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Effect of different biochars on antibiotic resistance genes and bacterial community during chicken manure composting

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HIGHLIGHTS

- MB addition resulted in a higher removal of ARGs, RSB yielded opposite results.
- RSB addition had a significant effect on three families of Firmicutes.
- The fate of ARGs might be influenced by pathogenic bacteria during composting.
- Positive correlations were found between ARGs and bio-available heavy metals.

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ABSTRACT

Rice straw biochar (RSB) and mushroom biochar (MB) were added to lab-scale chicken manure composting to evaluate their effects on the behaviors of antibiotic resistance genes (ARGs) and on total and bio-available heavy metals (Cu, Zn and As). The associated bacterial community was characterized by 16S rRNA high-throughput sequencing. The abundance of pathogenic bacteria was also calculated. At the end of the control composting experiment, the average removal rate of ARGs was 0.86 log units and the removal rate of pathogenic bacteria was 57.1%. MB addition resulted in a higher removal rate than that in the control composting experiment. However, RSB addition yielded opposite results, which may be due to the higher abundance of Erysipelotrichaceae, Lactobacillaceae, Family_XI_Incertae_Sedis (belonging to Firmicutes carrying and disseminating ARGs) and pathogenic bacteria carrying ARGs. Furthermore, the correlations between bio-available heavy metals and ARGs were more obvious than those between total heavy metals and ARGs.

1. Introduction

Antibiotics have been widely used in animal husbandry for both prophylactic and therapeutic purposes since the early 1990s. However, 30–90% of the administered antibiotics are excreted in feces and urine as non-metabolized parent compounds, making the animal manure a significant source of antibiotics and causing the development of antibiotic resistant microbes (Heuer et al., 2011). Furthermore, unlike human manure, waste from chicken farms does not undergo tertiary wastewater treatment (Selvam et al., 2012a). Manure carrying antibiotic resistance genes (ARGs) and antibiotic residues could enter soil following directly land application (Fang et al., 2015), which subsequently exerts detrimental effects on human health. Therefore, removal of ARGs from chicken manure before their application to soil has emerged as an environmental issue.

As a bioremediation technology, composting has been shown to be effective in significantly reducing the levels of antibiotics (Selvam et al., 2012a; Hu et al., 2011; Wu et al., 2011; Wang et al., 2012). Selvam et al. (2012b) studied tetracycline, sulfonamide and fluoroquinolone resistance genes during the composting of swine manure spiked with different levels of antibiotics, and found that after 56 days of composting, the selected ARGs, except *par*C, were undetectable in the composting mass. Wang et al. (2012) also observed that tetracycline and erythromycin resistance genes, except *erm*(B) and *tet*(A/C), reduced by at least several orders of magnitude using the simulated composting (55 °C, with modest aeration). However, although administration of chloramphenicol to food-producing animals has been banned in the European Union (EU) since 1994 because of its adverse side-effects, significantly high abundance of its resistance genes (*cml*A, *flo*R, *fex*A, *cfr* and *fex*B) were detected in the wastewater

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from swine feedlots. The similar results were also observed in broiler feedlots that included the manure/litter, soil, sediment, and water samples (Li et al., 2013; He et al., 2014). To date, only few studies have been conducted on chicken manure composting and there is little information available about the fate of chloramphenicol resistance genes during manure composting. In addition, pathogenic bacteria species carrying ARGs were detected in chicken manure (Fang et al., 2015), thus making it necessary to assess the change of these pathogenic bacteria during chicken manure composting.

Biochar is a good bulking agent that regulates moisture content and provides optimum free air space and suitable habitat for microorganisms in the composting mixture (Ogawa and Okimori, 2010). On the other hand, it could accelerate organic matter degradation and ammonium formation during the thermophilic phase and enhance nitrification during the maturation phase (Sánchez-García et al., 2015). Anders et al. (2013) found a marked shift in the microbial communities in the soil after the addition of biochar. Different biochars induced different changes in microbial community structures (Muhammad et al., 2014). Moreover, the dynamic of ARGs was strongly affected by bacterial phylogenetic compositions during sewage sludge composting (Su et al., 2015). Thus, the addition of different biochars to the composting may had different effects on ARGs.

Although previous studies demonstrated that ARGs were strongly affected by some heavy metals (e.g. As, Cu, Hg) in manure samples (Zhu et al., 2013; Ji et al., 2012), there is little information about the correlation between bio-available heavy metals and ARGs. Bio-available heavy metals may influence the microbial community because they could penetrate cell envelopes and exert biological effects (Roosa et al., 2014). Furthermore, the addition of biochar reduced the bioavailability and mobility of heavy metals (Zeng et al., 2015). Thus, while accounting for the fate of ARGs, the concentrations of both total heavy metals and bio-available heavy metals need to be considered.

We hypothesized that the addition of different biochars to the composting mixture (chicken manure and saw dust), even at a low rate, would induce different effects on ARGs. Therefore, in the present work, we chose two different biochars made from rice straw and mushroom residue that were called rice straw biaochar (RSB) and mushroom biochar (MB), respectively. Changes in the relative abundance of three types of ARGs (*tet* genes: *tet*A, *tet*B, *tet*L, *tet*M, *tet*W, *tet*Q, *tet*O and *tet*X; *sul* genes: *sul*1 and *sul*2; chloramphenicol resistance genes: *fex*A, *flo*R, *cml*A, *cfr* and *fex*B) and integrase gene *intI*1 were evaluated by real time fluorescence quantitative PCR (qRCR). In addition, the associated bacterial community was characterized by Ion proton sequencing of bacterial 16S rRNA gene, and the relative abundance of pathogenic bacteria was quantified. The concentrations of total and bio-available heavy metals (such as Cu, Zn and As) were also determined during composting. The objectives of this study were (1) to characterize the fate of selected ARGs during lab-scale composting; (2) to investigate the effect of addition of RSB and MB on bacterial community composition and on the relative abundance of ARGs and pathogenic bacteria; (3) to determine correlations between bio-available heavy metals and ARGs.

2. Methods

2.1. Description of raw materials

Composting materials comprised a mixture of chicken manure, saw dust (2 mm mesh size), with two different biochars, including rice straw biaochar (RSB) and mushroom biochar (MB), used as compost amendments. Chicken manure was collected from dried feces at a selected farm in September 2014 in Yuhang District of Hangzhou, China. The collected manures were stored in the lab at −20 °C for analysis before composting. The physicochemical properties of the raw materials are mentioned in Table S1.

2.2. Composting process

Three lab-scale composting experiments, which contained 5 kg mixture (chicken manure: saw dust = 3:2, v/v) and *C*/*N* ratio of nearly 20, were carried out. A control composting experiment was prepared without the addition of biochar, while RSB and MB were respectively added in the other two composting experiments at the rate of 5% dry weight. Accordingly, three composting experiments were labeled as CM (chicken manure + saw dust), CM + RSB (chicken manure + saw dust + 5% RSB), CM + MB (chicken manure + saw dust + 5% MB). All composting experiments were conducted for 42 days in 29 L rectangular foam containers (44 cm × 31 cm × 21 cm; Fig. S1). Each container was perforated with one hole (2 cm × 2 cm) on the side of the insulation wall, along with an air pump and a flow meter to achieve an aeration rate of 0.45 m^3/kg DW/h. The temperature at the center of each composting was measured at 9:00 am and 4:00 pm daily using thermometer. The temperature in the lab was also recorded daily at the same time. During the composting, ddH_2O was added irregularly to ensure that the moisture was maintained between 53% and 60%.

2.3. Sample collection

Samples were collected by mixing subsamples from the upper, central, and lower portions of the compost after rolling the containers to achieve high representativeness (Su et al., 2015). The samples collected on day 0 were designated as the initial sample, and a set of samples collected on days 5, 13, 22, and 42 were designated, respectively, as mesophilic phase sample, thermophilic phase sample, cooling phase sample, and maturation phase sample. Each sample was split into two parts: one part was stored at 4 °C for later chemical analysis and heavy metal extraction, and another part was stored at −80 °C for subsequent DNA extraction and further analysis after freeze-drying and sieving through a 2 mm mesh.

2.4. Analytical methods

2.4.1. Chemical analyses

Moisture content was measured by drying in an oven at 100 °C for 24 h, or until no change in dry weight was observed. The pH was determined on a 1:10 compost: water (w/v) suspension using the visionPlus pH6175 (Jenco, American) after 1 h equilibrium with shaking on an end-over-end shaker. The analysis of TC, TN and C/N ratio of the raw materials were analyzed by using automatic C/N analyzer (Flash EA 1112, Thermo Finnigan). For total heavy metals, weighed amounts of dried manures were digested with hydrofluoric acid followed by Aqua Regia (nitric acid + hydrochloric acid; 1:3 volume ratio). Meanwhile, the 4-step sequential extractions protocol with the modified BCR approach (Bureau of Reference of the European Community) was also applied to quantify the different fractions (exchangeable fraction; reducible fraction; oxidizable fraction and residual fraction) of heavy metal (Rauret et al., 1999). The sum of the first two fractions was defined as bio-available heavy metal because these fractions are potentially the most bioavailable (Roosa et al., 2014). An AFS-230E atomic fluorescence spectrophotometer (Beijing Kechuang Haiguang Instrument Company, China) was used to quantify As concentrations. The other two heavy metals were measured by a SHIMADZU AA-6300 atomic absorption spectrophotometer (AAS).