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Effects of co-composting of lincomycin mycelia dregs with furfural slag on lincomycin degradation, degradation products, antibiotic resistance genes and bacterial community

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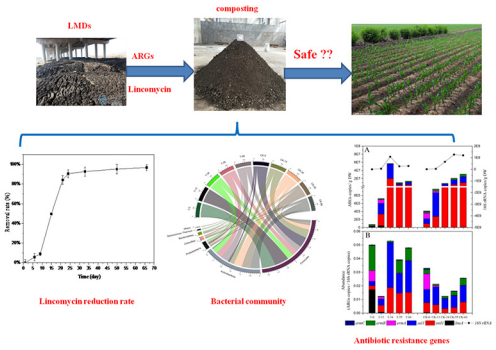
GRAPHICAL ABSTRACT



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ABSTRACT

This study explored the effects of co-composting of lincomycin mycelia dregs (LMDs) with furfural slag on variations in antibiotic resistance genes (ARGs) and the bacterial community. The results showed that more than 99% lincomycin was reduced after composting. Moreover, the total absolute and relative abundance of ARGs increased by 180 and 5 times, respectively. The gene *lnuA* was detected in the LMDs compost and it was proved to participate in lincomycin biodegradation based on the analysis of Pearson's correlation and the lincomycin degradation byproducts. Redundancy analysis showed the succession of the bacterial community had a greater influence than the environmental parameters (residual lincomycin, C/N, pH and temperature) on the variation of ARGs during composting. Composting was not effective in reducing most of the ARGs and *intI1* and thus the LMDs compost is dangerous to the ecological environment.

1. Introduction

As the largest producer of antibiotics in the world, China produced about 248,000 tons of antibiotics in 2013, accounting for 70% of the total global production (Zhang et al., 2015a). Correspondingly, as one of the most important by-products, millions of tons of antibiotics mycelia dregs (AMDs) are being generated each year (Zhang et al., 2015b). Although AMDs are rich in nutrients, they also contain high levels of

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residual antibiotics (Li et al., 2015). Improper treatments of AMDs would result in the dissemination of antibiotics to the environment (Chen et al., 2015a; Zhang et al., 2016a). So AMDs' safe disposal has been becoming a pressing problem.

Aerobic composting was considered one of the most promising treatments to deal with organic pollutants (Zhang et al., 2011; Yin et al., 2016). Many papers have suggested that most of the antibiotics from livestock manure or other materials could be degraded via composting (Liu et al., 2016; Ravindran and Mnkeni, 2017; Selvam et al., 2013; Yang et al., 2017). However, a significant issue with AMDs was the potential risk of increasing antibiotic resistance genes (ARGs) in the AMDs compost. The increased ARGs may pose a risk to the soil, water, air, plants and animals etc. (Gao et al., 2018; Qian et al., 2018; Guo et al., 2018; Fang et al., 2015; Sivagami et al., 2018; Su et al., 2017; Wang et al., 2018a; Zhu et al., 2013), which eventually threaten to human health. Therefore, to convert AMDs into a valuable resource, the removal of antibiotics was not enough, the ARGs' reduction might be more important. Many papers evaluated the feasibility of AMDs via composting just using simple physicochemical parameters, little is known about the variations of bacterial community and evolutions ARGs, particularly for the relationship between ARGs and bacterial community (Zhang et al., 2015c; Yang et al., 2016).

There are three known mechanisms responsible for resistance to lincomycin: (1) cellular protection through *23S rRNA* methylases encoded by *erm* genes, (2) efflux pumps through lincomycin export encoded by *vag* genes and (3) deactivation through phosphorylation or nucleotidylation encoded by *lnu* genes (Li et al., 2013). However, few investigations have searched for lincomycin resistance genes during LMDs composting. In the meanwhile, many studies have suggested that integron genes were the carriers of ARGs and they facilitate the horizontal transfer of ARGs between environmental bacteria and human pathogens (Duan et al., 2018; Subirats et al., 2018; Zhu et al., 2017). In addition, little information is known about the mechanisms of lincomycin degradation and its degradation products during composting. Based on these researches above, LMDs were co-composted with furfural slag. The aims of this study were: (1) to analyze the lincomycin degradation and its decomposition products, (2) to investigate the variations of ARGs, integron genes and bacterial community during LMDs and furfural slug composting, (3) to investigate the relationships among the environmental factors (pH, lincomycin residue, temperature and C/N), bacterial community, AGRs and integron genes. An overall and reliable evaluation of the security of LMDs co-composting with furfural slag was acquired.

2. Materials and methods

2.1. Composting materials and experimental setup

LMDs were obtained from a local biological pharmaceutical industry, Henan, China. Furfural slag was collected from a furfural plant in Henan province. Plant ash was collected from a biological power plant (Henan, China).

the composting experiments were performed in the composting area at Henan Xinxiang Hua Xing Pharmaceutical Factory, China. Furfural slag's pH was very low because of the extraction of sulphuric acid (Wang et al., 2013). In order to adjust pH, plant ash was mixed with furfural slag to adjust the pH of furfural slag. According to the previous study, the addition of about 20% plant ash (w/w) could adjust furfural slag's pH to 6.5–7.0 which was suitable for the growth of bacteria during composting. So the ratio of these two materials was set 1:0.2 (w/w). In order to simulate the actual composting conditions, pilot experiments were designed. Briefly, A total of 840 kg of a mixture of LMDs, furfural slag and plant ash at a ratio of 3:1:0.2 (w/w) was used. Furfural slag was added to adjust the initial C/N to approximately 25:1. Meanwhile, the same condition of a mixture of sewage sludge, furfural slag and plant ash was performed as the control. The composting pile of LMDs and furfural slag was called as T, and the control pile using sewage sludge and furfural slag without LMDs was designated as CK. Each pile was turned and mixed every three days manually, and the moisture was kept between 55% and 60% with tap water during all the composting time. The samples were collected from five longitudinal sections and different depths. After mixed, these collected samples were divided into two parts: one was freeze-dried with a vacuum freeze dryer (FD-1A-50, boyikang, China) and then preserved at −20 °C for the determination of lincomycin concentrations, 16S rRNA sequencing and quantitative PCR. The other part was stored at 4 °C for physicochemical analysis such as moisture, pH and C/N.

2.2. Determination of environmental factors

The temperature was recorded every day at a constant depth of 45 cm during composting. Moisture content was measured by drying the fresh samples in an oven at 105 °C until a constant weight. The pH determination was detected in the supernatant of 1:10 (w/v) water-soluble extract using a pH electrode (METTLER TOLEDO, Swiss). The C/N was measured with the method provided by Zeng et al. (2011). The determination of lincomycin was performed by HPLC according to our previous publication (Ren et al., 2018) and the lincomycin degradation percentage (D) was expressed as following formula:

$$D(\%) = 100 \times (C_n - C_0)/C_0$$

Where:
C_0: the concentration of lincomycin in the compost on day 0;
C_n: the concentration of lincomycin in the compost on day n.

2.3. Identification of lincomycin degradation products

HPLC (Water) coupled to an ion trap mass spectrometer (Micromass Q-TOF Micro TM) with a C18 column (250 mm × 4.6 mm, 5 μm, Discovery) was used to determinate the biodegradation products of lincomycin. Positive mode of electrospray ionization source was chosen for MS measurements with a capillary voltage of 4.0kv, nebulizer temperature of 350 °C. Potential lincomycin degradation products were separated by a 1.0 mL/min mobile phase at 30 °C in a thermostat. Mobile phase (A) was H_2O with 0.1% formic acid and (B) was methanol. Isocratic elution in the ratio of A/B = 20:80. Mass spectra were collected in the range of 110–1000 *m/z* using full scan mode. Firstly, lincomycin biodegradation products were identified in the total ions chromatograph by extracting *m/z*. Then the second stage of MS/MS was used to further analyze the product ions. The degradation products of lincomycin were finally identified according to MS analysis and related papers. The instrument control, data acquisition and evaluation were conducted with Alliance HPLC and Waters Masslynx TM 4.0 (for MS) software. The molecular structures were conducted with ChemDraw Ultra 7.0 software.

2.4. DNA extraction and qPCR

DNA was extracted from the compost samples using a fastDNA kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions. Standard PCR was performed to determine the presence of 9 lincomycin resistance genes (*lnuA*, *lnuB*, *lnuC*, *ermA*, *ermB*, *ermC*, *ImrA*, *vgaA*, *vgaD*), 2 sulfonamide resistances genes (*sul1* and *sul2*) and the integrase gene of class 1 integrons (*intI1*). 1.5% (w/v) agarose gel electrophoresis was used to analyze the PCR products. Five antibiotic resistance genes (*lnuA*, *sul1*, *ermA*, *ermB*, *ermC*) and *intI1* were detected. So they were analyzed further by qPCR using lightcycler480 Real-Time PCR detection system (Roche). The qPCR reaction system comprised a volume of 10 μL containing 1 μL of DNA template, 5 μL of 2X SybrGreen qPCR Master mix (Roche), 0.2 μL of each 10 μM primer (ShengGong, China), and 3.6 μL of double distilled water. The thermal cycling steps for qPCR amplification were as follows: (1) 95 °C for 3 min; (2) 95 °C for