WATER QUALITY

DNA Fingerprinting as a Means for Tracing the Source of *E. coli* Contamination

Final Report

Our current research has successfully shown that random amplified polymorphic DNA (RAPD) may be used to distinguish human from non-human *E. coli*. We have narrowed down the primers needed for RAPD analysis to three. We have created a database consisting of 160 DNA fingerprints from fecal isolates from human and animal sources (cow, horse and goose). For identification purposes, a database library was established. Within the library, DNA fingerprints for each host species was subdivided into units (patterns) such that members of the same unit share at least 75% similarity with the mean (composite) pattern.

Currently, we are examining a new and powerful resource made available to us from DuPont Qualicon, Wilmington, DE. The RiboPrinter Microbial Characterization System is an automated ribotyping system that generates fingerprints of ribosomal DNA. This technique is rapid and very sensitive. This system is currently being used to identify food pathogenic bacteria sources. We intend to develop a new application for tracking the source of *E. coli* contamination using the same system. This is a new approach and the key to its success will be finding the suitable restriction enzymes required to generate host specific *E. coli* ribotypes.

Major Goals and Objectives

- To test the hypothesis that human and animal *E. coli* strains are distinguishable
- To screen and select appropriate primers
- To build a small RAPD (random amplified polymorphic DNA) fingerprint database for *E. coli* from human and nonhuman sources.
- To identify landmark DNA patterns
- To determine the sensitivity and applicability of this technology

Summary of Progress

To date, the first three objectives have been achieved. The forth objective was modified because of the availability of the BioNumericâ software (Applied Maths, Belgium) for discriminant analysis and identification. To achieve the fifth objective, we are now collecting environmental samples for testing the sensitivity and applicability this technology.

After screening over 40 primers, three (primers 2, 1247, 1283) were selected. Each of these primers was used to generate a DNA band pattern. The band patterns from all three primers were combined to form a composite DNA fingerprint for each *E. coli* isolate. A database of RAPD of over 400 *E. coli* isolates from human and nonhumans has been constructed. Discriminant analysis showed that *E. coli* from humans and nonhumans are distinguishable. We have now over 50 environmental *E. coli* isolates from beach sand and lake water. RAPD fingerprints of all of these environmental samples have been prepared.

In addition to the above planned study, we have initiated a new approach: use of an automated RiboPrinterâ for tracking the source of *E. coli.* This new method is equally promising. Our ultimate goal is to use both databases (RAPD and ribotyping) for a consensus identification. In order to complete the two paralleled studies, we recently submitted two grant proposals: The first proposal, "Use of an automated ribotyping system for tracking the source of *E. coli* contamination," was submitted to the National Sea Grant Technology

Program. This proposal has been approved. The second proposal, "Tracking the source of *E. coli* by RAPD analysis," was submitted to Illinois-Indiana Sea Grant College Program for completing the second half of this study. This proposal is now pending.

Accomplishments

- Over 600 *E. coli* samples from human and non-human sources have been collected.
- Over forty primers were screened and three primers were selected for RAPD analysis.
- A RAPD database of over 400 *E. coli* isolates was generated.
- Discriminant analysis of RAPD fingerprints of over 400 isolates from human, cow, horse, goose, and seagull was performed.
- Seven papers were presented at conferences (2000 and 2001).

Applications/Benefits

The central focus of this study is to track the source of *E. coli* contamination in water. Water contamination is a major environmental problem. In Lake Michigan, the safety of water is important to both tourism and regional residents who depend on the lake as the source of drinking water. *E. coli* counts are routinely used by environmental regulatory agencies to monitor the water quality. During the summer, high levels of *E. coli* are the main cause of area beach closures. To control the water contamination problem and to analyze the risk of transmission of infectious diseases, it is necessary to trace the bacterial source of fecal pollution. Our long-term goal is to establish comprehensive *E. coli* DNA databases (RAPD and ribotyping). The technology for the tracking the source of *E. coli* contamination with the established databases will be available for environmental regulatory agencies (including EPA) through technology transfer as well as through service contract. The potential application is not just limited to Illinois and Indiana but can be broadened nationwide.

Narrative Report

Since March 1, 2000, the funding support of the current Sea Grant has enabled us to carry out intensive research on *E. coli* RAPD analysis. To-date, over 600 *E. coli* samples from human and non-human sources have been isolated and identified by the BBL CrystalTM Rapid Stool Enteric ID Kit (Becton Dickinson Cockeysville, MD). Three primers, (primer 2: 5'GTTTCGCTCC3'; primer 1247: 5'AAGAGCCCGT3'; and primer 1283: 5'GCGATCCCCA3') were selected for RAPD reaction. DNA of over 400 *E. coli* isolates from five host species (human, cow, horse, goose, and seagull) and environmental samples were isolated using GenomicPrepâ DNA Isolation Kits (Amersham-Pharmacia Biotech, Piscataway, NJ) followed by RAPD reaction with RAPD Analysis Beads (Ready-To-Goâ from Amersham-Pharmacia Biotech). *E. coli* isolates were characterized by the combined DNA patterns based on all three primers.

The UPGMA (unweighted pair group method using arithmetic averages) method was used for cluster analysis and generating dendrograms. A RAPD library was built for discriminant analysis using the BioNumericsâ software. Figure 1 shows a two-dimensional plot of the results of Manova discriminant analysis for 415 *E. coli* isolates from human (yellow, 160 from feces, 30 from urine, and 30 from blood) cow (red, 57), horse (green, 53), goose (light blue, 55) and seagull (lavender, 30). Each dot represents the RAPD fingerprint of a single *E. coli* isolate. The results show that the RAPD fingerprints of human *E. coli* are clustered as a group separating well from those of cow and overlapping only slightly with those of the other three host species. The horse *E. coli* group is generally distinct from others. The fingerprints of goose *E. coli* are more heterogeneous, overlapping somewhat with those of seagull and cow.

Based on the maximum similarity method of Pearson discriminant analysis, the rate of correct classification (CRC) is 85% for human *E. coli* and 79% for nonhuman *E. coli*.

In a paralleled study, the RiboPrinterâ Microbial Characterization System was used for automated ribotyping. Various restriction enzymes (*Cla* I, *Eco*R I, *Hin*d III, *Mlu* I, and *Pvu* II) were evaluated for generating useful ribotypes for bacterial source tracking. *Hin*d III was determined as the most appropriate enzyme for automated ribotyping. To-date, 194 isolates from five host species (human: 40; cow: 39, horse: 41, goose: 40; and seagull: 33) have been ribotyped. The results were promising.

In a separate study, the reproducibility and the consistency of both methods were evaluated. Multiple *E. coli* isolates from five family members were used for both RAPD and ribotyping analyses. The five members are father, mother, grandmother, and two sons. The results show that 1) multiple isolates of the same sample has identical bacterial DNA patterns, 2) the *E. coli* DNA fingerprints of the father and mother are identical, 3) the bacterial DNA fingerprints of grandmother and younger son are very close, and 4) the older son, who is rarely home, has a distinct *E. coli* DNA fingerprint.

Several points could be made from these results. First, the comparable resulting DNA fingerprints from both RAPD and ribotyping indicate that both are reliable techniques for such a purpose. The data generated by two techniques can be used for mutual validation, and the combined database should form a powerful library for bacterial source tracking. Second, identical and similar bacterial DNA fingerprints of four members and the distinct pattern from a son who is rarely home suggested that, within the same host species, diet might play a role in determining the strain(s) of *E. coli* in a particular individual. Third, we have always emphasized the use of a single *E. coli* isolate per sample per individual to avoid having duplicate patterns from the same sample. This study confirms our hypothesis that multiple isolates from the same fecal sample may show an identical *E. coli* DNA pattern. Therefore, for discriminant analysis using the maximum similarity method, multiple isolates from the same sample should not be used.

Brief Summary

The main goal of this project is to use the RAPD fingerprinting method for tracking the source of *E. coli* contamination in water. *E. coli* is an indicator of fecal pollution. The abundance of *E. coli* signifies the conditions that may pose a threat to human health and force beach closures. To understand and control the fecal contamination problem and to analyze the risk of transmission of bacterial diseases to humans, it is necessary to identify the sources of contaminants.

We have now a collection of over 600 *E. coli* isolates from human, cow, horse, goose, and seagull. A database library of RADP fingerprints of 415 *E. coli* isolates has been established. Discrimination analysis has shown that the RAPD fingerprints of human *E. coli* are generally distinguishable from nonhuman *E. coli*. We are now collecting and preparing DNA fingerprints of environmental *E. coli* samples to be tested against the library database. In addition to the RAPD analysis, we have initiated a companion study using an automated ribotyping system, which has shown to be useful for validating the RAPD data and for developing a consensus identification. The current study represents the first phase of a four-year project. In the second and final phase, we intent to build two comprehensive *E. coli* DNA libraries with 1200 bacterial isolates each. Our ultimate goal is to transfer this technology to environmental agencies for tracking the source of *E. coli* contamination.

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- **Initiation Date**: March 1, 2000
- **Completion Date** March 1, 2002
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