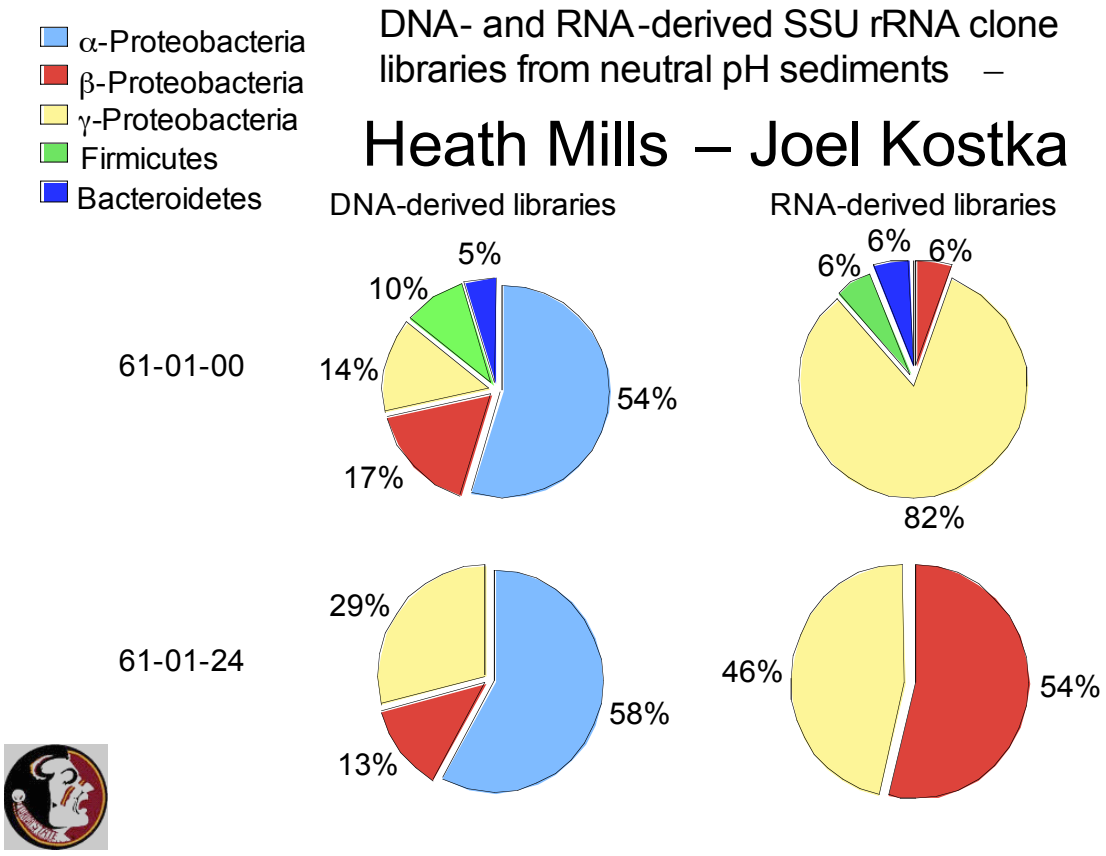

Figure 2

Functional gene array results at enzyme category level

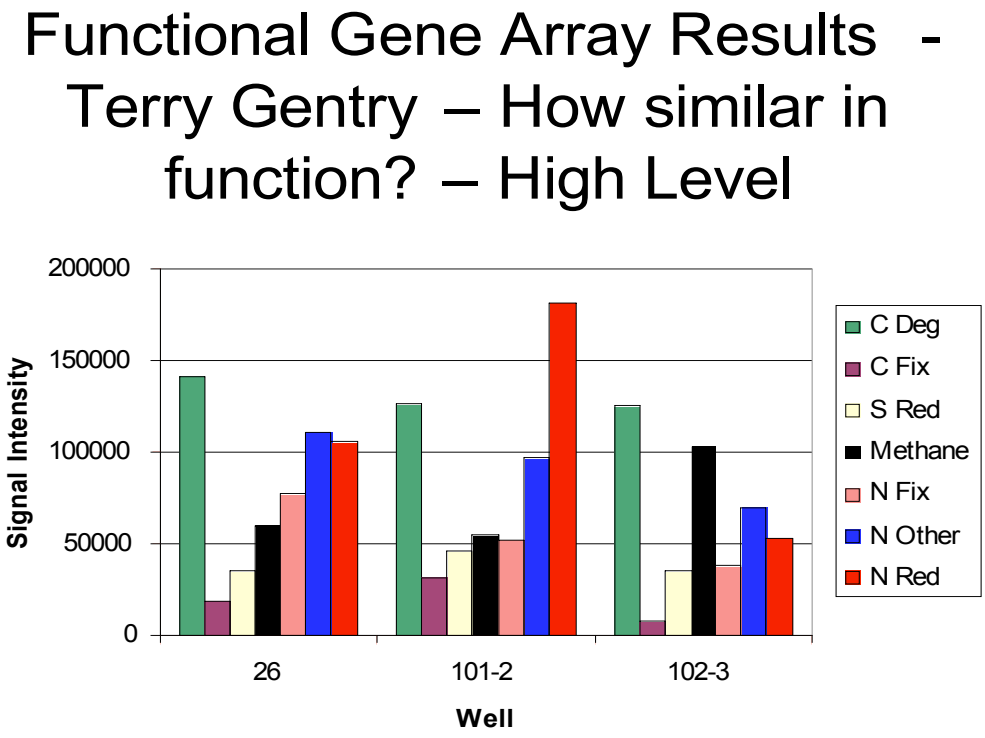

Figure 3

Functional gene array results at individual gene level

Chris Schadt – Microarray results
How similar at clone level?

	FW300	FW003	FW021	FW010	FW024
FW300	61(20)	189(36)	174(35)	80(21)	111(23)
FW003		25(11)	144(35)	61(17)	84(20)
FW021			10(5)	64(20)	90(24)
FW010				6(5)	118(37)
FW024					30(16)
Total Genes Detected	302	219	192	130	190
Simpson's diversity ind ex (1/D)	125.5	67.1	26.6	17.4	35.7
FRC Area Location (B=background)	B	2	1	3	3
Specific Conductivity (mS/cm)	0.33	1.60	11.40	36.00	15.83
pH	6.7	6.0	3.4	3.5	3.6
Aluminum (ICP -mg/L)	0.2	0.4	398.0	1120.0	527.4
Chloride (IC -mg/L)	2.4	124.7	220.2	686.4	281.4
Nitrate (IC -mg/L)	2.6	1015	8823	43019	8481
Sodium (ICP -mg/L)	2.0	44.0	522.0	849.0	950.1
Sulfate (IC -mg/L)	6.40	16.31	122.10	8.28	987.13
Uranium (ICPMS -mg/L)	0	0.10	12.2	0.17	44.81
Technetium (LSC -pCi/L)	0	141	30974	7190	36956
Tricloroethene -GSMS (u g/L)	<5	<5	NA	42.00	84.75

Figure 4

Different representations of the relationships among samples based on clone data