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Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure

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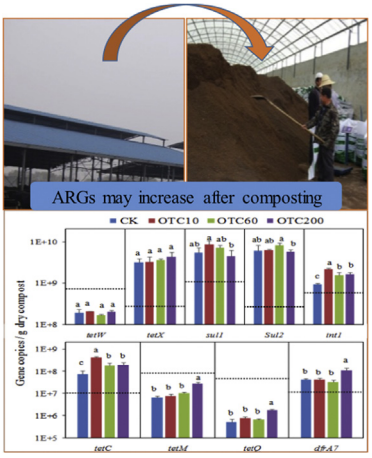
HIGHLIGHTS

- Relative abundance (RA) of ARGs in compost was similar with four OTC treatment levels.
- RAs of *tetC*, *tetX*, *sul1*, *sul2*, and *intI1* increased by 2–43 times after composting.
- OTC at 200 mg/kg increased the absolute abundances of 5/8 ARGs and *intI*.
- Changes in ARGs during composting associated with bacterial community succession.
- Composting did not remove most ARGs and the compost remained a reservoir of ARGs.

GRAPHICAL ABSTRACT



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ABSTRACT

Livestock manure is often subjected to aerobic composting but little is known about the variation in antibiotic resistance genes (ARGs) during the composting process under different concentrations of antibiotics. This study compared the effects of three concentrations of oxytetracycline (OTC; 10, 60, and 200 mg/kg) on ARGs and the succession of the bacterial community during composting. Very similar trends were observed in the relative abundances (RAs) of each ARG among the OTC treatments and the control during composting. After composting, the RAs of *tetC*, *tetX*, *sul1*, *sul2*, and *intI1* increased 2–43 times, whereas those of *tetQ*, *tetM*, and *tetW* declined by 44–99%. OTC addition significantly increased the absolute abundances and RAs of *tetC* and *intI1*, while 200 mg/kg OTC also enhanced those of *tetM*, *tetQ*, and *drfA7*. The bacterial community could be grouped according to the composting time under different treatments. The highest concentration of OTC had a more persistent effect on the bacterial community. In the present study, the succession of the bacterial community appeared to have a greater influence on the variation of ARGs during composting than the presence of antibiotics. Aerobic composting

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was not effective in reducing most of the ARGs, and thus the compost product should be considered as an important reservoir for ARGs.

1. Introduction

Aerobic composting is one of the main methods for the disposal and reuse of livestock manure. However, high concentrations of antibiotics and antibiotic resistance genes (ARGs) may be found in livestock manure due to the use of antibiotics for disease treatment and growth promotion [1–3]. The compost product is used widely as an organic fertilizer or soil amendment, and thus it is the main source of antibiotics and ARGs in the environment [4,5]. Therefore, there is growing concern about the risk of ARGs spreading to pathogens via horizontal gene transfer (HGT), which may make antibiotics ineffective [6–8].

Previous studies have shown that composting can effectively reduce the abundance of most classes of antibiotics, where the degradation occurs mainly in the first 2 weeks [9–11] and temperature is an important factor for the reduction of antibiotics via both degradation and absorption [12,13]. The presence of antibiotics may generate a selection pressure for ARGs, and thus some ARGs may persist even when the antibiotic pressure is gone [14,15]. Therefore, it has gradually been recognized that the removal of ARGs is as important as eliminating antibiotics, which is necessary for reducing the environmental risk related to the agricultural use of livestock manure [16]. Several studies have shown that the composting process is effective for reducing the abundance of several ARGs, but it is still unclear how and why the abundances of ARGs change during composting [9,17].

The presence of residual antibiotics in livestock manure may alter the microbial community during composting, thereby affecting the composting process and the quality of the compost product [18]. The types and concentrations of residual antibiotics vary greatly in animal manure [19,20], which may have diverse effects on the microbial community. Selvam et al. [9] found that the addition of 100, 20, and 20 mg/kg of chlortetracycline, sulfadiazine, and ciprofloxacin, respectively, led to transient perturbations of the bacterial community but the bacterial diversity recovered during the later stage of composting. Microbes are the main carriers of ARGs, so changes in the succession of the bacterial community may lead to variations in ARGs [21]. Therefore, exploring the relationship between the microbial community and ARGs is important for understanding the variation in ARGs during composting.

In this study, we tested the effects of oxytetracycline (OTC), which is employed frequently in livestock production as a typical antibiotic [19,20], using 16S rDNA sequencing and quantitative PCR (qPCR) methods, where we compared the effects of three concentrations of OTC on the bacterial community and ARGs. The results obtained provide insights into the changes in ARGs during the composting process and they may facilitate the development of appropriate measures to improve the performance of ARG reduction by composting, thereby decreasing the environment risk associated with the compost product.

2. Materials and methods

2.1. Experimental setup

The manure used in this study was collected from a medium-sized dairy farm in Yangling, Shaanxi, China. The dairy stopped using antibiotics for therapeutic or non-therapeutic purposes at 2 weeks before the manure was collected. The fresh manure was mixed, air dried to a water content <30%, crushed, and sieved through a 5-mm mesh. Wheat straw was cut into pieces measuring about 1 cm. The cow manure had a pH of 7.8, organic carbon content of 410.8 g/kg, and an organic nitrogen content of 1.91 g/kg. The wheat straw had an organic carbon content of 417.6 g/kg and an organic nitrogen content of 6.09 g/kg.

The composting experiment was performed in the composting area at Northwest A&F University. The compost reactors comprised 12 identical bubble boxes, which measured: length × width × height × thickness = 45 × 27.5 × 51.5 × 3.5 cm. There were 2 × 2 cm holes on the top, bottom, and wall faces to facilitate aeration. A stock solution of OTC (50 mg/mL) was prepared and diluted with an appropriate volume of sterile water. The diluted OTC solution was then mixed thoroughly with the cow manure. The cow manure was spiked with 0 (CK), 10 (OTC10), 60 (OTC60), or 200 (OTC200) mg/kg of OTC (purity >98%, Sigma) on a dry weight (DW) basis and allowed to equilibrate for 2 h. The concentration levels were set according to the residual concentrations of OTC reported previously in animal manure [12,19]. The manure was mixed with wheat straw (4:1 DW) and the moisture content was adjusted to 55%. Each treatment was repeated in triplicate. The compost was allowed to process for 40 days and turning was performed on days 1, 3, 5, 8, 13, and 21.

2.2. Sampling

The compost material was mixed homogeneously before sampling and ca 1 kg was sampled on days 1, 3, 8, 21, and 40 for each treatment. The initial mixture (CK) was sampled on day 0. All samples were analyzed for total culturable bacteria (TCB), OTC-resistant bacteria (ORB), and ARGs, but only the samples collected on days 3, 21, and 40 were used for 16S rDNA sequencing. The sample was divided into two parts, where one was cut on the benchtop to enumerate the bacterial content in 7 days, and the second was freeze dried using a vacuum freeze dryer (Songyuan, China), milled to 1 mm with an ultra-centrifugal mill (Retsch Z200, Germany), and stored at −80 °C before DNA extraction.

2.3. Enumeration of TCB and ORB

Five grams of each compost sample were suspended in 45 mL of phosphate-buffered saline and shaken for 30 min at 200 rpm. Control plates were also enumerated without the addition of compost samples. The supernatant was serially diluted to 10^{-3} using sterile saline dilution medium. Next, 150 μL of each diluted suspension was inoculated into Luria Bertani medium containing 0 or 50 μg/mL OTC to enumerate the TCB and ORB, respectively [22]. The numbers of colony-forming units were counted after incubating at 37 °C for 36 h. Only plates with 20–200 colonies were used for enumeration. All of the analyses were performed using triplicate samples.

2.4. Isolation and identification of culturable ORB and sulfamethazine-resistant bacteria

The compost samples collected from all of the treatments after 40 days were mixed and used to isolate ORB and sulfamethazine-resistant bacteria. The same procedure was used to enumerate the