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Metagenomic analysis of bacterial community composition and antibiotic resistance genes in a wastewater treatment plant and its receiving surface water

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ABSTRACT

The presence of pathogenic bacteria and the dissemination of antibiotic resistance genes (ARGs) may pose big risks to the rivers that receive the effluent from municipal wastewater treatment plants (WWTPs). In this study, we investigated the changes of bacterial community and ARGs along treatment processes of one WWTP, and examined the effects of the effluent discharge on the bacterial community and ARGs in the receiving river. Pyrosequencing was applied to reveal bacterial community composition including potential bacterial pathogen, and Illumina high-throughput sequencing was used for profiling ARGs. The results showed that the WWTP had good removal efficiency on potential pathogenic bacteria (especially *Arcobacter butzleri*) and ARGs. Moreover, the bacterial communities of downstream and upstream of the river showed no significant difference. However, the increase in the abundance of potential pathogens and ARGs at effluent outfall was observed, indicating that WWTP effluent might contribute to the dissemination of potential pathogenic bacteria and ARGs in the receiving river.

1. Introduction

Streams and rivers are important to human culture, welfare and development, but they have suffered from anthropogenic pollution for a long time, owing to the discharge of the untreated or crudely treated wastewater effluent from wastewater treatment plants (WWTPs) (Guecker et al., 2011). The WWTP effluent has the potential to pose a threat to the health of the receiving water bodies and alter the physico-chemical environment and microbial composition (Drury et al., 2013). Previous studies have documented that the WWTPs effluent could cause high polluting power (BOD, TOC, COD and suspended solids), nutrient pollution (phosphate, nitrate and ammonium) (Waiser et al., 2011; Gucker et al., 2006), and temporary oxygen deficits (Rueda et al., 2002), which may have detrimental impacts on ecological communities and functions of the aquatic ecosystems. To assess the impacts of effluent from a modern WWTP on the receiving river health, microbiota could be used as informative indicators of the health of aquatic ecosystems, not only because the microbes are ubiquitously and abundantly present in the aquatic ecosystems (Lawrence et al., 2005), but also because the microbial communities are the foundation of biogeochemical cycles (Azam, 1998). It is well known that most bacteria in the wastewater, including coliforms, can be removed by the treatment of modern WWTPs. However, the incomplete treatment of recalcitrant bacteria, especially some pathogens and bacteria carrying antibiotic resistance genes (ARGs) will put the receiving surface water at risk (Kumaraswamy et al., 2014; Marti et al., 2013). Owing to their high virulence and rapid airborne or water transmission, bacterial pathogens are disastrous and detrimental for human health (Cai and Zhang, 2013; Ahmed et al., 2014; Gomi et al., 2015). On the other hand, due to the overuse and misuse of antibiotics, antibiotic resistance has represented a significant global health problem, causing ARGs as new emerging pollutants (Port et al., 2012). Moreover, some previous studies have shown that WWTPs may contribute to the occurrence, spread and persistence of antibiotic-resistant bacteria

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(Marti et al., 2013; Port et al., 2012; Marti et al., 2014). Therefore, it is necessary to comprehensively assess the impacts of WWTP effluent on the receiving surface water, especially in catchments where the human communities place increasing pressure upon WWTP infrastructure and the health of freshwater ecosystems. In general, it is essential to identify the composition and abundance of the microorganisms and ARGs within wastewater treatment systems and their distribution in receiving surface water.

In the past, assessments of river health were widely based on the use of macroinvertebrates as long-term indicators (Canobbio et al., 2009). While, with the development of cultural and molecular methods, more and more studies have been conducted to use microbial community structure to reflect the river health (Langworthy et al., 1998; Tian et al., 2008; Yergeau et al., 2012). Although the cultivation-based methods could determine the microbial community directly and effectively (Sander and Kalff, 1993), quite considerable bacteria in the natural environment could not be successfully cultured or isolated in artificial medium (Hugenholtz et al., 1998). Previous limitations on cultural approaches have been largely overcome by advances in molecular biology such as molecular cloning (Frank et al., 2007), terminal restriction fragment length polymorphism (Liu et al., 1997), denaturing gradient gel electrophoresis (Muyzer et al., 1993), and fluorescent in situ hybridization (Erhart et al., 1997). The molecular approaches significantly improved our understanding of the microbial communities. However, those approaches were far away from revealing the panorama of the bacterial communities in complex environmental samples due to PCR bias and low throughput. Recently, the next generation high-throughput sequencing (HTS) techniques such as 454 pyrosequencing and Illumina high-throughput sequencing have shown great advantages on analyzing the microbial community more completely and accurately for their unprecedented sequencing depth (Li et al., 2015; Chiu et al., 2014). Besides, the recent advent of HTS-based metagenomic sequencing, i.e. directly sequencing of the genomic DNA extracted from the environmental samples, could also provide comprehensive information about the diversity and abundance of ARGs (Port et al., 2012; Chen et al., 2013; Yang et al., 2014).

In this study, we used 454 pyrosequencing and Illumina high-throughput sequencing techniques to evaluate the microbial community composition and ARGs in different treatment sections of WWTP and the river receiving WWTP effluent. We conducted bacterial 16S rRNA gene amplicons sequencing on 454 pyrosequencing platform to analyze the microbial community structures, and metagenomic sequencing on Illumina HiSeq 2500 platform to analyze the diversity and abundance of ARGs. The comparison between the WWTPs treatment process and the receiving river revealed overall profiles of bacterial communities and ARGs responding to the discharge of WWTP effluent in the receiving river.

2. Materials and methods

2.1. Sampling and DNA extraction

In this study, wastewater and sludge samples were collected from the Lucun WWTP, including sewage influent (SI), activated sludge (AS) and final effluent (FE). With a treatment capacity of 300,000 m^3/day, Lucun WWTP is located at the side of Beijing-Hangzhou Grand Canal in the southwest suburb of Wuxi, China. An integrated anaerobic/anoxic/oxic (A^2/O) treatment process was applied in this WWTP. Detailed information about this WWTP is shown in Table 1. The Beijing-Hangzhou Grand Canal received the treated FE from Lucun WWTP. Water samples were taken from five locations along the receiving river. One sample was taken from the upstream circa 20 m away from the effluent outfall (U20). Another one was taken from the river section of effluent outfall (EO0). The other three samples were the downstream circa 50 m (EO50), 250 m (EO250), 450 m (EO450) away from the effluent outfall. Three subsamples were collected from surface water of each section (one subsample at middle of the river and two subsamples with about 5 m distance to each side), and equal volume of the three subsamples from each river section were mixed to prepare one sample. To avoid the temporal variation, the sampling process was repeated for three times on March 14, April 8 and April 14 in 2012 and a total of 24 samples were obtained. The concentrations of total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) in the water samples were determined following the standard methods (APHA, 2012). The microorganisms in the water samples were collected by filtration using cellulose esters membrane with a pore size of 0.45 μm and stored at −20 °C before DNA extraction. Genomic DNA was extracted from the cells by using the FastDNA SPIN Kit for Soil (MP Biomedicals, CA, USA) according to the recommended protocol. The concentration and quality of the extracted DNA were measured with microspectrophotometry (NanoDrop®ND-2000, NanoDrop Technologies, Willmington, DE, USA).

Table 1
Information about the operational parameters and wastewater quality of the WWTP.

	Parameter	Value
WWTP	Flow rate	300,000 m^3/d
	Sludge loading rate (F/M)	0.09–0.11 kgBOD_5/(kgMLSS · d)
	Mixed liquor suspended solids (MLSS)	3.3–4 g/L
	pH	6.72–7.55
	Temperature	13.1–20.7 °C
	Influent COD_{Cr}	70–150 mg/L
	Influent ammonium nitrogen	20.76–25.15 mg/L
	Influent total nitrogen	22.90–39.48 mg/L
	Influent total phosphorus	5.61–8.97 mg/L
	Effluent COD	6.6–8.7 mg/L
	Effluent ammonium nitrogen	0.20–0.55 mg/L
	Effluent total nitrogen	8.76–10.74 mg/L
	Effluent total phosphorus	0.23–0.52 mg/L
Receiving river	Flow rate	720–980 m^3/s
	Water depth	1.7–2.4 m
	River width	70–80 m
	COD	6.0–8.2 mg/L
	Total nitrogen	6.58–9.44 mg/L
	Total phosphorus	0.16–0.25 mg/L

2.2. 454 pyrosequencing

The bacterial 16S rRNA gene was amplified by PCR with a set of primers targeting the hypervariable V3-V4 region (about 460 bp) of the 16S rRNA gene. The forward primer was 5′-AGAGTTTGATCCTGGCTCAG-3′, and the reverse primer was 5′-TTACCGCGGCTGCTGGCAC-3′. Barcodes that allow sample multiplexing during pyrosequencing were incorporated between the 454 adapter and the forward primers. The PCR products were sequenced on a Roche 454 FLX Titanium sequencer at MAJORBIO (Shanghai, China). After pyrosequencing, the sequences were then denoised by Mothur (Schloss et al., 2009). The generated raw sequences were processed to trim off the adapters and barcodes (ten nucleotide) and then filtered with following procedures: 1)'trim.seqs' command was used to remove sequences of low quality and limit the length of sequences ranging from 300 to 600 bp; 2) 'pre.cluster' command was used to remove the sequences that are likely due to pyrosequencing errors (Huse et al., 2010); 3) 'chimera.