TEXAS WATER RESOURCES INSTITUTE

Statewide Bacterial Source Tracking Program for FY 2015
FY 2015 Workplan 15-52

Quarter no. 5 From 9/1/15 Through 11/30/15

I. Abstract

AgriLife SCSC initiated evaluation of soil and water *E. coli* previously isolated from Riesel to see if the similarity/dissimilarity of these isolates provides evidence for naturalized *E. coli* at the site. UTSPH EP explored the classification of cosmopolitan *E. coli* isolates and effect on library identification accuracy. UTSPH EP also sequenced several GenBac PCR products from hogs, goats and deer to investigate DNA sequence diversity and identification of possible host-specific PCR markers. Outreach activities included (1) AgriLife SCSC attending the Soil Science Society of America Meetings in Minneapolis, MN and (2) the Fall 2015 txH_2O highlighting the BST Program in the story titled *"A decade of solving water quality mysteries."*

II. Overall Progress and Results by Task

Task 1 Project Administration

Subtask 1.1 TWRI will prepare electronic quarterly progress reports (QPRs) for submission to the TSSWCB. QPRs shall document all activities performed within a quarter and shall be submitted by the 15th of March, June, September, and December. QPRs shall be distributed to all Project Partners and posted on the project website.

The following actions have been completed during this reporting period:

a. The 5th quarterly progress report was submitted on December 15, 2015.

83% Complete

Subtask 1.2 TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly.

The following actions have been completed during this reporting period:

a. To date, $179,921 of the $215,842 has been expended.

83% Complete

Subtask 1.3 TWRI will host coordination meetings or conference calls with the TSSWCB, UTSPH EP, and AgriLife SCSC at least quarterly to discuss project activities, project schedule, communication needs, deliverables, and other requirements. TWRI will develop lists of action items needed following each project coordination meeting and distribute to project personnel.

The following actions have been completed during this reporting period:

a. A coordination meeting was held on November 23, 2015 to discuss meeting with TCEQ regarding BST.

83% Complete

Subtask 1.4 TWRI will work with AgriLife SCSC and UTSPHEP to develop a Final Report that summarizes activities completed, conclusions reached during the project, and the extent to which project goals and measures of success have been achieved.

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Task 2 Quality Assurance

Subtask 2.1 TWRI will work with UTSPH EP, AgriLife SCSC, and IRNR to develop a QAPP for activities in Tasks 3-5 consistent with EPA Requirements for Quality Assurance Project Plans (QA/R-5) (May 2006) and the TSSWCB Environmental Data Quality Management Plan (August 2007).

The following actions have been completed during this reporting period:

a. The QAPP was approved on May 1, 2015.

100% Complete

Subtask 2.2 TWRI will submit revisions and necessary amendments to the QAPP as needed.

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

83% Complete

Subtask 2.3 AgriLife SCSC and UTSPH EP will maintain and update, at least annually, the 7 statewide BST template-SOPs for collection of fecal samples for BST, isolation of E. coli, archival of E. coli isolates, ERIC-PCR, RP, pre-processing of water samples for Bacteroidales PCR, and Bacteroidales PCR consistent with EPA Guidance for Preparing Standard Operating Procedures (SOPs) (QA/G-6) and the TSSWCB Environmental Data Quality Management Plan so that they include the most recent advances in BST science, methodologies, markers and technologies.

The following actions have been completed during this reporting period:

a. AgriLife SCSC's efforts to evaluate a different DNA extraction method have been slowed due to a centrifuge malfunction. Repairs have been scheduled and the method comparison will occur during the next quarter.
b. UTSPH EP has been evaluating the use of a laboratory refrigerator with slide out shelves for performing ERIC-PCR gel electrophoresis. Initial results appear promising and this set up may provide a more feasible approach than using a walk-in cold room. If successful, the ERIC-PCR SOP will be revised.

90% Complete

Subtask 2.4 AgriLife SCSC and UTSPHEP will coordinate to ensure that needed personnel training is kept on par between the groups to ensure congruity statewide.

The following actions have been completed during this reporting period:

a. AgriLife SCSC and UTSPHEP continue to routinely converse via email and phone to discuss the congruency of lab methods.

90% Complete

Task 3 Analytical Laboratory Capacity, Library Exploration and Refinement, and Methods Development

Subtask 3.1 UTSPH EP and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment. This includes securing maintenance contracts, replacement parts, and expendable supplies.

The following actions have been completed during this reporting period:

a. DuPont has completed RiboPrinter preventative maintenance visits for both labs.
b. A -80° C freezer at UTSPHEP has broken down and a repair visit is pending. All samples were moved to another freezer for temporary storage.
c. AgriLife SCSC's benchtop centrifuge malfunctioned (for the 3rd time in the past two years). A dedicated electrical circuit and a new centrifuge motherboard will be installed next quarter to hopefully fix these issues.
d. See Subtask 2.3b above.

83% Complete

Subtask 3.2 UTSPH EP and AgriLife SCSC will retain (or hire) lab personnel, Graduate Students, and/or Postdoctoral Research Associates to 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) aid in the evaluation, expansion and maintenance of the Texas E. coli BST Library, 3) evaluate library-independent methods and markers, and 4) provide support on TSSWCB projects.

The following actions have been completed during this reporting period:

a. UTSPHEP has retained Joy Truesdale and Elizabeth Casarez to assist with project activities.
b. AgriLife SCSC hired Maitreyee Mukherjee as a postdoctoral research associate in July 2015.

90% Complete

Subtask 3.3 In order to quantify and characterize the possibility of naturalized E. coli populations occurring in soil and ultimately runoff, AgriLife SCSC, with assistance from TWRI, will install four small exclosures (built from plastic barrels, or similar) in each of 3 designated catchments (un-grazed rangeland, cropland, managed hay pasture) at the USDA-ARS Grassland Research Center in Riesel. Small, mesh-covered windows will be installed in each plastic container to allow for gas exchange. The open end of each exclosure will be buried in the soil to exclude inputs of E. coli from animals or water. One month after installation, four individual soil samples will be collected and composited from inside each exclosure. Four soil samples will also be collected and composited from outside of each exclosure. E. coli will be enumerated for each sample using EPA Method 1603. For each sample containing E. coli, up to 5

E. coli isolates will be isolated, verified, and archived. In FY16, these isolates will be analyzed by ERIC-RP for comparison to the Texas E. coli BST Library. A total of 25 presumptive naturalized E. coli isolates will also be characterized with ERIC-RP through collaborative work with the City of Houston.

The following actions have been completed during this reporting period:

a. Since the last several soil sampling events at Riesel failed to yield any detectable *E. coli*, AgriLife SCSC is evaluating the soil and water *E. coli* previously isolated from Riesel (as part of TSSWCB Project No. 13-56) to see if the similarity/dissimilarity of these isolates provides any evidence for naturalized *E. coli* at the site.

70% Complete

Subtask 3.4 UTSPH EP and AgriLife SCSC will collaborate to evaluate the geographical and temporal stability, composition, average rates of correct classification (accuracy), diversity of source specific isolates, and further development and refinement needs of the Texas E. coli BST library, as the library is updated with new known-source isolates.

The following actions have been completed during this reporting period:

a. UTSPH EP has been exploring the classification of cosmopolitan *E. coli* isolates and effect on library identification accuracy.

20% Complete

Subtask 3.5 Using known source fecal material, AgriLife SCSC and UTSPH EP will utilize the best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers. Specifically, efforts will be made to evaluate 1) additional wildlife known source fecal samples for human Bacteroidales HF183 marker, 2) additional deer fecal samples from across the state analyzed for the Bacteroidales HF 183 marker, and 3) addition of library-independent qPCR markers to the Texas BST toolbox. These fecal samples will primarily have been collected and archived as part of previous studies including the Arroyo Colorado project. Depending on the outcome of the Arroyo Colorado sample collection, additional samples may be needed for specific animal groups (i.e., avian wildlife). If more samples are needed, TWRI will collect and provide samples to AgriLife SCSC and UTSPH EP, as needed.

The following actions have been completed during this reporting period:

a. UTSPH EP sequenced several GenBac PCR products from hogs, goats and deer to investigate DNA sequence diversity and identification of possible host-specific PCR markers.
b. Dr. Elaine Moriarty, Institute of Environmental Science and Research (ESR), Christchurch Science Centre (New Zealand) offered to provide deer fecal DNA samples for the evaluation of *Bacteroidales* qPCR markers.

25% Complete

Subtask 3.6 TWRI, AgriLife SCSC and UTSPH EP will cooperate with other entities nationwide to ensure that the most up-to-date and accurate BST approaches are implemented in Texas by attending and participating in BST-related meetings, seminars and workshops, as appropriate, to learn of new and improved BST methods being employed elsewhere.

The following actions have been completed during this reporting period:

a. AgriLife SCSC attended the Soil Science Society of America Meetings in Minneapolis, MN, 15-18 November 2015.

83% Complete

Task 4 Targeted BST Analysis

Subtask 4.1 UTSPH EP will perform targeted BST analysis to support the Arroyo Colorado watershed protection plan development efforts.

The following actions have been completed during this reporting period:

a. The final field samples for the project were collected in May and all analyses are complete.

100% Complete

Subtask 4.2 AgriLife SCSC will perform targeted BST analysis to support watershed protection plan development efforts as directed by the TSSWCB.

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

0% Complete

Task 5 Outreach on Bacterial Source Tracking

Subtask 5.1 IRNR will host and maintain the http://texasbst.tamu.edu website to disseminate educational materials, project updates, science updates, notify readers about educational opportunities, and other outreach efforts to advance the science and application of BST in Texas and nationally.

The following actions have been completed during this reporting period:

a. TWRI continues to host and maintain the Texas BST Library website. Between 9/1/15 – 11/30/15, there were **65** visits to the website by **36** unique visitors.

83% Complete

Subtask 5.2 TWRI, UTSPH EP, and AgriLife SCSC will promote the use of and provide resources on BST by participating in meetings, conferences, workshops, seminars, and other appropriate venues. TWRI, UTSPH EP, and AgriLife SCSC will distribute educational brochures developed. As needed, TWRI, UTSPH EP, and AgriLife SCSC will develop additional flyers, one-pagers, tri-folds or other appropriate printed media, that can be used to 1) discuss the appropriate application of BST in identifying fecal contamination sources and 2) promote the analytical laboratory capability of public BST labs which the State has invested. As appropriate, TWRI will include information about BST in general, and this project specifically, in the txH2O magazine and Conservation Matters e-mail newsletter. Finally, TWRI, UTSPH EP, and AgriLife SCSC will periodically meet with natural resource agencies to advance the general knowledge and understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the use of BST in Texas.

The following actions have been completed during this reporting period:

a. The Fall 2015 txH_2O highlighted the BST Program in the story titled *"A decade of solving water quality mysteries."*

83% Complete

Subtask 5.3 TWRI, UTSPH EP, and AgriLife SCSC will work with public and private laboratories and other researchers/academia across the state which are exploring the use of BST or engaged in BST in Texas about the methods and approaches recommended by the Task Force and being implemented by the State. UTSPH EP and AgriLife SCSC will work to ensure that methodologies and QA/QC mechanisms adopted by these other laboratories are as congruent as possible with SOPs utilized by UTSPH EP and AgriLife SCSC (subtask 2.1).

The following actions have been completed during this reporting period:

a. No activity to report this quarter.

83% Complete

III. Projected Work for Next Quarter

- UTSPH EP will continue work the analysis of library independent BST marker data.
- UTSPH EP will continue to evaluate the use of a modified laboratory refrigerator for ERIC-PCR gel electrophoresis.
- SCSC will continue work to evaluate naturalized *E. coli* populations at Riesel and UTSPH EP will begin analysis of archived Houston *E. coli* isolates
- SCSC will evaluate alternate method for DNA extraction from water samples.