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The abundance and diversity of antibiotic resistance genes in the atmospheric environment of composting plants

Min Gao, Tianlei Qiu, Yanmei Sun, Xuming Wang*

Beijing Agro-Biotechnology Research Center, Beijing Key Laboratory of Agricultural Genetic Resources and Biotechnology, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

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ABSTRACT

Composting is considered to reduce the introduction of antimicrobial resistance genes (ARGs) into the environment through land application of manure; however, the possible pollution of ARGs in the atmospheric environment of composting plants is unknown. In this study, 29 air samples including up- and downwind, composting, packaging, and office areas from 4 composting plants were collected. Dynamic concentrations of 22 subtypes of ARGs, class 1 integron (*intl1*), and 2 potential human pathogenic bacteria (HPB), and bacterial communities were investigated using droplet digital PCR and 16S rRNA gene sequencing, respectively. In this study, *intl1* and 22 subtypes of ARGs (except *tetQ*) were detected in air of composting, packaging, office, and downwind areas. The highest concentration of 15 out of 22 subtypes of ARGs was detected in the packaging areas, and *intl1* also had the maximum average concentration of 10^4 copies/m^3, with up to $(1.78 \pm 0.49) \times 10^{-2}$ copies/16S rRNA copy. Non-metric multi-dimensional scaling of ARGs, potential HPBs, and bacterial components all indicated that the bioaerosol pollutant pattern in packaging areas was most similar to that in composting areas, followed by office, downwind, and upwind areas. The co-occurrence between ARGs and bacterial taxa assessed by Procrustes test, mantel test, and network analysis implied that aerosolized ARG fragments from composting and packaging areas contributed to the compositions of ARG aerosols in office and downwind areas. The results presented here show that atmoshperic environments of composting plants harbor abundant and diverse ARGs, which highlight the urgent need for comprehensive evaluation of potential human health and ecological risks of composts during both production as well as land application.

1. Introduction

Composting is a controlled aerobic process in which animal manure is degraded to stable material, with the resident microbial community mediating the biodegradation and conversion (Neher et al., 2013). It results in a stabilized product rich in humic substances that can be used as fertilizer (Sykes et al., 2007). During movement of the composting material, including shredding, compost pile turning, and compost screening (Wery, 2014), microorganisms can be aerosolized and thus released into the atmospheric environment (Taha et al., 2006; Mbareche et al., 2017). Bioaerosols are airborne biological particles that consist of fungi, bacteria, pollen, and fragments, constituents, and byproducts of cells (Walser et al., 2015). Exposure to composting bioaerosol can affect the health of workers (van Kampen et al., 2016). Accordingly, there is an increased public concern regarding potential health impacts on communities surrounding composting sites (Walser et al., 2015).

Animal manure is a major source of antibiotic resistance genes (ARGs), which are becoming recognized as environmental contaminants (Xie et al., 2016; Zhu et al., 2013). Although some studies have indicated that composting can potentially reduce ARGs (Selvam et al., 2012; Wang et al., 2015; Xu et al., 2016), potential human pathogenic bacteria (HPBs), and integrons (Qian et al., 2016) in manure, other studies have reported that the abundance of some ARGs remained nearly the same or even increased (Su et al., 2015) because composting failed to prevent the proliferation (Wang et al., 2015) and concentration of ARGs (Zhu et al., 2013). As a newly recognized environmental pollutant, aerosolized ARGs may travel considerable distances and pose a potential ecological risk in atmospheric environments. Inhalation of aerosol may represent a major pathway for exposure to ARGs and pathogens (Létourneau et al., 2010), thus the abundance and the transport of ARGs via aerosol deserves more attention. However, currently, few studies on the pollution characteristics of ARGs in the air in and around composting facilities are available.

* Corresponding author at: Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, No.9 Shuguang Middle Road, Haidian District, Beijing 100097, China.
E-mail address: wangxuming@baafs.net.cn (X. Wang).

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Over the last two decades, real-time quantitative PCR (qPCR) has been widely used for the detection and quantification of microorganisms in air (Hospodsky et al., 2010; Peccia and Hernandez, 2006; Yamamoto et al., 2012), including bioaerosols from composting plants (Le Goff et al., 2012; Le Goff et al., 2011; Wery, 2014). However, qPCR is subject to bias introduced by the reliance on quantitative standards (Cao et al., 2015). A promising new method for bioaerosol quantification is droplet digital PCR (ddPCR) (Hindson et al., 2011). This technology utilizes a water-oil emulsion system in which the sample is fractionated into thousands of nanoliter droplets to enable high-throughput digital PCR (Fröhlich-Nowoisky et al., 2016). ddPCR has higher precision and tolerance to inhibitors than qPCR (Cao et al., 2015; Sanders et al., 2011; Whale et al., 2013). Recently, ddPCR has increasingly been used for the quantification of DNA targets in complex environmental samples, i.e., ARGs in soils and organic residues (Cavé et al., 2016), and HPBs in feces (Verhaegen et al., 2016) and water (Cao et al., 2015; Rothrock et al., 2013).

To address the current knowledge gaps in the occurrence of ARGs in aerosols at composting facilities, in the current study, ddPCR was used to detect the concentrations of 26 genes, including 16S rRNA, 7 β-lactam resistance genes, 10 tetracycline resistance genes, 4 sulfonamide resistance genes, and 1 erythromycin resistance gene, 2 HPBs, and 1 class 1 integron, in air samples from 4 composting plants. Bacterial communities were also investigated by 16S rRNA gene sequencing. The purposes of this study were to characterize the abundance and patterns of ARGs in aerosols emitted at composting plants and to identify potential factors influencing ARGs transfer to surrounding atmospheric environments.

2. Materials and methods

2.1. Sample collection

Sampling was conducted from October 2014 to October 2015 on 4 commercial composting companies located in the Pinggu, Huairou, and Mentougou districts in Beijing, China. These 4 companies produce aerobic and thermophilic commercial composts using a mixture of manures from cattle, poultry, and swine, with mushroom residue and corn straw as the bulking agent. All composting operations lasted for approximately 1 month, with a thermophilic phase (temperature 55–65 °C) of approximately 15 days. In total, 29 air samples were collected in the upwind area (U, 1 sample), composting area (C, 9 samples), packaging area (P, 7 samples), office area (O, 8 samples), and downwind area (D, 4 samples) The up- and downwind areas were approximately 250 m away from the composting plants, respectively.

Bioaerosols were sampled using total suspended particulate (TSP) impactors (Applied Technical Institute of Liaoyang, China), in which airborne particles are collected on a quartz-fiber substrate(Ahlstrom Munktell, NO.420065, Falun, Sweden) with a diameter of 90 mm. The samplers were calibrated prior to each sampling and were operated continuously for 24 h at each sampling site and at an air flow rate of 100 l/min. After sampling, the filter was removed and placed in a low-temperature sampling box, then quickly returned to the laboratory and stored at −80 °C until analysis. The filters were baked in a muffle furnace at 500 °C for 5 h and each sterilized filter was kept in a sterilized plastic box until being loaded into the sampler, as described previously (Gao et al., 2017). All tools used were cleaned with 75% ethanol or autoclaved after each use.

2.2. DNA extraction and bacterial 16S rRNA gene sequencing

DNA was extracted from the loaded quartz-fiber filters using a Power Max Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), as previously described (Gao et al., 2017). The V3–V4 region of bacterial 16S rRNA was amplified using bacterial universal 338F and 806R primers. The amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and were quantified using a QuantiFluor™-ST fluorimeter (Promega, Madison, WI, USA), per the manufacturers' protocols. The purified amplicons were paired-end sequenced (2 × 300) in an Illumina MiSeq PE300 platform. The sequence data of the bacterial 16S rRNA genes have been deposited in the NCBI Sequence Read Archive database under accession no. SRP079950. The raw reads in fastq files were de-multiplexed and quality-filtered using QIIME (version 1.17) (Caporaso et al., 2010). The reads (average length, 436 bp) were truncated at any site receiving an average quality score of < 20 over a 50-bp sliding window. The reads containing ambiguous characters were removed for exact barcode matching. Sequences that overlapped by > 10 bp were assembled based on their overlap sequence. Operational taxonomic unit (OTUs) with a 97% similarity cutoff were clustered by UPARSE (version 7.1, http://drive5.com/uparse/) (Edgar, 2013). Chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011). The RDP Classifier (Wang et al., 2007) against the Silva (SSU115) 16S rRNA database (Quast et al., 2013) was employed to analyze the taxonomy of each 16S rRNA gene sequence using a confidence threshold of 70%. Normalization for read depth was performed during data processing.

2.3. ddPCR

ddPCRs were run on a QX200 Droplet Digital™ PCR system (Bio-Rad, Hercules, CA) following previously published protocols (Cavé et al., 2016; Rothrock et al., 2013; Cao et al., 2015). DNA (diluted 1:10,000) was used as a template for PCR. In this study, 4 “ARG types” including 22 “ARG subtypes” were analyzed. For example, “tetracycline resistance genes” is one example of an ARG type, whereas “*tetQ*” is one of the tetracycline resistance subtypes. The annealing temperatures for the primer pairs used to amplify 16S rRNA, 2 potential human pathogenic bacteria (*Escherichia coli* and *Staphylococcus* spp.), 1 class 1 integron (*intl1*), 7 subtypes β-lactam resistance genes (*blaCARB-4*, *blaOXA-18*, *blaOXA1*, *blaOXAII*, *blaOXAIII*, *blaPSE*, and *blaTEM*), 10 tetracycline resistance genes (*tetQ*, *tetM*, *tetS*, *tetT*, *tetW*, *tetA/P*, *tetG*, *tetL*, *tetZ*, and *tetX*), 4 subtypes sulfonamides resistance genes (*sul1*, *sul2*, *sul3*, and *dfrA1*), and 1 subtype erythromycin resistance gene (*ermB*) are listed in Table S2. Each reaction (20 μl) contained QX200 ddPCR EvaGreen Supermix (Bio-Rad), 100 nM of each primer, and 1 μl of sample DNA. The reaction mixture was mixed and loaded into a 8-channel disposable droplet generator cartridge (Bio-Rad) with 70 μl oil. The cartridge was placed into the droplet generator (Bio-Rad) to generate 12,000–20,000 droplets, which were manually transferred to a standard 96-well PCR plate. The PCR plate was heat-sealed with a foil plate seal (Bio-Rad) and placed in the CFX96 Touch™ Real-Time PCR Detection System for PCR amplification using the following thermal conditions (ramping rate 2.5 °C/s): 10 min at 95 °C, 40 cycles of 30 s at 95 °C, 60 s at the respective annealing temperatures listed in Table S2, and 30 s at 72 °C, 5 min at 4 °C, and 5 min at 90 °C.

Upon completion of PCR, the 96-well plate was transferred to a Droplet Reader (Bio-Rad) for data acquisition. Fluorescence in each droplet and in each well (~2 min per well) was automatically measured, recorded, and analyzed using the QuantaSoft™ Software 1.7.4.0917 (Bio-Rad). Quality controls, including “no template control” (NTC) wells, exclusion of wells with < 12,000 accepted droplets, distribution of the number of accepted droplets per reaction, fluorescence amplitude of positive and negative droplets, and Poisson mean estimates, were checked.

2.4. Data analysis and statistical analysis

Box frames and bar charts were generated in OriginPro 8.5. The correlations within 22 subtypes ARGs, as well as between 22 subtypes ARGs and 40 dominant genera were analyzed using the abundance of ARG per cells and relative abundance of 40 dominant genera (16S rRNA gene OTU data). Spearman's correlation coefficient ($r > 0.8$ and the *P*-