WATER QUALITY

Genomic Typing of *Escherichia coli* Isolates from Human, Animal, and Environmental Sources by Random Amplified Polymorphic DNA (RAPD) Analysis

Final Report

High *E. coli* counts is the major cause of Lake Michigan beach closures. *E. coli*, the common inhabitant of human and animal intestines, is widely used as an indicator for fecal pollution. High levels of the bacteria, therefore, indicate fecal contamination. The current method of *E. coli* enumeration, however, does not identify the source of the bacteria. The aim of this research is to develop a molecular method, DNA fingerprinting of *E. coli*, to trace the source of the bacterial contamination. The first phase of this study, the evaluation of primers for generating useful *E. coli* DNA fingerprints, has been completed. This portion of work will serve as a basis for establishing a model *E. coli* DNA fingerprint database for identifying the sources of bacterial contamination.

Objectives

The major goals are 1) to establish a DNA fingerprint database of *E. coli* from humans and several animal species, 2) to use the database for tracing the source *E. coli* from environmental samples, and 3) to transfer the technology to environmental agencies and organizations.

The immediate objectives are to standardize the technique of RAPD analysis, and 2) to evaluate and select potentially useful primers of generating informative *E. coli* DNA fingerprint database from humans and animal species.

Summary of Progress

One hundred twenty *E. coli* isolates from fecal samples of humans and animals have been obtained. The RAPD analysis technique for *E. coli* has been standardized. Over 40 primers were evaluated. Three of them have been identified to be useful in generating informative *E. coli* DNA fingerprint database, several others are also promising. Cluster analysis of *E. coli* DNA fingerprints from 120 isolates has been carried out, and potentially useful methods for the identification of *E. coli* from environmental samples have been explored.

Accomplishments

1. *Grant proposal submitted*--The preliminary work has led to a formal grant proposal entitled, “DNA fingerprinting as a means for determining the source of *E. coli* contamination”. The proposal was submitted and approved by the Illinois-Indiana Sea Grant College Program for funding beginning March 2000.
2. *Conference paper published*--A paper entitled, “A comparative study of RAPD fingerprints of *Escherichia coli* was presented at the 99th American Society for Microbiology (ASM) General Conference, Chicago, IL, May 1999 and the abstract was published in the Abstract of the 99th General Meeting of American Society for Microbiology, p.546, 1999
3. *Manuscript preparation*--A manuscript on the *E. coli* RAPD fingerprints of *E. coli* is being prepared for publication.

Potential Applications/Benefits

The technology will be used to identify the source of *E. coli* contamination, thus supporting the goals to solve the problem of *E. coli* related closures of Lake Michigan beaches in NW Indiana.

Narrative Report

Escherichia coli is an indicator for fecal pollution. High *E. coli* counts indicate fecal contamination of water. The long-term goal of this research is to use a molecular technique, the RAPD fingerprinting analysis, to trace the source of *E. coli* contamination.

The current research is supported by an initial research grant from the Illinois-Indiana Sea Grant College Program. The initial funding is mainly used to conduct the first portion (evaluation primers) of a comprehensive project entitled, "Genomic typing of *Escherichia coli* isolates from human, animal, and environmental sources by random amplified polymorphic DNA (RAPD) analysis". The following is a brief account of this preliminary work.

To generate the DNA fingerprints for tracing the source of *E. coli*, the procedure involves 1) sample collection, 2) *E. coli* isolation and identification, 3) DNA isolation and purification, 3) evaluation of primers, 4) RAPD reaction (PCR), 5) gel electrophoresis, 6) gel analysis and development of database, and 7) application of the database.

RAPD analysis uses a short (10-mer) primers to amplify the genomic DNA. Each primer can generative a specific DNA profile per sample. Since virtually unlimited number of primers can be synthesized, a large number of DNA fingerprints can be originated from just one *E. coli* isolate. However, not all primers are useful for generating the DNA fingerprints. Screening of primers and optimization of PCR conditions are needed prior to the comprehensive genomic typing work. The most useful primers so far identified are primers 2 (5'GTTTCGCTCC3'), 1247 (5'AAGAGCCCCGT3'), and 1283 (5'GCGATCCCCA3').

Thirty *E. coli* isolates from each of the four host species (humans, cows, horses, and geese) were analyzed using these three primers. Cluster analysis using the UPGMA method was carried out to generate a dendrogram, which was based on the combined DNA fingerprints using all three primers.

In the dendrogram, *E. coli* isolates from humans are distributed in three groups. The largest group consists of 24 (out of 30) isolates. *E. coli* isolates from cows are arranged in two groups. *E. coli* isolates from horses also forms two groups.> *E. coli* isolates from geese are more heterogeneous, some are in groups and others are scattered throughout the dendrogram.

For identification purpose, a library of each host species is established using a Molecular AnalystTM softerware (BioRed, Hercules, CA). Each library is subdivided into units. A mean pattern of all members of the unit is generated. The unit is constructed in such a way that each unit consists of the isolates having at least 50% correlation with the mean pattern of the unit.

To evaluate the potential applicability of the library and its units for tracing the sources of samples, RAPD fingerprints of 65 human *E. coli* (3 from feces, 30 from urine, and 32 from blood) and 3 cow (fecal) *E. coli* outside the database were analyzed. All three human fecal isolates were correctly identified as human *E. coli* and all three cow *E. coli* samples were also correctly identify as cow *E. coli*. Among the 30 human urine and 32 human blood isolates, 28 and 29, respectively, were determined to be human *E. coli*. These results were promising. It is expected that as the database is expanded, the accuracy and applicability will be further improved.

The above preliminary work has exceeded our original plan. The next phase to expand the database by including more isolates from humans and animals. Potentially useful primers will further be evaluated, so that more informative fingerprints will be included in the database. It is hoped that a useful database can be established for tracing the source of *E. coli* contamination. The ultimate goal is to transfer this technology is environmental agencies.

Back to Research Project List (../../research_waterquality.php)

Research Information

- **Principal Investigator**: W.T. Evert Ting and Charles Tseng
- **Initiation Date**: November 9, 1998
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- **Affiliation**: Purdue University Calumet

Contacts

Tomas Höök (../../staff/hook.php)
Associate Director of Research
765-496-6799
thook@purdue.edu (mailto:thook@purdue.edu)

Carolyn Foley (../../staff/foley.php)
Assistant Research Coordinator
765-494-3601
cfoley@purdue.edu (mailto:cfoley@purdue.edu)

Leslie Dorworth (../../staff/dorworth.php)
Aquatic Ecology Specialist
219-989-2726
dorworth@calumet.purdue.edu (mailto:dorworth@calumet.purdue.edu)

Paris Collingsworth (../../staff/collingsworth.php)
Great Lakes Ecosystem Speciatlist
312-866-7449
Collingsworth.Paris@epa.gov (mailto:Collingsworth.Paris@epa.gov)

Topics

- ☐ AQUACULTURE (/topic_aquaculture.php)
- ☐ AQUATIC INVASIVE SPECIES (/topic_ais.php)
- ☐ CLIMATE CHANGE (/topic_climate.php)
- ☐ COASTAL RESTORATION (/topic_coastal.php)
- ☐ GREAT LAKES ECOSYSTEMS (/topic_glecosystems.php)
- ☐ GREAT LAKES LITERACY (/education.php)
- ☐ MEDICINE DISPOSAL (http://web.extension.illinois.edu/unusedmeds/)
- ☐ NATURAL LAWN CARE (/l2l.php)
- ☐ NUTRIENTS (/topic_nutrients.php)
- ☐ RECREATION AND FISHERIES (/topic_recreation.php)
- ☐ RESILIENT COMMUNITIES (/topic_resilient.php)

- WATER RESOURCES (/topic_water.php)

Products

- AQUACULTURE (/products_aquaculture.php)
- AQUATIC INVASIVE SPECIES (/products_ais.php)
- CLIMATE CHANGE (/products_climate.php)
- COASTAL RESTORATION (/products_coastal.php)
- EDUCATION (/products_education.php)
- FISH CONSUMPTION (/products_fishcon.php)
- GREAT LAKES HEALTH (/products_glhealth.php)
- LAND USE PLANNING (/products_landuse.php)
- MEDICINE DISPOSAL (/products_gros.php)
- PROGRAM (/products_program.php)
- NATURAL LAWN CARE (/products_lawncare.php)
- WATER RESOURCES (/products_water.php)

Resources

- ABOUT US (/about.php)
- CHICAGO WATER WALK APP (http://www.chicagowaterwalk.org/)
- FUNDING (/funding.php)
- NEWSROOM (/newsroom)
- OTHER WEBSITES (/other_sites.php)
- PEOPLE (/staff.php)
- PHOTOS (http://iisg.photoshelter.com/)
- SOCIAL SCIENCE (/glssn.php)
- TEACHERS (/education.php)

Careers

- TEACHER TRAINING (/education.php)
- FELLOWSHIPS (/fellowships.php)
- INTERNSHIPS (/internship.php)
- SOCIAL SCIENCE (/glssn.php)

Illinois-Indiana Sea Grant
Purdue University
195 Marsteller Street
West Lafayette, IN 47907-2033

765-496-6009
iisg@purdue.edu (mailto:iisg@purdue.edu?subject=IISG Inquiry)

(http://web.extension.uiuc.edu/state/)

(http://www.seagrant.noaa.gov/)

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