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Metagenomic insights into ultraviolet disinfection effects on antibiotic resistome in biologically treated wastewater

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ABSTRACT

High-throughput sequencing-based metagenomic approaches were used to comprehensively investigate ultraviolet effects on the microbial community structure, and diversity and abundance of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in biologically treated wastewater. After ultraviolet radiation, some dominant genera, like *Aeromonas* and *Halomonas*, in the wastewater almost disappeared, while the relative abundance of some minor genera including *Pseudomonas* and *Bacillus* increased dozens of times. Metagenomic analysis showed that 159 ARGs within 14 types were detectable in the samples, and the radiation at 500 mJ/cm^2 obviously increased their total relative abundance from 31.68 ppm to 190.78 ppm, which was supported by quantitative real time PCR. As the dominant persistent ARGs, multidrug resistance genes carried by *Pseudomonas* and bacitracin resistance gene *bac*A carried by *Bacillus* mainly contributed to the ARGs abundance increase. Bacterial community shift and MGEs replication induced by the radiation might drive the resistome alteration. The findings may shed new light on the mechanism behind the ultraviolet radiation effects on antibiotic resistance in wastewater.

1. Introduction

The misuse or overuse of antibiotics for human health protection, animal breading and aquaculture has caused release of amounts of residual antibiotics into surface waters (Kummerer, 2009), groundwater (Luo et al., 2011) and sediments (Kim and Carlson, 2007). This has resulted in wide distribution of the emerging antibiotic resistant bacteria (ARB) and resistance genes (ARGs) in the environment (Kim and Carlson, 2007). The spread of the ARB and ARGs may lead to antibiotic resistance in human infections and pose serious threats to public health (Pruden, 2013).

Growing evidence has shown that sewage is an important reservoir for ARB and ARGs (Munir et al., 2011). Although ARB can be partially eliminated by conventional sewage treatment processes including activated sludge process and oxidation ditch (Da Costa et al., 2006; Dodd, 2012), various ARB and ARGs are still present in the biologically treated wastewater (Pruden et al., 2006). To ensure microbial safety, some disinfection technologies, e.g. chlorine (Li and Zhang, 2013), ozone (Luddeke et al., 2015) and ultraviolet (UV) (Guo et al., 2013), have been applied in sewage treatment plants (STPs) to disinfect the effluent. UV is considered as a promising approach for wastewater disinfection since UV-C light at a wavelength of 254 nm has a germicidal effect on most bacteria (Hijnen et al., 2006). It was reported that UV disinfection could damage some ARGs in bacterial cells (McKinney and Pruden, 2012) and reduce their abundance in wastewater (Munir et al., 2011). UV radiation is also known to accelerate horizontal gene transfer (HGT) (Aminov 2011) by mobile genetic elements (MGEs), which is considered as the main factor driving resistome alteration in drinking water (Chao et al., 2013), ocean sediments (Chen et al., 2013) and swine manure (Zhu et al., 2013).

Some previous studies have been conducted to investigate the effects of UV disinfection on specific ARB and ARGs. For example, Guo et al. (2013) studied the selectivity of UV treatment on

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antibiotic-resistant heterotrophic bacteria. Biswal et al. (2014) focused their study on the virulence and antimicrobial resistance genes in uropathogenic *Escherichia coli*. McKinney and Pruden (2012) analyzed the potential damage of UV disinfection on ARGs both in extracellular form and present within host ARB. However, few studies have been conducted to comprehensively investigate the UV disinfection effects on the bacterial community structure and ARG diversity and abundance. Also, research is lacking to reveal the underlying relationships among the bacterial community shift, antibiotic resistance alteration and MGE changes.

Furthermore, previous studies mainly used conventional molecular methods, e.g. polymerase chain reaction-denaturing gradient gel electrophoresis to profile microbial community (Demba Diallo et al., 2004) and quantitative real-time PCR (q-PCR) was used to quantify specific ARGs in environments (Pei et al., 2006). These low-throughput methods cannot comprehensively characterize the microbial community and targeted only a limited number of resistance genes due to the limited availability of PCR primers. Recent studies have shown that the next generation sequencing technologies have high sequencing depth and accuracy to cover complex bacterial communities (Chao et al., 2013; Ye et al., 2012). 16S rRNA gene amplicon sequencing has been widely used to characterize microbial community structure (Kozich et al., 2013). Annotation of shotgun reads generated by Illumina sequencing is considered as a reliable method for analyzing ARGs in complicated environmental samples including drinking water (Chao et al., 2013), deep ocean sediments (Chen et al., 2013) and activated sludge (Yang et al., 2013).

In this study, high-throughput sequencing-based metagenomic approaches and q-PCR were applied to comprehensively investigate the UV effects on the ARGs and MGEs diversity and abundance as well as the microbial community structure in biologically treated wastewater. We also characterized the roles of microbial community and MGEs in the changes in the ARGs diversity and abundance under the UV radiation. The findings of this study may help to understand UV effects on microbial antibiotic resistance and the underlying molecular ecological mechanisms.

2. Materials and methods

2.1. UV disinfection, bacterial viability and DNA extraction

Treated wastewater samples were collected from the outlet of an oxidation ditch of Dachang Municipal Wastewater Treatment Plant (Nanjing, China) without disinfection facilities. After being collected in sterile polyethylene containers, the water samples were immediately stored on ice and transported to the Environmental Biotechnology Laboratory of Nanjing University, and the disinfection process started within 4 h after the sampling. The dissolved organic carbon, chemical oxygen demand, total nitrogen and ammonia nitrogen concentration of the water samples were 5.91 ± 0.62 mg/L, 39.10 ± 2.31 mg/L, 9.73 ± 1.21 mg/L and 1.56 ± 0.16 mg/L, respectively. The pH value and the absorbance of the water at 254 nm were 7.0–7.2 and 0.13, respectively.

The radius of the container was 5.5 cm and the height of the container was 20.0 cm. 1.8 L of water sample was added into the reactor (a cylinder made of Plexiglas) and mixed gently by a magnetic stir bar at 300 rpm. A quartz sleeve with a low-pressure (30 W) mercury vapor UV-C lamp (254 nm, Philips, USA) inside was placed in the center of the reactor. The radiance value was 16.0 mW/cm^2 measured by radiometry equipped with a UV_{254} detector (Beijing Normal University Optical Instrument Factory). The value of UV dose was calculated by the formula $D = 10.0\ t$, where D represents the UV dose (mJ/cm^2) and t indicates the exposure time (s). More details about the UV dose calculation were provided in the Methods section of the Supplementary Materials. Before disinfection experiment, the apparatus was warmed up for 5 min to ensure stable UV radiation fluence. In China, the fluences of 20–80 mJ/cm^2 are generally required for disinfection of the secondary effluent of STPs (Guo et al., 2013), while USA requires the minimum UV dose of 160 J/m^2 for treatment of drinking water delivered to households (Linden et al., 2002). Thus, in this study we chose the UV radiation time of 0, 5, 50 and 200 s, with the corresponding UV fluences at 0, 50, 500 and 2000 mJ/cm^2, respectively (the samples were named as RW, UV50, UV500 and UV2000, respectively). The water samples were placed in the brown bottle and the radiation experiment was conducted in the dark at room temperature (25 ± 2 °C). After exposure, each sample was immediately concentrated for DNA extraction. For each UV dose, triplicate DNA samples were prepared for the following analysis. The detailed procedures are shown in the Methods section of the Supplementary Materials.

To investigate the photoreactivation effect, the remaining samples were transferred to transparent bottles, which were placed under the natural light for 4 h before DNA extraction (Linden et al., 2002), and the photoreactivation samples were named as UV50-4 h, UV500-4 h and UV2000-4 h.

Bacterial viabilities in all the samples were determined immediately after radiation and photoreactivation. The Live/Dead BacLight™ bacterial viability stain was used according to the manufacturer's instructions (Life Technologies, Carlsbad, CA, U.S.). SYTO® 9 green-fluorescent nucleic acid stains and the red-fluorescent propidium iodide were used to determine the ratio of activated and inactivated bacteria in the water samples (Craik et al., 2000). The detailed procedures are shown in the Methods section of the Supplementary Materials.

For DNA extraction, the irradiated and photoreactivated wastewater samples were concentrated through 0.22-μm-pore-size membrane (FDJHJS, Jinteng, China) by a vacuum filtration apparatus until the filter clogged. Total genomic DNA extraction was performed using the FastDNA@ Spin Kit for Soil (MP Biomedicals, CA, USA) following the recommended protocol. The DNA concentration and quality were measured by microspectrophotometry (NanoDrop®ND-2000, NanoDrop Technologies, Wilmington, DE).

2.2. Illumina HiSeq sequencing and metagenomic analysis

Equal mass of metagenomic DNA from each of the triplicate samples at each fluence was mixed together to minimize the potential variation. The mixed DNA samples (10 μg each) were sent out to Jiangsu Zhongyijinda Analytical & Testing Co., Ltd for Illumina high-throughput sequencing using HiSeq 2500 platform (Illumina, USA). The shotgun sequencing strategy of 101 PE (Paired-End sequencing, 101-bp reads) was applied to generate raw sequences. The raw sequences contaminated by adapter or containing three or more unknown nucleotides ('N') were firstly removed. A strict filtration strategy was then conducted using Galaxy (https://usegalaxy.org/). 'FASTQ Groomer' tool was used to convert quality formats, and 'Filter by quality' tool was then used to remove low quality sequences to ensure that more than 75% bases of each filtered read possessed Illumina quality scores greater than 30. Furthermore, quality-filtered 'N' was simultaneously removed. The metagenomics data were submitted to National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra) under accession number of SRA281039.

In order to explore the distribution patterns of ARGs and MGEs, the quality-filtered Illumina reads of different wastewater samples were separately aligned using BLAST software to the databases