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Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation

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ABSTRACT

The dynamics of bacterial and eukaryotic community associated with each step of a water purification plant in China was investigated using 454 pyrosequencing and qPCR based approaches. Analysis of pyrosequencing revealed that a high degree diversity of bacterial and eukaryotic communities is present in the drinking water treatment process before sand filtration. In addition, the microbial compositions of the biofilm in the sand filters and those of the water of the putatively clear tanks were distinct, suggesting that sand filtration and chlorination treatments played primary roles in removing exposed microbial communities. Potential pathogens including *Acinetobacter*, *Clostridium*, *Legionella*, and *Mycobacterium*, co-occurred with protozoa such as Rhizopoda (*Hartmannellidae*), and fungi such as *Penicillium* and *Aspergillus*. Furthermore, this study supported the ideas based on molecular level that biofilm communities were different from those in corresponding water samples, and that the concentrations of *Mycobacterium* spp., *Legionella* spp., and *Naegleria* spp. in the water samples declined with each step of the water treatment process by qPCR. Overall, this study provides the first detailed evaluation of bacterial and eukaryotic diversity at each step of an individual potable water treatment process located in China.

1. Introduction

The supply of safe drinking water to the public is one of the great technological advancements of the 19th century, as well as a major technological challenge (Berry et al., 2006; Kormas et al., 2010; Poitelon et al., 2010). It is well known that microorganisms are widely present in the drinking water treatment system, e.g. in the storage tanks, filter systems, and the interior of pipe walls (Bonadonna et al., 2009). Uncontrolled and excessive microbial growth not only leads to the deterioration in water quality and the associated undesirable tastes, odors, and visual turbidity, but can also cause process malfunctioning such as clogging of filters, bio-fouling and bio-corrosion (Hammes et al., 2008). In particular, the occurrence of pathogens such as enteropathogenic *Escherichia coli* O157, *Helicobacter pylori*, *Legionella pneumophila*, and *Mycobacterium avium* may cause waterborne illnesses (Aw and Rose, 2012). Among these, *Legionella* and *Mycobacterium* were most commonly

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detected in the drinking water systems, although typically present in trace quantities (Vaerewijck et al., 2005; Feazel et al., 2009; Felföldi et al., 2010; Marciano-Cabral et al., 2010). For instance, sequences related to *Legionella* spp. were detected in our previous studies of the biofilms developing on household taps (Lin et al., 2013). Eukaryotic microorganisms such as protozoa, algae, and fungi are also ubiquitous in freshwater environments. The presence of free-living pathogenic amoebae such as *Acanthamoeba*, *Hartmannella*, and *Naegleria* in potable water, increases the possibility of infections (Poitelon et al., 2009b; Marciano-Cabral et al., 2010; Koubar et al., 2011). Surprisingly, the diversity of eukaryotes in drinking water treatment system has received limited attention based on molecular technology.

In general, conventional water treatment system includes coagulation–flocculation, sedimentation, sand filtration, and disinfection processes (Abbaszadegan et al., 1997). However, still comparatively little is known of how each process impacts of the water microbial community and specifically how each step impacts on the species composition and relative abundance particular with regards biofilms. Hence, a thorough understanding of the community structure in biofilms may facilitate the management of drinking water production system and the control of biofilm (Simoes et al., 2007; Elhariry et al., 2012).

To date most of what we know with regards microbial community compositions and their dynamics in the drinking water treatment system has come from studies employing traditional culture methods, community fingerprinting techniques, and low-throughput molecular approaches (Eichler et al., 2006; Roeder et al., 2010). Recent advances in sequencing technology have enabled high-throughput sequencing of microbial communities, e.g. 454 pyrosequencing, and this now enables microbial detailed community structure analysis to be achieved at high taxonomic resolution (Andersson et al., 2010). Pyrosequencing is an ideal tool for investigating microorganisms that constitute the bulk of the community (Gobet et al., 2012). However, it is not well suited for quantitatively tracking key functional members of the community (Zhang et al., 2011). Conversely, quantitative real-time PCR (qPCR) has been demonstrated to be a useful tool for quantitative analysis of specific microorganisms in environmental samples (Brinkman et al., 2003). By integrating high-throughput pyrosequencing with qPCR, the composition of whole microbial communities can be assessed whilst quantifying key functionally important members.

This study is the first to unveil the dynamics of complex microbial communities in water samples undergoing different stages of treatment as well as in biofilms associated with key treatment steps including coagulation–flocculation, sedimentation, sand filtration, and disinfection by 454 pyrosequencing. In addition, qPCR has been used for quantitative analysis of potentially pathogenic microbes and free-living amoebae.

2. Materials and methods

2.1. Drinking water treatment process

The drinking water treatment plant is located in a small town of Hubei province, China. The water source came from the Mingfeng River (Source 1, supplying about 10%) and the East Trunk Canal (Source 2, supplying about 90%), both of which are branches of Yangtze River. This drinking water treatment plant produces nearly 8000 m^3/day of water, and serves almost 40,000 people. In brief, the surface water is first subjected to coagulation in reaction tanks, which then flows into settling ponds, resulting in sedimentation. Water is successively filtered through the sand. To ensure of the treated drinking water is safe to drink, ClO_2 (free residual chlorine: >0.1 mg L^{-1} after 30 min contact time) is added at a minimal residual concentration (>0.02 mg L^{-1}) prior to distribution to households (Fig. 1). Biofilms can grow on the walls of treatment facilities which are composed of cement, resulting in some nuisance, such as increasing the opportunity for pathogens to survive and persist. Hence, the walls of the reaction tanks and settling ponds are regularly cleaned at intervals of 15 days. In addition, the sand filtration is backwashed every 24 h to maintain downstream water quality.

2.2. Sampling and nucleic acid extraction

Biofilm and water samples were taken from each of the water treatment steps, including two raw water source areas, reaction tank, settling pond, sand filtration, and the clear water tank (Fig. 1). Each original sample was a mixture of three independent samples (Zhang et al., 2012). Water samples were collected at each treatment step and processed within 12 h. Microbial biomass was harvested from approximately 3 L water samples using 0.22 μm polycarbonate membranes (47 mm diameter, Millipore, USA) (Poitelon et al., 2009a). Meanwhile, the water in the reaction tanks, settling ponds, and clear water tanks was ordinarily drained in order to provide access to biofilms growing on the walls which were mechanically removed with a sterile scraper (Joh and Lee, 2011). To obtain the biofilm samples from the sand filters, backwashed water sample was collected and then treated as detailed above. For each sampling point, the physico-chemical and microbiological water quality parameters were determined. *In situ* water quality parameters (temperature and pH) were measured using a multi-parameters water quality monitoring sonde (YSI Professional Plus, USA). Other analyses included assessment of turbidity, COD_{Mn}, nitrate, nitrite, ammonia and total plate count which were performed according to Chinese standards (GB: 5749-2006) by Pony Testing International Group (Beijing, China). Samples were stored in an ice box before being transported to the laboratory, and then kept at −70 °C for further usage. Filtered membranes with microbes were cut into pieces with a sterilized cutter, and total DNA extracted using FastDNA SPIN Kit (MP Biomedicals, USA), following the manufacturer's protocol. For biofilm samples (~0.5 g), the same kit was used for extraction of total DNA.

2.3. PCR amplification and 454 Pyrosequencing

Bacterial V1–V3 region of the 16S rRNA gene was amplified using forward primer 8F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 533R: 5′- TTACCGCGGCTGCTGGCAC-3′. For eukaryotes, a PCR strategy was designed to amplify the variable V4 region of the 18S rRNA gene. The universal