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Compost-bulking agents reduce the reservoir of antibiotics and antibiotic resistance genes in manures by modifying bacterial microbiota

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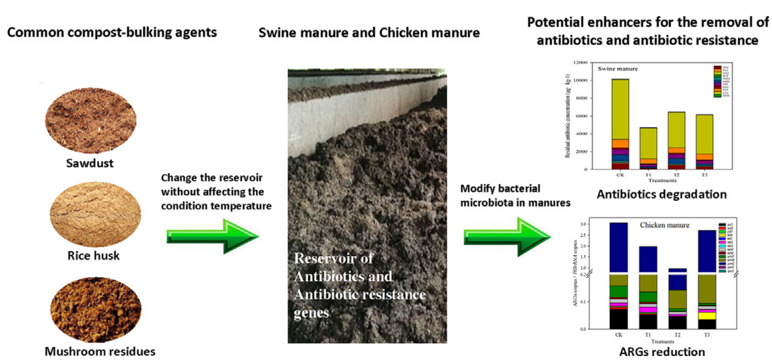
HIGHLIGHTS

- Bulking agents enhance antibiotics and ARGs removal without affecting temperature.
- The effects of bulking agents on antibiotics and ARGs fates vary with manure types.
- Sawdust is the most efficient enhancer for antibiotic removal in manures.
- Rice husk added into chicken manure effectively reduces ARGs.
- Antibiotics degrading and ARGs carrying responders to bulking agents were identified.

GRAPHICAL ABSTRACT



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ABSTRACT

Sawdust, rice husk, and mushroom residues are commonly used as bulking agents during manure composting; this work evaluated their potentiality for enhancing the removal of antibiotics and antibiotic resistance genes (ARGs) under a temperature-consistent condition. Results indicated that the addition of all the compost-bulking agents increased antibiotic removal in swine manure with increasing rates of 14.9%–33.4%; however they showed less effect on the reduction of residual antibiotics in chicken manure where fluoroquinolone (FQs) antibiotics are the dominant antibiotics, partly owing to the weak promoting effects of bulking agents on FQs degradation. The addition of bulking agents somehow hindered the reduction of ARGs within swine manure, whereas there were obvious reductions in the total relative abundance of ARGs in chicken manure with bulking agents added. Among the three bulking agents, sawdust was the most efficient enhancer for antibiotic removal in both manures, and rice husk exhibited the best performance on ARGs reduction in chicken manure. The relationship between antibiotics, ARGs and bacteria communities was subsequently delineated. Proteobacteria was proposed to play key roles on the effect of bulking agent addition on antibiotics and ARGs in swine manure. Particularly, the increased Xanthomonadaceae contributed much to the promoted antibiotic degradation as well as the high level of ARGs in swine manure with sawdust added. By contrast, the changes in dominant bacterial families by the addition of bulking agents into chicken manure were not strong enough to effectively enhance antibiotic removal, but largely influenced the ARGs abundance. The large reductions of Paenibacillaceae

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and Staphylococcaceae by rice husk addition were closely related to the reduced ARGs in chicken manure with rice husk added.

1. Introduction

Animal manure and its products have become important reservoirs of antibiotics and antibiotic resistance genes (ARGs) owing to the overuse of antibiotics in the livestock industry (Heuer et al., 2011; Zhu et al., 2013). Following the land application of animal manures that are rich in antibiotic and ARGs, the antibiotic resistance level of farmland soil increases and thus poses health and environmental problems (Sarmah et al., 2006). Mounting evidences suggests that the environmental release of antibiotic residues and antibiotic resistance agents is the main driver behind the increased risk of emergence of multi-resistant animal pathogens (Wright, 2010). Technologies such as anaerobic digest and aerobic composting have being used to reduce antibiotics residues and antibiotic resistance agents such as ARGs in manure based products, before their land use (Wei et al., 2016). Particularly, in manure-based organic fertilizer production, aerobic composting is well recognized as an efficient, simple, and economically feasible management to reduce antibiotic residues and antibiotic resistance in the products (Arikan et al., 2009; Wang et al., 2015). However, the removal efficiencies of residual antibiotics, and particularly ARGs within manure, by current reported technologies greatly varies among different animal manures, different treatment processes, and even different sites, being quite low in some cases (Zhao et al., 2010; Zhu et al., 2013). Researches on improving the antibiotics and ARGs removal efficiency of manure management technologies is a meaningful topic, which could involve the process optimization, the isolation of antibiotic degrading strains and the exploration of less-cost materials capable of enhancing the reduction of antibiotics and ARGs (Cui et al., 2016; Duan et al., 2017).

The addition of bulking agent is common during animal manure composting as it adjusts the humidity and the ratio of carbon to nitrogen of manure to an optimal condition for composting, which usually causes a rapid rise of compost temperature. Thus, most current works on the fates of antibiotics and ARGs during manure composting are performed under the condition with an addition of bulking agent (Selvam et al., 2013; Chai et al., 2016). It is believed that the addition of bulking agent during composting is able to enhance antibiotic degradation and inhibit ARGs spread within manure. Direct evidence is the work of Qiu et al. (2012) which showed the addition of rice straw and sawdust into manure resulted in an increased removal of sulfonamide antibiotic residues after composting. The materials of bulking agents are diverse, and agricultural wastes are the most common used owing to its less-cost and the necessity of recycling. This work tried to explore efficient enhancers from agricultural wastes that are frequently used as bulking agents in composting, for the removal of antibiotic residues and their ARGs. Sawdust, rice husk and mushroom residues are three commonly used bulking materials for the manure-based organic fertilizer production in Zhejiang Province, P.R. China, and their effects on the removal of residual antibiotics and ARGs in swine and chicken manures were compared herein.

The increase in compost temperature or high temperature retention is usually supposed as the key reason for bulking agents (e.g. rice straw, biochar, red mud) to favor antibiotic degradation (Qiu et al., 2012) and ARGs removal (Wang et al., 2016; Li et al., 2017) in composting as most of antibiotics are unstable under a thermophilic condition. Despite this, further investigations are still required to understand in what ways the bulking agents enhance antibiotics degradation and ARGs removal during composting. For example, whether bulking agents can change antibiotics and ARGs persistence within manure without affecting the temperature was still an outstanding question. As a result, this study investigated the effects of bulking agent addition on the persistence of antibiotics and ARGs under a temperature-consistent condition, in contrast to previous related reports (Qiu et al., 2012; Li et al., 2017). Temperature varies a lot during manure composting. A conventional composting process often contains the high-temperature phase (thermophilic-phase) and the low-temperature (mesophilic- or mature-phase) phase. This work putted focus on the fates of antibiotic and ARGs at a relatively low temperature of 30 °C as well as the roles of mesophilic bacteria on the removal of antibiotic and ARGs after bulking agent added. By setting a temperature-consistent condition in this study, we may also understand the existence of other enhancing mechanisms responsible for antibiotic degradation and ARG removal in composting with bulking agent added. The potential antibiotic degrading responders as well as the ARGs carrying responders to the addition of bulking agents were also explored by delineating the relationship between antibiotics, target ARGs and bacteria communities. The results would provide new insights into the control of antibiotic and antibiotic resistance pollution.

2. Materials and methods

2.1. Raw materials

Fresh swine and chicken manure samples were collected from a livestock farm in Yiwu City, Zhejiang Province, P.R. China, and immediately stored at 4 °C. Sawdust, rice husk, and mushroom residues were obtained from a local market in Yiwu City. The physicochemical properties of the above-mentioned materials were shown in Table S1.

2.2. Experimental design and sample collection

Four treatments, with or without bulking agent addition, were designed for each manure type: (1) CK: control treatment, only manure without the addition of bulking agent; (2) T1: manure added with 10% (w/w) sawdust; (3) T2: manure added with 10% (w/w) rice husk; (4) T3: manure added with 10% (w/w) mushroom residues. Each treatment had three replicates. For preparing the mixed samples, fresh manure sample was crushed and mixed homogeneously with bulking material at a rate of 10% (w/w), and then the manure-bulking agent mixture was packed in a sterilized shallow tray. The fresh weights of samples in each tray were 35 g for the treatments of chicken manure and 40 g for the treatments of swine manure. The physicochemical properties of the samples prepared with different treatments were shown in Table S2, and there was no big difference in physicochemical properties among different treatments of the same manure type. Finally, all treatments were subjected to solid-state incubation at 30 °C and 50% humidity for 5 days. After incubation, the samples in each tray were divided into two parts: one was air-dried for chemical analysis, and the other was stored at −20 °C or below for antibiotic analysis and genomic DNA extraction.

2.3. Determination of antibiotic concentration

Twenty sulfonamide antibiotics (SAs), four tetracycline antibiotics (TCs), nineteen fluoroquinolone antibiotics (FQs) and eleven macrolide antibiotics (MLSs) were determined in all samples. Antibiotic residues were extracted and purified from manure samples, and determined by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), according to the procedures as previously described (Qian et al., 2016; Lin et al., 2017). SAs, TCs, FQs and MLSs were extracted using the mixtures of acetonitrile with Na_2EDTA-McIlvaine