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Effects of coal gasification slag on antibiotic resistance genes and the bacterial community during swine manure composting

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ABSTRACT

This study investigated the effects of the addition of coal gasification slag (CGS) at three levels (0%, 5%, and 10% w/w) on antibiotic resistance genes (ARGs) and the bacterial community during composting. The addition of CGS effectively facilitated the removal of ARGs and mobile genetic elements (MGEs), where it significantly reduced the relative abundances of 5/11 ARGs and three MGEs in the swine manure composting product. In addition, the enrichment of ARGs and *intI1* was lower under the addition of 10% CGS compared with 0% CGS. The bacterial community was distributed according to the composting time under different treatments. Redundancy analysis showed that bacterial community succession and MGE-mediated horizontal gene transfer played important roles in the variations in ARGs. Network analysis indicated the co-occurrence of ARGs and MGEs with specific microorganisms. Thus, 10% CGS may be a suitable additive for reducing the risks of ARGs in compost products.

1. Introduction

Due to the increasing demand for animal-derived foods, the livestock and poultry industry have become increasingly intensive, large-scale, and specialized, thereby producing a large among of livestock manure, which is an important source of environmental pollution in China (Hu et al., 2017). A recent survey showed that China produces an estimated 618 billion kg of swine manure annually (Zhu et al., 2013). In modern intensive animal husbandry, antibiotics are used extensively for disease treatment and as growth promoters, thereby leading to a high abundance of antibiotic resistance genes (ARGs) in animal manure. Qian et al. (2017) detected 109 ARGs in chicken, pig, and bovine manure using high-throughput quantitative PCR (H-qPCR). Thus, livestock manure is considered as a reservoir for a wide range of ARGs. As new environmental pollutants, ARGs can be transferred and transmitted among microorganisms via mobile genetic elements (MGEs), and they are difficult to control and eliminate (Binh et al., 2008). Many studies have demonstrated that the direct application of untreated manure to agricultural soils can enrich the ARGs present in the soil (Zhu et al., 2013; Gou et al., 2018; Su et al., 2014). There is a high risk of these manure-derived ARGs spreading into the food chain because they would pose a great threat to human health (Zhu et al., 2016). Therefore, there is an urgent need to find a suitable method for controlling the contamination of ARGs in livestock manure.

Composting is an environmentally friendly method for the treatment and disposal of livestock manure, which can drastically reduce the amounts of pathogens in manure and produce organic fertilizers that can be used as soil conditioners (Cáceres et al., 2017). Previous studies have investigated the effects of composting on the ARGs present in livestock manure. For instance, Duan et al. (2017) found that the absolute abundances of *intI1*, *intI2*, and ARGs were reduced by 41.7% after composting. However, Wang et al. (2015) evaluated changes in tetracycline resistance genes during composting and found that while some remained static, others significantly increased. Conventional aerobic composting has a limited effect on the removal of ARGs and MGEs (Peng et al., 2018). Thus, it is necessary to explore a better way of improving the removal efficiency for ARGs during composting.

The use of additives in compost is important for enhancing the product quality, controlling odor emissions, passivating heavy metals, and reducing nitrogen losses (Jain et al., 2018; Zhang et al., 2017c; Wang et al., 2016b; Wang et al., 2018b). Some previous studies have applied additives in compost to improve the removal efficiency of ARGs. Li et al. (2017) investigated the effects of different proportions of biochar (0%, 5%, 10%, and 20%) on ARGs during chicken manure composting and suggested that the addition of 10% biochar could remove most of the ARGs during composting in a cost-effective manner. Peng et al. (2018) found that chicken manure composting with added zeolite could remove 86.5% of the ARGs and accelerate the removal of

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pathogenic bacteria. Coal gasification slag (CGS) is an inevitable by-product of the coal gasification process, and this carbonaceous material has high porosity and large specific surface area (Pan et al., 2016). The physical properties of CGS are similar to those of biochar and zeolite. In addition, CGS is a waste product so it is inexpensive and readily available. Xiang et al. (2018) showed that modifying sewage sludge with CGS pretreatment before soil application had positive effects on the cultivation of *Alhagi sparsifolia* Shap. However, the effects of the addition of CGS during swine manure composting on ARGs have not been reported previously. Thus, the present study tested whether CGS is an appropriate additive for the effective removal of ARGs in compost.

In the present study, three different proportions of CGS (0%, 5%, and 10%) were added to swine manure compost. Real-time qPCR and high-throughput sequencing methods were used to analyze the changes in ARGS, MGEs, and bacterial communities, and thus the effects of CGS. To obtain insights into the potential mechanisms that might drive the changes in ARGs during swine composting, the relationships among environmental factors, MGEs, ARGs, and the bacterial community were analyzed. The results obtained provide a reference to help reduce the abundances of ARGs during manure composting.

2. Materials and methods

2.1. Experimental setup

The compost materials involved in this study comprised swine manure, wheat straw, and CGS. The swine manure were collected from a pig farm at Northwest A & F University, Shaanxi, China. CGS was obtained from Materials Science and Engineering, Chang'an University, Shaanxi, China.

The composting experiment was conducted at the composting area of Northwest A & F University, PR China. Each composting box comprised a foam box (30 cm long × 30 cm wide × 51 cm tall, wall thickness = 3 cm) with a working volume of about 45 L and two 2 cm × 2 cm holes on each side to provide natural ventilation. The swine manure was mixed with wheat straw, and the C:N ratio was adjusted to 25:1 and the water content to 60%. A control compost box was prepared without the addition of CGS, and 5% CGS and 10% CGS were added separately to the other two compost boxes (based on dry weight). The three composting boxes were designated as CK = swine manure + wheat straw, L = swine manure + wheat straw + 5% CGS, and H = swine manure + wheat straw + 10% CGS. A thermometer was used to measure the temperature in the center of each composting box at 9:00 am and 5:00 pm each day, and the ambient temperature was also measured at the same time. The composting process was conducted for 30 days and the compost was turned over during sampling.

2.2. Sample collection

After composting for 0 days, 3 days, 7 days, 14 days, and 30 days, samples were taken from the upper, middle, and lower parts of each compost pile. After mixing, each sample was divided into two parts for storage. One part was stored at 4 °C for chemical analysis and the other was stored at −80 °C for DNA extraction, and subsequent analysis. D0 denotes the initial mixture without added CGS.

2.3. Determination of the chemical properties

The pH of each compost sample was determined in a fresh sample suspension (solid:water ratio = 1:10, w/v) using a pH meter (Sartorius, Göttingen, Germany). The moisture content was measured as the loss after heating at 105 °C for 8 h. The total nitrogen contents of the samples were analyzed according to the Kjeldahl method (Automatic Kjeldahl Apparatus, FOSS, Denmark). Total carbon was measured with a TOC Analyzer (TOC-UCPH, Shimadzu, Japan). The heavy metal bioavailable fractions (bio-Cu and bio-Zn) were determined as described by Zhang et al. (2017a) using the diethylenetriaminepentaacetic acid (DTPA) extraction method (Flame atomic absorption spectrometer, Hitachi, Japan). DTPA-extractable heavy metals were defined as bio-available heavy metals (Roosa et al., 2014).

2.4. DNA extraction and qPCR

DNA was extracted from 0.100 g of each sample using a Fast DNA SPIN Kit for Soil (MP Biomedical, USA) according to the manufacturer's instructions. Eleven tetracycline resistance genes (tet: *tetA*, *tetB*, *tetC*, *tetE*, *tetG*, *tetM*, *tetO*, *tetQ*, *tetT*, *tetW*, and *tetX*), three sulfonamide resistance genes (sul: *sul1*, *sul2* and *dfrA7*), five fluoroquinolone resistance genes (qnr: *aac(6′)-Ib-cr*, *qnrA*, *parC*, *qnrC*, and *qnrS*), four macrolide resistance genes (erm: *ermB*, *ermF*, *ermQ*, and *ermX*), and four MGEs (*intI1*, *intI2*, *ISCR1*, and *Tn916/1545*) were analyzed by PCR and agarose electrophoresis. The detected ARGs, MGEs, and 16S rRNA gene were analyzed further by qPCR.

The qPCR reaction mixture contained 1 μL of DNA template, 0.25 μL of each 20 pM primer (Sheng Gong, China), 10 μL of SuperReal PreMix Plus (Tian Gen, China), and 8.5 μL of RNase-free water. qPCR analysis was conducted according to the following settings: initial denaturation for 15 min at 95 °C, followed by 40 cycles at 95 °C for 10 s, annealing for 20 s at specific temperatures, and extension at 72 °C for 32 s. The extracted DNA was checked by qPCR using serially diluted samples to minimize PCR inhibition. The squared correlation coefficient (R^2) > 0.99 and amplification efficiency ranged between 90% and 110% for the standard curve, which was used to calculate the copy numbers of ARGs. The qPCR detection limit for ARGs was 10^4 copies/g solid. The relative abundances (RAs) of the ARGs were calculated as: copy number of an ARG/MGE copy number of 16S rRNA. Melting curves were generated and analyzed to detect nonspecific amplification.

2.5. 16S rRNA gene sequencing

The 16S rRNA gene high-throughput sequencing procedure was performed by Novogene (Beijing, China) using the Illumina HiSeq platform. The 16S V4 region was amplified using the primers: 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). The raw data were analyzed using QIIME software and the UPARSE pipeline. The UPARSE pipeline was employed for taxonomic assignment based on ≥97% similarity. Taxonomic classification was performed using the RDP classifier.

2.6. Statistical analysis

Spearman's correlation coefficients and analysis of variance/least significant difference tests ($P < 0.05$) were performed using SPSS Statistics 19.0. Principal components analysis and heatmap analysis were conducted with R3.1.0. Redundancy analysis (RDA) was performed using CANOCO 4.5. Network analyses based on the Spearman's correlation coefficients ($P < 0.01$) between ARGs, MGEs, and genera with RAs > 1% were performed using Cytoscope 3.4.0.

3. Results and discussion

3.1. Variations in ARGs and MGEs during composting

Eleven ARGs were detected during composting. The RAs of the ARGs detected in this study ranged from 10^{-6} to 10^{-2} gene copies/16S rRNA copies in the initial material, and *dfrA7* was the most abundant ARG (5.87×10^{-2} gene copies/16S rRNA copies) in the initial material. Pei et al. (2015) found that the abundance of *sul* genes was high in most areas affected by human activities. The RAs of all the detected ARGs were reduced to different extents (decreased by 5.1–95.9%) in the three treatments during the thermophilic phase. Many previous studies