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Community shift of biofilms developed in a full-scale drinking water distribution system switching from different water sources

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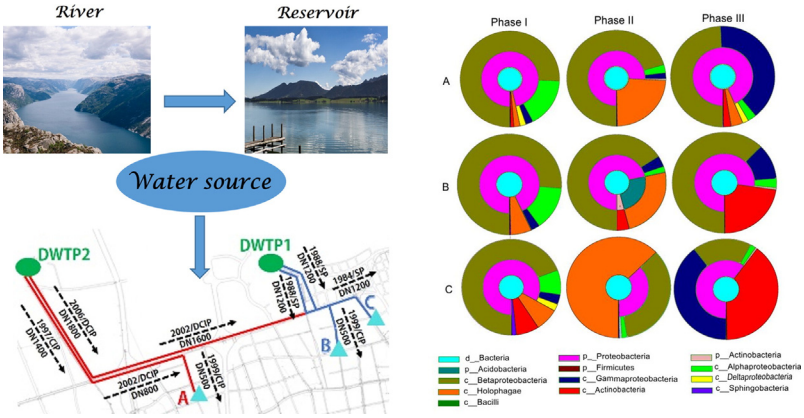
HIGHLIGHTS

- Biofilms sampled from a full-scale drinking water distribution system.
- Drinking water distribution system successively suffered two kinds of water sources with different grades.
- High-throughput sequencing of biofilm community
- Water source switching produced substantial impact on the biofilm community.

GRAPHICAL ABSTRACT



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ABSTRACT

The bacterial community of biofilms in drinking water distribution systems (DWDS) with various water sources has been rarely reported. In this research, biofilms were sampled at three points (A, B, and C) during the river water source phase (phase I), the interim period (phase II) and the reservoir water source phase (phase III), and the biofilm community was determined using the 454-pyrosequencing method. Results showed that microbial diversity declined in phase II but increased in phase III. The primary phylum was Proteobacteria during three phases, while the dominant class at points A and B was Betaproteobacteria (>49%) during all phases, but that changed to Holophagae in phase II (62.7%) and Actinobacteria in phase III (35.6%) for point C, which was closely related to its water quality. More remarkable community shift was found at the genus level. In addition, analysis results showed that water quality could significantly affect microbial diversity together, while the nutrient composition (e.g. C/N ration) of the water environment might determine the microbial community. Furthermore, *Mycobacterium* spp. and *Pseudomonas* spp. were detected in the biofilm, which should give rise to attention. This study revealed that water source switching produced substantial impact on the biofilm community.

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1. Introduction

Human health is closely related to drinking water quality, and access to safe, reliable drinking water in sufficient quantities is fundamental for good health and well-being (Bain et al., 2014). In China, there were 271 drinking water contamination incidents except Tibet autonomous region, of which 63% were classified as biological during 1996–2006 (Zeng et al., 2015). According to the World Health Organization, diarrheal diseases (including cholera) kill 1.8 million people every year, 90% of whom are children under five years of age, and 88% of the cases are attributed to unsafe water supply, inadequate sanitation or hygiene (Lee, 2004). In the United States, decreasing numbers of waterborne outbreaks reported per year since 1982, but an increasing percentage was attributed to drinking water distribution system (DWDS) issues (NRC, 2006). Therefore, investigation of microbes survived in the DWDS is of great significance in terms of drinking water safety.

As one of the world's greatest technological advancements of the 20th century, DWDS play an important role on keeping drinking water safety as it not only significantly affected drinking water quality but also drastically decreased the opportunity of human contraction of infectious waterborne diseases. However, the water quality would fluctuate due to water age, temperature, hydraulic conditions, decaying of residual disinfectants and bacterial regrowth when finished water is transported through a DWDS, whereby the biofilm was gradually developed in the DWDS (Chu et al., 2005; Manuel et al., 2007). Actually, the potential threat to human health derived from biofilms (Flemming and Wingender, 2010) in the DWDS had never been completely eliminated, and microbes in the biofilm have been generally recognized as the primary source of microorganisms in DWDS (Berry et al., 2006), and the potential threat to human health derived from biofilms (Flemming and Wingender, 2010) has never been completely eliminated. It is true that many types of opportunistic pathogens, such as *Legionella pneumophila* (Thomas and Ashbolt, 2011), *Nontuberculosis mycobacteria* (NTM) (Falkinham, 2009), and *Pseudomonas aeruginosa* (von Baum et al., 2010), and *Bacteroides* (Douterelo et al., 2014) have been detected in DWDS. Therefore, gaining insight into the bacterial community structure of biofilms was of great significance.

Besides DWDS, high-quality water sources also play an important role on guaranteeing drinking-water safety. In China, many drinking water treatment plants (DWTP) are changing water sources due to the population increase in cities as well as the rapid development of economy. Water source switching, however, could cause striking effects on the water quality of the DWDS. In 2008, Beijing attempted to replace its local water source with a long-distance water source transported from a neighboring province. Nevertheless, heavy red water occurred in the pipes transporting ground water when 80% of local source water was replaced. This problem was studied subsequently and the results showed that corrosion products contributed by specific bacteria were responsible for this issue (Zhu et al., 2014). Although the reasons of heavy red water was clear, the effects of water source switching on microbial community structure of biofilms need to be further studied.

Due to the difficulty of sampling, research on biofilms in DWDS generally focused on simulating DWDS (Douterelo et al., 2013; Wang et al., 2012b; Zhu et al., 2014). However, various factors (Janjaroen et al., 2013) affecting the microbial community of biofilms in the practical DWDS were difficult to simulate comprehensively. Consequently investigation of practical DWDS was worth conducting. Both culture-based and culture-independent methods have been adopted in previous studies, but growing evidence has shown that culture-independent methods (i.e., high-throughput sequencing) can be a powerful tool for a comprehensive overview of microbial communities in various environmental samples (Huang et al., 2014; Li et al., 2015; Ye and Zhang, 2011), so culture-independent method was selected in our research.

Our objective of this study was to seek information about the microbial community shift of pipe biofilms under the condition of various types of surface source water of varying quality. The full-scale DWDS studied in this research was characterized by multiple pipe materials and a long running time. Based on the growing pattern of biofilms and a strict sampling schedule, this study was conducted over the course of three years. The microbial community of biofilms was determined by using the 454-pyrosequencing method and was compared between different phases. This research provided insights into the influence of water source switching on the microbial community of pipe biofilms.

2. Materials and methods

2.1. Description of water sources and water treatment plants

The relative positions of DWTP and sampling points were illustrated in Fig. 1. Based on water sources, water treatment processes (Table 1) and water quality (Table S1), our research covered three phases. During phase I (03/2012–02/2013), the DWDS was supplied by DWTP 1 and DWTP 2, which received water from river water of grade III-V. Moreover, DWTP1 stopped supplying B and C at the end of this phase and they were supplied by DWTP 2 simultaneously. At the beginning of phase II, the water source supplying DWTP2 was switched from river water of grades III-V to reservoir water of grade II at the end of phase I. Water quality of bulk water samples in the practical DWDS varied to the largest extent during this phase, especially for total organic carbon (TOC) and conductivity (Table S1). Therefore, phase II (03/2013–02/2014) was regarded as the interim period. During phase III (03/2014–02/2015), the DWDS only was supplied by DWTP2 receiving water from reservoir water of grade II. It should be noted that the water treatment techniques applied in DWTP1 and DWTP 2 were different. DWTP2 added ozonation and granular activated carbon filtration before the disinfection process on the base of the conventional treatment process (Coagulation + Sedimentation + Filtration + Disinfection), whereas DWTP1 employed only the conventional treatment process. Considering these conditions, the effects of water source switching on the diversity and relative abundance of the microbial community in the biofilm developed in the practical DWDS was primarily discussed in this research, whereas the water treatment process was also considered to determine the difference between phase I and phase II.

2.2. Biofilm sampling device

Three sampling points (A, B, C) were selected in the practical DWDS of city Y in south China (Fig. 1). To facilitate biofilm sampling in the full-scale DWDS, a new valve well ($L \times B \times H = 3\ m \times 2\ m \times 1.2\ m$) equipped with biofilm sampling device was built at each sampling point. The schematic diagram of biofilm sampling device was shown in Fig. 2(a). In brief, a main pipe, a bypass pipe, three valves and one tap were installed in the valve well. Two valves were situated at the inlet (V_i) and outlet (V_o) of the main pipe, and the third valve (V_m) was equipped at the bypass pipe and six coupons and a tap were installed between V_i and V_o of the main pipe. The coupon [Fig. 2(b)] surface was cement mortar material with an area of about 2.9 cm^2. Biofilms developed when V_m was closed and V_i and V_o were kept opening.

2.3. Sampling

Tap water and biofilms were collected from each sampling point. Firstly, the tap was opened about two minutes to drain the stagnant water in the pipe, then the residual chlorine of water sample was determined on the spot (PC II, HACH, USA) and the bulk water (2 L) was collected subsequently. The tap was then sterilized with lighted alcohol cotton, and bulk water (1 L) was sampled again in a sterilized glass bottle to determine the heterotrophic plate counts (HPC). Upon arrival at the lab, bulk water was used to analyze pH, conductivity (PE20, METTLER, Switzerland), turbidity (2100Q, HACH, USA), and TOC (TOC4100, Shimadzu, Japan), and ammonia, nitrate, and nitrite were