

# **Comparison of Bacteroides human markers for pollution diagnostics in recreational waters**

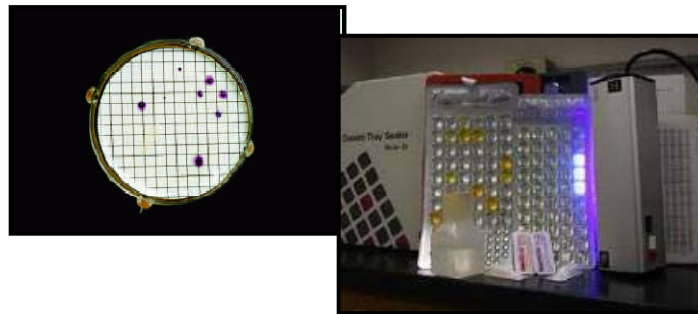
Asli Aslan and Joan B. Rose  
aayilmaz@msu.edu

- 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Aslan-Yilmaz, (unpublished data)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***

$$Ct = -(-3.2547(\log \text{ copies}) + 46.239)$$

Amplification efficacy:1.99

***B. thetaiotaomicron alpha mannanase***

$$Ct = -(-3.5423(\log \text{ copies}) + 40.1)$$

Amplification efficacy:1.91

# Quantification

## Comparison of different Bacteroides markers by qPCR:

Sample type	Treatment	<i>B. fragilis</i> copies/100 ml	<i>B. thetaiotaomicron</i> copies/100 ml
Raw sewage	None	3.84E+08	4.90E+07
Combined sewer overflow influent	Before flocculation	1.04E+08	1.76E+07
Combined sewer overflow effluent	chemically treated no disinfection	7.22E+07	5.09E+03
Biosolid	anerobic digestion	2.15E+08	1.55E+04
Biosolid	aerobic digestion	3.71E+08	5.25E+04

- 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.

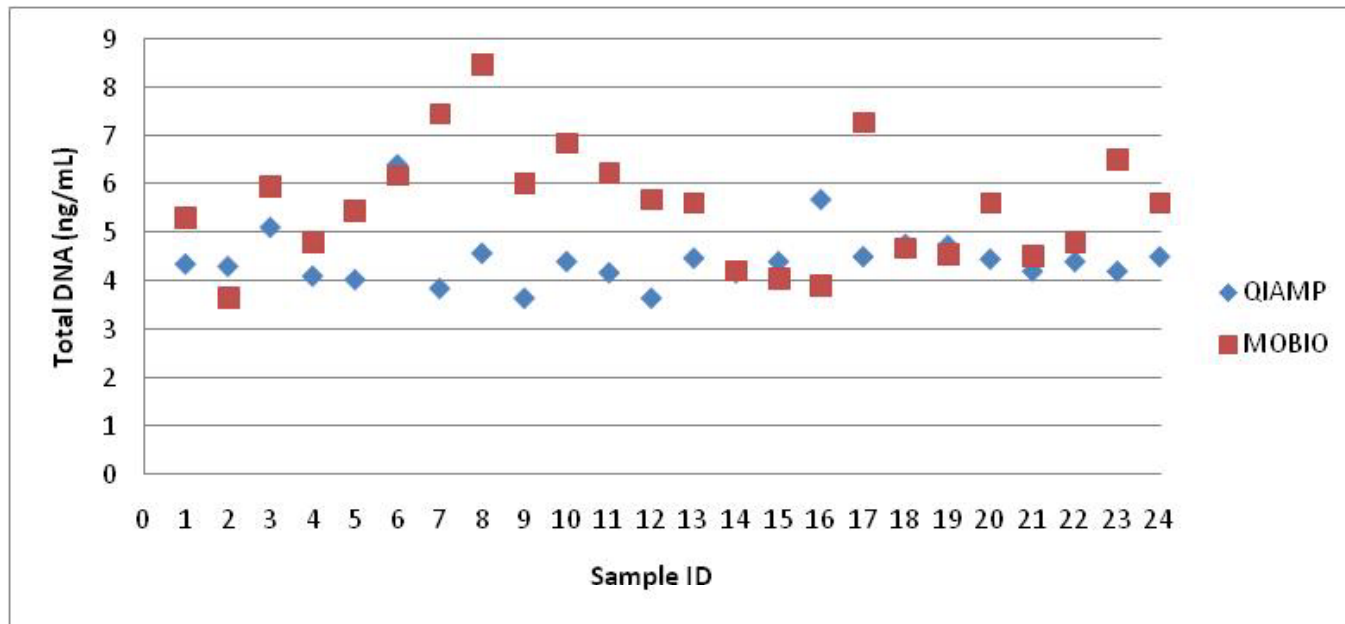
100 ml sample for each site was filtered.  
And transported to Michigan, MSU.

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

Results were measured by qPCR for *B. thetathiomicon*.



## Testing different nucleic acid extraction methods



MOBIO showed higher DNA concentrations compared to QIAMP ( $p < 0.001$ )

*B. thetathiomicon* (copies/100 ml)

	MOBIO	EPA	QIAMP
Muskegon Beach	BDL	BDL	BDL
Muskegon Lake	1.87E+05	4.52E+05	6.72E+04
Muskegon River	5.16E+04	3.44E+05	BDL
Grand Haven Beach	5.20E+05	7.91E+05	6.88E+04
Grand Haven Lake	1.81E+05	4.60E+05	BDL
Grand Haven River	2.43E+06	2.16E+06	1.18E+05
Saugatuck Beach	2.77E+05	3.23E+05	BDL
Saugatuck Lake	6.28E+05	2.78E+05	6.04E+04
Saugatuck River	5.28E+05	BDL	3.71E+04
Park Lake	BDL	6.24E+05	BDL
Lake Lansing	3.72E+05	1.64E+06	9.20E+03
Red Cedar River	BDL	2.64E+05	BDL
ELWWTP	5.80E+07	1.66E+08	3.64E+07

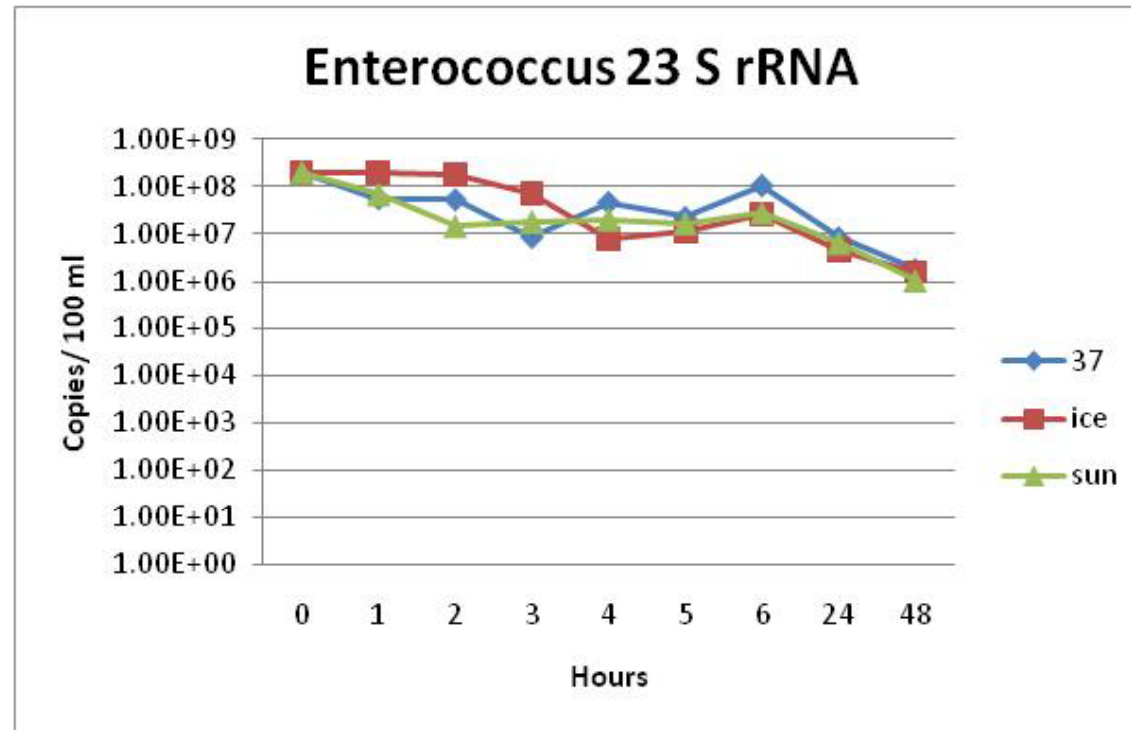
## Testing sampling transportation conditions

### Study Design:

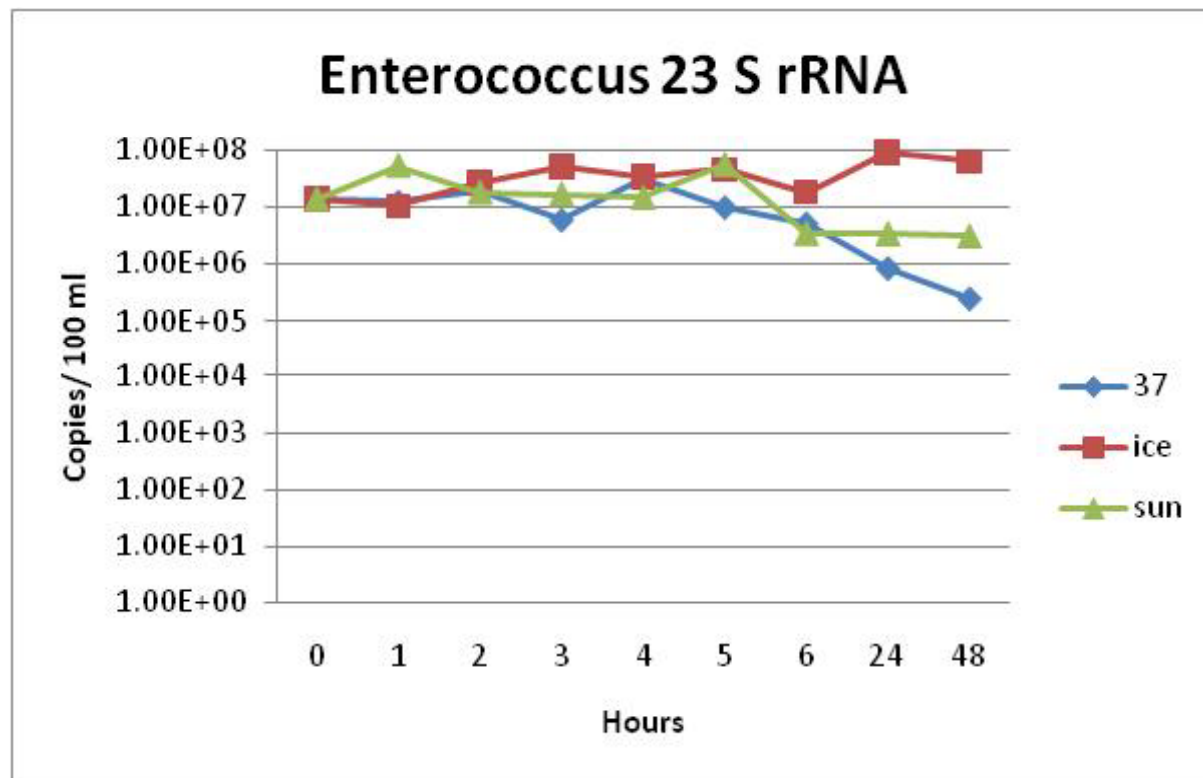
- Raw wastewater were collected into a 15 L sterile jar from ELWWTP
- Distributed into 1L sterile bottles
- Put under direct sunlight, on ice or in 37 °C incubator
- Examined at hours 0, 1, 2, 3, 4, 5, 6, 24, 48.

## Test sampling transportation conditions

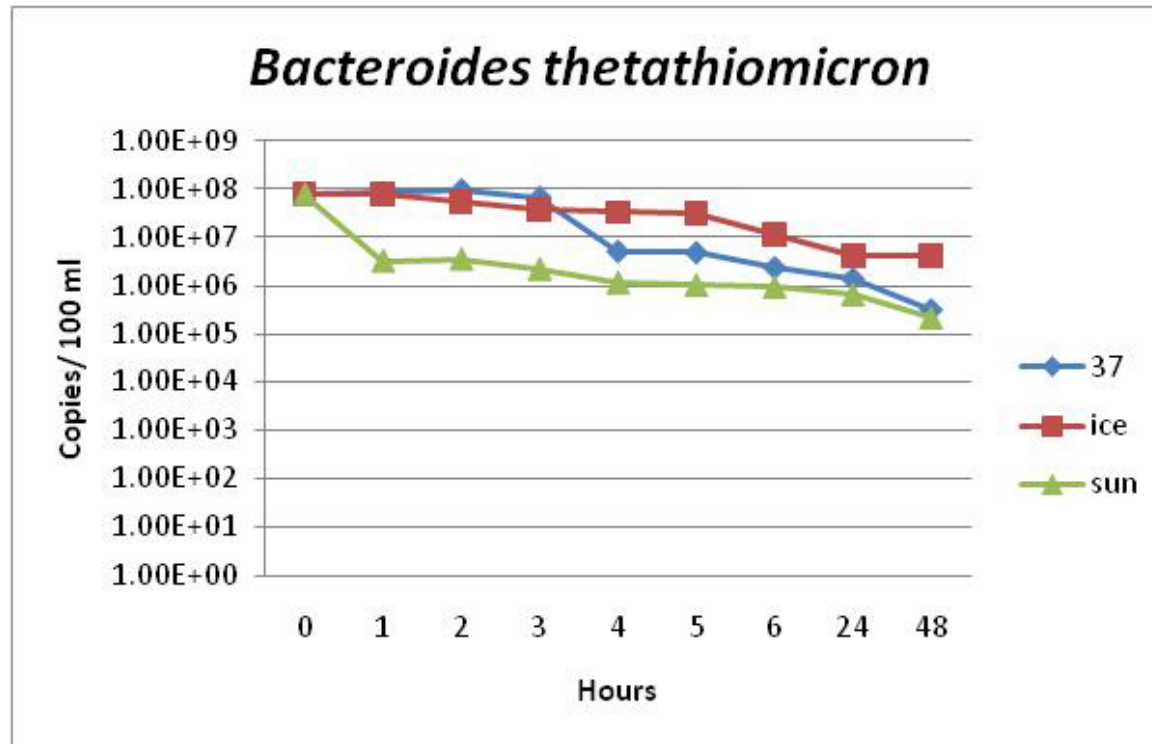
*E. faecalis* ATCC culture



## Wastewater



## Wastewater



# Conclusions

- ▶ ***Bacteriodes thetathiomicon* alpha mannanase 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 and 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

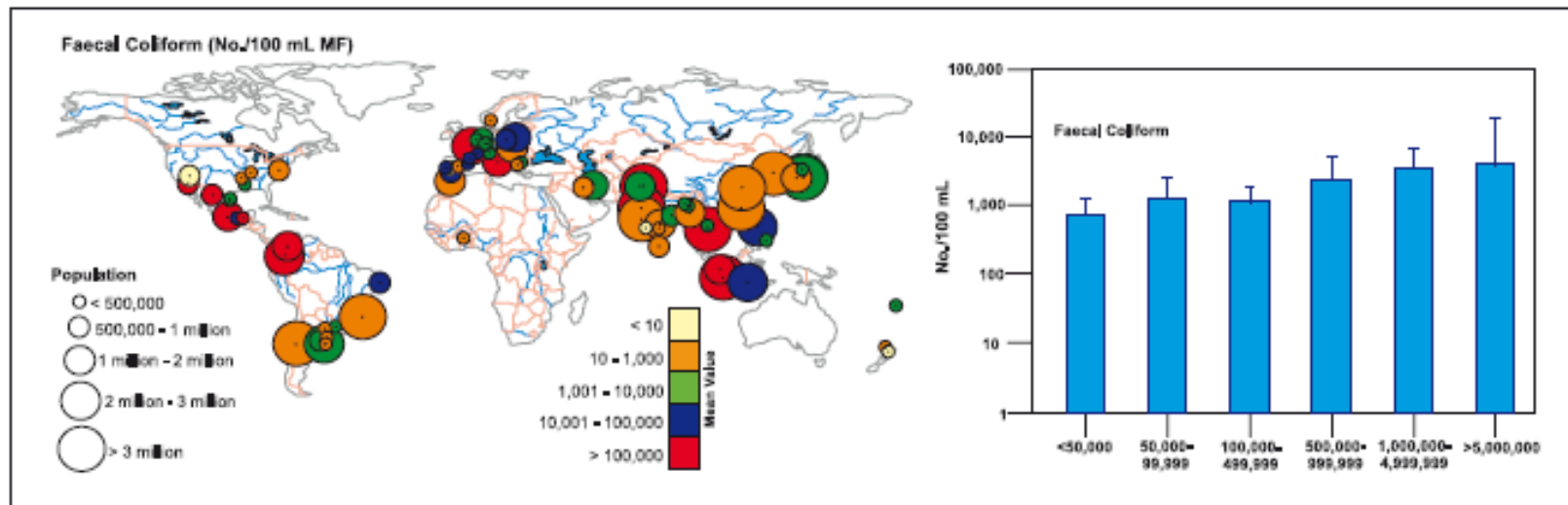
Forecasting maps

Risk assessment maps



# Next Steps

## Fecal Pollution in World's Water Sources



**Figure 38.** Faecal coliform concentrations in river monitoring stations located near to major cities, plotted according to population size (top figure). Bottom figure shows mean ( $\pm 1$  standard error) faecal coliform concentrations separated by population size class of nearby cities.

The levels of faecal 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

<http://www.cws.msu.edu/ic-sewage/>

**MICHIGAN STATE UNIVERSITY** | International Collaboratory for Sewage: IC-Sewage

Search

↑ MICROBIAL SOURCE TRACKING GOALS ORGANIZATION REFERENCES PROTOCOLS BECOME A MEMBER

### 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, sewerage, 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

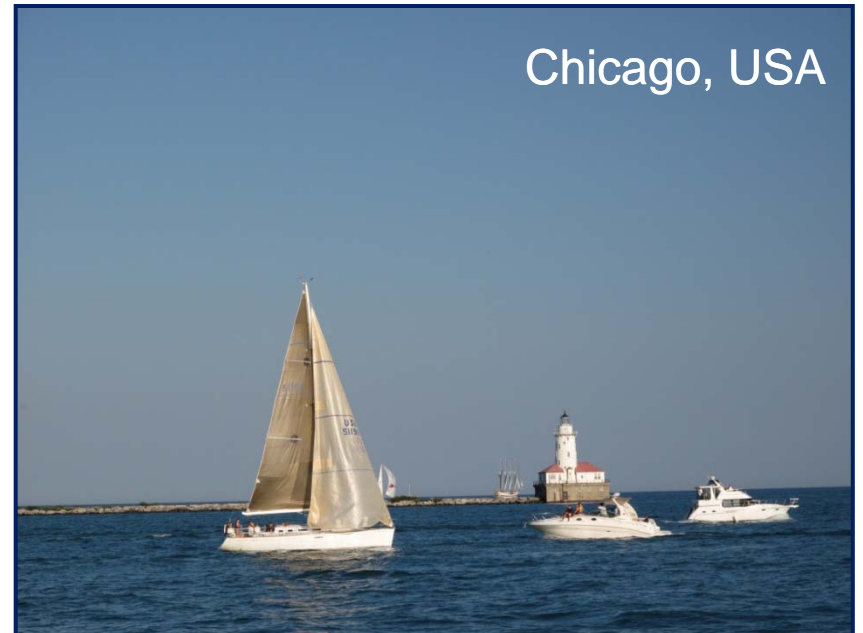
The 16th International Symposium on Health-Related Water Microbiology, WaterMicro 2011, will be held September 18-23, 2011 at the Rotorua Energy Center in Rotorua, New Zealand



Marmaris, Turkey



Chicago, USA



Lilongwe, Malawi



CWS  
Center for Water Sciences