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# Dynamic changes of the dominant functioning microbial community in the compost of a 90-m<sup>3</sup> aerobic solid state fermentor revealed by integrated meta-omics



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#### HIGHLIGHTS

- The dynamic changes of fermentor communities were analyzed by integrated meta-omics.
- Thermobifida fusca was the dominant lignocellulose degrader in cow dung composts.
- Control of the temperature and oxygen increased the fermentation efficiency.
- A 90-m<sup>3</sup> industrial-scale fermentor was used for artificial control of composting.

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#### ABSTRACT

The dynamic changes in the composition and function of both bacterial and fungal communities over time and at various depths in the compost of a 90-m³ industrial-scale fermentor were explored using integrated meta-omics. The microbial communities in the middle layer (1.2 m) of the compost developed a stable and simple structure over time, which was mainly composed of *Thermobifida*, *Bacillus*, *Thermomyces* and *Aspergillus*. According to the metaproteomic results, the bacterial community was more focused on cellulose degradation, characterized by 44% of the cellulases that were secreted by *Thermobifida*, while the fungal community was more likely to degrade hemicellulose, mainly via *Thermomyces* and *Aspergillus*. The results revealed that, under artificial control of the temperature and oxygen concentration, the efficiency of organic waste degradation was greatly increased and the fermentation cycle was shortened to 11 days.

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#### 1. Introduction

Organic wastes have become a focus of research due to the threat they pose to the environment and human health in modern society. Worldwide production of agrowaste amounts to more than hundreds of megatons every year, and a large portion of these agricultural residues is disposed of as waste or directly burned (Sarkar et al., 2012), further intensifying climate warming and air pollution. Composting can convert a variety of organic wastes into simple materials, and represents one of the most cost-effective and environmentally benign methodologies for the sustainable processing of such wastes (López-González et al., 2015).

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Composting is an aerobic process driven by the activities of microbial communities (de Gannes et al., 2013), which are affected by the types of substrates present and physico-chemical conditions, such as temperature, oxygen concentration, moisture content and pH etc. (Lei and Vandergheynst, 2000; Liang et al., 2003; Schütte et al., 2008). As the key players in promoting composting, these microbial communities have received considerable attention from researchers in related fields (Nayak et al., 2007; Peters et al., 2000; Ryckeboer et al., 2003). With the development of highthroughput sequencing technology, the composition and dynamics of microbial communities in various composts have been investigated (de Gannes et al., 2013; Zhang et al., 2015). Using a metaproteomic approach, the dominant microorganisms that degrade lignocellulose can be located and identified (Johnson-Rollings et al., 2014). Such studies are mainly focused on spontaneous changes within the microbial communities in composts in the natural environment. Our knowledge of the large-scale utilization of

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these dominant communities to improve compost efficiency and shorten the compost cycle is still limited.

The process of natural composting occurs over a long time period comprised of three phases, the mesophilic, thermophilic and mature phases (de Gannes et al., 2013; Zhang et al., 2014). To promote the efficient degradation and utilization of lignocellulosic biomass, the composting process is expected to be continuously in the thermophilic phase. To best realize this objective, compost conditions such as the temperature and oxygen concentration that affect the growth of microbial communities should be well established (Miyatake and Iwabuchi, 2006; Zhang et al., 2015), and this can be addressed by using a fermentor apparatus (Martins et al., 2013; Wei et al., 2012). However, we still have a limited understanding of the dynamic changes in composition and function of the microbial communities that occur with temporal and spatial variation. Additionally, from an industrial application perspective. ways of controlling the compost conditions to better enrich the dominant communities that play important roles in lignocellulose degradation have yet to be determined.

The aim of this study was to examine the dynamic changes in diversity and function of the microbial communities over time and at various depths in a fermentor compost under manual control. To fulfill this objective, this study investigated the temporal and spatial variations in the composition of the microbial communities in a 90-m³ industrial-scale fermentor corn cob compost using a pyrosequencing approach. To identify the dominant communities that participate in lignocellulose degradation, a metaproteomic approach was applied.

#### 2. Methods

#### 2.1. Compost composition and sampling

The compost study was performed in a  $90\text{-m}^3$  cylindrical fermentor (diameter, 5.4 m; height, 4 m, Fig. 1) in Shandong Province, China. The agricultural waste was corn cobs that were ground and mixed with fresh cow dung to obtain a C:N of 25-35:1, and this

**Table 1**Sample origin and physico-chemical parameters of the compost samples.

Sample	Sampling height (m)	Day	Temperature (°C)	pН	EC (mS/cm)
S7	3.3	7	25-30	6.3 ± 0.5	3.4 ± 0.1
U7	3.2	7	55-60	$7.2 \pm 0.3$	$2.9 \pm 0.4$
T7	1.8	7	70-75	$7.2 \pm 0.1$	$3.6 \pm 0.3$
M7	1.2	7	65-70	$6.5 \pm 0.1$	$5.9 \pm 0.6$
B7	0.1	7	50-55	$6.8 \pm 0.2$	$4.3 \pm 0.4$
M0	1.2	0	5-10	$5.9 \pm 0.3$	$3.3 \pm 0.1$
M4	1.2	4	60-65	$6.7 \pm 0.1$	$3.9 \pm 0.5$
M11	1.2	11	60-65	$6.5 \pm 0.1$	$5.1 \pm 0.5$
C7	0.2	7	60-65	$6.9 \pm 0.2$	$4.6 \pm 0.6$

mixture was used as the substrate for composting. Composted materials obtained from the mature phase of a natural maize straw compost were added to inoculate microorganisms. The final weight of the compost was over 100 tons and its height was 3.3 m in the apparatus. The initial water content was controlled within the range of 60–65% with the addition of water. The water content of samples was measured by drying them overnight in an oven. Once the inner temperature of the compost was over 50 °C and it had entered the thermophilic phase, the blower at the bottom of the fermentor was activated to aerate the compost.

After three consecutive months of the studying in fermentor composts, the best optimized experiment which lasted for 11 days was chosen for the further research reported in this paper. As listed in Table 1, a total of nine samples were collected from the compost at different heights and times. A sample taken from the mixture at day 0 was referred to as M0. Samples taken from days 4, 7 and 11 at a height of 1.2 m were referred to as M4, M7 and M11, respectively. In addition, as shown in Fig. 1A, samples were taken from five different layers at day 7 and were referred to as S7 (3.3 m), U7 (3.2 m), T7 (1.8 m), M7 (1.2 m) and B7 (0.1 m). A sample taken from a natural compost (the same substrate in a pile in the environment) at day 7 was referred to as C7. All samples were mixtures of three subsamples within the pile from a depth of *ca*. 30 cm and were stored at  $-80\,^{\circ}\text{C}$  before use.

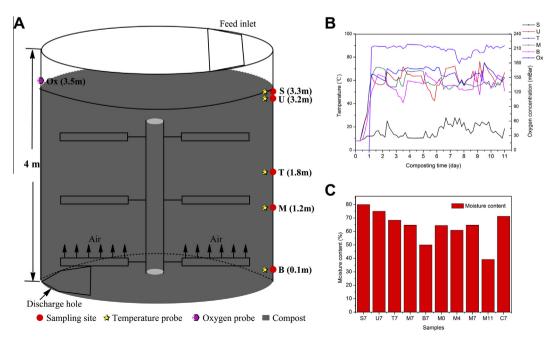


Fig. 1. Compost setup and physical properties. (A) Schematic diagram of the fermentor apparatus; (B) changes in temperature and oxygen concentration over time; (C) moisture contents of compost samples.

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