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Short Communication

Role of earthworms' mucus in vermicomposting system: Biodegradation tests based on humification and microbial activity

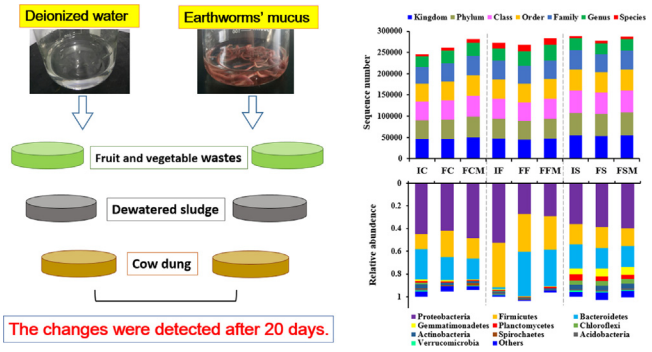
Kui Huang *, Hui Xia

School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou, 730070, China

HIGHLIGHTS

- Role of earthworms' mucus in vermicomposting systems was investigated.
- Earthworms' mucus facilitated the mineralization and humification of organics.
- Mucus led to the greatest increases of microbial activity in the FVW systems.
- Mucus positively stimulated Proteobacteria, but negatively affected Firmicutes.

GRAPHICAL ABSTRACT



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ABSTRACT

During vermicomposting, the organic wastes can be recycled into high-value products as mediated by earthworms through gut digestion, burrowing, casting and mucus excretion. However, to date, few studies have been done on the role of mucus in vermicomposting system compared to the effects of the other activities. Hence, this study investigated the potential role of earthworms' mucus in the decomposition and humification of organic wastes. For this, the mucus of *Eisenia fetida* was extracted and inoculated into three vermicomposting substrates using cow dung (CD), fruit and vegetable wastes (FVW), and sewage sludge (SS). The results obtained after a 20 day experiment showed that the mucus could accelerate the mineralization and humification rates of organic components. The dissolved carbon showed 9.8%–37.5% increase in treatments containing mucus, higher than those in substrates without mucus. Moreover, the mucus significantly stimulated the microbial activity and bacterial abundance, showing the greatest increases in FVW treatments. In addition, the mucus positively stimulated growth of Proteobacteria, but negatively affected the Firmicutes during decomposition. This result suggests that the earthworms' mucus significantly accelerated the decomposition and humification of vermicomposting materials, and could even promote microbial activity, growth, and increase community diversity in vermicomposting systems.

1. Introduction

Vermicomposting is a natural process of biochemical decomposition of organic wastes through the metabolic processing of both earthworms and microorganisms, allowing the bioconversion of organic wastes into bio-fertilizer for soil improvement. As a green and environment-

* Corresponding author.
E-mail address: huangkui@mail.lzjtu.cn (K. Huang).

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friendly technology, several organic wastes have been shown to be vermicomposted by earthworms (Lim et al., 2016). Earthworms, as a key driver, directly regulate the activity, abundance, community composition, as well as the habitat of the microbial community through their activities such as digestion, burrowing, mucus excretion, and casting, thus accelerating decomposition process (Brown et al., 2000; Domínguez et al., 2010). In addition, their metabolic activities and behavior also indirectly affect the features of microbial pools by converting the raw substrate into forms that can be readily assimilated, building a new hotspot for the microbiota (Domínguez et al., 2010; Kuzyakov and Blagodatskaya, 2015). Consequently, separately studying each earthworms' behavior in the drilosphere would allow illuminating and distinguishing the interactions and interrelations between earthworms and their microbiota.

To date, several studies have shown that the digestion, burrowing, and casting of earthworms could exert considerable influences on microbial communities in vermicomposting system (Domínguez et al., 2010; Huang et al., 2013), though the true extent of these influences are still to be ascertained. For example, after digestion in the gut of the earthworms, the total carbon and nitrogen contents decreased significantly, while the dissolved substances increased (Domínguez et al., 2010; Hussain et al., 2016). Moreover, earthworm burrows are considered as microbial hotspots, where enzyme activities and dissolved organic carbon and nitrogen tend to be higher (Parkin and Berry, 1999; Hoang et al., 2016). Similarly, earthworm casts can be enriched in mineral nitrogen, relative to the surrounding soil (Parkin and Berry, 1994; Parkin and Berry, 1999). However, compared to the other activities of earthworms in vermicomposting systems, studies on the role of the earthworms' mucus in the decomposition is still limited.

The earthworms' mucus contains a mixture of carbohydrates and protein-like substances that have multiple ecological functions in soil drilosphere. It does not only have drag-reducing characteristics in soil that benefit the movement of earthworms (Zhang et al., 2016), but also serves as nutrient source for plants (Zhang et al., 2009). Further, the earthworms' mucus had strong influences on the mobility and speciation of arsenic in contaminated soils (Sizmur et al., 2011). Accordingly, Salmon (2001) found that the earthworms' mucus could affect the community distribution of springtails in forest soils. Moreover, it has priming effect on the stimulation of microbial activity and decomposition of plant residuals in soil (Marichal et al., 2011; Bityutskii et al., 2012). To date, although some studies started investigating the effects of earthworms' mucus in the soil system, little information is available on the mucus' roles in vermicomposting systems. In contrast to the soil system, vermicomposts display much more complex characteristics with higher organic matter and microbial community diversity. Previous studies on soil systems showed that the mucus, as one of the products produced by the earthworms, may exert significant effects on the quality of vermicomposting products. Thus, to improve the quality of vermicomposts, it is of utmost importance to assess the effects of earthworms' mucus on vermicomposting system, particularly on the humification index and microbial community diversity.

This study investigated (1) the roles of the earthworms' mucus on organic matter decomposition and humification, and (2) the changes on the microbial pool when stimulated by the earthworms' mucus. Considering that different vermicomposting substrates may cause dissimilar effects, three common vermicomposting materials including fresh fruit and vegetable wastes (FVW), cow dung (CD), and dewatered sewage sludge (SS) were used as raw substrates.

2. Methods

2.1. Experimental setup

The fresh FVW, CD, and SS were separately collected from the local Hualian supermarket, cattle farm of Lanzhou Agricultural University, and Qilihe wastewater treatment plant in Anning District, Lanzhou City, respectively. All raw materials were stabilized and turned over in laboratory for one week before use. The earthworms' mucus was collected from the epigeic species of *Eisenia fetida*. After two-month culturing using dewatered sludge in the laboratory, adult earthworms with individual weights of 0.5 g were collected and then washed with tap water. To remove the gut content, the earthworms were not fed for 48 h and then rinsed with distilled water. Then, each 30 active earthworms were separately placed into 50 ml distilled water to excrete the mucus solution. In total, 600 earthworms were used to prepare the mucus. Because dying earthworms could cause sudden changes in the pH and electrical conductivity, the excretion was continued for 8 h (detailed in Supplemental Fig. S1). After which earthworms were picked out and their corresponding mucus solutions were mixed and used for subsequent experiments. Totally, 1000 ml of mucus were prepared. Physicochemical characteristics of the mucus are summarized in Supplemental Tables S1 and S2. Subsequently, 20 ml mucus was separately added into each 12 cm petri dish containing 300 g fresh substrate. For comparison, the same set-up but with 20 ml distilled water was used as the control. In this study, each treatment was carried out in triplicates. After gently mixing, all petri dishes were incubated at 25 °C in a vacuum drying oven. To maintain the moisture, 10 ml distilled water was added into each petri dish every 5 days. Since previous studies showed that the greatest effects of mucus manifested in the first 20 days (Bityutskii et al., 2012), this experiment was terminated on the 20th day, and samples were stored in −20 °C before use.

2.2. Analytical methods

The pH, electrical conductivity, dissolved phosphorous (DP), ammonia nitrogen, nitrate nitrogen, and dehydrogenase activity of the samples were measured using the methods described by Fu et al. (2016). Briefly, total carbon and nitrogen of dry samples were simultaneously determined using elemental analyzer (EuroVector, EA3000). The dissolved carbon (DC) and nitrogen (DN) were measured by TOC instrument (TNM-L, SHIMADZU) while amino acids were quantified using Cecil AA4300. Three-dimensional fluorescence excitation emission matrix spectroscopy for analyzing the organic forms of vermicomposts by the fluorescence spectrophotometers (RF-5300PC, SHIMADZU), following the methods of Huang et al. (2017).

The genomic DNA was extracted directly from the soil using the DNA isolation kit (MO BIO Laboratories, USA). Then, the abundance of bacterial 16S rDNA V3 region amplified by the primers of 341f/518r was quantified with the SYBR® *Premix Ex Taq*™ (TaKaRa) using the Thermal Cycler Dice (TP900, TAKARA), based on the methods of Huang et al. (2013). The bacterial 16S rDNA V4 region amplified the universal primers 515f/806r for pyrosequencing carried out at the Novogene Bioinformatics Technology Co., Ltd. Additional details on the PCR primers are given in the Supporting information (Table S3). The pyrosequencing was carried out in an Illumina MiSeq 2 × 250 platform, according to protocols described by Caporaso et al. (2012). High quality sequences were clustered into operational taxonomic units (OTUs) using UCLUST at 97% similarity level. The resulting data was used to calculate for alpha and beta diversities, and observed species on OTUs, as analyzed in QIIME (Version 1.7.0). Cluster analysis of the unweighted and weighted unifrac distance metrics of OTUs was performed by principal component analysis (PCA) and Principal coordinate analysis (PCoA). A student's *t*-test was used to compare differences in physicochemical properties at the P value of 0.05.

3. Results and discussion

Compared to the control without mucus, all treatments displayed lower total carbon and total nitrogen levels, but not significantly different (Table 1). In contrast, Bityutskii et al. (2012) found a significant decrease on total carbon in soil and plant residues with the addition of mucus. This difference could probably be due to the substrates used