# DEVELOPMENT AND APPLICATIONS OF MICROBIAL ECOGENOMIC INDICATORS FOR MONITORING WATER QUALITY: REPORT OF A WORKSHOP ASSESSING THE STATE OF THE SCIENCE, RESEARCH NEEDS AND FUTURE DIRECTIONS

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**Abstract:**
This article brings forth recommendations from a workshop sponsored by the U.S. Environmental Protection Agency's Science to Achieve Results (STAR) and Environmental Monitoring and Assessment (EMAP) Programs and by the Council of State Governments, held during May 2002 in Kansas City, Kansas. The workshop assembled microbial ecologists and environmental scientists to determine what research and science is needed to bring existing molecular biological approaches and newer technologies arising from microbial genomic research into environmental monitoring and water quality assessments. Development of genomics and proteomics technologies for environmental science is a very new area having potential to improve environmental water quality assessments. The workshop participants noted that microbial ecologists are already using molecular biological methods well suited for monitoring and water quality assessments and anticipate that genomics-enabled technologies could be made available for monitoring within a decade. Recommendations arising from the workshop include needs for (i) identification of informative microbial gene sequences, (ii) improved understandings of linkages between indicator taxa, gene expression and environmental condition, (iii) technological advancements towards field application, and (iv) development of the appropriate databases.
**Keywords:** ecogenomics, genomics, microbiology, proteomics, source tracking

## Article:

### *1. Introduction*

Clean water is one of our most valuable natural resources. In addition to providing safe drinking water it assures functional ecosystems that support fisheries and recreation. Human population growth and the associated increased demands on water pose risks to maintaining acceptable water quality. Government agencies oversee environmental management to maintain water quality, assure the public health and preserve the environment. Key to these responsibilities is assessment of source waters and the aquatic systems that receive inputs from industrial waste and sewage treatment plants, storm water systems and runoff from urban and agricultural lands. Rapid and confident assessments of aquatic resources form the basis for sound environmental management.

In this article, we use "environmental monitoring" to include assessments of both water quality and the ecological condition of aquatic habitats. Environmental monitoring includes measurements of physical characteristics (e.g. pH, temperature, conductivity), chemical parameters (e.g. oxygen, alkalinity, nitrogen and phosphorus compounds), and abundance of certain biological taxa. Bioindicator taxa range from the microscopic, such as Escherichia coli or enteric viruses for fecal contamination and various algal taxa for trophic status, to macroorganisms such as insects and fish for pollutant or temperature effects and trophic status (US EPA, 1990). Monitoring could also include assays of biological activity such as alkaline phosphatase (Overbeck and Chrost, 1990), tests for toxins such as microcystins and direct measurements of pollutants such

as heavy metals or hydrocarbons.

The importance of microorganisms in monitoring for threats to public health and the value of developing new methods for that purpose have been discussed in a recent report from the American Academy of Microbiology (Rose and Grimes, 2001) and will not be extensively covered here. Nonetheless, there is significant overlap between public health and ecological assessments in both the use of micro-biological indicators and in the opportunity to improve environmental monitoring with technologies emerging from genomics and proteomics.

Microbes can be very informative for environmental monitoring since their short generation times allow them to respond rapidly to changing environmental conditions. Molecular methods are commonly used in environmental microbiology research, but have not gained routine use for water quality assessments in support of environmental management. In fact, no molecular biological based method is currently approved by the EPA to monitor water for fecal contamination although work in this area is now rapidly progressing (Santo Domingo et al., 2003; Dick and Field, 2004; Haugland et al., 2005). Presently available molecular methods, especially those that can detect specific indicator microorganisms or pathogens with remarkable speed and sensitivity, could be brought into routine monitoring programs with relatively little further developmental research. Development of real-time, multi-parameter, remote sensing based on these methods is envisaged as a near term possibility. However, there is an overriding need to first identify where molecular methods can improve assessments and then to validate the methods in the context of what they can tell us of environmental condition (Fisher et al., 2003).

Incorporating molecular microbiological methods into environmental monitoring may have also been slowed by the need for trained personnel, the expense of equipping a laboratory and the higher cost of molecular assays compared to microbiological culture tests. Skilled personnel are now more available since molecular methods have become integral to microbiology curricula. Expenses for molecular biology equipment and reagents continue to decline. As more molecular methods become adopted for environmental monitoring, interests in commercializing them will increase making them ever more available at lower costs.

The workshop was organized to assess the state of the science, to identify research needs and to evaluate the prospects for molecular methods, in particular those arising from advances in microbial proteomics- and genomics-enabled technology, to improve water quality assessments. Here we provide our findings from the workshop discussions.

## 2. Molecular Methods in Environmental Microbiology

Molecular biological methods are routinely used in environmental microbiology research. Environmental microbiologists have developed many assays that take advantage of the speed, specificity and sensitivity of the polymerase chain reaction (PCR) to identify isolated strains of microorganisms. Moreover, molecular methods are widely utilized by microbial ecologists because only a very small percentage of microorganisms present in the environment can actually be grown in the laboratory. Growth of bacteria on agar plates or in liquid cultures has traditionally been pre-requisite to their detection and identification. Pace and his colleagues (Olsen et al., 1986; Pace et al., 1986) first suggested that DNA in environmental samples could yield sequences of genes from microorganisms to both identify the microorganisms and learn of their genetic capabilities without ever having to grow them. Over the last 15 years, large data bases of DNA sequences have been compiled. Genes such as those coding for ribosomal RNA (rRNA), DNA gyrase, and protein synthesis elongation factors from known, well characterized strains provide information on identity and phylogenetic (evolutionary) relationship, while functional genes, such as those coding for enzymes in the nitrogen and carbon cycles, biodegradative enzymes and pathogenic determinants give information on physiological capacity. At the same time, gene sequences obtained from samples collected in many different environments have helped to fill out the databases and provide an overview of microbial diversity. Although microbial diversity is vast, comparison of new sequences to sequences in databases provides information that can identify specific microbial groups and assess microbial diversity in relation to environmental conditions.

Molecular methods used to identify specific microorganisms and to assess microbial community diversity using DNA sequences are listed in Table I. These methods are applicable not only for identifying isolated microorganisms, but also for detecting specific microorganisms (e.g. fecal indicators, pathogens) and examining community composition using microbial community DNA. Specific and sensitive PCR detection of pathogens and toxin-producing harmful algae in freshwater and marine systems is a routine and common practice in microbial ecology research.

TABLE I

Status of current genomic tools that can be used to assess water quality [adapted from American Academy of Microbiology (2000)]

| Tool | Description | Current status | Reference |
|---|---|---|---|
| Direct hybridization | Detection of genes, gene expression | In wide use requires sequence knowledge and DNA to label for probe | Barkay *et al.*, 1985; Paul *et al.*, 1999 |
| PCR, RT-PCR | Rapid, sensitive detection of specific taxa, genes, or gene expression (RT-PCR) | In wide use, requires DNA sequence information | DeLeon, 1990; Girones *et al.*, 1995; Zehr *et al.*, 2001 |
| RAPD, RFLP, AFLP, AP-PCR, DGGE, T-RFLP, RFLP, ARDA | PCR-based, provides "fingerprint" of microbial community or the genome of microbial strains. Useful for description of community composition. Useful for source tracking | Widely used | Muyzer *et al.*, 1993; Ferris et al, 1997; Bernhard and Field, 2000a; Kerkhof *et al.*, 2000; Marsh *et al.*, 2000; Fuhrman *et al.*, 2002; Moyer *et al.*, 2001; Schafer and Muyzer, 2001 |
| Q-PCR, real-time PCR | Rapid, sensitive, quantitative detection of target | Use increasing rapidly, requires sequence knowledge | Rose *et al.*, 1997, Gruntzig *et al.*, 2001; Suzuki *et al.*, 2000 |
| Fluorescent *in situ* hybridization (FISH) | Allows detection, visualization of individual cells | Widely used, requires actively growing cells. Can be combined with activity assays | Amann *et al.*, 1995; Ouverney and Fuhrman, 2000 |
| NASBA | Isothermal RNA amplification; pathogen detection | Under development | Kozwich *et al.*, 2000; Fox *et al.*, 2002 |
| Microarrays | Community gene analysis, community functional analysis, community activity | Under development | Wu *et al.*, 2001; Cho and Tiedje, 2002; Adamczyk *et al.*, 2003 |
| Solid-state biochip | Real-time detection of cells and cell products | Under development | |

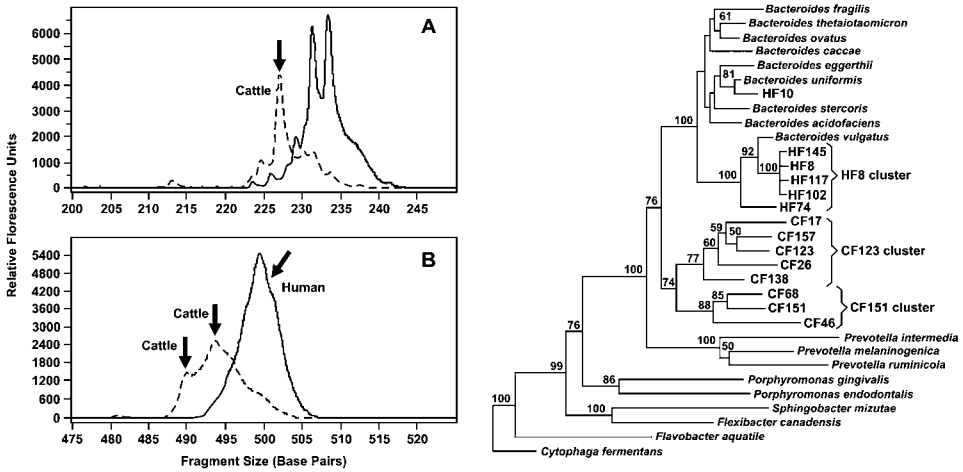


*Figure 1.* Fecal source identification with molecular methods. Left, T-RFLP analysis of rRN Agenes to differentiate Bacteroidetes DNA fragments isolated from human and cattle feces. Arrows indicate host-specific markers. Right, phylogenetic tree of Bacteroidetes sequences identified with the T-RFLP approach, showing unique groups associated with specific hosts. Sequences were used to design host-specific PCR primers that could identify cattle and human feces in water (Bernhard and Field, 2000a, b).

Differences between homologous genes can be rapidly distinguished by restriction fragment length polymorphisms (RFLP) rather than nucleotide sequence determinations. An RFLP approach, termed terminal restriction fragment length polymorphism (T-RFLP) analysis, provides information on the composition of microbial communities. This is a PCR-based analysis in which PCR products from microbial community DNA (labeled at one end with a fluorescent dye), are cut with a restriction enzyme, the resulting pieces are then separated according to size, and the terminal restriction fragments are visualized by their fluorescence yielding a

snapshot of microbial community composition. Information on microorganism identification and community diversity is provided by comparing lengths of the fluorescent labeled fragments with fragment lengths determined from computer analysis of known sequences in a database (Liu et al., 1997; Marsh et al., 2000). Figure 1 is a simple T-RFLP analysis of rRNA gene PCR products that uses enteric bacteria to distinguish between feces originating from either humans or cows. The method may be used with microbial community DNA, and eliminates the need for incubating samples to achieve bacterial growth (Bernhard and Field, 2000a, b). Clearly, the ability to identify sources of fecal pollution (e.g., sewer systems or farmland), and to rapidly do so without having to culture indicator bacteria would be a significant advance in water quality monitoring. Genomic DNA fingerprinting methods such as randomly amplified polymorphic DNA (RAPD), arbitrarily primed-(AP-PCR) or interspersed repetitive sequence PCR (rep-PCR), ribotyping, and amplified fragment length polymorphism (AFLP) are usually employed with pure cultures and have the potential to differentiate between very closely related strains of the same bacterial species. As such, these fingerprinting methods also have potential for determining the source of fecal pollution (e.g., human vs. non-human).

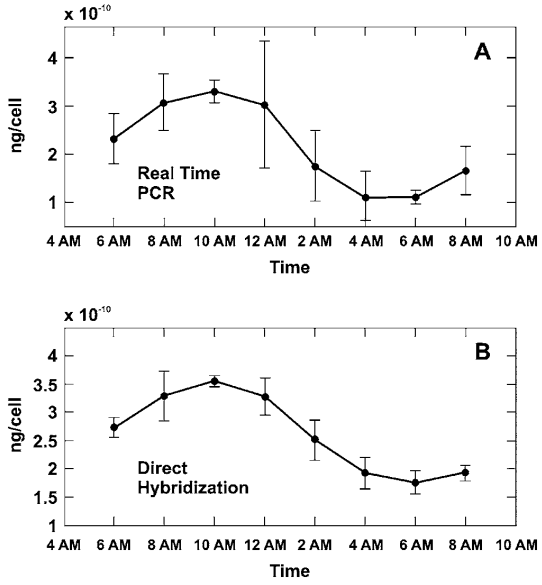


*Figure 2*. Detection of *Phaeodactylum tricornutum rcbl* (RUBISCO) mRNA in open ocean water. *P. tricornutum* rbcL gene expression was quantified over a diurnal cycle by real time PCR (A) and by hybridization of RNA immobilized on membranes with $^{35}$S-labeled probes (B). One challenge of ecological applications of genomics will be to account for variability in natural systems such as the diurnal activity of RUBISCO 3 (J. Paul, Univ. South Florida).

While analysis of DNA sequences can be used to gain information on the standing crop of microorganisms, the active fractions of microbial communities may be assessed with methods employing microbial community mRNA to measure gene expression. Development of RNA-based technologies has been slower because of the technical challenges of working with RNA, which is more labile than DNA. However, much has been accomplished with 16S rRNA, which in essence is an RNA transcript, and those results to some extent can be interpreted as community activity measurements. Methods using mRNA extracted from environmental samples have advanced and provide meaningful results on microbial gene expression (Wawrik et al., 2002). mRNA can be hybridized with probes for quantification of broad groups or specific types, or reverse transcribed and amplified by PCR (RT-PCR) with specific primer sets to provide quantitative information on microbial gene expression (Figure 2). Products from RT-PCR can be cloned then sequenced or cut with restriction enzymes for analyses of gene diversity.

Individual microbial cells can be directly identified in environmental samples by in situ hybridization with fluorescent dye labeled oligonucleotide probes (Amann et al., 1995) and by in situ PCR (Chen et al., 1997). In situ single cell detection can be combined with activity assays using $^{14}$C-labeled carbon substrates (Ouverney and Fuhrman, 2000) or bromodeoxyuridine, a thymidine analog (Pernthaler, et al., 2002) to enable simultaneous identification of individual microorganisms and determination of active ones. Microorganisms detected using cellular in situ molecular methods can be quantified using flow cytometry or microscopy coupled with image analysis.

## 3. An Overview of Genomics in Environmental Microbiology

While advances from genomics may have been most visible in the biomedical and related sciences, they have likewise been of immense benefit to microbiology. Microbial genomes were the first to be completely sequenced. Several hundred genome sequences from Bacteria and Archaea, and a few from single-celled Eukarya, are presently available (www.ornl.gov/microbialgenomes). Initial efforts, focused towards understanding evolution of the primary prokaryotic lineages, revealed a surprising amount of horizontal gene transfer across distant phyla (National Science and Technology Council, 2001). The identification of open reading frames coding for proteins of unknown function spurred active microbiological proteomic research. Microbial genomic analysis has revealed novel metabolic path-ways and genes in diverse organisms. New drug discoveries and novel approaches to managing disease are expected to come from the investigation of microbial genomes. An understanding of how organisms and their metabolic pathways have evolved, a history of lateral gene transfer throughout evolution, and fundamental information for applied microbiology (e.g., agriculture, bioremediation) are all anticipated benefits of microbial genomics.

The field of genomics has already matured into disciplines that approach organisms from different perspectives. These include functional genomics, transcriptomics and proteomics, bioinformatics and ecogenomics. These disciplines focus on different aspects of organisms, yield different types of information and use different approaches.

Functional genomics seeks to understand how many genes are present in an organism, what the functions of the genes are, how they are regulated and how the genome is organized and operates. The relatively small bacterial genomes of bacteria provided a good starting point for genomic research.

Transcriptomics and proteomics determine which fractions of genomes are expressed by focusing on gene transcripts and expressed proteins, respectively.

Genomics produces tremendous amounts of data that require extensive database development and sophisticated analytical procedures. This marriage of biology and computer science is called Bioinformatics. The inability to analyze databases in biologically meaningful ways is a limitation. For example, although gene detection algorithms can accurately find ~99% of the genes present in a genome, assigning a function to these (genome annotation) in bacteria is usually only successful for 40–60% of the open reading frames identified (National Science and Technology Council, 2001). Additional challenges are the lack of uniformity in databases and the need for better ways to interpret hybridization array data and other types of genomic data sets. Bioinformatics will be an important component of applications in environmental science.

Ecogenomics, the application of genomics to answer environmental questions, is a new science. Microbial communities represent the collection of gene functions distributed amongst its members. How genes that determine community activity (and function) are regulated in response to environmental stimuli is a long-term goal of ecological genomic studies.

In microbial ecology, the use of large insert clone libraries and bioinformatics have greatly increased our understanding of the genetic potential of marine and soil microbes (Beja et al., 2000, 2001). One promising application of genomics is the ability to discern the presence and activity of many genes simultaneously through the application of microarray technology. Microarray technology was originally described for genome-wide expression studies of individual organisms in response to stress or metabolic shifts (Schema et al., 1995; Derisi et al., 1997) or to interrogate single nucleotide polymorphisms and genetic diversity (Huber et al., 200 1). Both of these applications have been used in the study of individual environmental microbial taxa. Examples are exploring patterns of gene expression by Shewanella (Thompson et al., 2002) and differentiating between Cryptosporidium strains based upon single nucleotide polymorphisms (Straub et al., 2002). For water quality assessments, application of array technology could enable simultaneous measurement of expression of a wide range of environmentally relevant genes, with the capability to encompass the diversity in these target genes for

a microbial community (Wu et al., 2001; Cho and Tiedje, 2002). The first arrays designed for microorganisms included nitrogen-metabolizing genes (nitrite reductase, nitrifying genes), methane metabolizing, and bioremediative genes. Microarrays have been used to detect toluene- and ethylbenzene-degrading bacteria (Koizumi et al., 2002), nitrifying bacteria (Urakawa et al., 2002), and closely related strains of Bacillus including B. anthracis (Liu et al., 2001). Second generation arrays are in development and envisaged to contain, for example, 1100 probes for nitrogen cycling genes.

## 4. Emerging Technologies

Recent advances in high throughput culturing techniques to isolate bacteria have coupled dilution culture in a microtiter plate format with automated, very efficient fluorescence-based screening (Connon and Giovannoni, 2002). This approach enabled several groups of previously uncultivated microorganisms to be isolated, including the environmentally dominant SAR11 clade (Rappé et al., 2002) which was among the first new groups of bacteria discovered when cultivation-independent methods were used to survey microbial diversity in nature. The SAR1 1 clade comprises up to one-third of all cells in the open ocean (Morris et al., 2002), making it a very important subject for biological oceanography. High-throughput culturing techniques enable the design of strategies to isolate ecologically important, previously uncultivated microbes for study. Once in culture, genome sequences of these novel microorganisms can identify functional genes, providing both physiological information and prospective targets for DNA hybridization probes or PCR primers.

Measuring gene expression at the protein level (proteomics) has the potential to reveal more information about environmental interactions in microbial communities than measuring expression at the mRNA level (Griffin et al., 2001a). Limitations of two-dimensional protein gel electrophoresis spurred development of mass-spectrometry-based proteome analysis. Methods such as matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) and tandem mass spectrometry (MS/MS), coupled to selective protein labeling and rapid database searches, now allow high-throughput, quantitative sample analysis with detection of even low-abundance proteins (Griffin et al., 2001b). These approaches are comparable to microarray approaches in that they provide large amounts of information from each sample. Proteomic analysis can quantitatively distinguish between activation states of microbial communities in the environment, for example in response to environmental stressors.

Novel nucleic acid detection strategies are being developed to miniaturize and simplify gene detection technology. Traditional nucleic acid hybridization methods use solid membrane supports to immobilize target genes which are probed with labeled radioisotopes, fluorescent dyes, or chemiluminescent reporters. New hybridization detection systems can use genoelectronics, or label-less systems that detect hybridization as a change in the electrochemical potential of DNA hybrids compared to single stranded DNA. This technology may encompass elaborate and expensive formats (e.g., gold chips Berggren et al., 1999; Fan et al., 2000) while other formats are simple and disposable (Marrazza et al., 1999). Some methods use fluorescence of targets that are bound to probes tethered to fiber optic cables (Jiang and Wang, 2001).

The combination of genoelectronics, microfluidics, and microelectromechanical (MEMS) technology represents an important future direction of environmental detection and monitoring. The goal of these technologies is to provide small, inexpensive sensors capable of real time or near real time monitoring. Such technology will be deployed in autonomous monitors for in situ detection and remote telemetry of the data. An existing platform is the Environmental Sample Processor (ESP) developed by Scholin and colleagues at the Monterrey Bay Aquarium Research Institute (www.mbari.org/MUSE/Participants/Scholin-DeLong.html). This system combines water filtration, nucleic acid extraction, hybridization and chemiluminescent detection to monitor harmful algal abundance in seawater. As a first generation autonomous sensor, this instrument is contained within a rather large housing as the development of more compact systems continues.

A wide range of technology already exists in autonomous underwater vehicles (AUVs) and remote moorings that transmit data to shore-based stations (Short et al., 2001). For example, the Physical Oceanographic Real Time System (PORTS) is a series of buoys that send autonomous real time data to shore based monitoring

systems by telemetry (http://ompl.marine.usf.edu/PORTS). Such platforms can easily accommodate genosensors and biosensors. The challenge is to develop such systems that can withstand the rigors of autonomous deployment.

## 5. Challenges

A number of considerations need to be taken into account in order to develop practical microarrays and other genomic-based tools for use in environmental monitoring. These include target selection, accounting for the genetic variability of microorganisms in the environment, and the technical difficulties associated with achieving sensitive, accurate, and precise detection of target sequences in environ-mental samples. Consideration of these is needed to transform measurements of microbial communities into meaningful assessment tools.

### *5. 1. TARGET SELECTION*

Selection of target genes for molecular methods and especially microarray approaches is an important first consideration. Ideal targets for water quality assessment should be relatively abundant, respond to their cogent environmental stressor, and be easy to amplify or detect. Microbes are naturally abundant in water ($10^5$–$10^6$ bacteriaml$^{-1}$, $10^3$–$10^5$ flagellates ml$^{-1}$, $10^2$–$10^5$ phytoplanktonml$^{-1}$, 10–$10^3$ cili-atesml$^{-1}$, 1–$10^2$ rotifers ml-1) and relatively small samples could provide sufficient copies of common target genes for assays.

A common approach for selecting target sequences is to choose bioindicator taxa or functional genes and then identify unique nucleic acid sequences to use as targets. This presumes that the sequences are known and that the taxa under investigation can be cultured in order to determine the appropriate target sequence. We know this is frequently not the case. High-throughput culturing approaches can be designed to formulate microbiological media needed to obtain the microorganisms with their target sequences. Alternatively, the genes can be obtained from the microbial community – an approach that requires screening or comparison with other communities to validate the utility of the target. Regardless of how the genes are obtained, DNA sequences must be determined and high throughput sequencing will be essential.

Proteins can also prove useful for environmental monitoring (Ogunseitan, 2000) and provide information on catalytic responses of microbial communities to environmental stressors (Ogunseitan, 1997; Ogunseitan et al., 2000). High-throughput protein analyses using mass spectrometry analysis will help identify proteins to quantitatively distinguish metabolic or activation states of cells in the environment, for example in response to stressors.

Finally, with the application of new methods come new challenges in data interpretation. Use of any particular target should include, as positive and negative controls, groups of targets that are likely to be found in nearly all environments in order to demonstrate that all collection, extraction, and analytical procedures are working properly. Similarly, there should be some duplication of indicator targets. That is, if a particular target provides information on response to an environmental parameter, there should be additional targets that are capable of reporting a response to the same parameter. The reason for this is two-fold. First, because ecological niches are complex, an unforeseen factor may preclude detection of a specific target from a particular organism under conditions that one might expect it would be found. Second, duplication provides an additional level of confidence to the analysis. Indeed, valid results may require that a certain level of duplication be achieved in practice.

### *5.2. SPECIFICITY*

Biological assessments address presence, absence, abundance or diversity of species. Molecular approaches to microbial systematics and taxonomy have provided considerable topics for discussion; among them the question of what constitutes a microbial species? In the context of environmental assessments, the question of what actually constitutes a microbial species is considerably less important than determining what level of genetic difference constitutes a functional difference among cells or strains. Distinguishable genetic differences at the nucleotide sequence level can be denoted as “operational taxonomic units” and used for, e.g., to calculate a

diversity index. It is likely that low levels of gene sequence divergence between microbial species will not significantly affect responses to environmental change. What is most important is ensuring the probe detects the intended target at the intended level, and does not detect non-targets. How species and higher microbial taxa respond to environmental stressors will likely reside within elements that regulate gene expression. Better understanding of these will come about through comparative analysis of microbial genomes.

### *5.3. SPATIAL AND TEMPORAL VARIABILITY*

Since microbes respond rapidly to changes in environmental conditions that favor or inhibit their growth, spatial and temporal variability are important considerations in the application of genomic tools and targets. For example, natural variability as a result of diurnal or seasonal cycles must be distinguishable from changes that result from anthropogenic stressors. Figure 2 shows the natural daily pattern of RUBISCO gene expression by Phaeodactylum tricornutum in the ocean. Similarly, factors leading to patchiness in the distribution of microbial communities across spatial scales, from millimeters to the landscape level, must first be better understood in order to determine if differences between communities indicate a significant response to an environmental stimulus. Microbial community compositions of lake pelagic and littoral zones are likely to be quite different, although they may indicate the same water quality. Similarly, a better understanding of how microbial communities are vertically distributed in the water column and sediments will be very significant in addressing issues related to nutrient cycles.

### *5.4. SENSITIVITY*

Several microbial array studies have encountered difficulties related to sensitivity (e.g. Lucchini et al., 2001; Ye et al., 2001). The difficulties may sometimes be traced to low concentrations of microbial DNA, inefficient nucleic acid extraction, or compounds in natural samples that inhibit hybridization or PCR. Thus, it will be necessary to develop strategies and control procedures that will signal false negatives and inhibition. Monitoring microbial abundances will be problematic in oligotrophic waters and extraction problems may be particularly acute in turbid waters and sediments. Continued research on optimizing nucleic extraction and purification techniques and concentrating nucleic acids from natural sample matrices is needed to improve sensitivity.

### *5.5. NORMALIZATION AND STANDARDIZATION*

As with any measurement used in environmental monitoring, development of genomics-based methods must include consideration of data quality control and quality assurance. Quantitative measurements are needed to standardize and normalize data for comparison, yet many of the genomics-based assays are not yet quantitative. For example, a study by Peplies and colleagues found that while microarrays targeting rRNAs of bacterioplankton identified the major groups present, the data were not quantitative (Peplies et al., 2004). A goal of monitoring efforts is to assess environmental quality from site to site and over time. In addition, environmental and public health decisions, which could lead to closing or opening waters for designated uses, must be based on measurable levels of indicators. As with other analytical methods, variability in environmental samples can affect the sensitivity of molecular methods and must be taken into consideration. For example, normalization of data obtained with molecular methods, such as microarray hybridizations, can either be accomplished by spiking samples with internal standards, applying targets in a range of concentrations, or by hybridization with control nucleic acids from defined organisms or communities. Variations in gene sequence diversity in the environment will produce variation in array signal intensity. Although current studies suggest that normalization and standardization are achievable, much remains to be learned about standardizing results from molecular methods. An alternative approach that may be valuable for environmental applications is to look at the relative abundance of targets by comparing signal intensities between a standard and a sample or between two samples. This could be accomplished, for example, by labeling the samples with different dyes (e.g., Cy 3 and Cy 5) followed by hybridizing on a single array (Cho and Tiedje, 2002). This would tell whether the target has increased or decreased in each sample relative to the other. Finally, consideration should be given towards how data from molecular methods would be related to environmental and public health standards established on the basis of methods presently in use.

### *5.6. DATA ANALYSIS*

Data analysis and interpretation also present challenges. Molecular methods, such as those using arrays or proteomics analyses, can generate large amounts of data. Interpreting those data in terms of ecological condition will be particularly challenging since the abundance or activity of each indicator taxon or molecule would be linked to multiple environmental variables. Conceivably, co-variation of microbial community composition, genes or proteins with environmental condition may provide greater insight into ecological condition than the presence or absence of any one taxon. The development of genomics-enabled methods for environmental monitoring should proceed in concert with the development of the bioinformatics and software necessary to interpret the microbiological data in the context of ecological assessment.

## 6. Outcomes

### *6. 1. UTILITY OF THESE ASSAYS*

The potential utility of genomics-enabled assays for environmental monitoring is great. However, there are many additional applications. These approaches can be used to monitor water supply lakes both to assure a generally safe supply and perhaps to fine-tune and reduce the cost of finishing water for human consumption. Such techniques could include blocks of indicators for human pathogens or even biological weapons. These approaches can also be used as early-warning indicators. If they are sensitive enough, they may be able to assess the introduction of toxics, pollutants, or exotic species at a point where management strategies can be employed to prevent significant degradation of the water body. Simple and rapid quantitative PCR assays might replace culture-based assays of bacterial pollution, resulting in beach closures and re-openings that more accurately track pollution and reduce exposure risks (Santo Domingo et al., 2003; Dick and Field, 2004). In the case of an ongoing problem, these approaches can be used to identify and eliminate the source of pollutant inputs. Arrays and other genomics-enabled techniques can also be designed for monitoring remediation and restoration efforts in water bodies. This may be of great value if alternate strategies are being considered or tested, as the assessment may allow decisions on which alternate to pursue or abandon, thus potentially saving capital. Finally, the scientific value of both development and application of genomics-enabled techniques for water quality assessment should not be undervalued. As they are developed and used, our understanding of the fundamental ecological processes will be greatly improved and will also provide powerful tools to address additional questions, including those with a level of complexity that is beyond the reach of aquatic ecologists using current tools. Indeed, just as the disciplines of cell biology, genetics, and medicine have made quantum leaps in the last decade through the genomics revolution, the development and application of genomics-enabled technologies in aquatic microbial ecology will usher in an era of rapid progress.

## 7. Specific Recommendations

The workshop participants identified research needs and made recommendations for research with the goal of bringing genomics into the realm of water quality assessments to improve environmental management decisions. An over-arching need is to increase sequence information for both prokaryotic and eukaryotic microorganisms. A major limitation to progress is a relatively small sequence database, especially in relation to non-culturable taxa that predominate in the natural environment. This recommendation has previously been made by other working group reports (American Academy of Microbiology, 2001; National Science and Technology Council, 2001; Stahl and Tiedje, 2002; Staley et al., 1997). Additional recommendations fall into three areas: targets, technology, and data.

### *7. 1. TARGETS*

– Identify microbial indicators of water quality and ecosystem health, including genes and proteins known to be involved in pathogenesis and response to an-thropogenic impacts (e.g., nutrients, toxic organic compounds, heavy metals) on which to focus sequencing efforts and methods development for environmental detection.
– Improve understanding of gene expression as related to biogeochemical and nutrient cycles and to the survival of indicator microorganisms in the environment.

– Improve understanding of linkage between specific gene expression and stress. Such work may identify candidate microbial taxa or functions that can act as sensitive sentinels of environmental change.

– Improve understanding of temporal and spatial distribution and activity of aquatic microbial communities. Such efforts are fundamental to successful application of genomics approaches to water quality assessment.

– Improve understanding of the genetic diversity within and between species to develop microbial indicators.

### *7.2. TECHNOLOGY*

– Adapt medically driven genomics research technology to field amenable, inexpensive devices. One aspect of this is to encourage interdisciplinary collaborations of aquatic microbial ecologists with oceanographers and engineers who currently use remotely operated data collection and analysis platforms.

– Explore the use of emerging high-throughput technologies, such as pyrosequencing and proteomics analysis, for microbial community analysis and environmental monitoring.

– Develop effective nucleic acid extraction methods across a wide range of environments and/or identification of best methods for specific environments.

– Develop methods for concentrating "dilute" environmental samples/targets. – Enhance sensitivity of arrays for environmental applications.

– Develop effective and reliable methods for archiving nucleic acids of collected samples.

– Develop procedures for normalization and standardization of data across different laboratories.

– Develop improved isolation methods for key taxa (especially those termed "non-culturable") for laboratory studies of physiological responses to environmental stress.

### *7.3. DATA*

– Develop databases that effectively integrate genomic information and environ-mental information, including: environmental stressor dose-response data on gene expression and the use of GIS and EMAP sampling strategies for assays of microbial communities.

– Develop bioinformatics algorithms for data interpretation of genomic applications in environmental assessments and molecular technologies.

## 8. Prospects

Microbiological genomics indicators of water quality, with development and testing, could become widely available in the next ten years. Technologies already used in genomics research laboratories, such as microarrays, would likely be among the first to come online. Although it is relatively easy to imagine that a microarray could be used to simultaneously assay for thousands of bioindicator organisms and functional genes at a time, it is difficult to predict precisely what form a future "microarray" assessment tool for water quality will actually take since the technology is developing so rapidly. For example, although gene arrays are currently manufactured on glass slides or other solid devices, rapid advances in microfluidics and nanotechnology may lead to quite different platforms within a decade. Clearly, even as there is an increasing effort in applications (e.g. Rudi et al., 2000; Small et al., 2001) improvements in array technology and development are ongoing (e.g. Bavykin et al., 200 1). In addition, other genomics-enabled techniques currently being developed may lead to equally important water quality assessment tools. Thus, the actual format of autonomous, real-time water quality assessment systems that may be in place within a decade could be quite different in terms of chemistry and physical dynamics than is even imaginable today. One thing that will not change, however, is the need for genetic information – target sequences – that can be used on such instruments.

The vision of autonomous, real time genomics-enabled sampling platforms is achievable within a decade, but it

will require considerable financial and human resources. Although the investment will be large, it should pay immense dividends as the cost of using these techniques for water quality assessments declines, and the breadth of applications expands. We have identified current challenges and a framework in which to proceed. We strongly encourage agencies that have stewardship of the environment within their mission to join together as partners to bring a genomics era to environmental assessments.

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