

Science Question

Recently developed microbial source tracking (MST) methods have been used to determine the sources of fecal pollution and pathogens in environmental waters. While several studies have reported the successful application of MST methods, most methods rely on the use of culture-based methods and on the use of genes not involved in host-microbial interactions.

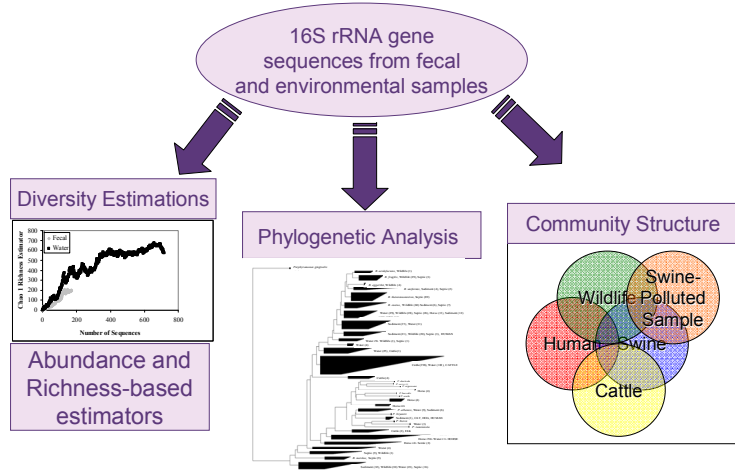
The proposed work focused in answering the following questions:

- Can metagenomic approaches be used to select for host-specific genes?
- Can phylogenetic genes be used to target avian sources of fecal pollution?
- What is the level of host specificity of novel 16S rDNA and metagenomic assays?
- What is the level of host distribution of novel assays?
- Are the genetic markers present in waters presumed to be impacted by targeted fecal sources?

Research Goals

The main goal of our research group was to develop and evaluate nonculture-based methods for environmental monitoring and risk assessment using 16S rRNA gene and fecal metagenomic sequences. Sequencing information for the molecular diversity of the 16S rRNA gene of fecal bacteria is somewhat limiting. Clone libraries were developed using universal primers and sequences were used to develop 16S rRNA gene PCR assays. In contrast, little information exists for functional genes involved in host-microbial interactions. To identify potential host-specific metagenomic markers the goal was to develop a novel competitive hybridization approach to enrich for metagenome fragments present in one fecal source and absent in other fecal sources. We called this approach genome fragment enrichment (GFE). Once genes were enriched the goal was to use bioinformatics to select for fecal bacterial genes and used them in PCR assay development. The performance of 16S rDNA- and metagenomic-based assays were tested using DNA extracts from targeted and nontargeted fecal sources and from fecally impacted waters.

Phylogenetic analysis of 16S rRNA gene sequences of fecal and environmental bacterial populations. This approach can be used using different taxonomic groups as target for assay development and for any type of fecal source.



Evaluation of 16S rRNA and mrcA gene swine-specific assays. Once a marker is identified, assays are tested for host-specificity, host-distribution, and detection limits using DNA extracted from fecal and water samples.

Fecal Type (origin) ⁰	General <i>Bacteroidales</i> (Bac32f/Bac708r)	Pig <i>Bacteroidales</i> (PF163f/Bac708r)	Pig Methanogen (P23-2f/P23-2r)	Pig <i>Prevotells</i> (Bac1f/Bac1r)
Pig Feces (DE)	1.00 (9/9) ^a	0.44 (4/9)	0.78 (7/9)	0.44 (4/9)
Pig Feces (OH)	1.00 (52/52)	0.98 (51/52)	0.42 (22/52)	0.81 (42/52)
Pig Feces (TX)	1.00 (7/7)	1.00 (7/7)	0.29 (2/7)	1.00 (7/7)
Pig Feces (TX)	1.00 (9/9)	0.89 (8/9)	0.22 (2/9)	1.00 (9/9)
Pig Feces (WV)	0.90 (18/20)	0.70 (14/20)	0.90 (18/20)	0.90 (18/20)
Pig Manure Pits (OH)	1.00 (3/3)	1.00 (3/3)	0.00 (0/3)	0.33 (1/3)
Pig Manure Pit (IL)	1.00 (1/1)	1.00 (1/1)	0.00 (0/1)	0.00 (0/1)
Pig Lagoons (IL)	1.00 (2/2)	1.00 (2/2)	0.00 (0/2)	0.00 (0/2)
Human Feces (WV)	1.00 (10/10)	0.30 (3/10)	0.30 (3/10)	0.60 (6/10)
Chicken (DE)	0.88 (7/8)	0.50 (4/8)	0.38 (3/8)	0.63 (5/8)
Raccoon (NE)	0.34 (23/68)	0.04 (3/68)	0.01 (1/68)	0.29 (20/68)
Horse (WV)	1.00 (12/12)	0.67 (8/12)	0.00 (0/12)	0.50 (6/12)
Cattle Lagoon (OH)	1.00 (1/1)	0.00 (0/1)	0.00 (0/1)	0.00 (0/1)

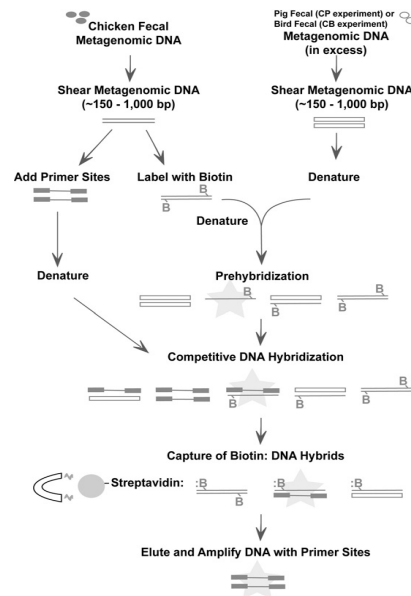
^a Percent positive PCR result using a given marker on a given source type. Numbers in parentheses indicate number positive PCR results divided by the total number of source type samples tested.

Sensitivity

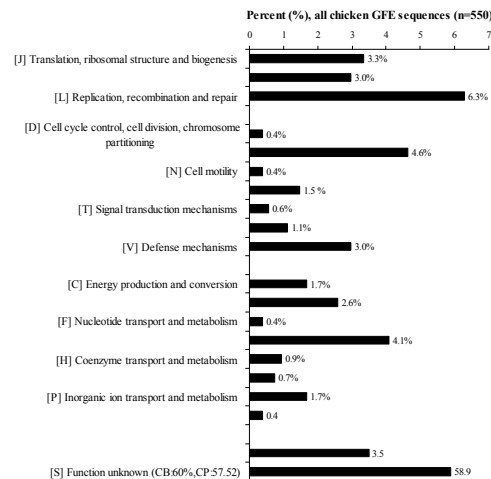
Specificity

Methods/Approach

Genome Fragment Enrichment (GFE) – Method used to enrich for host-specific metagenomic markers. Any DNA extract can be used as target and blocker



Bioinformatics are used to select for genes involved in host microbial interactions, and sequences from enriched DNA can then be used for PCR assay development

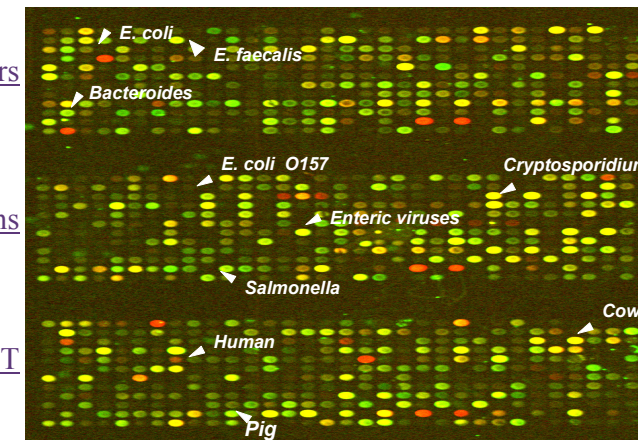


Genomic markers for microbial water quality assessment. Using a multi-tier approach it might be possible to look at indicators, pathogens, and fecal sources.

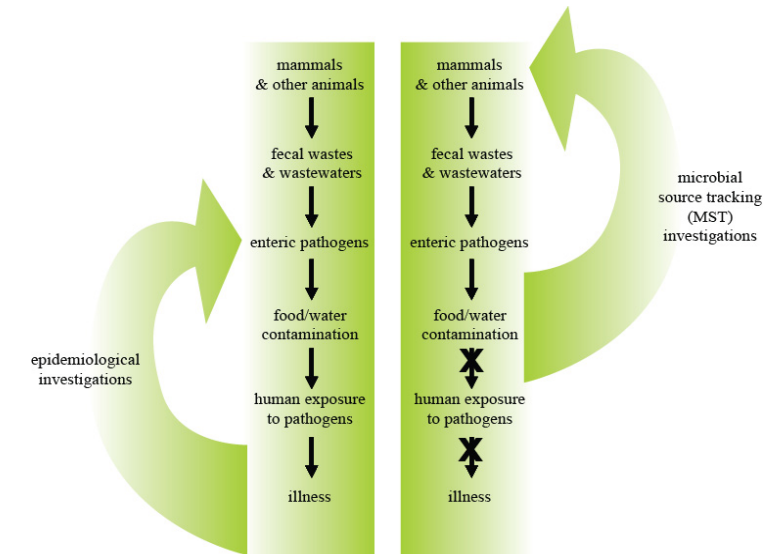
Indicators

Pathogens

MST



Multiple markers for microbial water quality assessment will allow us to monitor health and ecosystem risks at earlier stages of the fecal pollution continuum. Similar approaches can be used for many other microbial-based processes.



Results/Conclusions

Thus far, we have developed metagenomic markers to track human, cattle, and poultry sources of fecal pollution in surface waters. Some of these assays are now being evaluated in recreational water studies. In addition, using sequencing analyses of 16S rDNA clone libraries we have identified several novel sequences for waterfowl (i.e., gulls and geese). We have applied the latter assays in samples collected from multiple sites in Lake Ontario. The results from these studies have confirmed the importance of waterfowl as primary sources of fecal pollution in that region.

Impact and Outcomes

The methods developed in this study will be useful in epidemiological studies focusing in measuring risks associated with human and nonhuman sources of fecal pollution. Additionally, the assays we have developed can be used in the evaluation of risk management practices designed to prevent, reduce, and eliminate fecal pollution in recreational waters and waters used as sources of drinking water.

Future Directions

In the future, we will use metagenomic approaches to develop multi-tier assays to measure for the presence of indicators of fecal pollution and their sources as well as microbial pathogens. Additionally, we will use similar approaches to better understand the impact of fecal pollution on biogeochemical cycles and ecosystem health.

References

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