Establishing whodunit: Application of molecular markers for fecal source tracking

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Introduction
Natural waterways are vulnerable to fecal contamination originating from many possible sources, including wildlife, livestock, and septic systems. For years the microbiological quality of water has been tested by enumerating levels of the culturable fecal indicator bacterium Escherichia coli but this does not reveal the animal source of the feces. Therefore, host-specific gut bacteria would be better utilized as fecal markers to identify source of fecal contamination. However, it is not possible to differentiate among such bacteria using culture-based techniques.

Amplification of molecular markers allows for the detection of species-specific bacteria without the need for culturing. The goal of this study was to determine our ability to detect the presence of human- and ruminant-specific bacteria in the order Bacteroidales in water sampled from Copeland Creek. Water from three locations along Copeland Creek (West Bridge, East Bridge & Upstream, Fig. 1) was collected, DNA extracted and PCR performed to verify the presence of feces-derived Bacteroidales that are associated with specific hosts.

Materials and Methods

Sample collection
250 ml water samples were collected from Copeland Creek on March 15, 2014 after heavy rain and filtered through a 0.45 µm filter (Fig. 2) following the removal of 50 ml for determination of E. coli density (IDEXX Quanti-Tray/2000). Fresh fecal sources were collected using cotton swabs. Samples on filters and swabs were stored at -20ºC until DNA extraction.

DNA extraction
Filter retantate was resuspended in water and DNA extracted by one of the following methods:
- Inexpensive method: TENS buffer (Tris HCl, pH8, 1 mM EDTA, 0.1 N NaOH, 0.5% SDS) and 3M NaOAc, followed by phenol-chloroform extraction.
- Powersoil DNA extraction kit (MoBio): Manufacturer’s protocol followed.

PCR reaction
Bacterial 16S ribosomal RNA (rRNA) genes were amplified using universal primers (27F/1492r) as a positive control for DNA extraction. Total bacteroidales 16S rRNA gene was amplified using universal primers (32F-708R).

Nested PCR was performed using universal bacteroidales 16S rRNA gene as a template and CF128-708R primers for ruminant-specific and HF183-708R primers for human-specific fecal detection.

Results
- DNA extraction using the inexpensive method yielded good quality DNA from human feces and water samples but not from cow feces. Powersoil DNA isolation kit (MoBio) worked well with all fecal samples and water samples with various levels of turbidity by removing PCR inhibitors that can yield a false negative result. Additionally, primer specificities were confirmed by product size (Fig. 3) and DNA sequencing (not shown).
- Fecal contamination of creek water was apparent from the finding of fecal source associated Bacteroidales markers in all creek samples (Fig. 4, data not shown) but, based on the geometric mean E. coli density of 62 mpn/100 ml, the contamination was at levels well below the US EPA regulatory limit for recreational waters (126 mpn/100 ml).
- Ruminant-specific Bacteroidales were detected in all three creek samples (Fig. 5).
- Human fecal contamination was found in the Upstream and West Bridge samples but not in the East Bridge sample (Fig. 5).

Conclusions and future prospects
- Ruminant fecal markers were found in all three creek water samples. This is not surprising since Copeland creek runs through lands grazed by cows, goats, sheep, and deer.
- Human fecal markers were confirmed in the Upstream and West bridge creek water samples.
- Fecal contamination of the creek water was found to be below the US EPA regulatory limit for recreational waters.
- The method could be applied to other Sonoma County waterways. Use of quantitative PCR-based techniques would allow for determination of fecal marker densities.

References

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