Identifying source of fecal pollution in 19 stream sites of the Lamoille River Basin with MST method and enlarge the ribotyping library to identify E. coli

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Abstract

A library – dependent Microbial Source Tracking (MST) method was used to identify the specific sources of fecal pollution in 19 tributary sites of the Lamoille River, VT. The *E. coli* were isolated by filtration and identified using a conformational method with MacConkey agar and MUG. Identifications were confirmed with the Enterotube II (BD Diagnostic Systems, Heidelberg, Germany). MST was conducted using a Riboprinter (DuPont Qualicon) to generate DNA "barcodes" for the different varieties of *E. coli* in stream samples and to run library samples of known sources to enlarge the reference library. Most of the *E. coli* came from non-human sources but with a large percent of human source in the populated areas. The following results examine how consistently genetic barcodes from the same known source species agree.

Introduction

The fecal pollution in water is a health concern, because it is associated with drinking water, recreational activities, and food production (Bukh, 2011). The Sources of fecal pollution include riparian warm-blooded animals, avifauna, pets, farm animals, and humans. Human sources are a concern to the scientific community, because of the potential for human pathogens associated to be human *E. coli* (Bukh, 2011).

The source of a fecal pollutant can be known by a variety of methods. A method that has been used for many years the library –dependent method that relies in the cultivation and isolation of the specimen (EPA, 2002). This method needs a reference library, that is built using isolates from known sources; usually the isolates are taken from a fecal sample directly from the animal or after the immediate fecal extraction (U.S. Environmental Protection Agency, 2005).

The library – dependent methods require a known source database to compare the field samples (U.S. Environmental Protection Agency, 2005). To conduct a good study the library should be large enough to agglomerate the total genetic diversity present in the population of the indicator bacteria of the host animal, and be of sufficient size so that the isolates can match to host origin. Also the library can have genetic diversity in indicators bacteria because of different strains of E. coli that result from animal host related to the feeding habit, food sources, diet variation in host animal group. A small library size makes comparisons very difficult, because the large number of unidentified strains (U.S. Environmental Protection Agency, 2005).

Method

E. coli from water samples

The process of collecting E. coli involved going to 19 streams tributaries of the Lamoille and Chittenden Counties, VT, and aseptically collect two replicate stream water samples for each site (U.S. Environmental Protection Agency, 2002). The samples were taken to the laboratory for the process of filtration on sterile 0.45 µm membrane filters, for the recollection of the bacteria. The filter was placed in a petri dish of 50×9mm, with an MI Broth solution; this Petri Dish was put in a incubator for 24 hours at 35 ℃. In the process of incubation total coliforms (TC) were fluorescent blue-white, *E. coli* where fluorescent blue-green, and the others where non-fluorescent (U.S. Environmental Protection Agency, 2002).

A random colony was selected and grown in 15mL tubes of LB Broth, placed on the shaker for 24 hours. To make 148 reading in the RiboPrinter®, the specimens were repetitively selected from the LB broth and streaked into MacConkey agar. Red colonies appeared after 24 hour of incubation (U.S. Environmental Protection Agency, 2002). If the MacConkey colonies where all red, the next step was to confirm the samples on the Enterotube, that indicates the presence of E.coli or another species of bacteria. When there was a match, the sample was taken to the RiboPrinter®(U.S. Environmental Protection Agency, 2002). The RiboPrinter® produces a genetic barcode that was used to identify the unknown source of the samples. To generate the analysis we used Dice's similarity at 1.7% tolerance and 1% optimization.

Results

Avobe 90% accuracy in RiboPrinter® Analysis

Number of individuals of the same species in clusters

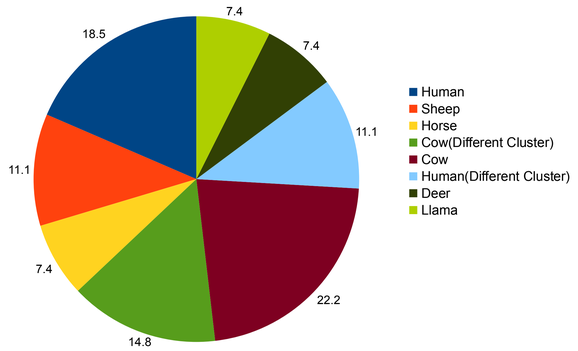

Figure 1

Identified isolates

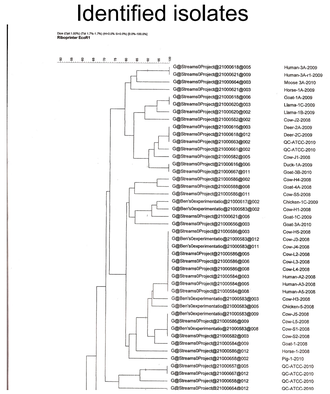

Figure 2

Sample analysis procedure

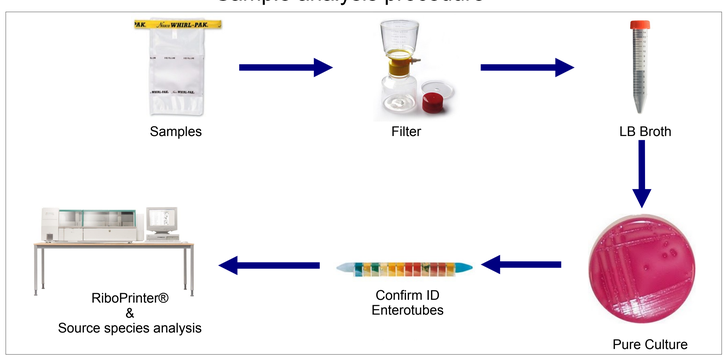

Figure 3

Conclusion

The RiboPrinter results were compared using the DuPont RiboPrinter library and the Johnson State College fecal source library. Looking into the results, some species cluster together in the 90% accuracy range. There were two clusters of human, and two clusters of cow genotypes, while sheep, deer, horse and llama produced single clusters. This means that the genetic barcode in the *E.coli* of a cow or human can have different strings even for members of the same species. In a perfect panorama all of the members of a same species would have to be clustered together forming a perfect pattern, because the library would have sufficient data of different strings of *E.coli*. The species that did not cluster together (goat, chicken and duck) are variations in the genetic barcode of the bacteria.

In future research the general idea will be, enlarge the known sources of samples, to give a large outlook for the unknown samples, so the genetic barcode can be match more perfectly.

References

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Acknowledgment

I thank the Team members of the Streams Project Staff, for their constant support. Also to my mentor Robert B. Genter for his knowledge and patience. I will also thank the University of Vermont for giving us the space to work, and Johnson State College for the materials in the laboratory. Equally I thank the Universidad Metropolitana for giving the funding to cover the expenses of the travel.