

## Coliforms

Coliforms were first used as indicators of sewage during the 19<sup>th</sup> century after *Escherichia coli* was first isolated (named *B. coli* at the time) and have since been staples for water quality assessment. The group is composed of gram negative, non-spore forming, rod-shaped bacteria that are capable of growth in the presence of bile salts and ferment lactose with the production of acid at 35±2°C within 24 - 48 hours. Because of the broad growth criteria, the coliforms include a host of genera including *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*. Although some genera have no correlation with fecal pollution, they can provide valuable information on process efficiency when combined with other fecal indicators. Furthermore, detection methods for coliforms are quite inexpensive and do not require high technical skill. This indicator is commonly used by our laboratory as part of the toolbox for characterization of fecal contamination in various waters.

### *Approaches used by the Rose WQEMM Laboratory*

- Membrane filtration
- USEPA Method 1604 (Total coliforms)
- IDEXX<sup>®</sup> (Total coliforms as part of Colilert<sup>®</sup>)

## *Escherichia coli*

*E. coli* is abundant in animal and human feces and is widely preferred as an index for fecal contamination in sewage, treated effluents, and all natural waters. What especially makes *E. coli* a good indicator is that it is found in higher abundance compared to the pathogens it indicative of and mostly being non-pathogenic to humans. Also, studies published by USEPA indicate significant correlation between *E. coli* in fresh water and the occurrence of gastroenteritis. Consequently, USEPA Method 1603 has been recommended for routine examination of *E. coli* in water treatment facilities. This indicator is commonly used by our laboratory as part of the toolbox for characterization of fecal contamination in various waters.

### *Approaches used by the Rose WQEMM Laboratory*

- Membrane filtration
- USEPA Methods 1603
- IDEXX<sup>®</sup>

## **Enterococci**

Enterococci is a gram-positive non-spore forming member of the Streptococci bacteria. Enterococci is commonly found in human and other warm blooded animal feces. While many strains are not harmful, the detection of enterococci in water does indicate a potential for the presence of enteric pathogens. Concentrations of colonies can be counted and reported as colony forming units/ volume of sample by using membrane filtration, selective medium, and incubation. Enterococci is used as an indicator of bacteriological quality of recreational waters because it was shown through epidemiological studies to have a high correlation with gastroenteric disease related to swimming in marine and fresh waters. This indicator is commonly used by our laboratory as part of the toolbox for characterization of surface waters in the Great Lakes.

### *Approaches used by the Rose WQEMM Laboratory*

- Membrane filtration
- IDEXX<sup>®</sup>

## ***Enterococcus faecium* surface protein (*esp*) gene**

Within the realms of microbial source tracking, genetic markers for specific host species are being increasingly used as library independent analyses. This is to circumvent the need for library-dependent methods, which can be costly and time consuming because of the need to build a reference database for the geographical area of interest. One genetic marker that has been rigorously tested for source tracking purposes is the *E. faecium* surface protein (*esp*) gene. This gene is a protein in the enterococci bacteria that is assumed to be human specific, and has been detected in untreated sewage 100% of the time in previous tests. Cultivation analysis for enterococci, DNA extraction, PCR, and gel electrophoresis reveals whether samples are positive or negative for the presence of the enterococci *esp gene*, providing information to the possible fecal inputs into a particular waterbody or sample area. This putatively human specific marker is used by our laboratory as part of the toolbox for characterizing sources of fecal contamination surface and ground waters through Michigan.

## ***Campylobacter***

The Rose WQEMM laboratory can detect thermotolerant *Campylobacter* species (*C. jejuni*, *C. coli*, *C. lari*, *C. upsalensis*), which are pathogenic to humans. The assay is a combination of methods of which were developed at the Institute of Environmental Science and Research Ltd. (Christchurch Science Centre, Christchurch, New Zealand). The method entails a double enrichment of the samples, extracting DNA by cell lysis, and enumeration by PCR (or QPCR). A portion of the enriched sample, after being streaked on specific media, also undergoes traditional biochemical analysis for additional confirmation.

## Bacterial Markers

The Rose WQEMM laboratory is capable of identifying and quantifying the bacterial sources of pollution in water. Recent microbial source tracking (MST) methods primarily utilize genetic markers which are library independent, and rely on molecular approaches that do not require cultivation of microorganisms. Currently, the following bacterial markers assays are available in the Rose WQEMM laboratory:

- putatively human-specific *esp* surface protein marker for *Enterococcus faecium*
- *alpha mannanase* gene targeted in *Bacteroides thetaiotaomicron*
- 16 S rRNA in *Bacteroides* in humans
- *E. coli* (*uid* gene)
- enterococci (23S rRNA)

*Sampling for specificity test for MST targets*  
(photo taken by Shika Singh, Michigan State University)

