Bacterial Source Tracking
Little Brazos River Tributaries
Bacteria Assessment Project

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Sources of Bacteria (*E. coli*)

- Sources of bacteria
What is BST?

Bacterial Source Tracking

• Data collection and analysis to determine the sources of fecal contamination in a waterbody

• Based on uniqueness of bacteria from individual sources – a variety of different methods are used

• Differs from modeling in that it is not a predictive tool and does not require calibration and validation of input variables
BST Methods

• A variety of different methods have been used

• Can be classified according to approach:
  • Phenotypic v. Genotypic
  • Library-dependent v. Library-independent
Phenotypic BST Methods

Methods:
• Kirby-Bauer Antibiotic resistance analysis (ARA)
• Carbon source utilization (CSU)

Advantages/Disadvantages:
• Less expensive
• Less discriminating
Genotypic BST Methods

Methods:

- DNA fingerprinting
  - Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)
- Ribotyping or RiboPrinting® (RP)
- Pulsed-field gel electrophoresis (PFGE)
Genotypic BST Methods

Methods:

Advantages/Disadvantages:
- More expensive
- More discriminating
Library-Dependent BST

Approach:
- Isolate *E. coli* from known sources
- Characterize isolates using phenotypic or genotypic techniques (e.g., ERIC-PCR, RP)
- Match unknown environmental isolates (water samples) against known-source library

Considerations:
- Library construction expensive
- Regional effects on library?
Library-Independent BST

Approach:

- Genotypic detection of microorganisms based on marker genes
- Does not require known-source library
- Most common approach targets *Bacteroidales*
What are *Bacteroidales*?

- More abundant in feces than *E. coli*
- Obligate anaerobes – less likely to multiply in environment
- Subgroups appear to be host specific
- Markers available for humans, ruminants, horse, swine
- Not pathogens
Library-Independent BST

Considerations:

• Rapid and less expensive than library-dependent methods
• Limited markers – human, ruminant, horse, swine for *Bacteroidales*
• New markers being developed
Which BST Methods Should be Used in Texas?

- Bacteria TMDL Task Force evaluated numerous methods

Direct comparison of four bacterial source tracking methods and use of composite data sets

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Keywords
antibiotic resistance, enterobacterial repetitive intergenic consensus polymerase chain reaction, Escherichia coli, pulsed-field gel electrophoresis, ribotyping, water quality, faecal pollution.

Abstract
Aims: Four bacterial source tracking (BST) methods, enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), automated ribotyping using HindIII, Kirby-Bauer antibiotic resistance analysis (KB-ARA) and pulsed-field gel electrophoresis (PFGE) were directly compared using the same collection of Escherichia coli isolates. The data sets from each BST method and from composite methods were compared for library accuracy and their ability to identify water isolates.
BST Methods Evaluated

Lowest Resolution

- Kirby-Bauer Antibiotic Resistance Analysis
- Ribotyping

Highest Resolution

- ERIC-PCR
- Pulsed Field Gel Electrophoresis
Which BST Methods Should be Used in Texas?

Recommended Methods:

• Library-dependent
  • Combination of methods (ERIC-RP, ERIC-ARA, or CSU-ARA)

• Library-independent
  • *Bacteroidales* PCR for human, ruminant, horse, and swine sources
BST for Little Brazos River Tributaries

• Tier 2 Analysis (Bacteria TMDL Task Force Report)
  – Targeted water quality monitoring
  – Land use analysis and modeling
  – Bacterial source tracking
    • Library-independent BST
    • Limited library-dependent BST
  – Analyze data with stakeholders
BST for Little Brazos River Tributaries

- Library independent
  - Analyze 50-100 water samples per segment (~250 total samples)
  - Bacteroidales PCR for human, ruminant, horse, and swine markers
BST for Little Brazos River Tributaries

- Limited library-dependent
  - Analyze *E. coli* from 50-100 water samples from across the entire study area
  - Confirmation as *E. coli* using biochemical tests
  - ERIC-RP fingerprinting
Next Steps for BST

• A sanitary survey design meeting will be held – outcomes will help AgriLife Research understand usefulness of existing known source library for BST

• Brazos River Authority will begin collecting water samples and AgriLife Research will begin BST on a subset of those samples

• AgriLife Research will be back at a stakeholder meeting in ~May 2009 to provide an update on the progress of BST

• BST should be completed ~February 2010
Questions?

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