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Antibiotic resistome promotion in drinking water during biological activated carbon treatment: Is it influenced by quorum sensing?

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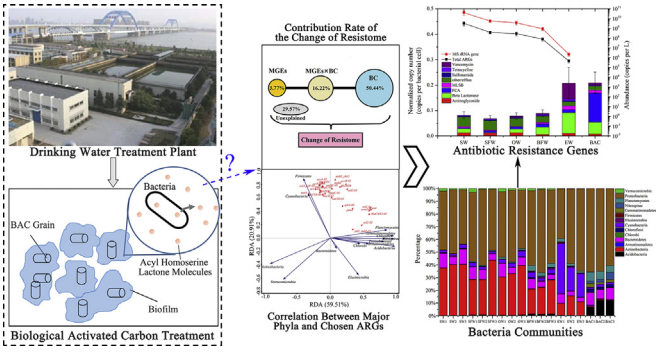
HIGHLIGHTS

- Antibiotic resistome promotion was observed after drinking water biological activated carbon treatment.
- 29 antibiotic resistance genes were identified as biofilm source and persisted in drinking water.
- Shift of bacterial communities was identified as key factor driving ARGs alteration.
- Acyl Homoserine Lactones promoted horizontal gene transfer in intragenus mating systems.

GRAPHICAL ABSTRACT



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ABSTRACT

The contamination of antibiotic resistance genes (ARGs) in drinking water may pose a direct threat to human health. This study applied high-throughput qPCR and sequencing to investigate the dynamics of ARGs and bacterial communities during the advanced treatment of drinking water using biological activated carbon. The promotion of ARGs was observed, and the normalized copy number of ARGs increased significantly after BAC treatment, raising the number of detected ARGs from 84 to 159. Twenty-nine ARGs were identified as biofilm-influencing sources in the BAC, and they persisted after chlorination. The shift of bacterial communities primarily had effects on the changes in resistome. Firmicutes, Cyanobacteria were related to persistent ARGs mostly in the BAC biofilm. Meanwhile, the Acyl-Homoserine Lactones (AHLs), quorum sensing molecules, and bacteria that produced AHLs were identified to understand the promotion of ARGs. The isolated AHL-producing bacteria belonged to the Proteobacteria, Firmicutes and Bacteroidetes phyla. Six detectable AHLs had an influence on plasmid-based horizontal gene transfer in the intragenus mating systems, indicating that the dynamics of ARGs were strongly affected by quorum sensing between specific bacteria in the biofilm. These results provide new insight into the mechanism of antibiotic resistome promotion in BAC biofilms.

1. Introduction

The emergence and spread of antibiotic resistant bacteria (ARB) are pressing public health problems worldwide, and antibiotic resistant genes (ARGs) have been recognized as emerging microbial contaminants (Levy and Marshall, 2004; Pruden et al., 2006). Increasingly, studies have shown that ARGs occur and spread among the edatope, aquatic and atmospheric environments (Zhu et al., 2013; Pal et al., 2016; L. Xu et al., 2016; Y. Xu et al., 2016). Moreover, the aquatic ecosystem is a significant reservoir for ARB and ARGs (Zhang et al., 2009). Due to the high prevalence of ARGs in surface and reservoir waters, the antibiotic

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resistance pollution of drinking water poses an intractable health issue for safe treatment (Schwartz et al., 2003; Jiang et al., 2013; Fernando et al., 2016). Drinking-water treatment plants (DWTPs) and distribution systems are reported to lack the ability to control ARG pollution, and tap water has been demonstrated to contain ARGs (Shi et al., 2013; Xi et al., 2009; Narciso-da-Rocha et al., 2013).

As an advanced drinking-water treatment technique, biological activated carbon (BAC) treatment can remove and biodegrade waterborne organic substances and pollutants, guaranteeing effluent quality (Simpson, 2008). However, according to several investigations in DWTPs, the diversity and abundance of ARGs can increase even after BAC treatment (Guo et al., 2014; Bai et al., 2015; L. Xu et al., 2016; Y. Xu et al., 2016). The biofilm generated on the surface of activated carbon during BAC processing might result in high concentrations of biomass and antibiotic contaminants in the effluent, and this biofilm may be an optimum site for ARG transfer in drinking water (Farkas et al., 2013; Bergeron et al., 2015). Usually, horizontal gene transfer (HGT) is the primary way of ARG transfer, which is mediated by mobile gene elements (MGEs) (Gyles and Boerlin, 2014). However, the specific modalities in which HGT works on biofilm remains unclear.

Biofilm formation is correlated with quorum sensing (QS), a communication system used by bacteria. Acyl-Homoserine Lactones (AHLs) are an important type of autoinducers functioning as QS signal molecules in the aquatic environment (Shrout and Nerenberg, 2012). QS can influence bacterial antibiotic resistance by regulating the expression of associated membrane proteins and the interference of general activity of QS might militate against ARGs in the environment (Jakobsen et al., 2012). Drinking-water biofilm is altered by AHLs secreting bacteria including *Klebsiella* and *Paenibacillus*, and those bacteria are reported as significant in the composition of BAC biofilm (Wu et al., 2014; Kim et al., 2014). Moreover, the association of antibiotic resistome and bacterial communities has been proven to a degree (Su et al., 2015; Jia et al., 2015). A key to understanding ARGs' HGT in biofilms can be the relationship among ARGs, AHLs and bacterial communities.

By combining high-throughput quantitative PCR and 16S rRNA gene high-throughput sequencing, this study characterized the effect of drinking-water BAC treatment on ARGs and bacterial communities. Based on those reevaluations, this study discusses the existence of AHLs and their producers in BAC biofilm and the influence of AHLs on the horizontal conjugative transfer of ARGs. The results may help explain antibiotic resistome promotion during BAC treatment and reveal the potential functional mechanism of quorum sensing on ARG transfer.

2. Materials and methods

2.1. Sample collection and DNA extraction

Water samples of source water (SW), sand-filtered water (SFW), ozone water (OW), BAC-filtered water (BFW), effluent water (EW) and BAC biofilm sample (BAC) from one representative DWTP were collected during June 2016 in Hangzhou city, eastern China (Fig. S1). The water supply capacity was 100,000 m^3/d, and normal water quality parameters within the DWTP are shown in Table S1. Samples were collected three times according to the backwash cycle in three replications. Each collection to obtain BAC biofilm samples occurred at the end of a backwash cycle (June 2nd, 13th, and 24th). Then, 20 g activated carbon was added to 100 mL sterile normal saline and was shaken ultrasonically for 20 min to suspend the biofilm's DNA in water (Buesing and Gessner, 2002). The genomic DNA of the water samples were extracted using FastDNA SPIN Kit (MP Bio, USA) according to the manufacturers' instructions. The concentration of purified DNA was quantified spectrophotometrically (NanoDrop ND-2000c, Thermo, USA) and stored at −85 °C until analysis.

2.2. High-throughput quantitative PCR (HT-qPCR)

The SmartChip Real-time PCR System (Warfergen Inc., USA) was used to perform high-throughput quantitative PCR (HT-qPCR) as previously described (Wang et al., 2014; Ouyang et al., 2015). There were 295 primer sets targeting 285 ARGs, 8 transposases, 1 integron and the 16S rRNA gene (Table S2). The results of the HT-qPCR were analyzed using SmartChip qPCR software (V2.7.0.1). For each primer set, an amplification efficiency beyond the range of 90%–110% was discarded, and amplification was confirmed with more than two positive replicates. The relative copy number of ARGs and MGEs was calculated by normalizing each gene's copy number and obtaining its ratio to 16S rRNA's normalized copy number (Looft et al., 2012; Schmittgen and Livak, 2008). The abundance of ARGs and MGEs was the product of the relative copy number and 16S rRNA's absolute copy number, which was determined by qPCR mentioned in previous research (Chen and Zhang, 2013).

2.3. 16S rRNA gene high-throughput sequencing

Bacterial community compositions in each sample were determined by performing 16S rRNA gene high-throughput sequencing on an Ion Torrent platform at the Analysis Center of Agrobiology and Environmental Sciences of Zhejiang University (Hangzhou, China). Barcoded PCR was conducted with universal primer pairs 515F (5′ - GTGCCAGC MGCCGCGG - 3′) and 907R (5′ - CCGTCAATTCMTTTRAGTTT - 3′) targeting the V4–V5 hypervariable region of the 16S rRNA gene (Turner et al., 1999). All sequences of each sample were trimmed for primers, barcodes and adaptor sequences by removing the low-quality reads. Sequence results were clustered into operation taxonomy units (OTUs) using UCLUST at the 97% similarity level. One representative sequence of one OTU was classified phylogenetically and assigned to a taxonomic identity using the Ribosomal Database Project (RDP) with a bootstrap confidence of 60 (Huang et al., 2015). The alpha diversity metrics were calculated using OTU numbers, and the beta diversity was estimated using phylum-level abundance. The difference among microbial communities was visualized by principal coordinate analysis (PCoA) in quantitative insights into microbial ecology (QIIME software V1.3.0).

2.4. AHLs and AHL-produced bacteria identification

Liquid–liquid extraction was used to extract the AHLs in each BAC biofilm sample (Buchholtz et al., 2006). First, 100 g activated carbon was added to 100 mL acidified ethyl acetate (0.5% acetic acid). The mixture was ultrasonically shaken for 20 min and then shaken on a shaking table at 180 rpm for 40 min with three replications. The supernatant ethyl acetate extract was collected using a decompressing filter and concentrated by rotary evaporation at 37 °C. The residues were resuspended in 0.5 mL methanol for analysis by HPLC-MS/MS (Agilent 6460, USA) (Wang et al., 2011). In this study, 12 types of AHLs and their characteristics were detected (Table S3). The AHL standards (Sigma, USA) were dissolved and diluted with methanol. All samples were applied to the HPLC column (Zorbax XDB C18, 2.1 × 150 mm, 3.5 μm) at a flow rate of 0.3 mL/min. The LC retention time, appearance of precursor ion *m/z* and two transition ions were the AHL characteristics used for the identification and quantification of each AHL.

To screen an AHL producer, 20 g of the BAC biofilm sample was processed by ultrasound for 20 min to obtain bacterial suspensions. After a series of bacterial suspension dilutions were obtained, 100 μL of the dilutions was spread in triplicate on R2A agar plates and then incubated at 25 °C for 7 d (Reasoner and Geldreich, 1985). Subsequently, the isolated strains were separated and the cultures purified. The AHL production of all target-separated bacteria was qualitatively detected using the report bacteria KYC55 test (Wu et al., 2014). *Agrobacterium tumefaciens* KYC55 was the indicator microorganism used for the detection of AHLs (Zhu et al., 2003). The isolated bacteria with specific chromogenic results