E. Coli Strains Inhabiting Interstitial Spaces of Soil Adjacent to Vermont Streams

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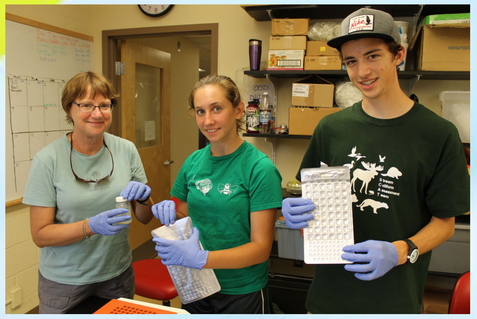
Introduction:

Fecal coliform bacteria like *E. coli* thrive in the digestive tracts of warm-blooded animals like mammals and birds. These bacteria, when excreted via the feces, can contaminate freshwater streams, rivers, and lakes. Much attention has been directed toward establishing water quality standards for fecal indicator bacteria (FIB), identifying the source of this microorganisms, and finding solutions to reduce the presence of FIB in freshwater drinking source, as many are potential pathogens (EPA). However, coliform bacteria have also been found in the interstitial spaces within soil and sediments, leading some to suggest that certain *E. coli* strains may actually reside in sediment (Kon *et al.* 2009; Korajkic *et al.* 2009). These bacteria may have become naturalized in the soil, and could grow and replicate for long periods of time, outside of their traditional home in the bodies of animals, thus representing an overlooked reservoir of potential bacterial contamination (Ishii, *et al*. 2006).

A 24-hour test (*Colilert*) can quickly indicate the presence or absence of *E. coli* in a sample. A more time consuming method, *microbial source tracking* (MST) is used to identify non-point sources of *E. coli*. Ribotyping, an MST method, is the matching of *genetic fingerprints* of isolated bacteria to known fingerprints from a source library (EPA).

Objectives:

The objectives of this project were to determine whether interstitial bacteria were present in soil adjacent to three streams in northern Vermont, and whether these bacteria were from known sources or if they represented a new strain previously unknown to the source trackers. As a corollary, a sterilization procedure was tested to create a sampling control, which had not been previously described in the literature.



Trowel Sterilization Procedure:

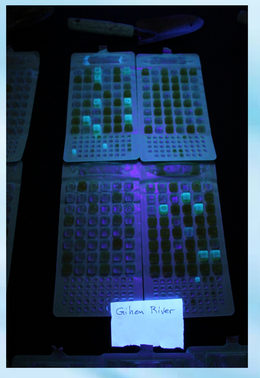
1. A small trowel was soaked in a canister of ethanol.
2. The trowel was shaken to remove any excess ethanol, and ignited with a lighter.
3. When the flame was completely eradicated from the trowel, it was cooled, then the E. coli collection procedure was initiated..

Quality Control Evaluation of Sterilization:

1. Distilled water was poured over a sterilized trowel.
2. All runoff water was caught in the sample bottle.
   a. After the water reached all areas of the trowel, the cover was quickly screwed on the sample bottle.

E. Coli Collection Procedure:

1. A sterilized trowel was used, and an area was selected in the stream bed one foot away from the stream.
2. Layers of soil were scraped away with the trowel until the hole filled with water.
3. When there was enough water, a sample bottle was inserted into the pool, until it filled with water. Then the cover was screwed on.
4. The bottle was labeled for its stream and sample number.



Colilert Test Procedure:

This work was done at Johnson State College JSC. The Colilert test is a rapid (24 hour) assessment for the presence/absence of *E. coli* and other coliform bacteria.

1. Contents of reagent were added to a 100 mL sample in a sterile, transparent, non-fluorescing vessel.
2. The vessel was and shaken.
3. It was then incubated at 35±0.5°C for 24 hours.
4. Low or no color change indicates no evidence of coliforms or *E. coli* . Yellow is a positive indicator for coliforms and fluorescent yellow is positive for *E. coli.*

Results:

JSC used a computer program to compare DNA samples from *E. coli f*ound in streams to a database of DNA samples from *E. coli* known to exist in the digestive tract of local fauna. The resulting data showed the DNA from the *E. coli* samples grouping with that of distinct strains of *E.Coli* found in a variety of animals, rather than grouping in clusters separate from known gastrointestinal *E.coli*. This indicates that the *E. coli* found in stream beds are not a distinct strain of *E. coli*, as hypothesized in this experiment, but rather closely related to or perhaps genetically identical to those found in animals. The trowel sterilization procedure worked, as no bacteria were found in the quality control samples.

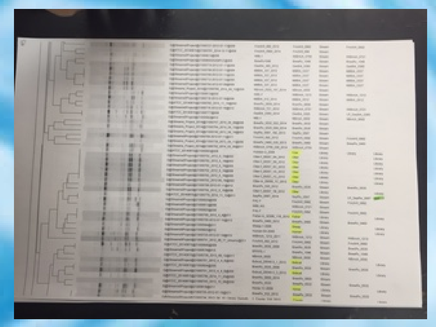
Ribotyping Procedure:

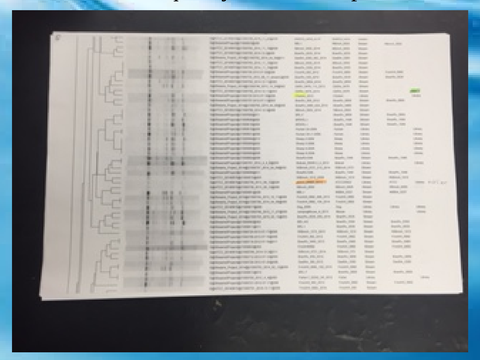
This work was done at the University of Vermont by the lab technician from JSC

1. The genomic DNA was extracted from each bacterial isolate
2. It then was cut with restriction enzyme ECO-R1.
3. The DNA was ran in an agarose gel by electrophoresis. DNA fragments were separated by size as they move through the gel.
4. The DNA fragment barcodes were compared to the known library of sources.

Rivers Used for Sampling:

French Hill Brook, Gihon River, Seymour River

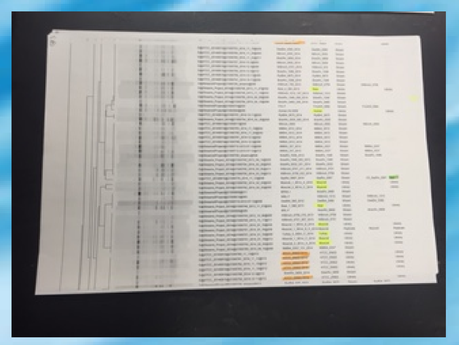




Discussion:

E. coli were found in the interstitial soil adjacent to the streams tested but these *E. coli* did not appear to be divergent strains. In conjunction, the sterilization procedure was successful, with negative results for contamination. The results of this experiment showed that the *E. coli* found living in soil near Vermont streams matched known sources of *E. coli* which inhabit warm-blooded animals.

These results raise more questions about the ecology of *E. coli.* Further experimentation might explore the possibility that these *E. coli* not only live, but replicate outside of a host body. Thus, rather than finding a conclusion to the original questions asked, only a discussion of further questions can be raised, those questions pondered, and eventually further tested.



Bibliography:

Ishii S, Ksoll WB, Hicks RE, Sadowsky MJ. Presence and Growth of Naturalized *Escherichia coli* in Temperate Soils from Lake Superior Watersheds. *Applied and Environmental Microbiology*. 2006;72(1):612-621. doi:10.1128/AEM.72.1.612-621.2006.

Colilert Test Kit Protocol. https://www.idexx.com/resource-library/water/colilert-procedure-en.pdf. Accessed 3/10/2015.

Korajkic, A., Badgley, B.D., Brownell, M.J. and Harwood, V.J. (2009), Application of microbial source tracking methods in a Gulf of Mexico field setting. *Journal of Applied Microbiology*. 107: 1518–1527. doi: 10.1111/j.1365-2672.2009.04351.x

Kon, T., Weir, S., Howell, E.T., Lee, H., Trevors, J.T. (2008) Repetitive element (REP)-polymerase chain reaction (PCR) analysis of *Escherichia coli* isolates from recreational waters of southeastern Lake Huron. *Canadian Journal of Microbiology*. 55(3): 269-276, 10.1139/W08-123

Ribotyping. https://courses.cit.cornell.edu/biomi290/microscopycases/methods/ribotype.htm. Accessed 3/10/15.

U.S. Environmental Protection Agency. 2005. Microbial Source Tracking Guide Document. United States Environmental Protection Agency, Office of Research and Development, Cincinnati, OH. EP/600-R-05-064

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