Science of the Total Environment 636 (2018) 717–726

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of nutrient addition on diversity and fate of fecal bacteria

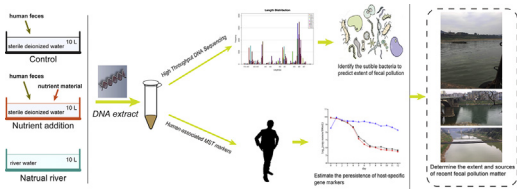
Yang Zhang [a,b], Renren Wu [b,c,*], Yimin Zhang [a,d,**], Guang Wang [b,c], Kaiming Li [b,c]

[a] College of Resources and Environment Engineering, Wuhan University of Technology, Wuhan 430070, PR China
[b] South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou 510530, PR China
[c] The key Laboratory of Water and Air Pollution Control of Guangdong Province, Guangzhou 510530, PR China
[d] College of Resources and Environment Engineering, Wuhan University of Science and Technology, Wuhan 430081, PR China

HIGHLIGHTS

- Competitive effects of nutrition had different effects on fecal bacteria.
- Lactobacillales, Clostridiales, Ruminococcaceae and Bacteroidales could assess recent fecal pollution.
- The human-specific marker decayed similarly across different treatments, and could predict presence of some anaerobic potential-pathogens.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 December 2017
Received in revised form 23 April 2018
Accepted 23 April 2018
Available online xxxx

Editor: Paola Verlicchi

Keywords:
High throughput DNA sequencing
Microbial source tracing
Bacterial community composition
Fecal indicators
Nutrient

ABSTRACT

Understanding the variations in the microorganisms associated with human fecal pollution in different types of water is necessary to manage water quality and predict human health risks. Using an Illumina sequencing method, we investigated variations in the fecal bacteria originating from fresh human feces and their decay trends in nutrient-supplemented water and natural river water. Nutrient addition contributed to the growth of heterotrophic bacteria like *Comamonadaceae*, *Cytophagaceae*, and *Sphingobacteriaceae*, but led to lower concentrations for *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*. This result suggests that the utilization of nutrients by high-activity bacteria may suppress other bacteria via depletion of the available nutrient resources. As we did not observe proliferation of *Bacteroidales*, *Lactobacillales*, *Clostridiales*, or *Ruminococcaceae* in either supplemented or river water, we consider these groups suitable for use as indicators to determine the level of fecal pollution. Moreover, we tested the persistence of *Bacteroidales* markers, including general-*Bacteroidales* marker GenBac and human-specific *Bacteroidales* marker qHS601, by quantitative PCR. We observed similar trends in the decay of the *Bacteroidales* markers GenBac and qHS601 in the nutrient-supplemented water and natural river water, and the high R^2 values of the GenBac ($R^2_{\text{nutrient-supplemented}} = 0.93$, $R^2_{\text{natural river}} = 0.81$) and qHS601 ($R^2_{\text{nutrient-supplemented}} = 0.93$, $R^2_{\text{natural river}} = 0.91$) suggests they are a good fit for the first-order decay model. We also found stronger correlations between the markers and potential pathogenic anaerobes in the different types of water, demonstrating the validity of the use of GenBac and qHS601 from *Bacteroidales* for the identification of human-associated pollution sources.

1. Introduction

Fecal pollution of water sources is a common cause of gastrointestinal disease outbreaks (Birden, 2004). Despite improvements in wastewater treatment plant (WWTP) technologies and management methods, fecal bacteria are frequently present in many water environments (Ahmed et al., 2015). To monitor and manage fecal

* Correspondence to: R. Wu, Ministry of Environmental Protection, South China Institute of Environmental Sciences, Ruihe Road, Luogang District, Guangzhou 510530, PR China.
** Correspondence to: Y. Zhang, College of Resource and Environmental Engineering, Wuhan University of Science and Technology, Wuhan 430081, PR China.
E-mail addresses: wurenren@scies.org (R. Wu), zym126135@126.com (Y. Zhang).

https://doi.org/10.1016/j.scitotenv.2018.04.312
0048-9697/

pollution, fecal indicator bacteria, such as total coliforms, fecal *coliforms*, *Escherichia coli*, and *enterococci*, have been monitored for many years (Lemarchand et al., 2004; Stewart et al., 2006; Stoeckel and Harwood, 2007). In particular, *E. coli* was regarded as the most effective indicators in freshwater due to their high abundance in warm-blooded animals (Villemur et al., 2015). However, these indicators can survive for extended periods outside of their hosts in suitable environments (Ishii et al., 2006; Luo et al., 2011), and cannot offer data related to the source of fecal pollution (Liang et al., 2015). Therefore, microbial source tracking (MST) has been developed as an effective method to determine the level and source of microbial pollution (Mattioli et al., 2016). To date, MST methods, including the use of qPCR of molecular markers, have mainly utilized the 16S rRNA genes of host-specific *Bacteroidales* (Brown et al., 2017). However, the utility of these markers is frequently questioned due to dissimilar sensitivity and specificity performances in different locations (Ebentier et al., 2013; Odagiri et al., 2015). In addition, using these methods, we cannot obtain information about the diversity and abundance of overall fecal bacteria in water environments. Understanding the profile of the fecal bacterial community and identifying potential pathogens in water are important for taking effective management to deal with the fecal pollution and protect human health (Sun et al., 2017).

High-throughput DNA sequencing technology facilitates the rapid acquisition of information on the microbial diversity and abundance, and bacterial community composition (BCC) in feces and natural rivers (Brown et al., 2017). Based on this information, researchers can construct a library of operational taxonomic units (OTUs) that are associated with different animal feces, in order to determine fecal pollution sources in water and evaluate pollution levels. For example, previous studies have identified fecal pollution from various animal sources, such as human, chicken, pig, cow, duck, and sheep, in different rivers using high-throughput DNA sequencing technology (Brown et al., 2017; Sun et al., 2017).

However, the application of this technology to water management is limited by several research gaps. Although high-throughput DNA sequencing technology has been utilized for the characterization of fecal bacterial communities and the determination of potential pathogens in rivers or WWTPs (Cai et al., 2014; Sun et al., 2017; Ye and Zhang, 2011), we lack knowledge about the variations in overall fecal bacteria in conditions with different nutrient levels. Abiotic factors (sun exposure, temperature, and salinity) and biotic factors (competition and predation) commonly influence bacteria (Korajkic et al., 2014; Sokolova et al., 2012; Wanjugi et al., 2016a). However, the nutrient environment may have a dramatic effect on the survival of fecal indicators (Wanjugi et al., 2016a). Many nutrients may enter the water along with the feces or manure liquid, from surface runoff, municipal WWTP effluents, and agricultural fertilizers (Chudoba et al., 2013), and the elevated levels of nutrients may alter the persistence patterns of the bacterial community (Lawes et al., 2016). Several studies have proposed that an abundance of nutrients containing organic carbon and phosphorus in ambient waters positively correlates with some fecal-associated bacteria, such as fecal *coliform* bacteria (Surbeck et al., 2010; Toothman et al., 2009), and that higher nutrient levels may extend the persistence period of *E. coli* (Wanjugi et al., 2016a). Therefore, the decay of bacteria in different types of water must be characterized to facilitate better measurements of fecal pollution levels and sources. Moreover, the relationship between potential pathogens and MST markers is critical for protecting human health, which hinders the development of management techniques for water sources in diverse regions (Ahmed et al., 2016).

In the present study, our overall objective was to (i) evaluate the influence of high nutrient levels on fecal-associated bacteria and MST markers, (ii) study the different persistence patterns of fecal-associated bacteria in manure liquid and natural rivers, and (iii) analyze the relationships between potential pathogens and MST markers. We combined high-throughput DNA sequencing and qPCR methods to characterize the variations in fecal bacterial communities in environments with different nutrient levels, including natural river water. Our approach may facilitate the analysis of the relationships between pathogens and other fecal indicators. Our findings will contribute to the rational design of water-quality monitoring programs.

2. Materials and methods

2.1. Microcosm design

Fresh human fecal samples were collected from 9 volunteers in Guangzhou, Guangdong Province, China. All fecal samples were transported immediately to the lab in sterile tubes on ice (4 °C) for use in the inoculation groups (IGs), including the control (CG) and nutrient-enriched groups (NG). All fecal samples (10 g) were mixed with 4 L sterile deionized water. To diminish interference by particles, we first passed the mixed samples through 37-μm, then 29-μm sterile filters. The mixed samples were divided into 2 equal parts to form the CG and the NG. Diluted LB-Bouillon culture medium (50 mL, 20 g/L) was added to the NG to elevate the levels of nutrients over those in the CG. Each treatment was diluted to 10 L with sterile deionized water. River water was collected from the Pearl River (113° 22′ 48″ N, 23° 6′ 50.4″ W) in Guangzhou. The total length of Guangzhou section of the Pearl River is about 23 km, the average depth is about 5.13 m. The mean annual velocity of runoff is 0.035 m/s, and the mean annual runoff is 88.5 m^3/s. The river passes through the center of Guangzhou and there are many residential and downtown business districts near the sampling site in the river. Therefore, the sampling site was mainly polluted by domestic sewage. The water samples were transported to the lab in a sterile barrel within 2 h, and prepared at the same time as the IGs. Particles in the water samples were removed by filtration through a 29-μm sterile filter, then the samples (10 L) were loaded with fecal material to form the microcosm (river water group, or RG).

Additional CG, NG, and RG treatments were synchronous established for parallel testing. During the experiments, the samples were mixed continuously with magnetic stirrers. All microcosms were maintained at ambient temperature (around 20 °C) and were exposed to diurnal sunlight for 12 days.

2.2. Determination of the physical–chemical index

In order to determine the nutrient levels and the environmental stressors in the samples, we assessed the level of NH_3-N, Chemical Oxygen Demand (COD), turbidity, dissolved oxygen, and pH daily during the experiment. We collected water samples (50 mL) from each group to determine their physical–chemical indexes (Fig. S1).

2.3. DNA extraction and qPCR

Triplicate 100 mL sample volumes from each treatment group were filtered through membranes with 0.22-μm pores to capture all bacteria; the membranes were stored at −20 °C for DNA extraction. We extracted genomic DNA from the water samples using a HiPure Water DNA Kit (Magen), according to the manufacturer's instructions. The extracted DNA solutions (40 μL) were stored at −20 °C until use for high-throughput sequencing or qPCR.

For the detection of fecal indicators, we conducted qPCR in triplicate on a Roche LightCycler® 480 II System. We used 3 fecal indicators, including an *Escherichia coli* indicator (EC23S857), a general-*Bacteroidales* indicator (GenBac) and a human-associated *Bacteroidales* indicator (qHS601). The details of these indicators were shown in Table S1. The qPCR mixtures (20 μL) contained 10 μL 2× Talent qPCR Premix (Tiangen), 2 μL DNA template, 0.8 μL of each primer, and 6.4 μL sterile water. The qPCR assay comprised initial denaturation (95 °C) for 30 min, followed by 40 cycles of denaturation (95 °C) for 5 s, then