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Escherichia coli, enterococci, and *Bacteroides thetaiotaomicron* qPCR signals through wastewater and septage treatment

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ABSTRACT

Fecal indicators such as *Escherichia coli* and enterococci are used as regulatory tools to monitor water with 24 h cultivation techniques for possible input of sewage or feces and presence of potential enteric pathogens yet their source (human or animal) cannot be determined with routine methods. This critical uncertainty has furthered water pollution science toward new molecular approaches. Members of *Bacteroides* genus, such as *Bacteroides thetaiotaomicron* are found to have features that allow their use as alternative fecal indicators and for Microbial Source Tracking (MST). The overall aim of this study was to evaluate the concentration and fate of *B. thetaiotaomicron*, throughout a wastewater treatment facility and septage treatment facility. A large number of samples were collected and tested for *E. coli* and enterococci by both cultivation and qPCR assays. *B. thetaiotaomicron* qPCR equivalent cells (mean: 1.8×10^7/100 mL) were present in significantly higher concentrations than *E. coli* or enterococci in raw sewage and at the same levels in raw septage. The removal of *B. thetaiotaomicron* target qPCR signals was similar to *E. coli* and enterococci DNA during the treatment of these wastes and ranged from 3 to 5 $\log_{10}$ for wastewater and was 7 $\log_{10}$ for the septage. A significant correlation was found between *B. thetaiotaomicron* marker and each of the conventional indicators throughout the waste treatment process for both raw sewage and septage. A greater variability was found with enterococci when compared to *E. coli*, and CFU and equivalent cells could be contrasted by various treatment processes to examine removal and inactivation via septage and wastewater treatment. These results are compared and contrasted with other qPCR studies and other targets in wastewater samples providing a view of DNA targets in such environments.

1. Introduction

Wastewater is the source of many human enteric pathogens (Nayak and Rose, 2007; Lee et al., 2006; Kamel et al., 2010; Robertson et al., 2006) and often associated with swimming-acquired illnesses in natural waters (Wade et al., 2006). Adequate wastewater treatment prior to effluent discharge plays a critical role in minimizing public health risks. On-site wastewater disposal using septic tanks has also been an issue regarding pathogen entry into and transmission through water particularly groundwater (Fong et al., 2007) and septage treatment and application on land has received little attention in regard to microbial quality. In most states, fecal coliform bacteria are still used to address wastewater treatment using

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the National Pollutant Discharge Elimination System permit programs and are disconnected from ambient water quality monitoring, in which coastal states are moving toward *Escherichia coli* (*E. coli*) and enterococci.

E. coli and enterococci have long been used as indicators of fecal pollution for recreational and drinking waters (USEPA, 2002, 2005a); and cultivation methods are used as the gold standard for the enumeration of these bacteria in water (Messer and Dufour, 1998). Recent advances in molecular biology such as polymerase chain reaction (PCR) and particularly quantitative PCR (qPCR) have revolutionized microbiology. Quantitative PCR has many advantages over standard cultivation methods, such as producing results rapidly (30 min–2 h), the ability to detect viable but non-cultivatable (VNBC) pathogens, and providing quantitative results with a wide detection range (10^0–10^8 copies/reaction). However, it is still recognized that both inactivated and live microbes will be detected, which is a disadvantage when evaluating disinfection processes. Never-the-less, evaluation and application of these qPCR methods for routine monitoring of fecal contamination in recreational waters is ongoing in the US (Haugland et al., 2005; Noble et al., 2006; Wade et al., 2006; Lavender and Kinzelman, 2009) as well as in wastewaters (Silkie and Nelson, 2009; Varma et al., 2009; Wery et al., 2008; Frahm and Obst, 2003).

One of the other disadvantages of using routine bacterial indicators is that the source cannot be identified while the specificity of molecular methods has lead to development of a field known as Microbial source tracking (MST) that has enabled the identification of animal or human sources of fecal contamination (Scott et al., 2002; Simpson et al., 2002; USEPA, 2005b). Many human fecal specific assays to address sewage discharges impacting water quality have targeted species in the *Bacteroides–Prevotella* group directed toward detection of 16S rRNA genes (Seurinck et al., 2005; Layton et al., 2006; Okabe et al., 2007). *Bacteroides* spp. are obligately anerobic, Gram negative, rod shaped, and non-endospore forming bacteria and are normally commensals that constitute the most numerous members of the intestinal flora of all warm blooded animals (Wexler, 2007). There are still concerns regarding cross reactivity of some of these genetic markers with feces from humans and other animals including cats and dogs (Sadowsky et al., 2007; Kildare et al., 2007). Some qPCR assays based on 16S rRNA genes have been reported to cross react with fish DNA (McLain et al., 2009). Moreover, the exact copy number of these 16S rRNA genes present in one cell is not known which makes conversion of qPCR copy number to cell equivalents rather difficult. Recently, Yampara-Iquise et al. (2008) examined a single copy putative mannanase 1-6 gene of *Bacteroides thetaiotaomicron* as a human fecal source tracking marker with good specificity.

The overall aim of this study was to evaluate the concentration and fate of *B. thetaiotaomicron*, throughout a wastewater treatment facility and septage treatment facility in contrast to *E. coli* and enterococci as measure by qPCR and cultivation. In this study, a qPCR assay targeting *uidA* gene for *E. coli* was developed and used, modified from Frahm and Obst (2003). Enterococci qPCR assay focused on the use of primers and probes designed for detection of the 23S rRNA gene sequences (Haugland et al., 2005; Silkie and Nelson, 2009). Samples were collected throughout the wastewater treatment processes as well as from septage before and after treatment. Cultivable counts of *E. coli* and enterococci were compared to the qPCR data generated and an automated DNA extractor was compared to a commercially available QIAmp DNA mini kit (Qiagen, Valencia, CA, USA).

2. Materials and methods

2.1. Samples

a) Wastewater treatment plant samples: Over 200 samples were collected from a municipal wastewater treatment plant, located in East Lansing, Michigan that serves a population of 90 000. The plant receives, on an average basis, a little less than 13.40 MGD (million gallons per day) wastewater inflow. Samples collected from this facility included:
   i) Raw sewage (RS)
   ii) Primary effluent (PE), after the solids have settled
   iii) Secondary effluent (SE pre-chlorination), after activated sludge process and secondary clarification
   iv) Secondary effluent (SE post-chlorination), after disinfection by chlorination, and
   v) Tertiary effluent (TE), effluent from secondary treatment post sodium bi-sulfite dechlorination and filtration through rapid sand filters.

For comparison of auto and manual DNA extraction, raw sewage ($n = 9$), and primary effluent samples ($n = 9$), secondary post-chlorinated effluent ($n = 9$), and tertiary effluents ($n = 9$) were used, which is a total of 36 samples from the wastewater environment.

For assessment of conventional indicators and *B. thetaiotaomicron* (α-mannanase gene) RS, PE, SE and TE samples were collected in triplicates during 18 sampling events from January 2009 to January 2010. During six sampling events within this time frame, secondary treated effluent prior to the chlorination step was also collected in triplicates. During each sampling event, one hundred milliliters of RS and PE, 500 mL of pre-chlorinated SE and 2 L of SE and TE were collected in triplicates. Chlorinated SE and TE samples were collected in bottles pre-loaded with sodium thiosulphate (1 mL of 10% solution) to neutralize any residual chlorine present in the effluents. All samples were transported on ice and processed within 2 h after collection.

b) Septage treatment plant samples: Samples were collected from a septage treatment plant located in Charveloix, Michigan. This treatment plant utilizes an aerobic biological treatment system to treat septage wastes (solid waste from septic tanks) and discharges the treated effluent to a municipal sewer system. Samples were collected during eight sampling events between January 2009 and November 2009. During each event, triplicates of 50 mL raw septage and 500 mL of septage effluent were collected, placed on ice and shipped to Water Quality and Health Laboratory at Michigan State University, East Lansing, MI.