USDA-CSREES 2006 National Water Quality Conference

A field test of library-independent genetic markers for bacterial source tracking

Bacterial source tracking (BST) is a process by which fecal water pollution is apportioned between host sources. For this project we tested a binary assay to detect multiple library-independent markers across multiple site visits. The underlying premise was that the most significant source of contamination will be detected most frequently. Most of the genetic markers used in this study were developed as part of a microarray-based screening study. PCR assays were developed for each of 15 markers and all markers were validated against fecal samples from a variety of sources. We included one other published marker (Scott et al. 2005) and all of the markers target enterococci. Standard methods were used to filter water samples, enumerate total enterococci, prepare template DNA, and conduct PCR tests. PCR was used to test for the presence of all 16 markers at 10 samples sites with 8 visits per site. Ten markers were either ubiquitous or mostly absent from the samples tested in this study. Using the remaining six markers, sites were ranked based on the total number of positive detection events. Rankings were low (=median score), or high (>median score). No gold standard exists by which to judge the diagnostic specificity and sensitivity of BST markers, but we can make some subjective judgments. One site surrounded by small cottages (JOJ6) and a site where we detected odor of raw sewage (SHER9) scored high for human markers. Areas experiencing the highest cattle grazing pressure (CR4, CR6, and CR6A; estimated 3-4k head collectively) scored high for cattle markers. Presence/absence detection of library-independent markers may be most useful for relative comparisons of fecal contamination based on multiple sites. Based on the experience from this study, however, it is clear that multiple markers will be needed to increase the probability of detecting a specific source.

Author: Douglas Call
Coauthor(s): Douglas Call Pete Plescia Edward Kuhn Marilyn Soule