Report on the NABIR Workshop

Application of Genomic Technology to Bioremediation

December 5-7, 1999
Arlington, VA

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PURPOSE

The mixed wastes containing metals, radionuclides, and solvents in many DOE contaminated field sites are an immediate and complex challenge to DOE waste management responsibilities. Although *in situ* bioremediation remains the most potent technology for treating such mixed wastes, a potential difficulty in implementing bioremediation is that the predictability of bioremediation process performance cannot be made with confidence. In some cases, predictability is limited by the lack of fundamental knowledge about microbial community structure, composition, functions, and dynamic changes under different environmental conditions; and in other cases, by the lack of accurate parameter estimation. Current methods for measuring and evaluating the effectiveness of bioremediation are too cumbersome. Rapid, simple, reliable, quantitative and cost-effective tools that can be operated in real-time and in field-scale heterogeneous environments for assessing bioremediation endpoints are needed.

The purpose of this NABIR workshop was to bring people with different technical backgrounds together, promote scientific exchange, discuss the theoretical and practical implications of advanced genomic technologies within a bioremediation context, and identify scientific needs.

STRUCTURE

The focus of this workshop was specifically on the recently developed genomic technologies (nucleic acid, whole-genome, proteome) that might provide more simple, near-real time, field-deployable, specific, sensitive, quantitative and cost-effective analytical tools and techniques for monitoring *in situ* microbial communities in natural environments. Four scientific sessions were arranged and 40 people from different fields attended this workshop (see Appendix for the program agenda and participant list). Readers are encouraged to contact speakers and participants directly for presentation copies or in-depth discussions. Readers can also contact the organizers for help in obtaining presentation copies.

Before the scientific discussion, Dr. Anna Palmisano gave a welcome remark and Dr. Jizhong Zhou highlighted the needs, goals and structure of this workshop. Dr. James M. Tiedje initiated Session I with a discussion of key issues and challenges for analyzing microbial ecology and community dynamics within the context of bioremediation.

Diversity, quantitation, sensitivity, function, cause and effect were identified as significant challenges. In subsequent sessions, speakers amplified on these themes and issues relative to environmental analyses. Speakers in Session I highlighted common molecular techniques employed by the environmental microbiology community for those participants that are not that familiar with existing nucleic acid techniques. Session II focused on the recent developments in microarray technology with emphasis on the merits and limitations of existing microarray printing, hybridization and analysis techniques. Included in Session II were industry participants, who provided a very balanced and well-rounded perspective of their own (and competing) microarray systems. Session III highlighted some successful examples of microarray technology for genomic studies, and exploratory studies for microbial community analysis. Session IV included a review of other genomic technologies and a roundtable discussion of key issues for advancing technology within NABIR, such as specificity, sensitivity, quantification and cost analysis of genomic technologies

MAJOR THEMES and CONCERNS

Diversity and *quantitation* were identified as the greatest challenges facing environmental microbiologists (Fig. 1). Other concerns related to sensitivity, specificity, activity, reproducibility, bias and functions were also addressed. Genomic technologies were discussed from the point of sample collection and nucleic acid extraction, through detection and the development of real-time, integrated systems to be utilized at the point of use. It was noted that different types of bioremediation require different types of microbial information, and that the relative importance of NABIR related concerns (Fig 1) is dependent upon the bioremediation scenario. That is, intrinsic bioremediation puts more emphasis on microbial presence, function, and detector sensitivities. Biostimulation requires quantitation of microbial diversity, competition, stability, and succession processes. Bioagumentation, on the other hand, requires quantitation for microbial tracking, dispersal, and activity (expression).

Several different microarray formats were also discussed, including planar arrays, suspension arrays, gel pad arrays, and various methods for hybridization enhancement. An important admission from all microarray presenters was that existing technology provides *relative*, not absolute, quantitation of target genes. Hence, all changes in gene expression or abundance are relative to a baseline condition. The importance of reproducible, quantitative nucleic acid extraction, amplification, labeling and detection techniques therefore became more obvious, and was discussed extensively in the roundtable sessions.

PCR-based techniques were discussed primarily within the context of assessing total microbial diversity or quantifying individual genes/microorganisms. The ability of PCR to quantitatively and faithfully reproduce *total* in situ microbial diversity is unknown; the implications of this uncertainty for microarray-based detection systems was a major focal point of the roundtable discussions. The greatest challenge is how to couple the sensitive, single gene-based PCR technology with the multiple genes-based microarrays hybridization for quantitatively assessing microbial community structure and activity in natural environments.

Major Themes and Discussion Topics

NABIR Concerns	Extraction	BAC Libraries	PCR	T-RFLP	qPCR	Planar Microarrays	Suspension Microarrays	Mass Spectrometry	Integrated Systems
Diversity	X	X	X	X	X				X
Quantitation	X		X	X	X	X	X		X
Sensitivity						X	X	X	X
Specificity						X	X		
Reproducibility			X	X	X	X	X		
Bias	X		X		X				
Function	X	X							
Cause/Effect	X	X							X
Field Applications	X				X			X	X

Genomic Technology

Figure 1. The spectrum of NABIR-related concerns against the workshop topic areas, with 'X' denoting areas of greatest discussion (and uncertainty).

Compared to conventional molecular methods, microarrays offer the additional advantages of high sensitivity, rapid detection, lower cost, automation, two-color hybridization detection (for simultaneous display of differential gene expression), and low background levels. Although the power of DNA microarrays to assess microbial diversity and activity was obvious, the following questions and concerns were expressed: (1) How are microarrays implemented in a bioremediation context. (2) What gene sequences should be targeted for microarray construction? (3) Are microarray hybridization quantitative? (4) If PCR is a limiting factor, can we develop microarrays to avoid PCR? (5) Can PCR-based quantitative methods be coupled with microarray hybridization for quantifying microbial community structure and activity when the biomass in environmental samples is low; (6) Can 16S rRNA gene-based oligonucleotide microarrays be designed and constructed for analyzing microbial community structure and activity with reliable specificity? (7) How do environmental contaminants affect microarray hybridization? (8) How do we rapidly compare microarray hybridization data with many different samples and extrapolate functional pathways?

SCIENTIFIC NEEDS

Genomics and microarray-based technology represent a potential revolution in biological sciences. Although genomic technologies are extremely powerful tools for

monitoring gene expression and detecting genetic polymorphisms, the concept and performances of microarray hybridization have not been tested rigorously with complicated environmental samples. Studies on microarray hybridization, especially oligonucleotide microarrays, are needed in terms of specificity, sensitivity, quantitation, activity and data analysis within the context of environmental samples.

Diversity issues have so far prevented any 'standardization' of genomic techniques for routine implementation in a bioremediation context. Therefore, the group suggested working towards generally accepted means of evaluating purification procedures; developing generally accepted lysis procedures; understanding humic substances and their interaction with nucleic acids; developing simultaneous DNA/RNA extraction methods; and establishing a set of microorganisms to benchmark extraction efficiencies across different methods and sample types. Because the adsorption properties of different samples are so different, the group also recommended 'qualifying' measures of extraction/detection efficiency with a measure of copies per μg extracted DNA or RNA, NOT copies per g of soil.

Techniques that show differences in community structure along spatial, temporal or chemical gradients are still useful. However, there was significant concern regarding the genes that are important to monitor in any given situation. This concern was related to our incomplete understanding of relevant microorganisms for any bioremediation activity. To advance genomic technologies into the areas of metabolic potential and function, cause and effect and in-field bioremediation applications, it was recognized that future developments in RNA analysis and automated, integrated systems will be required. Finally, accuracy and absolute quantitation will be important because bioremediation is a field dominated by engineers, and genomic information must be correlated with chemical/physical measures obtained by other analytical methods.

Appendix A: Program Agenda

US Department of Energy NABIR Workshop

Application of Genomic Technology to Bioremediation

Doubletree Hotel, Arlington, VA
December 5–7, 1999

Goals

- To discuss and assess genomic technologies that offer the promise and potentials for providing large scale, real time, and field-applicable tools for microbial community analysis and characterization.

- To discuss and identify the problems, challenges and strategies of applying genomic technology to in situ bioremediation.

Program agenda

Sunday, December 5, 1999			
Arrival			
Monday, December 6, 1999			
7:00–8:00	**Breakfast**		
8:00–8:05	Welcoming remarks	Anna Palmisano	DOE
8:05–8:15	Workshop structure, goals, and desired products	Jizhong Zhou	Oak Ridge National Laboratory
	Session I: Introduction and overview of molecular methods for microbial community analysis		
8:15–8:45	Key issues and challenges in studying microbial ecology and community dynamics	James M. Tiedje	Michigan State University
8:45–9:15	Kinetics effects in the amplification of mixed populations of homologs by the polymerase chain reaction	Stephen Giovannoni	Oregon State University
9:15–9:45	Systematic community analysis using T-RFLP and DGGE	Allison Murray / Terry Marsh	Michigan State University
9:45–10:15	Analysis of large genomic fragments derived from complex microbial assemblages: applications for identification, quantification, and characterization of uncultivated microorganisms	Ed DeLong	Monterey Bay Aquarium Research Institute
10:15–10:45	Quantitative PCR	Andy Ogram	University of Florida
10:45–11:00	Break		
	Session II: Microarray Technology		
11:00–11:30	Printing technologies for microarray construction	Don Rose	Cartesian Technology
11:30–12:00	Analysis of microarrays labeled with multiple fluors using a confocal, four laser scanner	Ernie Kawasaki	GSI Lumonics
12:00–12:30	Genosensor-based ecotoxicity response assessment	Ken Beattie	Oak Ridge National Laboratory
12:30–1:30	**Lunch**		

Session III: Applications and potentials of microarray technology for genomic and environmental studies

Time	Topic	Speaker	Affiliation
1:30–2:00	DNA microarray technology development	Mark Schena	Stanford University.
2:00–2:30	DNA microarrays for monitoring gene expression in mycobacterium tuberculosis	Marty Voskuil	Stanford University
2:30–3:00	DNA Microarray for Monitoring the Stage of Bioremediation	Mary Lowe	Loyola College, Maryland
3:00–3:30	Break		
3:30–4:00	Oligonucleotide microarrays and direct nucleic acid detection from environmental samples (i.e., no PCR)	Darrell Chandler	Pacific Northwest National Laboratory
4:00–4:30	Oligonucleotide microarrays for distinguishing nitrifiers	John Kelly	Northwestern University
4:30–5:00	DNA microarrays for microbial community characterization	Jizhong Zhou	Oak Ridge National Laboratory

Tuesday, December 7, 1999

7:00–8:00	**Breakfast**		

Session IV: Roundtable discussion (A) Other genomic technology; (B) key issues to NABIR

Time	Topic	Speaker	Affiliation
8:00–8:05	Other genomic technology	Darrell Chandler	Pacific Northwest National Laboratory
8:05–8:30	PCR on chips	Robert Foote	Oak Ridge National Laboratory
8:30–8:55	Laser desorption mass spectrometry for microbial DNA analysis	C.H. Chen	Oak Ridge National Laboratory
8:55–9:20	Overview of other microarray technologies	Darrell Chandler	Pacific Northwest National Laboratory
9:20–9:45	Integrated Systems	Fred Brockman	Pacific Northwest National Laboratory
9:45–10:00	**Break**		
10:00-10:05	Key issues to NABIR	Fred Brockman	Pacific Northwest National Laboratory
10:05–10:45	Nucleic acid extraction and purification from environmental matrices	Andy Ogram / Richard Hurt	University of Florida/Oak Ridge National Laboratory
10:45–11:55	Specificity, sensitivity, quantitation and data processing	Jizhong Zhou	Oak Ridge National Laboratory
11:55–12:35	Cost analysis, service center and collaboration	Darrell Chandler	University of Idaho/ Pacific Northwest National Laboratory
12:35-	Adjourn	Jizhong Zhou	Oak Ridge National Laboratory

Appendix B: List of Participants

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