

Application of Microbial Source Tracking Methods (MST) in Tributary Pollution Assessment

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Water Quality

- **Drinking Water**
- **Recreational Water**
- **Wastewater Treatment**
- **Reclaimed Water**

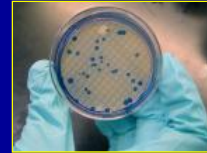
➤ **Assessed by indicator organisms (IOs)**

- Found in GI tract of humans and animals
- Should be present when pathogens are present and absent when pathogens are absent

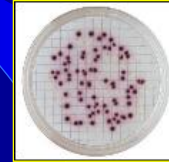


Currently Recognized IOs

- Fecal coliforms – thermolerant coliforms of fecal origin
- *Escherichia coli* – the dominant fecal coliform species
- Enterococci – also of fecal origin; much more salt-tolerant than fecal coliforms



mFC



mTEC



mEI

Possible Sources of Indicator Organisms



- Aging central sewer infrastructure
- Onsite wastewater disposal systems (septic)
 - Surface runoff
 - Agriculture
 - Wild animals
 - Soil/sediments



What Do We Gain from Knowledge of Source of IOs?

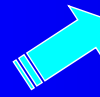
- Source matters!
- More accurate risk assessment
- Ability to remediate pollution
- Necessary for total maximum daily load calculations



The Basis of Microbial Source Tracking (MST)



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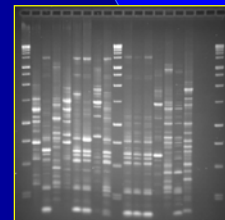
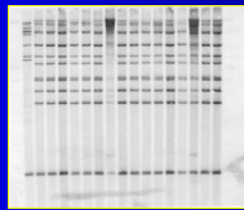
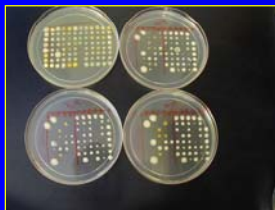
The Central Hypothesis

- Certain microbial species or types are associated with the gastrointestinal tract of specific animal hosts.
- This association can be used to “track” the fecal microorganism back to its host.



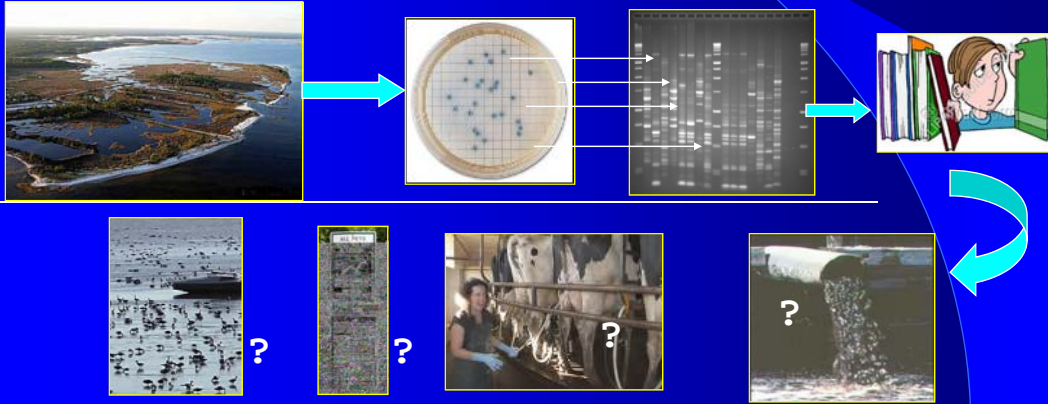
The Library Concept

- A database of patterns corresponding to IOs isolated from the feces of various host animals.
- Phenotypic or genotypic



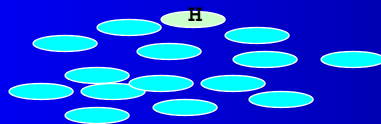
Library-dependent MST

Match patterns of isolates from water to those in library to determine sources of bacteria in water



Library-Independent Methods

- Detect one specific trait or set of traits (marker) found only in microorganisms associated with a particular host.



Concerns

Library-dependent

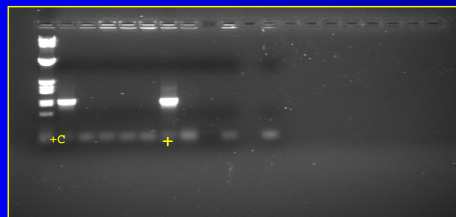
- **Applicability to large geographical area**
- **Temporal stability**
- **Size**

Library-independent

- **Methods are not available for all host species**
- **Specificity**
- **Sensitivity**

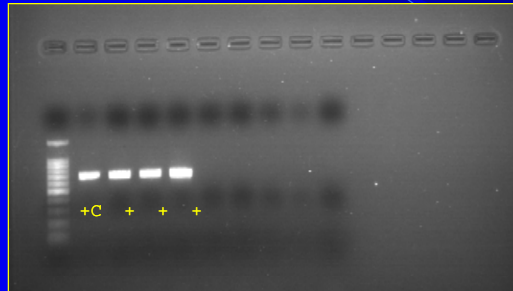
PCR-based Library-independent MST Methods Used in the Study

Ent. faecalis



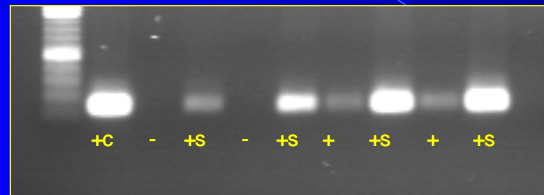
- 16 S rRNA
- ~ 50% of *Enterococcus* spp. isolated from sewage are *Ent. faecalis*

esp



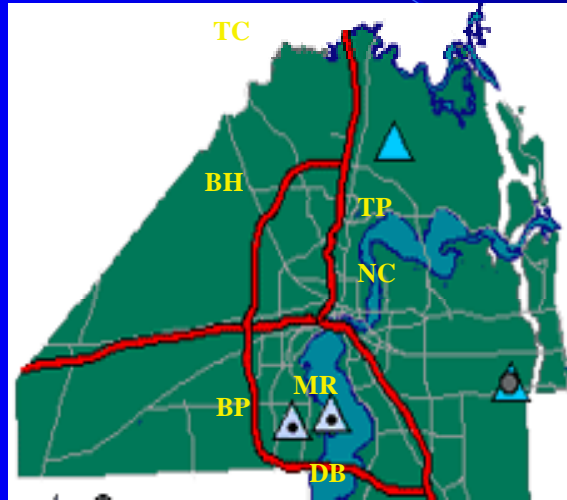
- *Ent. faecium* surface protein
- Virulence factor of bacterium associated with humans

Bacteroides

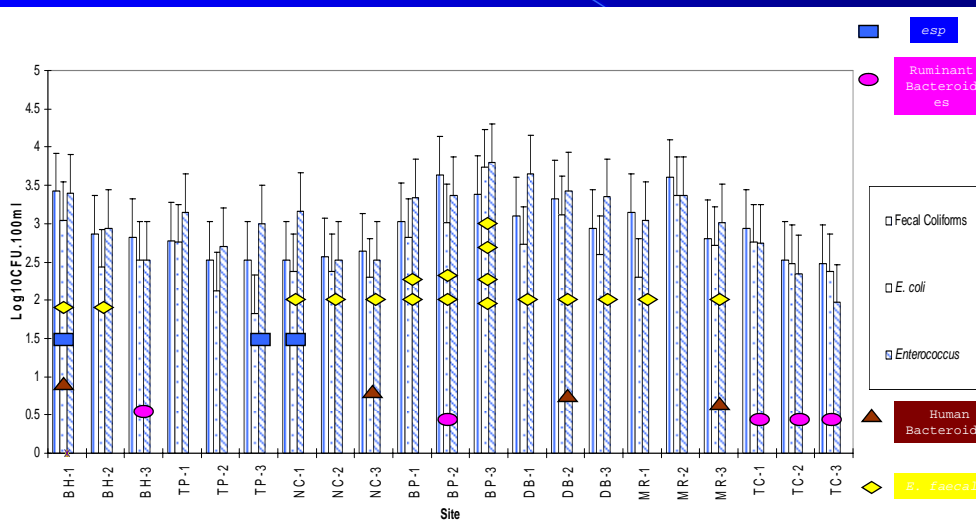


- 16S rRNA
- Human and ruminant
- Fecal anaerobe that tends to co-evolve with the host

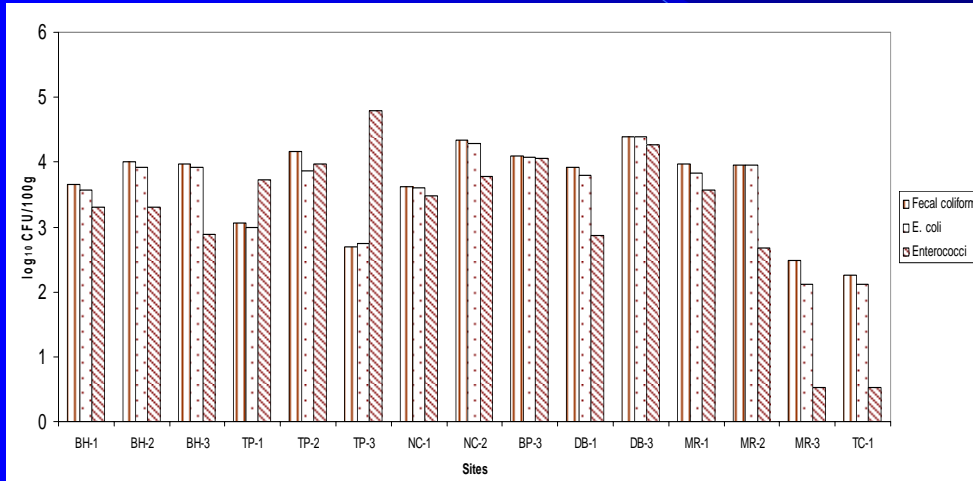
Map of sampling sites



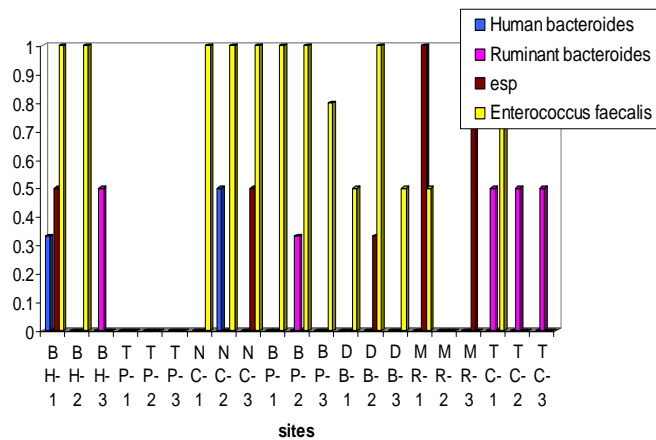
Mean IO concentration in the Water Column (07/2005-02/2006)



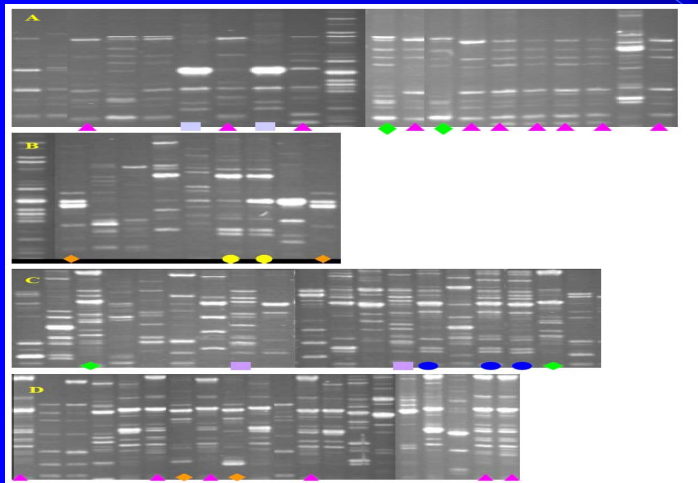
Mean IO Concentration the Sediment (07/2005-02 /2006)



Frequency Distribution of Human-associated Markers



Library-dependent MST

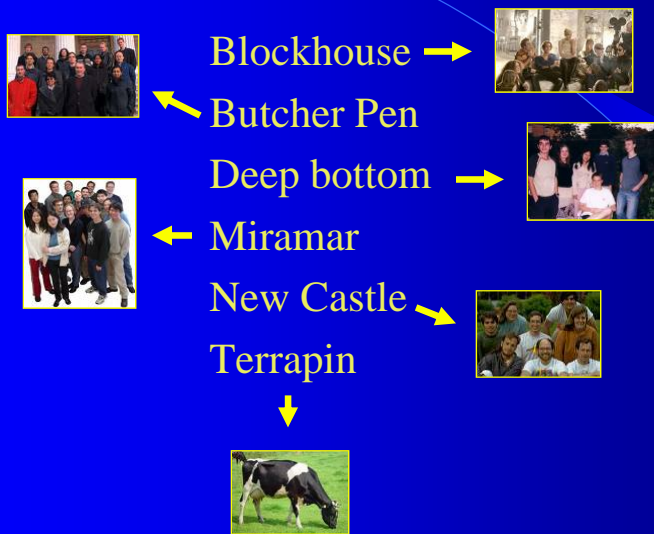


- (A) Terrapin Creek sediments *
- (B) Terrapin Creek water column*
- (C) Butcher Pen sediments**
- (D) Butcher Pen water column **

• *Enterococci patterns from sediments more clonal compared to the water column, suggesting that original source of contamination (dairy farm) was severed

• **Enterococci patterns from the water column and sediments show high diversity suggesting ongoing contamination

Conclusions



Acknowledgments

- PBS&J
- Dr Valerie Harwood
- Harwood lab members

