Introduction to Microbial Source Tracking

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One of the major challenges in environmental and regulatory microbiology is to identify sources of fecal microbial pollution that impact bodies of water. The few successful methods developed over the years have gradually evolved from simple phenotypic methods based on characteristics of the microbe to relatively sophisticated molecular (genotypic) methods based on DNA sequences. Microbial source tracking (MST) techniques differentiate between particular characteristics of the pathogens excreted by potential sources. This article focuses on the MST technique using bacteria.

The MST method is based on two principles. The first is that the genetic structure of the bacterial population is clonal: bacteria divide by binary fission. The two daughter cells that are generated as a result of this cell division are virtually identical in all aspects, and all descendents of a common ancestral cell are genetically related to each other. MST makes use of the clonal population structure of bacteria to classify organisms based on their genetic fingerprints into groups of clonal descent.

The second principle behind MST methodology is the assumption that within a given species of bacteria, various members have adapted to conditions in specific hosts or environments. Consequently, there is a high degree of host specificity among bacterial strains seen in the environment. A bacterial strain that has adapted to a particular environment or host (such as an animal's intestinal tract) is capable of colonizing that environment and competing favorably with members of its indigenous flora. Such a bacterial strain is called a *resident strain*. Resident strains are usually shed from their host over a long period of time, providing a characteristic signature of their source. A *transient strain* is a bacterial strain introduced into a new environment or host that cannot colonize and persist there. A host sampled over time for a given species of bacteria will show that a few resident strains are consistently being shed while a large number of transient strains are shed for brief lengths of time. A study by Hartl and Dykhuizen (1984) showed that, of fecal samples from a single subject collected over 11 months, two *E. coli* isolates were resident strains, appearing 252 times, while 548 were transient strains.

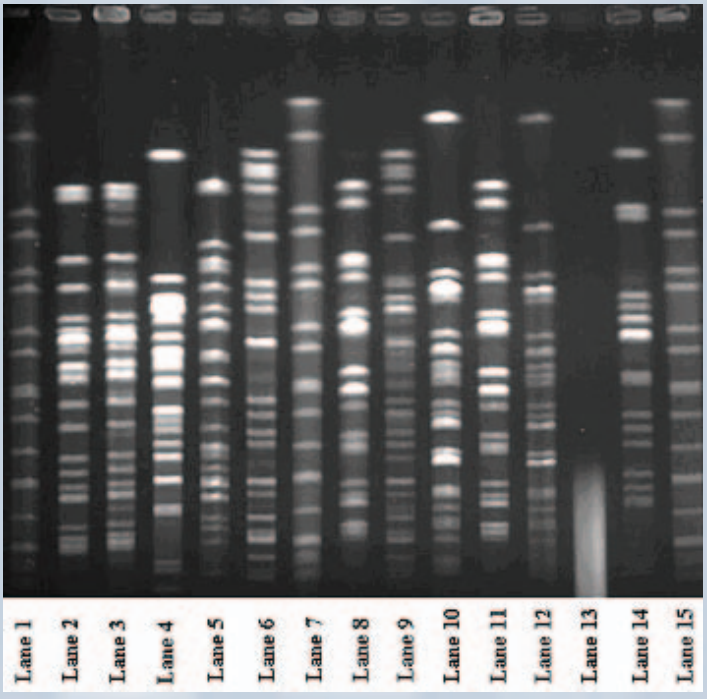
Quantitative MST methodologies recognize that in any given pollution scenario there are multiple contributing animal sources of microbial pollution, each having unique clones of bacteria that constitute their normal flora.

Two broad categories of MST methodologies are used: quantitative (otherwise known as library-based) and qualitative (known also as presence/absence [P/A] methods). The quantitative MST methodologies can be divided into genotypic- and phenotypic-based methods. Qualitative methods rely on collection of bacterial strains of known origin from a target species (the source library) to identify the origin of the bacterial strains that have been isolated in the study sites. These methods rely on the identification of target organisms that can be associated with specific sources of microbial pollution.

Quantitative MST methods

Quantitative MST methodologies recognize that in any given pollution scenario there are multiple contributing animal sources of microbial pollution, each having unique clones of bacteria that constitute their normal flora. Isolates from appropriate bacterial species are collected from the polluted sites and from the suspected animal sources of pollution, which are identified through a sanitary survey of the area surrounding the polluted site. An appropriate molecular subtyping or phenotypic characterization method is then used to characterize all bacteria in the collections. Finally, the genetic fingerprints or phenotypic characteristics of the bacterial isolates from the polluted site are compared to those of the bacteria from the suspected animal sources (the source libraries).

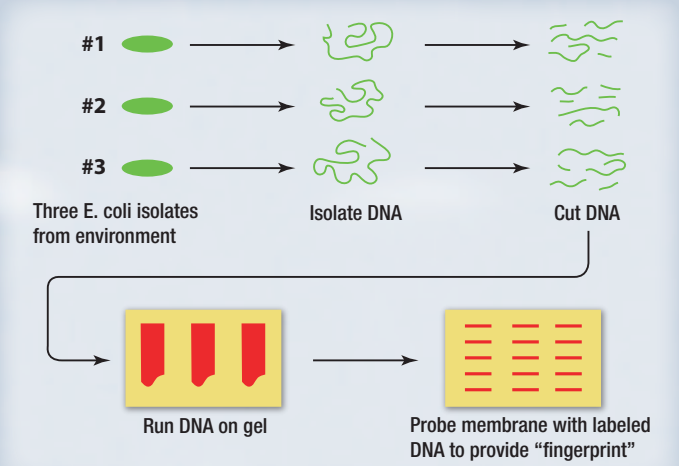
When a strain of bacteria with an identical genetic fingerprint or with identical phenotypic characteristics is isolated



PFGE analysis of E. coli *strains isolated from various source samples.*

from both a polluted site and a suspected animal source, the animal is implicated as a contributor of that specific clone of bacteria to the polluted site. The figure below left shows the genetic fingerprints of *E. coli* strains isolated from various sources and determined by the PFGE method; ribotyping (right) is also used.

Ribotyping and PFGE are commonly known as DNA fingerprinting methods. Ribotyping exploits the variations in DNA sequences that produce ribosomal RNA, while in PFGE the entire bacterial chromosome is cut into a small number of large fragments; the fragments are then size-separated by electrophoresis and visualized.



Steps in the ribotyping process. If sample 1 from a human can be matched to sample 2 or 3 from the environment, for example, a source can be implicated. Diagram courtesy of Peter G. Hartel, University of Georgia.

Qualitative MST

The qualitative method concept is based on the assumption that microbial species (bacteria, bacteriophages, viruses) or biotypes within a species (groups that produce a given type of toxin): a) have host-species specificity, and b) have a target organism/molecule/gene that can be detected in the water samples. The challenge of this approach is that since bodies of water are impacted by numerous sources, a large number of host-specific targets must be identified and validated. Another challenge is the need to develop quantitative approaches to correlate the levels of the targets detected in P/A methods to levels of indicator organisms, the prime targets of anyMST-based methods.

Target organisms for MST studies

Since microbiological standards for water quality are based on numbers of indicator organisms, those organisms are a natural target for source-tracking studies. Decisions to conduct MST studies are based mostly on finding elevated levels of indicator organisms. Among those commonly targeted are *E. coli*, fecal coliforms, fecal streptococci, and enterococci.

For library-based methods, the choice of target organism is crucial, since it impacts both the study design (number of samples taken and number of isolates to be analyzed), and the size of the library. For instance, *E. coli* has just a single species, whereas enterococci or fecal coliforms have several. A larger number of samples is needed for multiple-species targets to achieve accurate representation of the target organism in the water samples.

MST at the Institute for Environmental Health

Most of the basic approaches for MST studies have been developed by the MST team at the Institute for Environmental Health. This team has conducted more than 150 MST studies in the United States and Canada. These studies have focused on beach pollution, drinking water sources, watershed assessment, TMDL studies, storm water systems, and the impacts of various water and land uses, including tourism in national parks, grazing and water quality, feedlots and groundwater, septic systems and water quality, and wastewater treatment plant discharges.

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Reference

Hartl, D.L., and D.E. Dykhuizen, 1984. The population genetics of Escherichia coli, Ann. Rev. Genet., 18: 31-68.