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Antibiotic resistome in landfill leachate from different cities of China deciphered by metagenomic analysis

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ABSTRACT

High throughput sequencing-based metagenomic analysis and network analysis were applied to investigate the broad-spectrum profiles of ARGs in landfill leachate from 12 cities in China. In total, 526 ARG subtypes belonging to 21 ARG types were detected with abundances ranging from 1.1×10^{-6} to 2.09×10^{-1} copy of ARG/copy of 16S rRNA gene. 68 ARG subtypes that accounted for 73.4%–93.4% of the total ARG abundances were shared by all leachate samples. The four most abundant ARGs, *sul*1, *sul*2, *aad*A and *bac*A can be served as ARG indicators to quantitatively predict the total abundances by linear functions ($r^2 = 0.577–0.819$, $P < 0.001$). No distinct regional distribution pattern of the ARGs was observed among different cities in China, while the ARG compositions of the leachate were clearly distinct from those of other environmental sample types. Nearly 90% ARG subtypes in the anaerobic digestion sludge from sewage treatment plants (STPADS) were shared by the leachate and the abundances of leachate and STPADS ARGs generalists accounted for 84.5% and 87.7% of total abundances in these two types of anaerobic samples, respectively. Furthermore, Procrustes analysis suggested that microbial community composition might be the determining factor of ARG compositions in landfill leachate. ARGs within the same type or among the different types showed higher incidences of non-random co-occurrence and 17 genera might be potential hosts of multiple ARGs. This study highlighted that landfill leachate is an important reservoir of various ARGs and provided a useful reference for the surveillance and risk management of ARGs in landfill environments.

1. Introduction

As emerging contaminants, the effect of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) is currently considered to be a serious environmental problem, which poses a serious threat to human and animal health on a global scale (WHO, 2014; Berendonk et al., 2015). So far, not only the medical settings but also environmental compartments, such as soil, municipal wastewater, pharmaceutical manufacturing effluents, aquaculture and animal husbandry facilities have been found to be hotspots of ARGs (Zhu et al., 2013; Bengtsson-Palme et al., 2014; Wang et al., 2015a; Cabello et al., 2016; Guo et al., 2017; Zhou et al., 2017).

Currently, landfilling is the most common and essential management strategy for the disposal of municipal solid waste (MSW) worldwide, especially in the developing countries (Foo and Hameed, 2009; Eggen et al., 2010). In the large and medium-sized cities of China, there were 657 landfills operating in 2016, which received about 60% of the total volume of MSW disposed (National Bureau of Statistics of China, 2017). Landfill is considered as a potential repository of antibiotics for receiving unwanted and unused drugs, illegal clinical wastes, pet feces, sludge, used baby diapers and toilet papers from clinics, hospitals and households (Eggen et al., 2010; Threedeach et al., 2012). Landfill leachate formed by the percolation of liquid through landfills is a combination of contaminants including organic matter, ammonia-

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nitrogen, heavy metals, chlorinated organics, inorganic salts as well as ARGs (Renou et al., 2008; Clarke et al., 2015; Wang et al., 2015b; Yi et al., 2017). ARGs in the leachate can transfer to the recipient environments and result in serious risk on ecosystem safety and human health (Zhang et al., 2016).

Several polymerase chain reaction (PCR)-based studies mainly focused on the occurrence and abundance of a few ARG subtypes in the leachate (Wang et al., 2015b; Wu et al., 2015; Yu et al., 2016). However, owing to limited availability of primers, little is known regarding the comprehensive ARG profiles in the leachate by PCR and quantitative PCR (qPCR) approaches (Yang et al., 2013). High-throughput sequencing (HTS)-based metagenomics analysis is a powerful tool to overcome the limitations of above methods (Schmieder and Edwards, 2012). It has been used to reveal the broad-spectrum profile of ARGs in various environmental samples (Christgen et al., 2015; Garner et al., 2016; Jia et al., 2017; Lau et al., 2017). Consequently, the HTS-based metagenomic analysis was used in this study to comprehensively understand the occurrence and abundance of ARGs in landfill leachate. In addition, the leachate quality is different in landfills located in varied climatic regions (Naveen et al., 2017), which may have an influence on the composition of microbial community and ARGs. However, the differences/similarities in composition of ARGs in landfill leachate from different regions have not been extensively investigated. Thus, 13 landfills from 12 Chinese cities located in North China, East China, Central South China and South China were selected to evaluate the broad-spectrum resistome profiles in the leachate. It is well known that landfill leachate and anaerobic digestion sludge from sewage treatment plants (STPADS) are formed under anaerobic conditions in two typical anaerobic engineering systems, respectively. Metagenomic analysis has revealed that a variety of ARGs existed in STPADS, which is also viewed as ARG hotspot (Christgen et al., 2015; Guo et al., 2017; Luo et al., 2017). However, no study has been reported to systematically evaluate the similarity and difference of resistome between the two typical anaerobic environments. Therefore, 16 public metagenomic datasets of STPADS were downloaded and reanalyzed using the same pipeline to conduct the detailed comparison in the present study.

The objectives of this study were (1) to comprehensively characterize the ARG profiles in the landfill leachate from different cities in China using the HTS-based metagenomic analysis; (2) to compare ARG profiles in two typical anaerobic engineering systems, i.e., landfill and municipal anaerobic digester; and (3) to identify the relationships of environmental factors and microbial community with ARGs and the co-occurrence patterns among ARGs and microbial taxa in landfill leachate using network analysis.

2. Material and methods

2.1. Sample collection and public metagenomics datasets collection

A total of 19 leachate samples were collected from 13 landfills in 12 Chinese cities over two months. Geographical distribution of the sampling cities was shown in Fig. S1. Moreover, one and three activated sludge (AS) samples were collected from the aeration tanks of leachate treatment plants (LTP) in Fenyi and Shenzhen, respectively. The sole anaerobic digestion sludge (ADS) sample was collected from the fermenter of LTP in Shenzhen. Detailed landfill leachate treatment processes in the LTP of Fenyi and Shenzhen were illustrated in Fig. S2. All the raw leachate samples from each landfill were taken without rainfall during at least 7 consecutive days to exclude the precipitation effect. Detailed sample information was summarized in Table S1 and Table S2. Leachate samples were stored in 1-L sterilized containers, while AS and ADS samples were stored in 50 mL sterilized polypropylene centrifuge tubes. All samples were mixed with 100% ethanol at a volume ratio of 1:1 immediately after collection for biomass fixation (Ju and Zhang, 2015). The fixed samples were placed into dry ice box and transferred to laboratory within 48 h. On arriving the laboratory, each leachate sample was filtrated by sterilized 0.22 μm polycarbonate membranes (47 mm diameter, Millipore, USA) and the microbial DNA in the membranes were then extracted. The fixed AS and ADS samples were centrifuged at 15000 rpm for 10 min to collect pellet as soon as arriving the laboratory, following by the DNA extraction. Simultaneously, another 1 L sample of each leachate without adding ethanol was also collected to conduct chemical analyses. These samples were centrifuged at 10000×g for 10 min and then the aqueous phase was filtered through 0.45 μm glass fiber filters (47 mm diameter, Millipore, USA) for further solid phase extraction (SPE) and other water quality parameter detection.

In the present study, 16 STPADS metagenomics datasets were downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Achieve and MG-RAST (Rapid Annotation using Subsystems Technology for Metagenomes). The detailed information of the datasets was summarized in Table S3.

2.2. Chemical analyses

The water quality parameters, including pH, electrical conductivity (EC), total nitrogen (TN), ammonia nitrogen (NH_3-N), total phosphorus (TP), phosphate (PO_4^{3-}), total organic carbon (TOC) and chemical oxygen demand (COD) of the leachate were analyzed following the standard methods (APHA, 2005). According to a recent survey which investigated national consumption, emissions, and multimedia fate of 36 frequently detected antibiotics in China (Zhang et al., 2015), seven classes of antibiotics, including 20 specific antibiotics, i.e., sulfamonomethoxine (SMM), sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfaquinoxaline (SQX), sulfamethazine (SMZ), ofloxacin (OFX), norfloxacin (NOR), enrofloxacin (ENR), ciprofloxacin (CIP), pefloxacin (PEF), tetracycline (TET), doxycycline (DOX), cefotaxime (CTX), cephalexin (CEL), ampicillin (AMP), erythromycin (ERY-H_2O), roxithromycin (RTM), lincomycin (LIN), chloramphenicol (CHL) and trimethoprim (TMP) (Table S4) were selected as the targets for SPE and ultra-performance liquid chromatography-tandem mass spectrometry detection using methods described by Li et al. (2009). All the chemical parameters were measured in triplicate for each sample.

2.3. DNA extraction

DNA was extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions. DNA quality was evaluated using gel electrophoresis (1% agarose) and DNA concentrations were measured using NanoDrop spectrophotometer (ND-One, Thermo Fisher Scientific, USA). The DNA samples were stored at −80 °C for further analysis.

2.4. High-throughput sequencing

2.4.1. Metagenomic sequencing

Approximately 5 μg of DNA for each sample were sent to Novogene (Tianjin, China) for library construction with an insert size of 350 bp. Sequencing was performed on Illumina Hiseq 4000 platform (150 bp paired-end strategy). The metagenomic data size for each sample was about 10 Gb, resulting in a total of over 240 Gb data output from 24 samples. All the metagenomic data sets were deposited in MG-RAST server under the project ID mgp21084 (http://metagenomics.anl.gov/mgmain.html?mgpage=project&project=mgp21084) and the related information for each sample were summarized in Table S5.