**Project:** Microbial Source Tracking by Cellular Fatty Acid Composition of Indicator Organisms

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**Summary:** Fecal contamination resulting from human, domestic animals, and wildlife poses a serious threat to human health and the environment. Presence of pathogens leads to human diseases and economic losses for industries that depend on water quality of lakes, rivers and streams. One of the factors preventing effective measures for controlling microbial pollution is the inability to determine the exact sources of fecal contamination. Sewer overflows, inefficient sewage treatment, failing septic systems, agricultural runoff, and wildlife are possible contributors to microbial pollution. Although recently developed microbial source tracking (MST) methods are relatively accurate, most of these methods tend to be costly and technically challenging to be widely applicable. In this research, fatty acid methyl ester (FAME) profiles of indicator organisms are studied as an innovative tool to predict the sources of microbial pollution in water environments. Identification and quantification of fatty acids extracted from the membranes of indicator organisms serve as phenotypic fingerprints showing statistically significant differences for different microbial species, in some cases for different strains of the same species.

The differences in the FAME profiles of total coliform (TC), fecal coliform (FC), *Escherichia coli*, and *Enterococci* isolated from the selected possible sources of fecal pollution are being investigated. The host categories include human (sewage samples), livestock (bovine, poultry, and swine), and wildlife (waterfowl and deer). Whole-cell fatty acids are identified and quantified by a gas chromatograph with flame ionization detector (GC/FID). The differences between the FAME profiles of different hosts are analyzed using multivariate statistical tools, mainly discriminant analysis (DA) and Principle Component Analysis (PCA).

The results indicate that the FAME profiles of TC, FC, and *E. coli* isolates contain statistically significant host-specific differences. The presence of three signature fatty acids in the isolates of human origin, 12:0 2OH, 12:0 3OH and 14:0 2OH, and other three in the isolates from the livestock samples, 15:0, 18:0 and 19:0 ISO, are the primary factors that allow accurate discrimination of the human isolates against the non-human sources when TC and FC are used as indicators. In the case of *E. coli* isolates, the presence of fatty acids 10:0, 16:1 ω5c, and 19:0 ISO suggest livestock sources fecal pollution. When individual hosts are pooled into larger categories of human, livestock, and wildlife, the known-source FC and *E. coli* isolates are classified into their respective host-categories at 95 and 89%, respectively. In a field testing study, a linear discriminant function based on the differences in FAME profiles of know-source FC and *E. coli* isolates predicted the predominant source fecal pollution in a natural pond as wildlife. The pond has a large population of Canada geese and there is no other apparent source of fecal matter. Thus, the prediction is considered highly accurate. These results indicate the accuracy of FAME profiling in predicting sources of fecal pollution in water environments and suggest that it may be an economical and easily applicable MST method that can be used to affectively asses microbial risks and implement more accurate Total Maximum Daily Load (TMDL) programs.

**Publication(s):**