Science of the Total Environment 506–507 (2015) 279–286

Contents lists available at ScienceDirect

Science of the Total Environment

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

Long-term application of fresh and composted manure increase tetracycline resistance in the arable soil of eastern China

Shuang Peng [a,b], Yiming Wang [a], Beibei Zhou [a,b], Xiangui Lin [a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 Beijing East Road, Nanjing, 210008 Jiangsu Province, PR China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

HIGHLIGHTS

- *tetO* was introduced with fresh and composted manure into soil.
- The abundance of *tetG*, *tetL*, and *tetB*(*P*) increased after fertilization with manure.
- Abundance of 4 genes decreased while that of 2 increased by compost manuring.
- *tetG* diversity was lower at the 5–10 cm soil depth in compost-manured soil.
- The dominant *tetG* genotypes shared strong homology with pathogenic bacteria.

ARTICLE INFO

Article history:
Received 3 May 2014
Received in revised form 4 November 2014
Accepted 4 November 2014
Available online 21 November 2014

Editor: E. Capri

Keywords:
Antibiotic resistance genes
Composting
Swine manure
Real-time PCR
Pollutant

ABSTRACT

The aim of this study was to compare the occurrence, abundance, and diversity of tetracycline resistance genes (*tet*) in agricultural soils after 6 years' application of fresh or composted swine manure. Soil samples were collected from fresh or composted manure-treated farmland at three depths (0–5 cm, 5–10 cm, and 10–20 cm). Nine classes of *tet* genes [*tetW*, *tetB*(*P*), *tetO*, *tetS*, *tetC*, *tetG*, *tetZ*, *tetL*, and *tetX*] were detected; *tetG*, *tetZ*, *tetL*, and *tetB*(*P*) were predominant in the manure-treated soil. The abundances of *tetB*(*P*), *tetW*, *tetC*, and *tetO* were reduced, while *tetG* and *tetL* were increased by fertilizing with composted versus fresh manure; thus, the total abundance of *tet* genes was not significantly reduced by compost manuring. *tetG* was the most abundant gene in manure-treated soil; the predominant *tetG* genotypes shared high homology with pathogenic bacteria. The *tetG* isolates were more diverse in soils treated with fresh versus composted manure, although the residual *tet* genes in composted manure remain a pollutant and produce a different influence on the *tet* gene resistome in field soil.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Antibiotic resistance is a growing global public health concern (Martínez, 2008). Antibiotics are used worldwide in concentrated animal feeding operations (CAFOs) to treat disease and promote growth. In China, over 8000 tons of antibiotics are used as feed additives each year (Ji et al., 2012; Ben et al., 2008). However, when used in this manner, antibiotics can select and enrich for resistant bacteria in the gastrointestinal tracts of production animals, providing a potential reservoir for the dissemination of resistant bacteria into the environment (Wu et al., 2010; Mackie et al., 2006). Bacteria can acquire antibiotic resistance genes (ARGs) via horizontal gene transfer or spontaneous mutation (Lee et al., 2010; Davies and Davies, 2010). While the number of ARGs found in bacteria continues to increase, the use of antibiotics in animal husbandry is causing concern that these ARGs can be transferred to community-associated pathogens via water and food webs, potentially contributing to the proliferation of antibiotic resistance (Negreanu et al., 2012).

Manure has become a reservoir of resistant bacteria and antibiotic compounds, and its application to agricultural soils is assumed to significantly increase the ARGs and resistant bacterial populations in soil (Heuer et al., 2011; Schmitt et al., 2006). Composting has been demonstrated to significantly reduce antibiotic levels (Hu et al., 2011; Selvam et al., 2012a; Wu et al., 2011), and may be used to reduce ARGs in manure (Selvam et al., 2012b; Yu et al., 2005). Although previous studies have addressed the influence of swine manure on the diversity of tetracycline resistance genes in soil microcosms (Schmitt et al., 2006), few studies have addressed the polluting capacity of manure after composting and repeated application to arable soil. Although application of composted manure in the field has become more common, little is known about its effort on ARG pollution.

* Corresponding author. Tel.: +86 25 86881589.
E-mail address: xglin@issas.ac.cn (X. Lin).

http://dx.doi.org/10.1016/j.scitotenv.2014.11.010
0048-9697/© 2014 Elsevier B.V. All rights reserved.

First discovered in the 1940s, tetracyclines has become one of the most commonly used therapeutics in human and veterinary medicine (Chopra and Roberts, 2001), by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site they can inhibit protein synthesis and sterilize gram positive and negative bacteria. At least 40 different tetracycline resistance (*tet*) genes have been characterized (Roberts, 2005), and three mechanisms have been identified: antibiotic efflux pumps, target modification with ribosomal protection protein, and antibiotic inactivation (Kumar and Schweizer, 2005; Lambert, 2005). More than 22 *tet* genes have been detected in environmental samples (Zhang X.X. et al., 2009), including sewage treatment plants, hospital wastewater, animal production, aquaculture areas, untreated sewage, natural water, sediments, and even treated drinking water. The main sources of resistance on farms include application of animal manure to agricultural soils and leakage from manure lagoons (Heuer et al., 2011). Therefore, it is necessary to uncover the true extent of agriculturally associated resistance in the soil resistome. It is equally important to reduce the dissemination of the ARGs during manure fertilization.

The objective of this study was to investigate the occurrence, abundance, and diversity of *tet* genes in soil samples collected from rice and wheat crop rotation farms after 6 years of composted manure treatment (CS). As a basis for comparison, we also characterized soils (FS) collected from farms treated with the same dry weight of fresh manure and control sites with no fertilizer (CK). Polymerase chain reaction (PCR) was used to determine the occurrence of 23 *tet* genes frequently reported in various environmental compartments. The abundance of the detected *tet* genes was determined by real-time quantitative PCR (qPCR). To understand the potential mechanisms of ARG distribution in manure and manure-treated soil, we used clone libraries to analyze *tetG* diversity because it is the most abundant gene in farmland soil. This study therefore represents a comprehensive assessment of *tet* genes in long-term manure-treated soil. Understanding the involvement of each of the three tetracycline resistance mechanisms could provide insight into how to control antibiotic resistance deriving from composted manure or agricultural activities.

2. Methods

2.1. Source of the swine manure and composted manure

The experiment was conducted at the Chinese Academy of Sciences Changshu Agriculture Ecology Experiment Station (31°33′ N, 123°38′ E), Jiangsu Province, China. Fresh swine manure was collected from a large-scale swine farm near the station; the composted manure was collected from a small organic fertilizer factory next to the swine farm, with an annual production of 3000–5000 tons. The factory uses manure from the same swine farm and the composted manure products are packaged and sold to local farmers as commercial organic fertilizer. On June 7, 2011, the manure samples were collected and stored at −40 °C for laboratory analysis. There was 10.62 mg/kg chlortetracycline in the fresh manure and 0.33 mg/kg chlortetracycline residue in the composted manure. We detected no tetracycline, oxytetracycline, or doxycycline in either manure type. The characteristics of *tet* genes identified in fresh and composted manure are shown in Table 2. We assessed the fresh manure samples for twenty-one classes of *tet* gene, which were then cloned, sequenced, and searched against GenBank with BLASTn (http://blast.ncbi.nlm.nih.gov).

2.2. Farms and sample collection

The farms had no manure application history from the founding of the station (1987). Farmlands with rice and wheat crop rotation were treated twice yearly (spring and fall) from 2006 to 2011 with 9.0 tons (dry weight)/ha fresh swine manure ($n = 4$) or composted manure ($n = 4$) prior to planting. The soil is classified as gleyic–stagnic anthrosols. On June 7, 2011, the manure was applied to the farms to be planted with rice. On November 7, 2011 (rice harvest), each farm was sampled. Fresh samples of a loamy clay soil (pH-KCl 7.5; approximately 4.5% organic matter; 28–42% moisture upon sampling), obtained from depths of 0–5 cm, 5–10 cm, and 10–20 cm were collected at six plots (Φ 3 cm) on each farm (30 m^2). The samples were mixed thoroughly. Control sites with no fertilizer were sampled by the same method. Plant residues and stones were removed before the soil samples were homogenized. Samples for molecular analysis and chemical determination were stored at −40 °C and 4 °C, respectively.

2.3. DNA extraction and PCR detection of tet genes

From each sample, 0.5 g moist soil was used for DNA extraction using a FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA) according to manufacturer instructions, and manure DNA was extracted using a FastDNA® SPIN Kit for feces samples. The extracted soil DNA was dissolved in 80 μL DES buffer and the DNA concentration was determined by microspectrophotometry (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE) before being stored at −20 °C until use.

We assayed 23 *tet* genes [eight for ribosomal protection: *tetB*(*P*), *tetM*, *tetO*, *OtrA*, *tetQ*, *tetS*, *tetT*, *and tetW*; 13 for efflux pumps: *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetH*, *tetJ*, *tetK*, *tetL*, *tetY*, *tetZ*, *and tet30*; one for antibiotic inactivation: *tetX*; and one for unknown mechanisms: *tetU*] using the primers listed in Table 1. PCR was performed in a 25-μL volume containing 12.5 μL *Premix EX Taq*™ mixture (TaKaRa), 2 pmol of each primer, and 150 ng template DNA. *tetX* and *tetG* were amplified under the following conditions: 35 cycles of 95 °C for 45 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Cycling conditions for the remaining 21 *tet* genes were as follows: 35 cycles of 94 °C for 15 s, annealing for 30 s at the temperatures in Table 1, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. PCR products were analyzed by 2% agarose gel electrophoresis in 1× TAE buffer. To confirm reproducibility, duplicate PCR reactions were performed for each sample. A *tet* plasmid sequence was used as the positive control and sterile water was used as the negative control for each assay. Table 1 PCR primers used in this study.

2.4. Quantification of tet genes

qPCR was used to determine the abundance of *tet* genes in the soil. Gene copy number was quantified by qPCR analysis with a C1000™ Thermal Cycler equipped with the CFX96™ Real-Time system (Bio-Rad, USA). Plasmids carrying each targeted *tet* gene (Section 2.1) were extracted and purified using the E.Z.N.A.™ Plasmid Mini Kit (OMEGA, USA). Plasmid concentrations were determined by NanoDrop, and the abundance of *tet* gene per microliter of plasmid solution was calculated according to Zhang T. et al. (2009) A 10-fold dilution series of plasmid DNA was made to generate a six-point calibration curve (Ct versus log of initial *tet* gene copy) for qPCR. Assays were set up using the SYBR Premix Ex Taq™ Kit (TaKaRa). The 20-μL reaction mixture contained 10 μL SYBR® Premix Ex Taq™, a primer set (0.5 μM each), and 1.0 μL template containing approximately 2–9 ng DNA. Blanks were always run with water as the template instead of soil DNA extract. Specific target gene amplification was confirmed by agarose gel electrophoresis and melt curve analysis. qPCR was performed in triplicate and amplification efficiencies of 95.4–102% were obtained with R^2 values of 0.990–1.000. Based on the calibration curves, the Ct value of a test sample was used to calculate the abundance of *tet* gene, and then normalized against the mass (ng) of the extracted DNA. To correct for variations in extraction efficiencies, eubacterial 16S rRNA genes were quantified as described by Biddle et al. (2008) so that *tet* abundance could be normalized to the total bacterial community.

Download English Version:

https://daneshyari.com/en/article/6327705

Download Persian Version:

https://daneshyari.com/article/6327705

Daneshyari.com