

# Introduction:

Fecal coliform bacteria like E. coli thrive in the digestive tracts of warm-Vermont EPSCoR CONDDD blooded animals like mammals and birds. These bacteria, when excreted via the feces, can contaminate freshwater streams, rivers, and lakes. Much **Center for Workforce Development & Diversit** 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 Study Conducted By: Elise Prehoda, Paul Nasca, 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  $\overline{E}$ . 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).



**Results:** 

## **Trowel Sterilization Procedure:**

. 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.

### JSC used a computer program to compare DNA samples from E. coli found 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 rowel sterilization procedure worked, as no bacteria **Discussion:**



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





### **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.

## **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.

*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.



# and Sharon Boardman

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.





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  $35\pm0.5^{\circ}$ 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*.

## **Rivers Used for Sampling:**

French Hill Brook, Gihon River, Seymour River

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Colilert Test Kit Protoco 3/10/2015



## **Objectives:**

### **Colilert Test Procedure:**



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