# Report as of FY2006 for 2006MN161B: "Development of a DNA Marker Gene System to Determine Sources of Fecal E. coli in Watersheds"

## Publications

- Articles in Refereed Scientific Journals:
  - Yan, T., M. Hamilton, and M. J. Sadowsky. 2007. High throughput and quantitative procedure for determining sources of Escherichia coli in waterways using host-specific DNA marker genes. Appl. Environ. Microbiol. 73:890-896

## Report Follows

**Development of a DNA Marker Gene System to Determine Sources of Human and Cow Fecal E. coli in Watersheds**

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**Progress Report**

Many of Minnesota's rivers, lakes, and streams do not meet the Clean Water Acts "swimmable" goal due to elevated numbers of fecal coliform bacteria. Sources of fecal coliform bacteria include runoff from feedlots and manure-amended agricultural land, wildlife, inadequate septic systems, urban runoff, and discharge from sewage systems. Moreover, high levels of fecal bacteria in Minnesota's watersheds threaten the use of these water resources for recreational use and drinking. In this study, we used pooled genomic tester and driver DNAs in suppression subtractive hybridizations to enrich for host source-specific DNA markers for *Escherichia coli* originating from cows and humans. Thus far in the project, we have concentrated our efforts on isolating human specific marker DNAs. Three separate subtractive hybridizations were done using 5-60 human *E. coli* strains as tester DNAs and 20-50 *E. coli* from other animals as driver DNAs. This generated 576 potential marker genes specific for human *E. coli.* Of these, 160 were screened by dot-blot Southern hybridization for reactivity to *E. coli* from humans, and 146 reacted with human *E. coli* control DNA. All the probes were tested for specificity in hybridization reactions with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Results of these analyses indicated that 21 cloned DNA fragments showed some hybridization specificity to DNA from *E. coli* isolated from humans. However, while our best probes identified greater than 50% of the 210 human *E. coli* strains tested, they also cross hybridized to a significant numbers of

non-human strains. Current studies are being done to increase the number of human *E. coli* strains identified with these probes and to increase hybridization specificity.

Over the past decade, several microbial source tracking (MST) methods have been intensively investigated, leading to the development of a wide variety of potential methods. Most methods to date, however, have suffered from low discriminatory power. In contrast, several genotypic-based methods have been found to be highly efficient in discriminating amongst bacteria originating from different animal hosts. We have developed a genetic marker based detection system (using DNA probes) for host-specific traits that are ecologically meaningful with respect to the microorganism studied. We have been using a multi-strain, genomic comparison approach to identify DNA fragments unique to *E. coli* strains isolated from a particular type of host source. Using this approach we have successfully developed DNA probes specific for *E. coli* strains originating from Canadian geese and ducks.

In our current studies we focused our efforts on the development of marker probes for *E. coli* strains originating from cows and humans. The prioritization of these two types of host sources was mainly due to their predominance as contributors to agricultural- and urban-derived fecal contamination in watersheds. To achieve our goals, we used the technique of subtraction suppressive hybridization (SSH) to identify DNAs that are specific for *E. coli* originating from humans and cows.

We used a multi-strain genomic comparison approach for the identification of host-specific DNA markers. The suppression subtractive hybridization (SSH) technique was used to enrich for DNA fragments unique to *E. coli* from each type of host sources. The *E. coli* strains used in SSH and subsequent specificity analyses were obtained from a library of unique isolates previously isolated from the feces of 12 known animal host sources (cats, chickens, cows, deer,

dogs, ducks, geese, goats, horses, pigs, sheep, and turkeys), and humans. Suppressive subtractive hybridizations were done using the CLONTECH PCR-Select™ Bacterial Genome Subtraction kit (BD Biosciences CLONTECH, Mountain View, CA). In initially, three different subtraction hybridizations were done; Human subtraction 1 used 5 human *E. coli* strains as tester DNA , and 5 goose E. coli strains as driver DNAs. Following transformed of subtraction products, 192 clones were picked. These were screened by dot-blot hybridization and 11 probes were found to be tester specific (all 11 were confirmed as specific by Southern Hybridization). All 11 probes were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. However, none of the probes reacted with a large number of human strains and cross hybridization with strains from other hosts was pronounced. This suggested that a larger number of tester and driver DNAs were needed.

In subsequent analyses, 20 human *E. coli* strains were used as tester DNAs and 20 *E. coli* from animals (5 cows, 5 geese, 5 pigs, 1 chicken, 1 dog, 1 cat, 1 horse, 1 sheep) were used as driver DNAs. Following transformed with the subtraction mixture, we picked 480 clones and screened 75 of these by dot blot Southern hybridization. All 75 clones had strong hybridization signal when probed with pooled DNAs from the tester strains (Figure 1) and only weakly hybridized when probed with driver strains. Sixty-four of these clones were confirmed as tester specific by Southern hybridization and restriction enzyme digestion analysis showed there were 41 unique probes. Of these 15 were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Nine probes were shown to react predominately with human strains, but only about 10% of the human strains reacted and the same

human strains reacted with probes (Figure 2). One probe reacted with 26 of 210 human strains and only 2 chickens, 2 horse, and 1 sheep strain. No further colony hybridizations were attempted because the same human strains were identified with almost all 15 probes.

Consequently, we tried an additional subtraction using 60 human strains as tester DNAs and same 20 animal strains as driver DNAs as discussed above. Following transformation of the subtraction products, we picked 576 clones and screened these by dot blot Southern hybridization. Of these clones, 74 were selected as being tester specific and 71 of the 74 were confirmed as being tester DNA specific by Southern hybridization. Twelve of these were tested by colony hybridization with same strains as discussed above, and all 12 tested hybridized with greater numbers of human strains than probes from the first or second human subtractions. Some of these 12 clones identified greater than 50% of the 210 human strains. Unfortunately, they also cross hybridized with significantly greater numbers of non-human strains, compared to probes from the first human subtraction (10-30% for several hosts). In one case, nearly 60% of cat strains cross reacted with the tested probes.

We are currently doing additional subtraction hybridizations to increase the number of human *E. coli* strains identified with these probes and to increase hybridization specificity. To achieve this goal, we will use 60 human *E. coli* strains as tester DNAs and approximately 50 animal strains as driver. The driver strains will be picked according to the results obtained from colony hybridizations done in the third subtraction above. We will also use more cat *E. coli* strains in the driver mix to reduce cross hybridizations. We are also in the process of determining the hybridization specificity of probes directed against *E. coli* from cows.

**Publications, Presentations, or Published Abstracts:**

*Publication*

Yan, T., M. Hamilton, and M. J. Sadowsky. 2007. High throughput and quantitative procedure for determining sources of Escherichia coli in waterways using host-specific DNA marker genes. *Appl. Environ. Microbiol.* 73:890–896.

*Presentations*

Sadowsky, M. J. 2006. Alternate source and sinks of Pathogens in the Environment. Annual Meetings of the American Society of Agronomy (ASA), Crop Science Society of America (CSSA), and Soil Science Society of America (SSSA), Indianapolis, IN.

Sadowsky, M. J. 2006. Development and Use of a High-Throughput Robotic Method to Determine Sources of *E. coli* in the Environment, University of South Florida, Tampa, FL.

Sadowsky, M. J. 2006. Has Human Activity Outstripped the Environments Ability to Rid Itself of Fecal Bacteria? Albrecht Lecture, Earth Day, University of Missouri, Columbia, MO.

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