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Comparison of Bacteroides Human Markers for Pollution Diagnostics in Recreational Waters

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Comparison of Bacteroides human markers for pollution diagnostics in recreational waters

Asli Aslan and Joan B. Rose
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• Rapid methods are ready for use to provide timely information to protect recreational-water users from waterborne diseases.

**Current fecal indicators**
(Culture based)
- Fecal coliforms, *Escherichia coli*
- *Enterococcus faecalis, E. faecium*
- *Clostridium perfringens*
- Bacteriophages

**Upcoming indicators**
(qPCR based)
- *Escherichia coli*
- *Enterococcus spp.*
- *Bacteroides*

In recent years 175 species from 96 different genera have been classified as emerging waterborne pathogens (WHO, 2003).

**Future tools:**
Norovirus? Adenovirus? Host specific markers?
Why Bacteroides genus?

- present in large concentrations in feces (25% of the anaerobic microbiota of the human colon)
- genome sequencing of two species are completed  
  - *B. thetaiotaomicron* (Xu et al. (2003))
  - *B. fragilis* (Cerdeno-Tarraga et al. (2005))
- have identifiable genetic host specificity that has been utilized for MST

*B. thetaiotaomicron alpha mannase gene:*

1 copy = 1 cell

**High Specificity**
Xu et al., (2003)
Yampara-Iquese et al., (2008)
Aslan-Yilmaz, (unpublished data)
**B. fragilis 16S rRNA**

Ct=-( -3.2547(log copies) + 46.239)

Amplification efficacy: 1.99

**B. thetaiotaomicron alpha mannanese**

Ct=-( -3.5423(log copies) + 40.1)

Amplification efficacy: 1.91
## Quantification

**Comparison of different Bacteroides markers by qPCR:**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Treatment</th>
<th>B. fragilis copies/100 ml</th>
<th>B. thetaiotaomicron copies/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sewage</td>
<td>None</td>
<td>3.84E+08</td>
<td>4.90E+07</td>
</tr>
<tr>
<td>Combined sewer overflow influent</td>
<td>Before flocculation</td>
<td>1.04E+08</td>
<td>1.76E+07</td>
</tr>
<tr>
<td>Combined sewer overflow effluent</td>
<td>chemically treated no disinfection</td>
<td>7.22E+07</td>
<td>5.09E+03</td>
</tr>
<tr>
<td>Biosolid</td>
<td>aerobic digestion</td>
<td>2.15E+08</td>
<td>1.55E+04</td>
</tr>
<tr>
<td>Biosolid</td>
<td>aerobic digestion</td>
<td>3.71E+08</td>
<td>5.25E+04</td>
</tr>
</tbody>
</table>
• **Specificity:**

• 226 samples of bovine, sheep, chicken, duck, swine, geese, horse

**B. fragilis 16s rRNA:**
All cow samples were positive. NOT specific for human.

**B. thetaiotaomicron alpha mannase gene:**
• 98% specific to human.
• However, 14% of swine samples were positive (all close to detection limit)
Study Design:

Samples were collected from various beaches, rivers and lakes around Michigan.

100 ml sample for each site was filtered. And transported to Michigan, MSU.

Filters were extracted using two commercially available extraction kit and bead beating technique (USEPA, 2010).

Results were measured by qPCR for *B. thetathiomicron*. 
Testing different nucleic acid extraction methods

MOBIO showed higher DNA concentrations compared to QIAMP ($p<0.001$)
### B. thetathiomicron (copies/100 ml)

<table>
<thead>
<tr>
<th>Location</th>
<th>MOBIO</th>
<th>EPA</th>
<th>QIAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muskegon Beach</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Muskegon Lake</td>
<td>1.87E+05</td>
<td>4.52E+05</td>
<td>6.72E+04</td>
</tr>
<tr>
<td>Muskegon River</td>
<td>5.16E+04</td>
<td>3.44E+05</td>
<td>BDL</td>
</tr>
<tr>
<td>Grand Haven Beach</td>
<td>5.20E+05</td>
<td>7.91E+05</td>
<td>6.88E+04</td>
</tr>
<tr>
<td>Grand Haven Lake</td>
<td>1.81E+05</td>
<td>4.60E+05</td>
<td>BDL</td>
</tr>
<tr>
<td>Grand Haven River</td>
<td>2.43E+06</td>
<td>2.16E+06</td>
<td>1.18E+05</td>
</tr>
<tr>
<td>Saugatuck Beach</td>
<td>2.77E+05</td>
<td>3.23E+05</td>
<td>BDL</td>
</tr>
<tr>
<td>Saugatuck Lake</td>
<td>6.28E+05</td>
<td>2.78E+05</td>
<td>6.04E+04</td>
</tr>
<tr>
<td>Saugatuck River</td>
<td>5.28E+05</td>
<td>BDL</td>
<td>3.71E+04</td>
</tr>
<tr>
<td>Park Lake</td>
<td>BDL</td>
<td>6.24E+05</td>
<td>BDL</td>
</tr>
<tr>
<td>Lake Lansing</td>
<td>3.72E+05</td>
<td>1.64E+06</td>
<td>9.20E+03</td>
</tr>
<tr>
<td>Red Cedar River</td>
<td>BDL</td>
<td>2.64E+05</td>
<td>BDL</td>
</tr>
<tr>
<td>ELWWTP</td>
<td>5.80E+07</td>
<td>1.66E+08</td>
<td>3.64E+07</td>
</tr>
</tbody>
</table>
Testing sampling transportation conditions

Study Design:

• Raw wastewater were collected into a 15 L sterile jar from ELWWTP

• Distributed into 1L sterile bottles

• Put under direct sunlight, on ice or in 37 °C incubator

• Examined at hours 0, 1, 2, 3, 4, 5, 6, 24, 48.
Test sampling transportation conditions

_E. faecalis_ ATCC culture

![Graph showing Enterococcus 23 S rRNA levels over time under different conditions.](image)
Wastewater

Enterococcus 23 S rRNA

Copies / 100 ml

Hours

- 37
- ice
- sun
Wastewater

Bacteroides thetathiomicron

Copies/100 ml

Hours

- 37
- ice
- sun
Conclusions

- *Bacteriodes thetathiomicron* alpha mannanese gene is a promising human sewage indicator.
  - Shanks et al. compared 10 different markers and only 3 of them were human specific.

- Sample storage up to 48 hours at hot temperature without ice leads to loss of signal,
  - There is a difference in different markers in terms of sample storage an transport to the laboratory.
  - Dick et al. (2010) reported the variation in decay rates of microorganisms in mesocosmos.
  - Further studies will be done to address this variability.

- The EPA bead beating procedure is thus far worked well for recovery of DNA in the samples studied.
  - More samples including different matrixes are needed for assessment.

- USEPA will be publishing the new criteria and health departments will be implementing these qPCR based methods. The source tracking markers will likely be the next round’s issue.
  - After the training program, health departments will also be able to adapt the source tracking methodologies in their laboratories and be ready ahead of time.
Next Steps

Great Lakes studies for water pollution monitoring

qPCR signal maps
Forecasting maps
Risk assessment maps
The levels of fecal coliform bacteria in rivers correlate with population size of cities located upstream of sampling points (GEMS, 2007). There is no such comprehensive data for *E. coli*. 
International Collaboration for Sewage (IC Sewage) is a consortia of 42 laboratories around the globe.

The overall mission:
“To advance our understanding of the impact of wastewater on water quality and health throughout the world, and to set the stage to meet and document improved sanitation, sewerage, and wastewater treatment for the global community”.

Specific objectives:
• Develop a diagnostic for sewage pollution to address public health risk
• Implement a technology transfer program
• Serve as a network connecting studies all over the world
• Create a global map of pollution
Welcome to the IC-Sewage Website!

The overall mission of the IC-Sewage is to advance our understanding of the impact of wastewater on water quality and health worldwide and to set the stage to meet and document improved sanitation, sewage, and wastewater treatment for the global community. As a part of this collaboration, the group is working on developing and demonstrating how new genomics tools such as microbial source tracking methodologies can be used to characterize and quantify human fecal pollution in water in order to advance understanding of the impacts of wastewater and sanitation on human health.

News
IC Sewage is preparing to be a workgroup in International Water Association

The article on the IC Sewage was published in the August issue of Water 21

The 16th International Symposium on Health-Related Water Microbiology, WaterMicro 2011, will be held September 19-23, 2011 at the Rotorua Energy Center in Rotorua, New Zealand.