United States
Environmental Protection
Agency

Wastewater Technology Fact Sheet

Bacterial Source Tracking

INTRODUCTION

Pathogens are a major pollutant of water bodies nationwide according to many states' Clean Water Act 303(d) reports. Various sources contribute pathogens to contaminated waters, including fecal pollution from humans, wildlife, and livestock. Besides being potential pathogens, fecal bacteria (such as *Escherichia coli*) can indicate the presence of other waterborne pathogens. Bacteria from human sources may indicate the presence of human viruses, while bacteria from wild and domestic animals may indicate the presence of the parasites *Giardia* or *Cryptosporidia*. The presence of any fecal bacteria in drinking water is considered a health hazard. Knowing the source(s) of bacteria in a water body or water supply is of great value in the remediation and prevention of further bacterial contamination. However, it can be difficult to address water quality impairment effectively without a reliable method to determine the source of contamination. Bacterial Source Tracking (BST) is new methodology used to determine the source of fecal pathogen contamination in environmental samples.

There are many BST methods available and more are under development. Interest in applying these techniques stems from EPA's recent implementation of the Total Maximum Daily Load (TMDL) study, as BST techniques appear to provide the best method to determine the origins of fecal contamination in water bodies. Projects to develop TMDLs for fecal coliforms and to design and implement best management practices (BMPs) to reduce fecal loading in water may benefit from BST technology (Hager, 2001). This fact sheet discusses BST methods and presents examples of BST application to TMDL development and implementation.

DESCRIPTION

Potential sources of fecal bacteria are generally grouped into three major categories: human, livestock, or wildlife. In more urban watersheds, a fourth category of pets or dogs may be added. Each source produces unique, identifiable strains of fecal bacteria because the intestinal environments and selective pressures to which the bacteria are subjected differ from source to source.

BST may use one of several methods to differentiate between potential sources of fecal contamination, all of which follow a common sequence of analysis. First, a differentiable characteristic, or fingerprint (such as antibiotic resistance or DNA), must be selected to identify various strains of bacteria. A representative library of bacterial strains and their fingerprints must then be generated from the human and animal sources that may impact the water body. Indicator bacteria fingerprints from the polluted water body are compared to those in the library and assigned to the appropriate source category based on fingerprint similarity. BST methods can be grouped as molecular or non-molecular methods, according to the characteristic used to identify or fingerprint the bacteria. Table 1 summarizes the classification of various BST methods.

Molecular methods are also referred to as "DNA fingerprinting" and are based on the unique genetic makeup of different strains of fecal bacteria. Molecular methods rely on genetic variation as the fingerprint to identify the source of fecal contamination. Three molecular BST methods are commonly used, including ribotyping (RT), polymerase chain reaction (PCR), and pulsed-field gel electrophoresis (PFGE). Procedures for the RT and PFGE methods are relatively similar among multiple studies, but substantially different variations are reported when using PCR methods (Hagedorn, 2001).

TABLE 1 CLASSIFICATION OF BST METHODS

Molecular methods (DNA fingerprinting)
Ribotyping (RT)
Polymerase chain reaction (PCR)
Pulsed-field gel electrophoresis (PFGE)
Non-molecular methods
Biochemical methods
Antibiotic resistance analysis (ARA)
Cell wall fatty acid methyl ester (FAME)
F-specific coliphage typing
Carbon utilization (BIOLOG)
Chemical methods
Caffeine detection
Optical brightener detection

Source: Parsons, 2001.

Non-molecular methods use non-genetic characteristics as the fingerprint or basis to differentiate the source of fecal bacteria, and may be further subdivided between biochemical and chemical methods. Biochemical methods are based on the ability of an organism's genes to actively produce a biochemical substance. The type and quantity of the substance(s) produced form the bacterial fingerprint. Antibiotic resistance analysis (ARA) is the most commonly used biochemical BST method. Other biochemical methods, such as cell wall analysis of fatty acid methyl ester (FAME), F-specific coliphage typing, and carbon source utilization (BIOLOG system), are under development. Chemical methods do not detect the presence of fecal bacteria, but rely on the identification of compounds that co-occur with fecal bacteria in human wastewater to differentiate the source of fecal pollution. Thus, chemical methods can only determine whether or not the source of fecal pathogens is human (Hagedorn, 2001). Examples of compounds used in chemical BST include caffeine and optical brighteners commonly used in laundry detergents.

APPLICABILITY

BST is intended to aid in identifying sources (e.g., human, livestock, or wildlife) of fecal contamination in water bodies. Several states have started to use DNA fingerprinting to target water quality problems and formulate a mitigation strategy (Pelley, 1998; Blankenship, 1996). These techniques can also be used to direct implementation of effective BMPs to remove or reduce fecal contamination. For example, two New Hampshire communities are performing BST surveys (using the RT method) to determine the contribution of bacterial contamination from several specific sources so that BMPs may be put in place to help rehabilitate water quality. The following is a summary of one representative survey.

Hampton Harbor, New Hampshire

Hampton Harbor is a tidally dominated, shallow estuary located at the extreme southeast corner of New Hampshire. The Hampton Harbor clamflats are closed for clam harvesting during September and October due to elevated fecal coliform levels. The flats are open from November through May but close temporarily if the rainfall exceeds 0.25 inches. These clamflats are popular, productive, and accessible to the public. Despite the construction of a new wastewater treatment facility in the Town of Seabrook, the bacteria levels often exceed the limits set by the New Hampshire Shellfish Program, resulting in flat closures and frustrated clam diggers. The potential sources of bacterial contamination include birds (cormorants, starlings, gulls), domestic animals (cats, dogs, goats, horses), sanitary wastewater from wastewater treatment plant failures, and wildlife. The intent of the survey was to provide information to support implementation of specific source controls and to reduce the bacterial contamination to a level that increases the number of days that the clamflats are open for recreational harvesting.

Source classification provided by BST is often used in the development and implementation of TMDL projects. The information can be used to assign load reduction allocations to sources in a watershed. For example, BST techniques have been very useful to regulatory officials in Virginia, where the ARA method

has been used in seven TMDL watershed projects to date.

Virginia Department of Conservation and Recreation TMDLs

Over 300 stream segments were listed on the Commonwealth of Virginia's 303(d) list for fecal coliform bacteria violations. The uncertainty inherent in identifying specific sources of fecal coliform bacteria in the streams has hindered development of the TMDLs. BST studies were applied to three stream segments (Accotink Creek, Blacks Run, and Christians Creek) to provide more accurate waste-load allocations and enhance the development and defensibility of the TMDLs. In each, the RT method was used to determine the dominant sources of fecal coliform in the impaired stream segments. The source-tracking distribution determined in each segment were used to modify and strengthen the waste-load allocations in the TMDL watershed model. In addition, DNA testing is underway in the Muddy Creek, Lower Dry River, Mill Creek, and Pleasant Valley watersheds as part of their TMDL implementation plans.

Cedar Creek, Hall Creek, Byers Creek, Hutton Creek, and Lower Blackwater River were also placed on the Commonwealth of Virginia's 303(d) list because of violations of the fecal coliform bacteria water quality standard. In fulfilling the state requirement to develop a TMDL Implementation Plan, a framework was established to reduce fecal coliform levels and achieve the water quality goals for which TMDL allocations were developed. BST analysis using the ARA method was performed as part of the TMDL implementation. Results indicated contributions of fecal coliform from livestock, human, and wildlife sources. The wildlife contribution alone was enough to push fecal coliform levels beyond the standard at five sampling sites, while human sources alone were high enough to violate the standard at five sampling sites. Livestock sources were sufficient to violate the standard at eight of eleven sampling sites. In the Cedar and Hutton Creek watersheds, livestock appeared to be an issue throughout the watershed, while in the Hall/Byers Creek watershed, livestock problems appeared limited to smaller tributaries (e.g. Indian Run and Tattle Branch). Human sources seemed most significant in the Hall/Byers and Hutton Creek watersheds. The quantity of control measures required during implementation was determined and progress toward end goals will be assessed during implementation through tracking control measure installations and continued water quality monitoring. Water quality monitoring will include fecal coliform enumeration and BST analysis. BST will provide an indication of the effectiveness of specific groups of control measures, specifically agricultural and urban. Implementation was scheduled to begin in July 2001, with the final goal being the delisting of the impaired segments from the Commonwealth of Virginia's 303(d) List of Impaired Waters by 2011.

ADVANTAGES AND DISADVANTAGES

In general, molecular BST methods may offer the most precise identification of specific types of sources, but are limited by high per-isolate costs and detailed, time-consuming procedures. They are also not yet suitable for assaying large numbers of samples in a reasonable time frame. Biochemical BST methods are simpler, faster, less expensive, and allow large numbers of samples to be assayed in a short period of time.

BST development is so new that little research comparing individual methods is complete. Results of initial studies should become available over the next few years.

The United States Department of Agriculture recently funded a two-year study to compare three BST methods using *E. coli* and *Enterococcus*: ARA, PFGE, and RT. The merits of these methods will be compared by a) accuracy, cost, and processing time; b) determining the geographic range of the libraries; and c) assessing the utility of each method in field experiments. This comparison and development of BST methodology is intended to refine BMP implementation and focus resources on pollution sources for water quality impairments.

The United States Geologic Survey is developing a program to identify sources of fecal bacteria in the waters of Berkeley County, West Virginia. At least five methods will be tested for their ability to determine animal sources of fecal bacteria in water samples (RT,

PFGE, ARA, PCR, and BIOLOG carbon-utilization). The three objectives of this project include building source libraries for the five methods, comparing methods to see which is best to determine sources, and using the best method to identify sources of bacteria in water resources of Berkeley County. This study will provide source libraries for five promising methods to identify bacteria sources, quantitative information on which method(s) works best, determination of bacteria sources for ten domestic wells that contain bacteria, and determination of bacteria sources for five large public-supply springs. The libraries and methods will be applicable to both surface and ground water in Berkeley County and surrounding areas.

PERFORMANCE

Many BST techniques are undergoing intensive research that leads to rapid change in existing methods and the creation of new methods. BST technologies are quickly becoming proven and should be used by federal and state regulatory agencies to address sources of fecal bacterial pollution in water. Although they are still experimental, BST methods represent the best tools available to determine pathogen TMDL load allocations and TMDL implementation plan development. The following are examples of BST technique performance in specific watershed studies.

Antibiotic Resistance Analysis (ARA) Method

Holmans Creek, Virginia

Holmans Creek watershed was listed on the Commonwealth of Virginia's 1998 303(d) TMDL Priority List of Impaired Waters based on violations of the fecal coliform bacteria water quality standard. There are several potential fecal coliform sources in this watershed, including the non-point sources of wildlife, livestock, individual residential sewerage systems, and land application of manure and litter. Beef cattle, poultry and dairy are the major livestock operations in the Holmans Creek watershed. Residential sewerage in the watershed consists of direct discharges from straight pipes (homes without facilities to treat their waste discharge), privies, and failing septic systems. BST analysis using the ARA method was used to classify sources of the fecal bacteria found in the polluted water. Results of the BST analysis suggest that the primary source of fecal pollution is human, constituting just under half of the total fecal coliform deposited into the waters of Holmans Creek. Wildlife and cattle sources each contribute approximately one-fourth of the total fecal coliform loads in the watershed. Poultry were determined to be a minor contributor to fecal coliform pollution in Holmans Creek, contributing one-tenth of the total fecal load.

Stevenson Creek, Florida

The Stevenson Creek basin encompasses approximately 6,000 acres in central Pinellas County, Florida. In keeping with the objectives of the Stevenson Creek Watershed Management Plan, a BST study was initiated to identify the dominant source(s) of fecal contamination to Stevenson Creek in Clearwater, Florida. The ARA method was chosen because it can assess the source of indicator organisms based on a much larger subset of the bacterial population than molecular methods can. The dominant sources of fecal coliform over the course of the study were wild animal, dog, and human, with the overall trend indicating that wild animal isolates comprised the majority of fecal coliforms obtained when colony forming units (CFU) counts exceeded the acceptable limit of 200 CFU per 100 mL. While human input was not the major cause of elevated fecal coliform levels for most of the samples analyzed for this study, the domination of some small populations by human isolates suggests that human sources contribute to low-level background contamination. This occurs when fecal coliform populations are low, near the transition to dry season, and perhaps few isolates are washed into surface waters from draining storm water. Lowering water tables may also draw wastewater from small, otherwise innocuous leaks. Overall, there was little evidence of acute human fecal contamination on a large scale; however, human sources may influence two sampling sites, detectable despite the presence of fecal coliforms from other sources. The human input alone for these two sites in one month was high enough to violate water quality standards.

Pulsed-Field Gel Electrophoresis (PFGE) Method

Eastern Shore, Virginia

DNA fingerprinting using PFGE proved helpful when an oyster farmer on Virginia's Eastern Shore was faced with the closure of his shellfish beds due to elevated levels of *E. coli*. Failing septic tanks were assumed to be the primary source of the fecal pollution, but a survey of septic systems in the sparsely populated watershed indicated that they were not the cause, and it became necessary to identify other potential sources. The highest levels of coliform bacteria were measured in the small tidal inlets and rivulets of the wetlands located upstream of local houses, shifting suspected sources from human to other sources. Researchers collected fecal samples from raccoon, waterfowl, otter, muskrat, deer, and humans in the area and used DNA fingerprinting to confirm the suspicion that the source was not anthropogenic in nature. Comparing *E. coli* from the shellfish beds against the fingerprints of known strains in the DNA library, the researchers linked the in-stream *E. coli* to deer and raccoon (mostly raccoon). Several hundred animals, including 180 raccoon, were removed from areas adjacent to the wetlands. *E. coli* levels subsequently declined by 1 to 2 orders of magnitude throughout the watershed, allowing threatened areas of the tidal creeks to be reopened to shellfishing.

Four Mile Run, Virginia

Four Mile Run is listed on the Commonwealth of Virginia's 303(d) listing for elevated levels of fecal coliform bacteria. The Northern Virginia Regional Commission is currently developing a TMDL for the Four Mile Run watershed, with the final draft to be submitted to Virginia Department of Environmental Quality by March 1, 2002. Four Mile Run is an urban stream with no agricultural runoff. The watershed is home to 183,000 people, just over 9,000 per square mile. The dominant land use in the watershed is medium to high density residential housing. Seven central business districts exist within this 20 square mile watershed, and two high-capacity interstates pass through the watershed along with numerous primary and secondary roadways. The watershed is approximately 40 percent impervious. A large pet population accompanies the dense human population in the watershed. As to potential fecal sources, there is little manufacturing industry to generate point source discharges and there are no combined sewers in the majority of the watershed. Sanitary sewers serve more than 99.9 percent of the watershed population. The number of septic systems in the watershed is believed to be less than 50. The PFGE method of BST analysis was conducted on *E. coli* DNA from seasonally varied stream and sediment samples in the watershed. Results of the analysis show that waterfowl contribute over one-third (38 percent) of the bacteria, humans and pets together account for over one-fourth (26 percent), and raccoons account for 15 percent of the contamination, with deer (9 percent) and rats (11 percent) also contributing. The predominant non-human sources include wildlife species with intimate association with the waterways.

Ribotyping (RT) method

Little Soos Creek, Washington

A BST survey was designed to help characterize sources of fecal coliform bacterial contamination in Little Soos Creek in southeast King County, Washington, in response to the impact of existing and anticipated urban development in the area. Little Soos Creek has historically been categorized as a Class A stream, but violates fecal coliform standards for this classification. The goal of the BST survey was to help determine the contribution to contamination of the stream from two potential sources: livestock on hobby farms and ranches adjacent to the stream and on-site septic systems close to the stream in highly permeable soils. Other animal sources were also considered. Genetic fingerprinting (using ribosomal RNA typing or RT) was performed on *E. coli* isolates to effectively match specific strains of *E. coli* from a contaminated site in the stream to its source. The intent was to provide information to support implementation of specific source controls. The study identified the sources of approximately three-fourths of the fecal coliform contamination, with the primary sources determined to be cows, dogs, and horses. Although septage was identified as a contributor to the contamination problem, it was not indicated as a major

source. However, even low levels of contribution from septage suggest the potential for Little Soos Creek to harbor a number of human viral, bacterial, and parasitic pathogens associated with human sources. For this reason, further investigation of the contribution from septic systems and of human exposure (particularly children) to the stream may be warranted.

Lower Boise River, Idaho

The Lower Boise River watershed from Lucky Peak Reservoir to the Snake River near Parma contains almost one-third of Idaho's population and four major municipalities, including the city of Boise. An arid climate (approximately 10 inches of annual rainfall) makes irrigation a requirement on most farmland. This irrigation coupled with reuse of pasture water on irrigated fields results in the contribution of non-point discharge of fecal coliform bacteria to the Lower Boise River. In 1994, the Idaho Department of Environmental Quality (IDEQ) placed the Lower Boise River on the 303(d) list for impairment of primary and secondary contact designated uses because fecal coliform levels exceeded state standards. A draft TMDL was completed and submitted to the USEPA on December 1998 and approved on January 2001 with implementation plan due July 2001. The TMDL indicates that bacteria discharge loads will require more than 95 percent reductions from non-point source bacteria loadings to meet the primary contact bacteria standard. A DNA fingerprinting of coliform bacteria was conducted to focus bacteria reduction improvement. *E. coli* cultures were grown from fecal samples of cows, sheep, humans, ducks, and geese, and DNA from these samples was identified. The major bacteria sources in the watershed identified using the RT method were waterfowl, humans, pets, and cattle/horses. Waterfowl were clearly the largest source. The major advantage of using the DNA fingerprinting tool is the ability to develop accurate control measures (BMPs) in terms of bacterial sources. Prior to this study, IDEQ knew there were bacteria problems, but did not know where to focus control measures. The results of the BST analysis identify the major sources, allowing IDEQ to strategically place BMPs.

University of Georgia/USDA RT comparison

BST methods, including RT, rely on a database of known source fingerprints to identify environmental isolates of fecal bacteria. It is not well understood to what degree these known source fingerprints are biogeographically variable. This is important because a fingerprint database developed for one state or region may or may not be applicable to another. The objective of a University of Georgia/USDA study (Hartel *et al*, 2002) was to use the RT method of BST analysis to determine the geographic variability of the fecal bacterium, *E. coli*, from one location in Idaho and three locations in Georgia for four animals: cattle, horse, swine, and poultry. The study identified distance from the source sample to the watershed as a key variable for cattle and horses, but not for swine and poultry. When the *E. coli* ribotypes among the animals were compared at one location, the relative percent difference between them was 86, 89, 81, and 79 for each of the four locations, suggesting good ribotype separation among host animal species at one location. Achieving a high degree of accuracy in matching environmental isolates of fecal bacteria to a host origin database depends on having a large number of isolates for comparison and using a distance of 175 km or less (at least for certain host animal species).

COSTS

Given the fact that many BST methods are still in the research and development phase, there is great variation for cost per sample (or per isolate) among different laboratories. Factors that affect cost include the following:

Analytical method - Molecular BST methods (e.g. RT) are generally more expensive than non-molecular methods. In addition, automated techniques are more expensive but less labor-intensive than manual techniques for the same method (such as RT).

Size of the database - It is not known what size database or library of bacterial isolates from known fecal sources is required for accurate source prediction in a given watershed. Considerations in the development of the BST library include the size of the watershed, the diversity of animal species and human

sources that may significantly impact water quality, and the heterogeneity of the population within a given source species. In many studies, the number of isolates required to develop the known source database may make up the majority of total isolates analyzed, constituting a large fraction of the total cost for the study.

Number of environmental isolates - The number of isolates that must be analyzed from the water body of interest varies among study sites. There may be multiple isolates from each water sample taken, with costs generally calculated per isolate.

Level of accuracy - Cost increases in proportion with accuracy or the percentage of isolates classified correctly. In some cases 80 percent is considered the lowest acceptable level of accuracy. More studies are needed to determine the level of accuracy achievable by each BST method.

The cost for BST analysis ranges from $25 to $100 per isolate using molecular methods and from $10 to $30 per isolate for non-molecular methods. These costs are based on classifying a sample within an accuracy range of 70 to 90 percent or higher. However, there is little firm guidance on the required number of reference fecal samples and isolates extracted from each sample, causes wide variance in the total cost for a fecal source tracking project. For example, the cost for TMDL developments for Accotink Creek, Blacks Run and Christians Creek in Virginia by the USGS Richmond office was approximately $617,000 (total for the three TMDLs), while the New Hampshire Department of Environmental Services spent approximately $225,000 to establish the ribotyping laboratory and partially support the two source tracking surveys. In two ongoing comparison studies, the cost of the San Juan Creek Watershed Bacteria Study (California) is $274,000 (excluding the expenses for laboratory analysis), while the USDA grant to compare three BST methods (RT, PFGE and ARA) is $310,000.

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Other EPA Fact Sheets can be found at the following web address:
http://www.epa.gov/owm/mtbfact.htm

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Other methods

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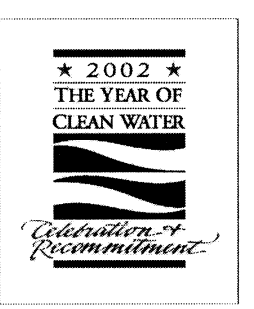

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