Transport of human and animal wastes into natural waters can result in contamination with fecal pathogens that are increasingly becoming a serious health risk. However, difficulties of differentiating sources of microbial pollutants limit the options to control the pollution. The goal of Microbial Source Tracking (MST) is to identify the source of microbial contamination in natural waters. My MST study was conducted using samples collected from The Upper Sugar Creek Watershed in Ohio. Samples were assayed using a PCR-based molecular method to detect and quantify the Bacteroides 16S rRNA gene. The Sugar Creek Watershed is a mixed-use watershed suitable to examine the source of microbial contamination from human and agricultural activity and/or wildlife. Host-specific Bacteroides assays (human, ruminant, horse, pig, and dog) were used to determine potential host sources. A quantitative PCR (qPCR) assay for general Bacteroidales was used to investigate the magnitude of fecal contamination. Viable counts of E. coli were determined for statistical comparison with the BacteroidesqPCR assay. These data were analyzed along with land management data. We found frequent human specific signals at residential land use areas and also observed high magnitude of general BacteroidalesqPCR signals at concentrated livestock operations, assayed in residential areas. The results indicate the potential application of the MST method to aid in making land management decisions to control microbial contamination at the watershed scale.

Materials and Methods

Study Site

In 2000 the Ohio Environmental Protection Agency (EPA) labeled the Sugar Creek Watershed as the second most impaired in Ohio. The upper Sugar Creek watershed contributes to the hypoxia in the Gulf of Mexico because it is located in the headwaters of the Muskingum Watershed, Ohio’s largest watershed flowing to the Ohio River. The Upper Sugar Creek Watershed (Fig. 1) has different land uses with potential contaminant sources such as residential areas, crop fields, livestock operations (dairy, sheep, horse, and swine), and natural forested areas. This mixed-use watershed is suitable to examine the source of microbial contamination from human and agricultural activity and/or wildlife.

Results - General Bacteroides qPCR

All samples except Sample Point 8 contained a detectable amount of Bacteroides marker (see Fig. 1 for the location of each sample site) with concentrations ranging from 10^3 to 10^9 DNA target segments per 100 ml sample. There was variation in marker concentrations among the three sampling events (Fig. 2). Recurrent high quantities were observed at Sample Points 14 and 21 (indicated with red circles in Fig. 2). This suggests a possible association between fecal contamination and land use. The sub-watershed of sampling point 14 had a residential area right above the sub-watershed. Sample Point 21 had a pasture for dairy cows and tile lines from this pasture contributed to the water at this sample point.

Sample filtration, DNA extraction and PCR amplification: Water samples (100 ml) were filtered (0.2 µm filter) to collect bacterial cells and then the DNA was extracted using the PowerSoil DNA Kit (MoBio, CA). PCR reactions were conducted using general primers to confirm the presence of Bacteroides in the samples.

Host specific PCR: Ruminant- and human-specific Bacteroides 16S rRNA gene markers were tested for host specificity and then used for host specific Bacteroides detection in water samples. All forward primers specific for each marker (CF128 and CF183f for ruminant specificity and HF134f and HF183f for human specificity) were paired with the general reverse primer, Bac708r. Ten individual cow feces, seven individual human feces, one water-treatment plant influent and one septic influent were used to test host specificity. Primer specificity was further investigated using pooled fecal DNA samples (pig, deer, sheep, dog, goose, and horse).

Quantitative PCR (qPCR): A general set of primers that targeted the 16S rRNA gene of Bacteroides markers were used to assess the magnitude of concentrations in the samples. For each qPCR run, all samples were analyzed in triplicate. PCR inhibitors in the samples were determined to be negligible based upon results obtained after 10-fold and 100-fold dilutions.

Results - General Bacteroides qPCR

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'Sample Point 8 was a community-maintained natural spring used as a drinking water supply.'
Results - Host Specificity and Sensitivity Study

Ruminant- and human-specific Bacteroidales 16S rRNA gene markers were tested for specificity and sensitivity. The two ruminant specific markers displayed 100% sensitivity for individual cow feces DNA. However, they also tested positive for other ruminant species (deer and sheep). In addition, one of the markers (CF128f) was positive for pig feces DNA (Fig. 3). The two human specific markers displayed 86% sensitivity towards individual human feces DNA and were positive for both wastewater influent DNA, and septic influent DNA, considered to be pools of human fecal DNA. No false positives were observed with other host DNA pools (Fig. 4).

Figure 3. Host specificity PCR results with two ruminant specific primers (CF128f, CF183f).

(A) ruminant specific primer distinguished cow fecal DNA from human fecal DNA, wastewater influent DNA (WWP), and septic influent DNA (SP). (B) Deer and sheep fecal DNA were also positive for both markers and pg fecal DNA was positive for CF128f.

Figure 4. Host specificity PCR results with two human specific primers (HF154f and HF183f). (A) False positive on cow fecal DNA was not observed. One out of 7 individual human fecal samples was falsely negative for both primers. Wastewater influent DNA (WWP), and septic influent DNA (SP) both tested positively. (B) No false positives were observed on other host DNA samples.

Results - Host Specific PCR

Ruminant- and host-specific PCR were conducted on all stream water samples. Two ruminant markers were strongly positive in samples from Sample Point 21 on July 21 and weakly positive in samples from Sample Points 5, 6, and 18 on August 6. Human host-specific PCR positive results were also observed in water samples from various sites including the Sample Points 14 and 21 where an intense general Bacteroidales qPCR signal was observed (Table 1).

Table 1. Host Specific PCR result for all samples. No host-specific positive found at site 4, 7, 8, 10, 13, and 16. +: Weak positive; **: Strong positive. The intensity of these positive signals were judged by comparison to reference positive controls on electrophoresis gel image.

Conclusion

E. coli is a culturable aerobic bacterium and is widely used as fecal indicator to regulate water quality. Bacteroidales, the subject of this study, is an anaerobic bacterium and is expected to have limited survival after release into the environment. We observed a positive correlation between E. coli and Bacteroidales numbers in environmental water samples. However, we believe Bacteroidales is a more sensitive and specific indicator of fecal contamination.

Host specific PCR analyses revealed the origin of Bacteroidales with the human specific marker clearly able to distinguish human fecal contamination from agricultural / wildlife fecal contamination. Ruminant specific analysis was not able to differentiate between cow and deer. However, a combination of host specific PCR analyses and land use data analyses will make the microbial source tracking method more accurate and a powerful tool in making land management decisions that affect microbial contamination at the watershed scale.

Supporting References


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