Report as of FY2006 for 2006CA173B: "Quantitative PCR Assays for Specific Host Sources of Fecal Pollution for Test Watersheds"

Publications

- Articles in Refereed Scientific Journals:
  - Ivanetich, Kathryn, Pei-Hsin Hsu, Kathleen M. Wunderlich, Evan V. Messenger, Ward G. Walkup IV, Troy M. Scott, Jerzy Lukasik, Jerry Davis, Microbial Source Tracking by DNA Sequence Analysis of the Escherichia coli malate dehydrogenase gene. Journal of Microbiological Methods 67, 507-526 (2006).

Report Follows

RESEARCH PROGRAM:

Project Summary: The purpose of this study is to develop and validate Quantitative PCR assays for host specific and reference targets and apply the assays to the San Pedro Creek Watershed in Pacifica, CA in order to identify sources of fecal pollution. Year 1 of the project has demonstrated significant methodology development and validation. In addition, sites in the San Pedro Creek Watershed with anticipated fecal pollution have been sampled, and over 96% contained elevated levels of fecal indicators, suggesting these sites significantly contribute toward watershed pollution. These samples are being processed for future analysis by QPCR.

Project Overview:

The purpose of this study is to develop and validate Quantitative PCR assays for host specific and reference targets and apply the assays to the San Pedro Creek Watershed in Pacifica, CA in order to identify sources of fecal pollution and focus remediation efforts.

Identifying the host sources of fecal pollution in watersheds is typically accomplished via microbial source tracking (MST), and is essential for effective remediation of watershed pollution. Host-specific microorganisms, which are found only or primarily in a single host species such as humans, are highly advantageous for MST. Since assays for host-specific microorganisms circumvent the need for the large host origin database that is required for most MST assays, they provide more specific, faster, and less expensive MST assays.

The San Pedro Creek Watershed in Pacifica, CA, is an important local recreational resource and provides steelhead rookery, has both rural and urban aspects, but has elevated levels of fecal pollution that contribute to coastal pollution and beach closures.

San Pedro Creek Watershed sampling focused on areas suspected of contributing fecal pollution to the main stem of the creek, namely sites on the North Fork, above the Park Mall Shopping Center culvert (Figure 1, upper right corner - Big Bend area), and sites associated with tributaries that feed into the main stem from the South, primarily between Adobe and Highway 1 (Figure 1, lower left area). Eight to 11 sites were sampled on each of six wet/rainy events, and E. coli, total coliform and Enterococcus levels were enumerated. Fifty (96.2%) of the watershed samples had elevated levels of Enterococcus (> 35 CFU/100 ml geomean), and were utilized for isolation of mixed Enterococcus cultures.

Four methods for isolation of genomic DNA were applied to a representative sampling of Enterococcus and E. coli cultures (Epicentre Masterpure, Qiagen Genomic Tip 100/G, Sigma GenElute 5 Minute Plasmid and Sigma Genomic DNA kit) and evaluated by DNA yield from bacterial cultures, and by assay cost, overall time and technical time. Because of its speed, the Sigma GenElute 5 Minute Plasmid kit method was modified with the inclusion of lysozyme or incubation at elevated temperatures, but although this method gave yields of 60-200 ng DNA from E. coli cultures (used as a positive control), yields from Enterococcus cultures were unacceptably low (0-16 ng DNA). The Epicentre Masterpure and Sigma genomic DNA kits provided the highest yields of genomic DNA and were of comparable low cost (ca. $1 per isolation). The Sigma method was preferred since it required only 3 hours, i.e. half as much time as the Epicentre method.

Enterococcus cultures were prepared and genomic DNA isolated from 12 fecal samples, 10 sewage samples, and 80 watershed samples. In addition, BCS on North Florida provided

isolated genomic DNA from 10 known bacterial species and strains and from Enterococcus cultures from 30 fecal, sewage and watershed samples.

An automated protocol for the quantification of Enterococcus genomic DNA with PicoGreen dye, for the Beckman Biomek 2000 was developed and optimized. The protocol performed all pipetting steps, except for transfer of 1 ul of DNA unknowns into the read plate, all mixing, and transfers, and calculations, including generation of the standard curve and calculation of the DNA concentration of unknown samples.

Significant progress has been made in the development and validation of Quantitative PCR (QPCR) assays for host-specific microorganism targets: (1) the esp gene of Enterococcus faecium, as a specific marker for human fecal pollution, and (2) two gene targets in Enterococcus faecalis as indicators for avian and human fecal pollution, plus two reference gene targets diagnostic for total Enterococcus, as indicators of total fecal pollution.

All QPCR assays were applied to fecal, sewage and environmental samples (Table 1). The human specific E. faecium target (Esp2b2) was positive for sewage and environmental samples with suspected human contamination, and did not react with avian, cow, dog, human or rabbit fecal samples. Furthermore, the human specific gene target identified 5 of 7 environmental samples with human fecal contamination (Table 2). All of the above data are fully consistent with the putative assay specificity, as the gene target is not found in all human individuals. In contrast, the Ace1a and Esp1a gene targets in E. faecalis, with putative specificity for human and/or bird hosts, reacted across all samples, and were non-specific with regard to host. These gene targets were therefore not useful for MST and were not studied further. The reference target (16Sb) reacted with all fecal, sewage and environmental samples, and exhibited similar levels of gene expression across all samples, confirming its efficacy as a reference marker for total Enterococci (Table 1).

In summary, in the period under review the project has demonstrated significant progress in assay development and validation, and methodology development. In addition, sites in the San Pedro Creek Watershed with anticipated fecal pollution have been sampled, and a high percentage (96.2%) of these watershed samples were found to contain elevated levels of fecal indicators, i.e. Enterococcus. These samples are being processed for future analysis by QPCR.

Final results of this study (to be completed with funding from the University of California Center for Water Resources) are anticipated to facilitate the identification of the sources of fecal pollution in watersheds, and thus enable more efficient watershed management and provide specific data on sources of fecal pollution in the San Pedro Creek. In particular, the focus on development of assays for human fecal pollution will impact the sources of pollution from which humans experience the highest health risk.

Professional Presentations:

Ivanetich, Kathryn, Status of California Watersheds and Novel Methods to Identify Host Sources of Fecal Pollution, California State University at Chico, Department of Chemistry and Biochemistry, April 2006.