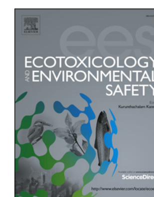
Ecotoxicology and Environmental Safety 158 (2018) 87–93

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Changes in bacterial community structure and antibiotic resistance genes in soil in the vicinity of a pharmaceutical factory☆

Youchao Zhu, Qi Zhang, Jiahui Xu, Qian Qu, Tao Lu, Benben Du, Mingjing Ke, Meng Zhang, Haifeng Qian*

College of Environment, Zhejiang University of Technology, Hangzhou 310032, PR of China

ARTICLE INFO

Keywords:
Antibiotic resistance genes
Soil bacterial community
Nutrients
Pharmaceutical factory

ABSTRACT

China is the largest global producer of antibiotics. With the demand for antibiotics increasing every year, it is necessary to assess potential environmental risks and the spread of antibiotic resistance genes (ARGs) associated with antibiotic production. Here, we investigated the occurrence and distribution of ARGs in soil in the vicinity of a pharmaceutical factory. The results showed that antibiotic concentrations were under the detection limit; however, ARGs were present in soil and tended to be enriched near the factory. A significant correlation between the relative abundance of *int*I-1 and tetracycline ARGs implied that horizontal gene transfer might play an important role in the spread of ARGs. The occurrence of these ARGs could be the results of previous antibiotic contamination. However, the soil bacterial community structure seemed to be more affected by nutrients or other factors than by antibiotics. Overall, this study supports the viewpoint that long-term pharmaceutical activity might have a negative effect on environmental health, thus, underscoring the need to regulate antibiotic production and management.

1. Introduction

Total annual antibiotic use, including those associated with medical and veterinary applications, has reached 100,000–200,000 t worldwide (Wang and Tang, 2010). China is the largest producer and consumer of antibiotics in the world (Hvistendahl, 2012). In 2013, antibiotic usage reached approximately 162,000 t in China, of which 77,760 and 84,240 t were consumed by humans and animals, respectively (Zhang et al., 2015). More than 150 antibiotics are in use today (von Nussbaum et al., 2006), and these include fluoroquinolones, macrolides, tetracyclines, and sulfonamides, which are commonly used in human and veterinary medicine (Giger et al., 2003; Golet et al., 2003).

Given that significant amounts of antibiotics are produced and subsequently released into different environmental constituents such as soil and surface and ground water (Andreozzi et al., 2004; Bartelt-Hunt et al., 2011; Boxall et al., 2004), the potential impact of antibiotic residues on various organisms is currently an important focus of research. Soil contains millions of microbes per gram (Torsvik et al., 1990), which play a fundamental role in soil functions such as element cycling, environmental pollutant degradation, and energy flow (Barros and Feijoo, 2003; Bremner and Blackmer, 1978; Lewis et al., 1999). Antibiotic residues in soil will greatly change bacterial community structures, and thus disturb soil functions (Fang et al., 2016; Pinna et al., 2012; Zhang et al., 2017). Understanding the fate and transport of antibiotics in the environment is fundamental to limit their spread and optimize their management. Antibiotics can enter the environment through different pathways. Adsorption was considered the major process that governs the mobility and transport of antibiotics in the environment, and bound-residue formation largely controls the long-term storage of antibiotics in soils and sediments (Carstens et al., 2013). Adsorption can be a limiting factor for the subsurface horizon transport and biodegradation of antibiotics by soil microorganisms and can also prevent antibiotics from undergoing biotic and abiotic degradation (Vasudevan et al., 2009). Antibiotics can also be released into the environment through the air and water near production sites. Pharmaceutical factories, in most cases, are not capable of completely removing these compounds from their wastewater (Gadipelly et al., 2014), which could contaminate the surrounding environment.

Antibiotics might not only disrupt the functions of soil microbial communities and possibly affect their growth, but also lead to the spread of antibiotic resistance genes (ARGs) (Zhu et al., 2013). The migration and transformation of ARGs in the soil environment is potentially more harmful than the occurrence of antibiotic residues in the environment (Arias and Murray, 2009; Ji et al., 2012; Perreten and

☆ This manuscript has been thoroughly edited by a native English speaker from an editing company (Editage by CACTUS). Editing Certificate will be provided upon request.

* Corresponding author.
E-mail address: hfqian@zjut.edu.cn (H. Qian).

https://doi.org/10.1016/j.ecoenv.2018.04.016
Received 18 January 2018; Received in revised form 4 April 2018; Accepted 6 April 2018
0147-6513/ © 2018 Elsevier Inc. All rights reserved.

Boerlin, 2003). Even at levels below the clinically determined minimum inhibitory concentrations, some antibiotics can stimulate the emergence of bacteria harboring ARGs (Jechalke et al., 2014). Horizontal gene transfer (HGT) is considered the main mechanism of ARG propagation (Thomas and Nielsen, 2005). Some ARGs have been shown to exist in transposons, integrons, or plasmids, which are mobilizable elements that can be transferred among bacteria (Martinez et al., 2007).

In this study, the soil in the vicinity of a pharmaceutical factory that had produced antibiotics for years, was collected to study soil chemical characteristics, bacterial community structure, ARG distribution, and the relationships among these parameters. The purpose of this study was to investigate the bacterial community structure changes the occurrence and distribution of ARGs based on distance from the factory and soil depth. To our best knowledge, this is the first study to investigate the occurrence and distribution of antibiotic resistance genes in soil in the vicinity of a pharmaceutical factory.

2. Materials and methods

2.1. Sampling sites

Soil sampling was conducted in November 2016 from various locations in the vicinity of a pharmaceutical factory in Shangyu, Zhejiang Province, China; the manufacture of bulk pharmaceutical chemicals is the principal business of this factory, and most of these are quinolones such as ofloxacin, levofloxacin, gatifloxacin, and ciprofloxacin. The annual yield of quinolones for this factory reaches 2900 t, and it has manufactured antibiotics for 12 years (up to soil sample collection). In addition, erythromycin derivatives such as azithromycin and roxithromycin are also main products of this factory. However, no tetracycline and sulfonamide antibiotics are produced by this factory. The pharmaceutical factory is located near a river. Soil samples were collected from the surface horizon (10 cm depth) and subsurface horizon (30 cm depth) at four locations along the river, across south-to-north horizon distances of 0, 100, 600, and 1000 m from the pharmaceutical factory. The sampling sites were approximately 3 m offshore, and covered by small grass, except for the site at 100 m. The soil collected from 100 m was sandier than soil from other distances. At 600 m, a plot of farmland is located on the other side of the river. There are some farmhouses at approximately 1.5 km north. No other factory is located within a 2 km radius. The soil samples collected from different spots were labeled accordingly; those collected from 0, 100, 600, and 1000 m distance and a 10 cm depth were marked as Z10, H10, S10, and T10. Similarly, Z30, H30, S30, and T30 was used for the 30 cm depth. A total of 32 soil samples with four replicates in each group were collected in this study. Each soil sample was immediately placed in an individual polyethylene bag with ice and transported to the laboratory. Stones, roots, and other organic debris were removed from the samples prior to storing at − 80 °C for physicochemical and biological analyses. Before each physicochemical determination, the soil samples were taken from the refrigerator, thawed, and air dried.

2.2. Soil chemical properties

To obtain the fundamental characteristics of the samples, soil chemical properties including total carbon (TC), total nitrogen (TN), total sulfur (TS), available phosphorus (aP), available potassium (aK), soil organic matter (SOM), pH, and electrical conductivity (EC) were determined. The soil chemical properties were measured according to previous studies. Briefly, a soil suspension in a soil: water ratio of 1:2.5 was obtained to measure the EC and pH using a conductivity meter and pH meter (Dong et al., 2009; Pietri and Brookes, 2008), respectively. Soil TC and SOM were determined using the Walkley–Black method (Nelson et al., 1996). Soil TN was measured using the Kjeldahl method (Bremner, 1960). Soil TS was determined by the turbidimetry method after the dry-ash oxidation procedure (Rossete et al., 2008). Soil aK was measured using the flame photometer method (Jackson, 1973). The content of aP was determined using the Olsen-P method (Poile et al., 1990).

2.3. Analysis of soil antibiotic concentrations

To extract antibiotics from the soil, approximately 1 g of the air dried sample was passed through a 0.3 mm sieve and added to 5 mL of 50% $Mg(NO_3)_2$–10% $NH_3{\cdot}H_2O$ (96:4, v: v). After oscillating (65 Hz, 25 °C) and sonicating (20 kHz and 100 W bath at 25 °C), both for 15 min, the slurry was centrifuged (4500 r/min, 4 °C) for 5 min (Tai et al., 2009). The supernatant was passed through an Oasis hydrophilic-lipophilic balance (HLB) polymer cartridge (Waters, Watford, UK) referring to the method described by Rossmann et al. (2014). The extracts were separated on an Xterra MSC18 (3.5 µm, 2.1 × 100 mm) column using the Dionex HPLC system (Dionex UltiMate 3000 system). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was carried out on the AB Sciex QTRAP® 5500 LC-MS/MS system. The specific chromatographic conditions were in accordance with the description of Rossmann et al. (2014).

2.4. DNA extraction and 16S rDNA amplicon sequencing

Total genome DNA was extracted from approximately 0.5 g of soil sample using the CTAB/SDS method and was monitored using 1% agarose gels; then, DNA was adjusted to 1 ng/µL using sterile water for library construction. The specific primer pair 341 F (CCTAYGGGRBGCASCAG) and 806 R (GGACTACNNGGGTATCTAAT) targeting V3–V4 hypervariable regions were used to amplify the extracted DNA. The amplified DNA was then detected on 2% agarose gels. Bright strips of 400–450 bp in length were selected and purified using Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were created using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations. The library was then sequenced using an IlluminaHiSeq. 2500 platform (Zhejiang Tian Ke hitech Development Co., Ltd. Hangzhou, China), and 250 bp paired-end reads were generated for further analysis. Paired-end reads were combined using FLASH (V1.2.11). The generated raw tags were then filtered using QIIME (V1.9.1) for quality control. Uparse software (Uparse V8.1.1861) was used to classify tags into operational taxonomic units (OTUs) based on sequences similarity (≥ 97%). Species annotation was completed using RDPclassifier (Version 11.4) based on the GreenGene Database. Alpha diversity including Chao1, Shannon, Simpson, abundance-based coverage estimators (ACE), Good-coverage and Beta diversity on both weighted and unweighted unifrac were calculated by QIIME (Version 1.9.1). Principal coordinate analysis (PCoA) and redundancy analysis (RDA) were performed using R software (Version 3.2.2).

2.5. Quantitative polymerase chain reaction assays

To further investigate the effects of antibiotics on geo-biological-chemical circulation including carbon cycle (C cycle), nitrogen cycle (N cycle), and phosphorus cycle (S cycle), based on soil microorganisms and variations in ARGs, real-time quantitative PCR (qPCR) was performed to survey the distribution of genes in soil microorganisms in the vicinity of the pharmaceutical factory using an Eppendorf Master Cycler®ep RealPlex4 system (Wesseling-Berzdorf, Germany). qPCR amplification was performed in a 10 µL reaction volume containing 5 µL SYBR Green Realtime PCR Master Mix (Toyobo), 0.4 µL forward primers, 0.4 µL reverse primers, 3.2 µL double-distilled water, and 1 µL soil genomic DNA as the template. PCR conditions were as follows: initial denaturation at 50 °C for 2 min and then 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 1 min; final extension at 72 °C for 10 min. Four replicates were performed in each group using the same PCR conditions. A total of 33 genes, namely, two C cycle-related

Download English Version:

https://daneshyari.com/en/article/8853770

Download Persian Version:

https://daneshyari.com/article/8853770

Daneshyari.com