

Bacterial Source Tracking Using the BIOLOG -Automated Identification System

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Amount of Funding Requested: \$8000.00

OBJECTIVE: To purchase an BIOLOG-Automated System for identification of fecal bacteria from non-point sources polluting watersheds, rivers, lakes, and streams.

Determining sources of fecal pollution in waters and the identification of nonpoint sources of pollution is a research area made possible by very recent developments in bacterial source tracking methodology. Watershed restoration is a high priority with state and federal regulatory agencies and fecal source typing is a critical part of restoration research. Funding for fecal source typing research has only become available in the last couple of years. Nationwide implementation of the total maximum daily loading (TMDL) concept by EPA is the driving force behind fecal source typing development. In an effort to implement the TMDL concept, fecal source typing has become very important in Texas.

Both molecular (genotype) and biochemical (phenotype) fecal source typing methods are under development. DNA fingerprinting has received the greatest publicity, but to date there are at least six different methods described in the scientific literature that show potential. It is reasonable to assume that some combination of source typing methods will be needed to provide the most accurate and reliable answers. It is doubtful that any one method will emerge as the "best" method for all situations.

Molecular methods (*e. g.*, DNA fingerprinting) offer the most precise source identification, but are limited by expense, detailed and time-consuming procedures, and are not yet suitable for assaying large numbers of samples in a reasonable time frame. Biochemical methods (*e. g.*, antibiotic resistance analysis (ARA), nutritional patterning (BIOLOG), fatty acid signature analysis) are simpler, quicker, less costly, and allow large numbers of samples to be assayed in a short period of time. Dr. Alexandria Graves, Post-Doctoral Research Associate with Dr. Richard W. Weaver has extensive experience using both ARA and BIOLOG. The best approach at present is to use a biochemical method to determine sources on large numbers of samples, and then confirm (validate) both the method and the results by assaying some subset of samples with a molecular procedure. Our ability to rapidly and accurately identify fecal bacteria using the BIOLOG system and determine sources on large numbers of samples enables us to confirm accuracy with a genotypic method (DNA fingerprinting), thus it will put us on the leading edge of fecal source typing and watershed restoration research.

Biological wastewater treatment systems are exposed to a broad range of contaminants, and serve as a rich example of microbial diversity. Unfortunately, most of the microorganisms present in biological wastewater treatment systems remain unidentified. Some of these microorganisms may yield multiple functions of value, such as high capacity biodegradative or specialized metabolic functions. In order to map the relationship between microbial community structure and function, it is necessary to identify microbial isolates by a method which is capable of distinguishing among species. The BIOLOG-Automated Identification System is a rapid and sound way to conduct these experiments.

EXPECTED RESULTS

Research benefits. We expect the TWRI grant to play a significant role in the continuance and expansion of this important research area. The requested bacterial identification equipment will allow us to pursue and be more competitive for sponsored projects with federal agencies. Many of these agencies have grant programs that directly support the types of research listed above such as watershed restoration (EPA, USGS, NSF), fecal organisms in the environment (NIH, EPA), and bioremediation (DOE, NSF, EPA, USDA). Also, several state and regional agencies regularly solicit RFPs where the requested equipment will support additional proposals.

The inclusion of improved bacterial identification into research areas of biological control, watershed restoration, bioremediation, and disease diagnostics will allow our group to be more productive in publication, but will contribute to other programs. Cooperators at TAMU using these facilities will also be more competitive and to build and maintain strong research programs that lead to private sector involvement in isolate databases.

We also expect this identification system to help attract, recruit, and retain better-qualified graduate students in the areas mentioned above. Some of our current graduate students and post-doctoral researchers can benefit from this equipment as well, particularly in projects requiring accurate microbial identification.

EQUIPMENT REQUESTED. One complete Automated Identification System-MicroStation System, and databases for aerobic bacteria (manufactured by Biolog). The Microstation has the capacity to read all Biolog MicroPlate configurations. The MicroPlate is placed in the reader at the appropriate time interval and analyzed via the MicroLog software. The MicroStation also allows the user to expand and create customized databases of their isolates. The system includes MicroLog software, Electronic User Guide, Reader, Pipetter, Turbidimeter, Colony Magnifier Lamp, Training, and a one year warranty. The BIOLOG-Automated Identification System identifies microorganisms based on the unique carbon source signature of each strain. Identification is performed by comparison against the carbon source signatures of known strains in the database, which currently contains signature profiles of over 2,000 microorganisms.

Identification of bacteria is critical to tracing non-point sources of fecal contamination of water, to identify potential candidates as biological control agents, and to identify plant pathogens. Methods for rapid and accurate identification by carbon source signatures (BIOLOG- Automated Identification System) will complement genotypic nucleic acid-based characterizations currently used in other TWRI bacterial source tracking projects. Automated identification systems are critical for rapidly handling large numbers of bacterial isolates and grouping them by carbon source phenotypes. Recent advances in database management and automation in the identification of microorganisms now allow large numbers of different microbes to be processed quickly. The requested automated system can perform over 100 automated analyses per day and can be used in a wide range of research projects where precise microbial identification is involved, as well as database creation of new microbes that do not fit the commercial databases. These identification and characterization techniques will allow intelligent selection of groups of bacteria for more complex genome-based DNA fingerprinting methodologies. Such equipment-based capabilities are an integral part of the research and teaching missions of the University. Granting agencies expect that our research laboratories have accurate and state-of-the-art microbial identification and characterization capabilities.

The equipment is a priority purchase for the Department of Soil and Crop Sciences because the Department will provide matching funds of \$10,000. In addition, \$15,000 in capital equipment budgeted on a TCEQ contract will be used as matching funds to make the purchase. The equipment will be immediately put to use in determining bacterial source loading of restricted river segments in Texas.

**TAES/TWRI
Water Resources Research
Project Budget Form**

| Expenditure Description | Amount Requested | Other Sources | Total |
|---|------------------|-----------------------|----------|
| Staffing Requirements: | | | |
| 1) | | | |
| 2) | | | |
| 3) | | | |
| Fringe Benefits | | | |
| Total Staff Costs | | | |
| Travel: | | | |
| Supplies and Materials: | | | |
| Capital Equipment (purchases over \$5,000) | \$8,000 | \$25,000 [*] | \$33,000 |
| Printing and Publications | | | |
| Other Direct Costs (describe in detail) | | | |
| Total Project Costs | | | |

* The \$25,000 will come from \$15,000 in a current 319 contract for capital equipment purchase and \$10,000 matching funds from the Department of Soil and Crop Sciences.