

Application of Molecular Microbial Technologies to USGS Science

Executive Summary

The Office of Water Quality sponsored a 2-day meeting March 13-14, 2001, at the USGS headquarters in Reston, Virginia. Invited attendees were USGS scientists who currently use a variety of molecular methods to address microbiological issues in USGS research and assessment programs. The goal of the meeting was to hold a wide-ranging discussion on current USGS capabilities in molecular microbiology and to explore ways of expanding the use of those technologies to answer questions in USGS science. Another objective of the meeting was to develop guidance for USGS leadership on the need for, and most efficient implementation of, methods in molecular microbiology to address hydrologic, geologic or biological resource assessment and research over the next 5 years.

The meeting identified a number of molecular methods currently available and identified those methods being used by a small group of USGS scientists in Districts, research centers, and science centers. These research methods have numerous applications in the scientific projects of the USGS. In many cases, these molecular methods constitute the only or the best approach to address microbiological questions. Therefore, every USGS microbiologist uses these methods to some extent. At the present time, however, the availability of molecular microbiological methods for use in research and National program applications is extremely limited within the USGS. The small District research, and science center laboratories currently using molecular microbiology methods simply do not have the capacity needed to apply a particular method to a National program. Funding is also limited for the acquisition of new instrumentation and other equipment needed for the research projects to keep up with this rapidly evolving field of science.

The meeting also identified a number of broad scientific questions that fit well with the mission and strategic plans of the USGS and would greatly benefit from the application of newer molecular methods. Questions of human and wildlife health, sources of contamination, and biochemical transformations are extremely complex and can only be addressed by a toolbox of scientific methods. Currently the USGS toolbox is very limited in molecular tools that can be used to solve the difficult questions that are integral to our mission and these tools must be available if microbiological issues are to be addressed.

The events of September 11, 2001, and the subsequent threats of bioterrorism have highlighted in a tragic way the need for expanded capability in the area of molecular microbiology. The need to identify and monitor for pathogens that could be used as terrorist weapons is not an emerging issue; it *has* emerged. The molecular methods outlined in this document, and the challenging microbiological issues to be faced, would be integral to any USGS effort to address biological weapons of terror. It is again

important to note that the USGS currently does not have the capacity to respond rapidly in a meaningful way to the needs of the country.

Background

In 1999 the USGS/BRD held a workshop on the application of genetics and molecular tools in the biological sciences at the Leetown Science Center. The overview of the white paper that emerged from this workshop, entitled *Genetics and Molecular Tools: Setting the Standard for Biological Science in the USGS*, begins with the following paragraph:

“Recent technological advances in genetics and molecular tools have revolutionized our basic understanding of biological and evolutionary processes. These contemporary applications have significantly enhanced our ability to delineate the finest level of biological diversity (i.e., genetic diversity) and provided remarkably sensitive techniques for diagnosing the presence and effects of environmental perturbations on a host of endangered, threatened, or at-risk species. Individual scientists within USGS have kept pace with the advancing technology, but the organization has not fully embraced the utility and capacity of these tools. Simultaneously there has been an exponential growth of critical environmental issues that demand relevant and efficient application of these state-of-the-art technologies.”

Within the USGS, similar technologies can be applied to hydrologic and geologic resource issues, but the target of such studies is typically microorganisms. It is clear that naturally occurring microorganisms affect, and in fact largely control, many inorganic and organic chemical processes in water, soils, and aquifer materials. In addition, the presence, fate and persistence of other microorganisms (especially those with human or animal health effects) are increasingly the target of USGS studies, as issues of human health become a more prominent part of USGS science.

As for the BRD scientists who attended the Genetics and Molecular Tools workshop, microbiologists throughout USGS recognize the need to apply technological advances in the biological sciences to broad and comprehensive problems in the hydrologic, geologic and environmental sciences. Furthermore, other agencies with which the USGS cooperates or collaborates are looking to the USGS for application of these technologies to answer critical resource assessment and protection issues in the hydrologic and geologic sciences, as well as in the biological sciences. Among these agencies are the DOE, DOD, CDC and USEPA, state and local departments of health or environmental quality, and water utilities. The number of USGS scientists currently using these technologies is small and infrastructure and equipment needs pose significant hurdles to further implementation of these rapidly developing technologies. Without correctly targeting investment in the development and application of these technologies throughout the USGS, the USGS may lose a significant leadership opportunity, and risks being unable to meet the basic resource information needs demanded by its cooperators and the Nation.

Most of the technologies and techniques talked about in this document have been largely developed outside of the USGS. Many of these technologies are considered to be the only or the best means of addressing certain microbiological questions. Therefore, every microbiologist in the USGS uses some of these technologies to some extent. Although microbiologists in NRP, WRD District offices and BRD and GD science centers use molecular technologies in their research and operational studies, the USGS is just beginning to incorporate studies of microbiology in its National and Regional programs, and has not embraced biotechnological applications in most of its programs. What is exciting, however, is that many of the state-of-the-science methods being developed outside of the USGS present tremendous opportunities for application in our programs to understand processes, and benefit scientific understanding of a wide array of natural resource issues. The USGS is in a unique position to apply a wide array of newly emerging techniques because of the wide range of questions that our science seeks to answer. If we don't capitalize on these opportunities to apply existing and rapidly developing microbial technologies, we miss the opportunity to dramatically expand the scope, reach, and value of our work at a relatively low cost. We also risk the potential loss of opportunity as cooperators and clients (EPA, states) go elsewhere to get current state-of-the-art methods.

The incorporation and development of microbiological studies in USGS programs is in an early stage of evolution, somewhat comparable to the status of water quality studies in the early 1970s. The USGS has become a leader in water-quality analytical chemistry and interpretation of data produced by these methods, and has found nationwide application of these methods in real life studies. Applications of these analytical chemistry techniques were made possible because a strong investment was made in the basic laboratory techniques that are now in routine use.

Current Methods in Molecular Microbiology

The meeting participants identified a large number of techniques currently available to the scientific community. A list of these methods, along with a brief description, is contained in the Glossary of Terms in Appendix III of this report. Some of these techniques are actively being applied by USGS scientists within USGS research and operational programs. Figure 1 depicts ways in which these technologies may be used to address questions about the occurrence, distribution, function, and ecology of microorganisms. Table 1 shows how information on the occurrence, distribution, function, and ecology of microorganisms is relevant to the USGS mission and identifies which methods are applicable to which questions. Appendix II identifies contacts for these applications. Creative application of these technologies is the key to their usefulness in addressing the complex scientific questions and to manage our natural resources.

Current and Future Information Needs

The meeting participants identified current and future needs for scientific information that could be addressed by various molecular microbiological technologies. Information needs fall under four broad categories:

- Impact of microorganisms on human and animal health
- Microbial bioremediation of hazardous waste sites
- Ecosystem chemical transformations driven by microbial processes (biogeochemistry)
- Fundamental science of microorganisms that provides the basis for understanding how they affect human and animal health and biogeochemical processes

Table 1 identifies some broad information needs that fit well with the current mission and structure of the USGS. These are urgent natural resource information needs for which data could be collected rapidly with the proper resources available and the commitment to use them. Included in this table are indications of where each of these information needs fits into WRD and USGS strategic plans. It is apparent that applications of molecular microbiology fit into most technical areas of the strategic plans. It must be noted that although the table indicates USGS scientists with capabilities for many current techniques, these scientists are located mostly in three categories of laboratory settings: small research labs in WRD, NRP, or GD; small research or applications labs in Districts; and the BRD National Wildlife Health Center. Of these, most are interested in applying these methods to many of the issues that drive USGS National Programs, but simply don't currently have the capacity to provide these services or capabilities at a larger scale to National programs.

Table 1.

Information Need	Potential Cooperators	USGS Mission (see footnote)	Best Available Technologies (see Appendix III for details and Appendix II for USGS scientists using these methods)
A. Source of indicator or pathogenic microorganisms	State & Federal Regulators, local health agencies; watershed councils; USDA; wastewater dischargers	3, 4, 9, 10 A, C, D	DNA-typing methods; microarrays; T-RFLP, hybridization, standard growth tests, ELISA tests
B. Occurrence and distribution of indicator microorganisms	DW Utilities; EPA; State/Federal regulators; USDA/state Ag; CDC; FDA; FWS	3, 10, 9, A, D	Culture-based (rapid methods); hybridization (p/a); PCR (p/a or quantitative); flow cytometry; MALDI-TOF (p/a)

C. Occurrence and distribution of human and wildlife pathogens	DW Utilities; EPA; State/Federal regulators; USDA/state Ag; CDC; FDA; FWS	3, 4, 7, 9, 10 D, A, C	Immunological; microarrays; culture-based (rapid methods); hybridization (p/a); PCR (p/a and quantitative); flow cytometry [R]; MALDI-TOF (p/a)
D. Number or kinds of microorganisms related to process	Predictive modelers; TMDL; recreation water managers; contaminant degradation models; DW suppliers; watershed mgrs.	3, 4, 5, 7, 9, 10 A, B, C, D	Culture-based (rapid methods); flow cytometry, T-RFLP; hybridization, microarray; MALDI-TOF; quantitative PCR
E. Differences in specific microbes among samples (taxonomic)	Water utilities; health agencies; DOD; DOE; FWS; CDC; EPA; NPS (microbial prospecting), states (acid mine drainage); mining petroleum quality; MMS; Agriculture	3, 4, 5, 7, 9, 10 A, B, C, D	Culture (taxon); metabolic (taxon); MALDI-TOF; T-RFLP; microarray; DNA-typing; in-situ hybridization
F. Differences in microbial communities (process)	Regulators of toxic or Superfund sites; water utilities; health agencies; Agriculture	4, 7, 9, 10 B, C, D	Hybridization: microradiography; Fluorescent In Situ Hybridization; in situ PCR for genetic or metabolic taxonomy; tetrazolium enzyme assays; live/dead analyses; other enzymes; T-RFLP; microarray

(p/a) - presence/absence

USGS mission is defined from the Bureau and Water Discipline strategic plans.

WRD plans:

1. urban and suburban growth
2. coastal zone growth
3. drinking water (source protection)
4. healthy aquatic habitats
5. waste management and contaminant remediation
6. hydrologic hazards
7. changing climate
8. single resource view of ground and surface water
9. watershed management
10. wildlife health

USGS plans:

- A. water availability and quality
- B. contaminated environments
- C. land and water use
- D. environmental effects on human health

Strengths and Weaknesses of Current USGS Capabilities in Molecular Microbiology

Strengths: These are strengths of the USGS as a whole---not of our current capability in molecular microbiology

- 1) National coverage (offices in every state)
- 2) Centralized water quality guidance by the Office of Water Quality, and at the regional level
- 3) Field capability
- 4) Expertise on hydrologic, geologic, and biologic systems
- 5) Already a small core group of microbiologists applying these methods to USGS science in three disciplines

Weaknesses:

- 1) Lack of support for regional or national application of newer microbiology methods in USGS research or operational programs
- 2) Lack of awareness of relevance of microbiological issues to National programs
- 3) Distributed, small-lab structure of microbiological expertise hampers effective communication and resource use.
- 4) Lack of personnel and infrastructure for large scale applications; e.g., for a national-scale monitoring program:
 - a. Incomplete guidance for regional or national application of newer microbiology methods
 - b. Laboratory and field equipment and personnel needs would be substantial in some cases
 - c. Little training available for non-microbiologists to learn proper collection and handling techniques, and expertise in even basic procedures such as membrane filtration techniques has been reduced since the decrease in the NASQAN program

Needs:

- 1) Inclusion of microbiological issues and techniques in national programs
- 2) Improved communication between microbiological researchers, other USGS scientists, and Program managers
- 3) More research personnel in the fields of microbiology
- 4) New and up-to-date equipment
- 5) Additional microbiology expertise in the Office of Water Quality
- 6) For National monitoring programs:
 - a. Approved labs to perform routine analyses
 - b. Training for non-microbiologists
 - c. Documented/approved methods

Future Directions for Incorporation of Molecular Microbiology in USGS Research and Operational Programs

The meeting participants outlined a set of broad scientific information needs that have relevance to science and social values. This list is not meant to be complete but is representative of the types of scientific information needs that have great relevance to the public and fall within the mission of USGS. Each of these questions requires the use of a variety of microbial and molecular techniques that could be substantially addressed in the next 5 years within the USGS if the proper resources were available.

I. Develop an understanding of the relationship between indicator organisms and pathogens

Why and How: New, and better indicator organism methods are being developed and tested. Indicator methods are in wide use throughout the country to infer the potential for contamination of water with pathogenic organisms that cannot easily be isolated from water samples. Indicator organism tests can, at best, show that the water has been contaminated with organisms from fecal sources. Innovative new methods for detecting pathogenic organisms are now possible with newer molecular technologies. These new methods will allow comparisons of the occurrence of pathogenic organisms to the occurrence of bacterial indicators. Application of these emerging methods on a wide-scale basis is an ideal role for the USGS, with its large infrastructure of water quality sites in the NAWQA, NASQAN, Toxics, and State Cooperative programs. A large regional or national assessment of waters for pathogenic organisms with various indicators collected simultaneously would be a tremendous contribution.

Does the Science Exist: Methods for indicator organisms are very well developed. Other methods for pathogenic organisms require molecular methods that exist only in small research labs within the USGS. The availability of samples from a wide variety of locations would help determine the reliability of newly developed methods.

Needed Elements: To substantially address this goal would require the availability of production laboratories with state-of-the-science equipment to process the number of samples needed. The existing research labs, with proper investments in new instrumentation, are well suited to develop and perfect methods that can be applied in a large-scale program.

Long-Term Issues: This goal would go a long way toward answering the important question of how well tests for indicator organisms protect the public and the environment.

II. Understand the source, transport, and fate of fecal contamination in ambient recreational waters and in drinking-water sources

Why and How: Addressing this goal requires the ability to track the sources of fecal organisms, both indicator and pathogenic. Great strides have been made in recent years using *E. coli* as an indicator of fecal contamination and using molecular techniques (see table 1) to indicate the source of the contamination. As with goal I, the resources of existing USGS programs, trained personnel, and distributed offices makes large-scale application of new technologies possible.

Does the Science Exist: Several promising techniques are now being applied in studies around the country. Because of the importance of this issue to the regulatory community we can expect further rapid developments of these techniques. The use of non-native trace organisms such as certain bacteriophage may be one approach that can be used to understand the fate of organisms in the environment.

Needed Elements: The questions of the fate of indicator or pathogenic organisms cannot be answered just by understanding the origins of the organisms. Complex issues of water quality, hydrology, geology, climate, and geography have a direct bearing on issues of fate and importance of the contamination to the environment. Holistic scientific approaches that are the hallmark of USGS science are required to provide relevant answers. Interdisciplinary studies already under way and addressing such issues as ground water/surface water interactions, coastal environments, aquifer storage and recovery, or aquifer recharge provide important opportunities to leverage existing information on hydrology, geology, or ecosystem processes that may affect microbial transport and fate. Likewise, existing studies addressing the fate of human or animal wastes (e.g., CAFO studies, the Toxic Substances Hydrology Program's Emerging Contaminants Reconnaissance, models of river transport to drinking source water intakes) again provide important opportunities to add significant information on microbial transport and fate while tracking sources using multiple methods. Along these lines, the Toxics Program has begun a plan to incorporate microbiological pathogens and microbial antibiotic resistance into its Emerging Contaminants Reconnaissance Program, and the Ground Water Resources Program has begun to explore how it might incorporate microbiological issues into its programs addressing ground-water sustainability.

The value of adding microbiological analyses to such programs has often been overlooked by Program managers, who are more typically trained in the physical sciences. The number of USGS scientists available to address these questions is now limited, and Program managers have difficulty identifying USGS scientists to participate in such programs. The distributed expertise in microbiology and multiple funding mechanisms makes it difficult for Program managers to bring this expertise to bear on their programs. The USGS must develop an improved communication network for addressing microbiological issues in many USGS programs and the

capacity to provide these methods on a more comprehensive basis to answer larger scale questions in many different USGS Programs.

Long-Term Issues: Current water quality regulations are driving the need to understand sources of contamination but it is clear that this issue will not go away and the complexities are such that answers obtained in one location may not be widely applicable to other locations.

III. Understand the mediating role of microbial community structure on the transport and transformation of organic and inorganic chemicals

How and Why: Much of the current work on this subject within the USGS is sponsored by the Toxics program and to a lesser extent the DODEC program. These are primarily aimed at fate and transport issues having to do with contaminants introduced into ground and surface water. Improvements in molecular technology have made further definition of biologically mediated transformations possible by identifying new forms of microbes and studying how microbial populations vary from one area to another. Initially, much of the work for this goal would be conducted at existing research sites where work already has been done on the hydrology and other associated conditions important to the interpretation of results.

Advanced microbial molecular techniques also can be used to investigate transport and transformation issues in uncontaminated river and ground-water systems. Understanding of chemical transformation in hydrologic systems requires knowledge of biological transformations. Molecular techniques can be used to define and understand microbial communities that mediate chemical changes.

Does the Science Exist: Yes, but this goal can only be addressed with a multidisciplinary approach involving hydrologists and chemists, as well as molecular biologists. Much of the background information on specific sites already is available at a number of USGS research sites.

Needed Elements: Much of the initial work on this goal will be aimed at inventorying microbial populations at specific sites. PCR, culture-based methods, and hybridization probes could be used. These capacities already exist within the USGS but more standardized methods need to be made available (e.g., the same methods need to be compared across sites). In addition, this is a research area that could profit from much more extensive application of new technologies in current development, but not currently being used by USGS scientists, such as microarrays, or technologies currently available in only one laboratory (e.g. T-RFLP).

Long-term Issues: This goal will be complicated by the wide range of environmental conditions, types of contaminants, and heterogeneity of most sites. It is hoped that a long-term commitment to this goal will lead to a more general understanding of the role of microbial communities in chemical transformations.

IV. Understanding the occurrence and environmental distribution of wildlife and fish diseases, events, and processes

How and Why: Recent scientific articles have drawn attention to emerging infectious diseases of wildlife in marine and freshwater settings, the probable climatic and anthropogenic causes of these diseases, and the likely threats to biodiversity. Some disease agents can be transferred between animals and humans (e.g., West Nile virus, *E. coli*), with or without disease in the animal host. The USGS has a special role to play in providing the information needed to protect trust and endangered species, wildlife, and biodiversity on Federal lands. There are significant opportunities for interdisciplinary USGS science to be brought to bear on questions of the environmental distribution of wildlife or fish disease, and the factors that affect this distribution. Some of these findings might serve as models for the distribution of human disease.

Does the Science Exist: Wildlife diseases are caused by the same types of microbiological agents (bacteria, protozoa, viruses) as are human diseases, therefore the technologies for addressing these microorganisms are the same. In some cases, however, much less is known about the animal disease agents (e.g., their identities, what genes are responsible for the disease) than about similar microorganisms that affect humans. Virtually nothing is known about the transmission of wildlife disease agents along complex hydrologic pathways in wildlife habitats (such as wetlands, or river hyporheic and riparian zones), although human contamination of aquatic resources is widely cited as a possible factor in emerging wildlife disease. Many important questions could be addressed by well-designed interdisciplinary USGS studies.

Needed Elements: BRD has established the National Wildlife Health Center, a Biosafety Level 3 laboratory and the only Federal facility dedicated to wildlife health, in Madison, Wisconsin. There are significant opportunities to leverage the capabilities of this facility with USGS capabilities in mapping, hydrology, biology, water quality, and even microbial ecology and biogeochemistry, in order to develop a much more profound understanding of the disease threats to USGS trust species and the implications for human health. As with other laboratories, larger-scale studies and more incorporation into USGS-wide programs will necessitate increased personnel and additional equipment.

Long-term Issues: Integration of baseline information on wildlife pathogens and disease occurrence with long-term modeling is sorely lacking. This issue has received much less attention than issues perceived to have more urgency, such as those directly related to human health. Interactions between wildlife health and human health must be more formally investigated.

V. Understanding the relation between ecosystem structure/function and microbial diversity

How and Why: It is currently recognized that science has only identified perhaps 1 to 10 percent of the extant microorganisms. For example, over 400,000 beetles have been identified, but only about 4,000 bacteria! Whenever new microbial technologies are applied, to either typical (e.g., agricultural soil) or new (e.g., Antarctica) environments, new findings are made about the types of microorganisms that exist in those environments. The USGS conducts research and assessments in virtually every ecosystem on earth, and acquires information on many varied ecosystem properties, but only rarely on microorganisms. Nevertheless, microorganisms are likely responsible for many of the biogeochemical transformations that transform toxic constituents, make nutrients available for other organisms in those ecosystems, and govern population dynamics through wildlife health and disease.

Does the Science Exist: Broad patterns in microbial diversity can be addressed with variations of many of the methods described in this document. Exciting new approaches allow fingerprinting of communities so that similarities and differences between the microorganisms that inhabit different environments can be readily determined. Then, the identities of these individual microorganisms can be sought. These methods include some in use in USGS labs, as well as methods in development that are not currently in use in USGS labs. As for several other issues, application of these technologies suffers due to lack of consideration at the Program level. For example, USGS should consider including a microbial community role for all future ecosystem studies.

Needed Elements: The primary needed element is simply to add new microbial technologies to existing interdisciplinary studies. However, the number of USGS labs with the capability to conduct microbial community studies is extremely limited, and additional scientists and equipment would be needed for larger-scale applications.

Long-term Issues: Microbiology should be part of new projects that look in unique hydrogeologic environments. There is a great potential for patents and advancement of basic ecosystem science.

The USGS is well positioned to address the broad goals outlined above.

Why Us?

- 1) No one else has the national field capability; no one else samples water/environment at the same level as the USGS (i.e., CDC, EPA, USDA, universities, or the private sector).
- 2) A national field capability, multidisciplinary approach is required; The USGS has modelers, transport specialists, sampling program designers.
- 3) USGS experience in hydrology, biology, and geology provides a multidisciplinary approach needed to support improved predictive models.

- 4) USGS has a large ongoing base of interdisciplinary projects to which microbiology could be added; the new information would improve our existing models and lead to models that better predict important chemical and biological processes.
- 5) USGS scientists are the stewards of fish and wildlife health; there is an existing integrated network with the Fish and Wildlife Service, and the National Park Service to address issues of trust species conservation and endangered species.
- 6) The USGS has interdisciplinary-critical skills, experience, and interests to address NRC themes of global climate change, state of the nations' ecosystems, and abiotic/biotic interdependence.

Why Now?

- 1) Information is by needed by EPA, CDC, and recreational water resource managers for maintaining public health by protection of drinking water sources.
- 2) Water managers need this information now because of emerging threats from land use change, climate change, and confined animal feeding operations, and regulatory needs such as TMDL.
- 3) Better predictive models are needed to assess transport of microbial contaminants in studies of bank infiltration, artificial recharge of aquifers, domestic waste disposal, inadvertent releases.
- 4) Tools with improved resolution are essential; existing models are inadequate for accurate predictive capability.
- 5) Molecular methods allow more rapid and selective identification of microbial agents allowing the design of more effective control strategies, and provide early warning of contamination in drinking water supplies.
- 6) With the addition of the Biological Discipline, the USGS is now in a position to more holistically address the critical issues.

Organizational Needs Required to Successfully Apply Molecular Microbiology Within the USGS

The USGS currently has “pockets” of expertise in microbiology in various locations across the country. All these microbiologists use molecular technologies to some extent. Some large instrumentation investments such as gene sequencers are also available in research labs. If the USGS is to move forward with applications of these technologies, molecular microbiology methods need to be made available on a larger scale to many USGS Programs. This means a larger investment in both personnel and equipment, better communication of the need for and the relevance of microbiology in a variety of investigations, and for some programs, the development of more routine and standardized methods. Two major efforts are needed:

1. A research program is needed to work closely with the operational program to develop more standardized methods that have broad applicability to some of the major scientific questions that we would like to answer. This requires a commitment of funds and person power dedicated to this effort. It is not practical to ask the research labs to make their research instruments available for large-scale application projects, nor can this effort substantially divert efforts in continuing basic research. One way of accomplishing these goals would be to establish a central facility for microbiology research and applications on the general model of the Methods Research and Development program at the National Water Quality Laboratory. Research at this facility would be dedicated to developing usable tools for broad-scale applications in USGS monitoring and assessment programs.
2. Closer coordination is needed between existing and developing USGS programs and existing USGS microbiologists, so that existing expertise and programs are leveraged to the fullest extent. Program managers should consider incorporating molecular microbiology in the design of major USGS initiatives. These tools need to be an integral part of our scientific toolbox that is effectively used to address major scientific questions. Planning efforts to incorporate substantial new technologies into large coordinated scientific studies such as the planned initiatives need to include thinking about molecular microbiology up front and not as an afterthought.

Appendix I

Meeting attendees:

Tom Byl, Tennessee District
Donna Francy, Ohio District
Ed Furlong, National Water Quality Laboratory, Denver
Sheridan Haack, Michigan District
Ron Harvey, National Research Program, Boulder
Dan Hippe, WRD, Northeast Region, Reston
Stephen Sorenson, Office of Water Quality, Reston
Mary Voytek, National Research Program, Reston
Janice Ward, Office of Water Quality, Reston
Mark Wolcott, BRD, Madison

Appendix II

List of current contacts for the methods now available within USGS. Each contact is listed with both topical categories for Table 1 and techniques.

Tom Byl, (tdbyl@usgs.gov) Tennessee District – Topical categories: A, B, C, D, E, F. Technologies: microbial culturing techniques, hybridization, PCR, microscopy, DNA-typing.

Donna Francy, (dsfrancy@usgs.gov) Ohio District – Topical categories: A, B, C. Technologies: microbial culturing techniques, ELISA, PCR.

Ed Furlong, (efurlong@usgs.gov) National Water Quality Laboratory, Denver - Topical categories: A, B, C. Technologies: spectrometry (MALDI-TOF), enzyme assays.

Dale Griffin, (dgriffin@usgs.gov) GD, St Petersburg, FL -- Topical categories: A, B, C, D, E, F. Technologies: microbial culturing techniques, hybridization, PCR, DNA-typing, ELISA, microscopy.

Sheridan Haack, (skhaack@usgs.gov) Michigan District – Topical categories: A, B, C, D, E, F. Technologies: hybridization, PCR, DNA-typing, enzyme assays, ELISA, microbial culturing techniques, microscopy.

Ron Harvey, (rwharvey@usgs.gov) National Research Program, Boulder – Topical categories: A, B, C, D, E, F. Technologies: hybridization, microbial culturing techniques, microscopy.

David Metge, (dwmtege@usgs.gov) National Research Program, Boulder – Topical categories: A, B, C, D, E, F. Technologies: hybridization, microbial culturing techniques, microscopy.

Ron Oremland, (roremlan@usgs.gov) National Research Program, Menlo Park – Topical categories: A, B, C, D, E, F. Technologies: microbial culturing techniques, hybridization, PCR, microscopy.

Don Stoeckel, (stoeckel@usgs.gov), Ohio District - Topical categories: A, B, C, D, E, F. Technologies: microbial culturing techniques, hybridization, PCR, DNA-typing.

Mary Voytek, (mavoytek@usgs.gov) National Research Program, Reston – Topical categories: A, B, C, D, E, F. Technologies: DNA-sequencing, T-RFLP, hybridization, PCR, microbial culturing techniques.

Mark Wolcott, (mark_Wolcott@usgs.gov) BRD, Madison – Topical categories: A, B, C. Technologies: microbial culturing techniques (especially pathogens), PCR, hybridization, DNA-typing.

Appendix III

Glossary of technical terms used in this report:

DNA (Deoxyribonucleic Acid): A polymeric molecule composed of deoxyribonucleotide units joined together in a specific sequence, through the formation of 3'-5' phosphodiester bonds.

DNA Polymerase: An enzyme that catalyzes DNA synthesis from a template molecule and deoxyribonucleotide triphosphates (dNTPs).

DNA Typing: A method used to identify genetic inheritance patterns. Can be used to study populations, determine source of microorganisms and perform epidemiology studies. Examples include:

- **Ribotyping** - where the target of method is the gene that encodes **ribosomal** RNA
- **Rep-PCR** - where the target of the method is any of several **repetitive** sequences in the DNA
- **RAPD**- where the target of the method is a **randomly amplified** sequences in the DNA
- **PFGE** - **pulsed field gel electrophoresis**, where the entire DNA is cut into smaller fragments and the pattern of these fragments after electrophoresis produces the DNA type

Electrophoresis: The separation of molecules using an electrical current, usually applied to a gel matrix. The gel matrix has a sieving effect, which allows molecules to be separated on the basis of size, while the electric field separates molecules on the basis of charge. A variant is

- **DGGE** - **denaturing gradient gel electrophoresis** in which the molecules are separated by sequence

Elisa: Enzyme linked immunosorbent assay. A method in which an enzyme assay is used to detect a specific immunologically-active constituent of a target organism, usually producing a color or fluorescent response.

Enzyme: A biological catalyst (either RNA or protein) that assists a specific chemical reaction. RNA enzymes are also known as ribozymes.

Enzyme Analysis: Detection of the presence of a particular enzyme by its function. Numerous colored or fluorometric enzyme substrates have been created. These can be detected singly or in multiple test arrays.

FAME: Analysis of the **fatty acid methyl esters** derived from the molecules that make up the cell membranes of microorganisms. FAME patterns may be characteristics of particular microorganisms.

Flow Cytometry: A process whereby cells in a sample are passed one-by-one across a detector that senses the presence of various indicator molecules in or attached to the cells. This method can count many cells in a sample and can sort the cells according to properties.

Gene: A segment of DNA that encodes a single functional (polypeptide, protein or RNA) molecule.

Genotype: The specific genes, or arrangement of genes, present in an organism.

Hybridization: The process of attaching a piece of DNA to its matching sequence in a nucleic acid extract. Detection of the match indicates the presence of the particular gene in the sample. A variant is in situ hybridization in which the hybridization process is carried out on individual cells under the microscope.

Immunodetection: Methods in which molecules on a microorganism's surface are used to detect its presence, based on specific reactions employing antibodies to those molecules. May be used in other methods such as flow cytometry, microscopic examination of samples and in some methods used to concentrate specific microorganisms from environmental samples.

MALDI-TOF: Matrix-assisted laser desorption/ionization---time-of-flight mass spectrometry. A method by which molecules within microorganisms produce a characteristic pattern of ions. This method can be used to analyze several types of molecules in cells, including nucleic acids and proteins.

Microarrays: Small "computer-chip"- sized templates holding molecules that can be used for many molecular methods. Such templates allow multiple and very rapid analyses. Detection is usually by micro-electronics.

Polymerase Chain Reaction (PCR): A repetitive process, usually aided by the action of a (heat-stable) DNA Polymerase enzyme, which copies a DNA template such that the number of copies increases exponentially. Used in many molecular methods to create more DNA for analysis.

Variants are:

- quantitative PCR (real-time PCR) in which the reaction rate is used to determine the number of molecules present in the original sample
- reverse-transcriptase PCR in which RNA is the target instead of DNA.

RNA (Ribonucleic Acid): A polymeric molecule composed of ribonucleotide units joined together in a specific sequence, through the formation of 3' → 5' phosphodiester bonds.

T-RFLP: Terminal restriction fragment length polymorphism analysis. A method based on publicly-available libraries of nucleic-acid sequences. Nucleic acid sequences representing selected genes are obtained for all microorganisms in a sample, then fragments of the nucleic acids are separated by size and the size is correlated with the (known) sequences in the library.

ENVIRONMENTAL SAMPLE

Isolate Individual Microorganisms (Microorganisms Must be Known)

Test Sample for Molecules that Indicate Selected Microorganisms (Microorganisms Must be Known)

Test Sample for Molecular Properties Associated with Environmental Factors (Microorganisms May be Unknown)

CULTURE-BASED

Test sample for the presence of particular microorganisms by growing them

Examples

EPA methods for e.g., *E. coli* or enterococci in recreational water

MOLECULAR-BASED

Test individual (isolated) microorganisms for genes that indicate e.g., source, function, and ability to cause disease

Examples

Source Determination

Ribotyping
Rep-PCR typing
RAPD typing
PFGE typing

Function

PCR or HYBRIDIZATION to detect genes associated with e.g., nitrate, arsenic or sulfate reduction or the ability to degrade organic contaminants.

Disease

PCR or HYBRIDIZATION to detect toxin genes in *E. coli*

Test a concentrated sample or an aggregate chemical extract for genes or other molecules that indicate function or ability to cause disease or presence of specific organisms.

Examples

Function

As for the nucleic acid extract from individual microorganisms, an aggregate chemical extract could be tested by PCR or HYBRIDIZATION to detect genes associated with e.g., nitrate, arsenic or sulfate reduction or the ability to degrade organic contaminants.

Disease

Again, the same tests could be performed as for individual microorganisms: e.g., PCR or HYBRIDIZATION to detect toxin genes in *E. coli*

Specific Microorganisms

Test sample for e.g., *Salmonella* (a disease-causing bacterium) using FLOW CYTOMETRY, IMMUNODETECTION, MALDI-TOF.

Test a concentrated sample or aggregate chemical extract for chemical/molecular patterns using:

T-RFLP

DGGE

MICROARRAYS

FAME

MALDI-TOF

ENZYME ANALYSIS

ECOSYSTEM FUNCTION OR BIOGEOCHEMICAL PROCESS

Test the aggregate extract for molecules that indicate groups of microorganisms that occur uniquely in association with measured environmental properties.

Examples

Soil samples that exhibit high rates of CO₂ emission

Contaminated aquifer samples that show evidence of bioremediation

Water samples with high levels of arsenic

WILDLIFE/HUMAN DISEASE

Test the aggregate extract for molecules that indicate microorganisms that occur uniquely in association with a disease outcome.

NEW MICROORGANISMS

Test the aggregate extract for molecules that indicate microorganisms that occur uniquely in a particular environment.