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TEXAS A&M UNIVERSITY
AGRICULTURE & LIFE SCIENCES
Department of Soil and Crop Sciences

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Nick Dornak
Plum Creek Watershed Coordinator
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Dear Nick:

Attached please find the bacterial source tracking (BST) results for the two Cypress Creek water samples collected on 08/07/2017. For BST, we DNA fingerprinted four isolates from each sample using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting. The fingerprints were compared against the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories using both 3-way and 7-way source splits.

The following pages detail: 1) the composition and performance of the current Texas *E. coli* BST library that was used to categorize your isolates and 2) a summary of the BST results for your isolates.

Please do not hesitate to contact me if you have any questions or would like to discuss the results further. We look forward to the continuation of this project and assisting you further with the characterization of your watershed.

Sincerely,

Terry J. Gentry
Associate Professor & Laboratory Director

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Bacterial Source Tracking Methods

From each water sample collected on 08/07/2017, a total of four isolates each were DNA fingerprinted using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting (ERIC-RP). The DNA fingerprints were compared to known-source fingerprints in the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories (Table 1). The current library contains 1,765 *E. coli* isolates from 1,554 different fecal samples representing over 50 animal subclasses. This is the result of collecting over 3,500 domestic sewage, wildlife, livestock, and pet fecal samples from 13 watersheds across Texas and screening over 6,000 isolates for clones and host specificity.

Table 1. Texas *E. coli* BST Library (ver. 5-15, cross-library validation) composition and rates of correct classification (RCCs) by Jackknife analysis of ERIC-RP composite data sets using an 80% similarity cutoff and 3- and 7-way splits.

Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio***	Left Unidentified (unique patterns)
HUMAN	**384**	**330**	**22%**	**100**	**4.5**	**6**
DOMESTIC ANIMALS	**532**	**495**	**30%**	**100**	**3.3**	**19**
Pets	83	74	5%	84	16.8	41
Cattle	232	216	13%	93	7.2	11
Avian Livestock	95	88	5%	89	17.8	26
Other Non-Avian Livestock	122	117	7%	94	13.4	15
WILDLIFE	**849**	**729**	**48%**	**100**	**2.1**	**16**
Avian Wildlife	273	250	15%	79	5.3	19
Non-Avian Wildlife	576	479	33%	91	2.8	15
Overall	**1765**	**1554**		**ARCC** ** **= 100% (3-way) 91% (7-way)**		**18%**

**ARCC = average rate of correct classification: the proportion of all identification attempts which were correctly identified to source class for the entire library, which is similar to the mean of the RCCs for all source classes when the number of isolates in each source class is similar.

***A RCC/Random Ratio greater than 1.0 indicates that the rate of correct classification is better than random. For example, the rate of correct classification for human is 4.5-fold greater than random chance.

Source Categories

With currently available BST methods, it is not possible to discriminate between all possible species of animal sources simultaneously, since each added category tends to decrease the accuracy of source classification. This is illustrated in Table 1 where the 7-way split has an overall lower average rate of correct classification (91%) than the 3-way split does (100%). Therefore, we currently combine most species into groups based upon similarity of their physiology and/or potential management. Below are the 3- and 7-way split categories that were used for categorizing your *E. coli* isolates and which we have most frequently used for characterizing watersheds:

3-way split

1. Domesticated animals and livestock (livestock and pets)
2. Wildlife (including feral hogs)
3. Humans

7-way split

1. Cattle
2. Other livestock, non-avian (non-avian livestock other than cattle; sheep, etc.)
3. Other livestock, avian (chickens, etc.)
4. Pets (dogs, cats)
5. Avian wildlife (ducks, geese, sparrows, etc.)
6. Non-avian wildlife (deer, feral hogs, coyotes, etc.)
7. Humans

For any *E. coli* isolate that could not be matched to a group in the Texas *E. coli* BST Library, its source category was designated as being "unidentified."

In Tables 2 & 3, the far right column lists the ID of the closest library match for each tested *E. coli* isolate. The ID of the closest library match for each isolate should be used for informational purposes only and not be interpreted as species-level source classification of the isolates since our current methods are not capable of doing this (e.g., they cannot distinguish between isolates from different species of non-avian wildlife, such as deer and feral hogs).

Bacterial Source Tracking Results

Results for samples 582571 and 582572 collected on 08/07/17 are shown in Tables 2 & 3. Overall out of the 8 isolates, 3 were classified as originating from livestock and domesticated animals, 3 from wildlife, and 1 isolate was identified to be from a human source, using a 3-way source split. Using the more detailed 7-way split, 3 of the isolates were classified as originating from cattle, 3 from non-avian wildlife, and 1 was identified as originating from a human source. The source could not be identified for 1 of the isolates.

Table 2. Classification of *E. coli* isolates from sample 582571 collected on 08/07/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-582571-8/7-A	Human	Human	Raw Sewage
Cypress Creek-582571-8/7-B	Unidentified	Unidentified	Cattle
Cypress Creek-582571-8/7-C	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582571-8/7-E	Wildlife	Wildlife, Non-Avian	Feral Hog

Table 3. Classification of *E. coli* isolates from sample 572572 collected on 08/07/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-582572-8/7-A	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582572-8/7-C	Wildlife	Wildlife, Non-Avian	Mouse
Cypress Creek-582572-8/7-D	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582572-8/7-E	Wildlife	Wildlife, Non-Avian	Feral Hog

* The ID of the closest library match for each isolate is provided for informational purposes only.

Notes

The BST results in this report should be interpreted cautiously since they represent only 4 *E. coli* isolates from each sample and the samples were collected at only a single time-point. Analysis of additional *E. coli* isolates from multiple sampling events may strengthen and further validate these initial results.

End of Report