A Quantitative Detection Method for Host-Specific Fecal Bacteria Based on Real-Time, qPCR

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A major challenge to quantifying E. coli using quantitative polymerase chain reaction (qPCR) is that the PCR reagents themselves are contaminated with E. coli DNA. In this project we developed a pretreatment step to eliminate the contaminant DNA, which allowed us to detect a single E. coli cell using qPCR.

Current detection methods and monitoring approaches are inadequate for developing successful and cost-effective strategies for reducing fecal pollution. One of the main limitations of the current culture-based detection methods is that they do not distinguish between bacteria that originate from different hosts, such as a human, cow or bird. Because any particular water (e.g., lake, river, estuary, or beach) is likely to receive pollution from multiple point and non-point sources, the inability to distinguish host-specific indicators significantly limits the ability to identify and target the main sources, placing a major constraint on the management of fecal pollution. A variety of new approaches that exploit host-specific fecal bacteria to conduct microbial source tracking (MST) studies are being explored. While many of the methods may eventually provide powerful tools for identifying the wide range of human and animal point and non-point sources that contribute fecal pollution to a water body, none of the molecular methods currently has the potential to provide a direct quantitative measure of the fractional contribution from each of the sources to the total concentration of fecal pollution.

Quantitative methods are needed to identify and target the dominant sources of pollution, to monitor changes in the concentration of fecal pollution and its sources over time, to assess the effectiveness of specific mitigation strategies, and to provide more information for evaluating the true public health risks. Therefore, the goal of our research is to develop a quantitative method for calculating the fractional contribution of fecal pollution from human and animal sources by measuring host-specific fecal indicator bacteria using real-time, quantitative polymerase chain reaction (qPCR).

E. coli is the most common fecal indicator organism for water quality assessment, yet a major impediment to the use of qPCR for detection of E. coli is that the PCR reagents themselves are contaminated with E. coli DNA. This contamination makes it impossible to measure low concentrations of E. coli in a water sample, which severely limits the utility of E. coli qPCR for environmental applications. Therefore, the major thrust of our research has been developing and validating a method to eliminate the contaminant DNA prior to qPCR. We successfully developed and compared two decontamination methods, one using an enzyme to degrade the DNA (DNase), and the other using ultrafiltration to physically remove the contaminant DNA. These decontamination methods will be useful beyond our specific application, as many researchers and microbiologists work on qPCR applications that suffer from DNA contamination.
reagent contamination. We then combined the decontamination methods with qPCR to develop a robust enumeration method for *E.coli*.

The next step in our project is to use our new qPCR method for *E.coli* along with similar methods that have been developed for other fecal indicator organisms as well as host-specific fecal bacteria. We will use these methods alongside traditional culture-based methods to understand what fraction of total fecal bacteria is contributed by each sub-group of bacteria in both human and animal fecal sources. This information is essential before the fractional contribution of different fecal sources can be determined. In addition, we are using these new qPCR methods to help identify the key pollution sources in Rodeo Lagoon, as described in the section below.

**Professional Presentations**


**Collaborative Efforts**

The support of UCCWR has resulted in several new collaborations. This fall we will apply the methods developed in our project to a new field site in the Golden Gate National Recreation Area, Marin County, CA. In collaboration with Prof. Mark Stacey (CEE, UC Berkeley), the project aims to identify sources of nutrient pollution in Rodeo Lagoon, which suffers from annual toxic blue-green algae blooms and fish kills, including the endangered tidewater goby. A grant from the National Park Service is supporting this research. We have also initiated a second collaboration with Prof. Kate Field at Oregon State University and Prof. Stefan Wuertz at UC Davis, who are both leaders in the development of quantitative, molecular methods for fecal source tracking. This research will help us understand how new molecular methods compare with traditional methods for measuring fecal bacterial contamination from human and animal fecal sources.

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