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# Spatiotemporal changes in bacterial community and microbial activity in a full-scale drinking water treatment plant

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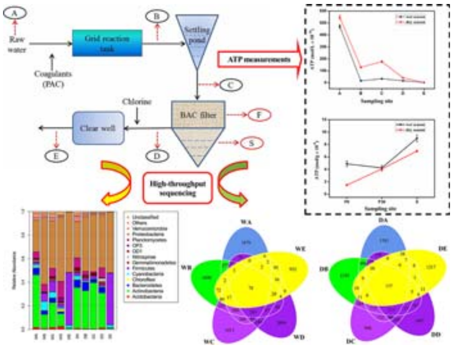
## HIGHLIGHTS

- Spatiotemporal variation in bacterial communities was investigated in a full-scale DWTP.
- Combination of Illumina Hiseq sequencing analyses and cultivation-based techniques was adopted.
- The bacterial communities present in finished water exhibited high stability.
- A substantial core microbiome persisted through the treatment process.
- *Mycobacterium* sp. and *Blastococcus* sp., were isolated from finished water during the dry season.

## GRAPHICAL ABSTRACT



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## ABSTRACT

To gain insight into the bacterial dynamics present in drinking water treatment (DWT) systems, the microbial community and activity in a full-scale DWT plant (DWTP) in Guangzhou, South China, were investigated using Illumina Hiseq sequencing analyses combined with cultivation-based techniques during the wet and dry seasons. Illumina sequencing analysis of 16S rRNA genes revealed a large shift in the proportion of *Actinobacteria*, *Proteobacteria* and *Firmicutes* during the treatment process, with the proportion of *Actinobacteria* decreased sharply, whereas that of *Proteobacteria* and *Firmicutes* increased and predominated in treated water. Both microbial activity and bacterial diversity during the treatment process showed obvious spatial variation, with higher levels observed during the dry season and lower levels during the wet season. Clustering analysis and principal component analysis indicated dramatic shifts in the bacterial community after chlorination, suggesting that chlorination was highly effective at influencing the bacterial community. The bacterial community structure of finished water primarily comprised *Pseudomonas*, *Citrobacter*, and *Acinetobacter*, and interestingly showed high similarity to biofilms on granular activated carbon. Additionally, the abundance of bacterial communities was relatively stable in finished water and did not change with the season. A large number of unique operational taxonomic units were shared during treatment steps, indicating the presence of a diverse core microbiome throughout the treatment process. Opportunistic pathogens, including *Pseudomonas*, *Acinetobacter*, *Citrobacter*, *Mycobacterium*, *Salmonella*,

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*Abbreviations:* ATP, adenosine triphosphate; AWCD, average well-color development; BAC, biological activated carbon; CCA, canonical correspondence analysis; COD, chemical oxygen demand; DO, dissolved oxygen; DWT, drinking water treatment; DWDS, drinking water distribution system; DWTP, drinking water treatment plant; DWTS, drinking water treatment system; GAC, granular activated carbon; HPC, heterotrophic bacterial counts; OTU, operational taxonomic unit; PCA, principal component analysis; PCR, polymerase chain reaction; TOC, total organic carbon.

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*Staphylococcus*, *Legionella*, *Streptococcus* and *Enterococcus*, were detected in water including finished water, suggesting a potential threat to drinking-water safety. We also detected bacteria isolated from each treatment step using the pure-culture method. In particular, two isolates, identified as *Mycobacterium* sp. and *Blastococcus* sp., which belong to the phylum *Actinobacteria*, were obtained from finished water during the dry season. Together, these results provided evidence of spatial and temporal variations in DWTPs and contributed to the beneficial manipulation of the drinking water microbiome.

## 1. Introduction

Bacterial communities are ubiquitous throughout drinking water treatment systems (DWTSs). They can play an important role in removing pollutants (e.g., organic carbon consumption in filtration systems) (Albers et al., 2015), but can also lead to water-quality deterioration or biological-safety problems (e.g., leakage and regrowth of opportunistic pathogens in treated water) (Gomezsmith et al., 2016; King et al., 2016). Therefore, bacterial communities in DWTSs are closely related to water quality and public health, suggesting that a comprehensive and thorough understanding of microbial community dynamics in DWTSs is crucial for security of water-quality and development of effective control strategies.

Several previous studies investigated the spatial heterogeneity of microbial quantity and communities in DWT plants (DWTPs) (Li et al., 2017; Lin et al., 2017; Pinto et al., 2014), although seasonal effects on bacterial communities remain under debate. For example, a large temporal shift in bacterial community structure was documented in DWTPs (Pinto et al., 2014); however, Li et al. (2017) reported that the primary dynamic pattern of variations in bacterial communities occurred during the treatment process rather than through the temporal fluctuations. A previous study also suggested that substantial seasonal dynamics in bacterial communities were observed in cold drinking water, whereas the hot-water bacterial communites remained stable across seasons (Henne et al., 2013). Other studies showed that spatial and temporal microbial dynamics during DWTPs are likely influenced by the specific characteristics of each system, including raw water characteristics, treatment processes and temperature (Hammes et al., 2008; Henne et al., 2013; Prest et al., 2016; Sun et al., 2014). However, most of earlier studies involving drinking water microbiology were undertaken in isolated model systems without establishing associations with realistic environmental parameters (Kwon et al., 2011; Lehtola et al., 2004; Liao et al., 2014). As a result, we have only a limited vision of the characteristics of bacteria community variation and its influential factors in DWTPs under realistic conditions.

Currently, >95% of waterworks in China continue to use conventional water treatment processes, including coagulation-flocculation, sedimentation, filtration, and disinfection, to purify drinking water (Feng et al., 2012). Although some studies have focused on microbial ecology present in the conventional water treatment processes, there remains a lack of information on the spatiotemporal microbial variations present during multistep treatment processes. Moreover, the dynamics of bacterial communities in dual-medium filters have received limited attention. Most studies have been based on the use of a single method, such as the pure-cultures method or 454 pyrosequencing, unable to represent the complete picture of microbial activity and bacterial communities in DWTPs. Therefore, actual microbial activity and bacterial communities need to be explored in more detail by high-throughput sequencing analyses combined with cultivation-based techniques. Moreover, the impact of treatment processes and how they shape the bacterial community structure in finished water and the presence of common microbial patterns associated with seasonal variations across different locations remain unknown. This information is vital for the management of DWTSs.

In this study, the microbial community diversity in a full-scale DWTP in Guangzhou, South China, was investigated by Illumina Hiseq sequencing analyses combined with cultivation-based techniques during the wet and dry seasons. This studied system is representative of water utility systems, with treatment processes including coagulation, sedimentation, filtration, and disinfection. The primary objectives of this study were to investigate the spatiotemporal variations in microbial biomass, diversity and community structure, identify persistent bacteria throughout the treatment process, and determine which treatment processes shape bacterial community structure in finished water. In addition to providing insight into the spatiotemporal microbial dynamics in DWTPs, the results of this study provide a data basis for practical applications.

## 2. Materials and methods

### 2.1. The full-scale DWTP and its treatment processes

The DWTP is located in Guangdong province, southern China and its raw water originates from the Beijiang River, which is a branch of the Pearl River. This DWTP has a daily water supply of 560, 000 tons and serves almost 1 million people. The treatment processes include coagulation, sedimentation, dual-media filtration, and chlorination (Fig. 1). Specifically, there is a 30-cm depth of granular activated carbon (GAC), with a ~80-cm layer of quartz sand on the bottom of the dual-media filter. The biological activated carbon (BAC) filtration is backwashed every 36 h to guarantee downstream water quality. Free chlorine is added to the BAC effluent at a concentration range of 1.5 mg/L to 2.0 mg/L.

### 2.2. Sample collection and processing

The DWTS was operational for many years prior to samples collection. Samples were collected during the wet and dry seasons in 2016, and water samples, including raw water (A), reactor-tank effluent (B), settling-pond effluent (C), BAC-filter effluent (D), and finished water (E), were collected into sterile containers during each of the water treatment steps. Moreover, GAC samples (F) were obtained from filters located at different depths of the activated carbon-filter layer (0 cm and 30 cm), and sand samples (S) were collected from the upper part of the quartz-sand layer (30 cm below the surface of the GAC layer). All the biofilm samples were sampled after backwashing. For each sampling

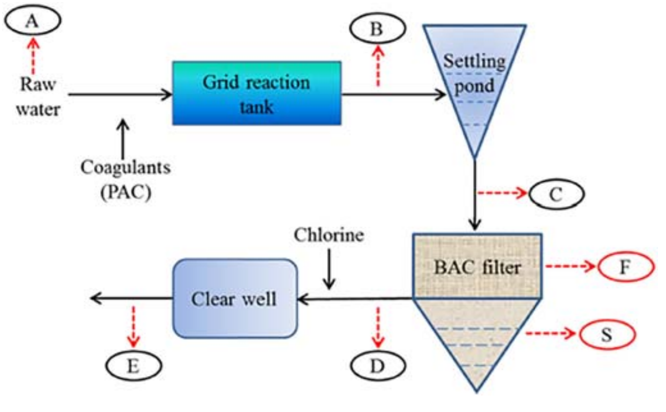

**Fig. 1.** Schematic diagram of the drinking water treatment process. A: raw water; B: grid reaction tank effluent; C: settling pond effluent; D: BAC filter effluent; E: finished water; F: GAC particles; S: quartz sand.