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The distribution of active β-glucosidase producing microbial communities in composting

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Abstract

The composting ecosystem is a suitable source for the discovery of novel microorganisms and secondary metabolites. Cellulose degradation is an important part of global carbon cycle, and β-Glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to glucose. This work analyzes the succession of β-glucosidase-producing microbial communities that persists throughout cattle manure-rice straw composting, evaluates their metabolic activities and community advantage during the various phases of composting. Fungal and bacterial β-glucosidase genes belonging to glycoside hydrolase families GH1 and GH3 amplified from DNA and gene abundance levels were analyzed. The major reservoirs of β-glucosidase genes were the fungal phylum *Ascomycota* and the bacterial phyla *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *DeinococcusThermus*. This indicates that a diverse microbial community utilized cellobiose. The succession of dominant bacteria was also detected during composting. *Firmicutes* was the dominant bacteria in the thermophilic phase of composting and shifted to *Actinomycetes* in the maturing stage. *Proteobacteria* accounted for the highest proportions during heating phase and thermophilic phase of composting. By contrast,

fungal phylum *Ascomycota* were minor microbial community constituents in thermophilic phase of composting.Combined with the analysis of the temperature, cellulose degradation rate and the CMCase and β-glucosidase activities, showed that the bacterial GH1 family β-glucosidase genes make greater contribution in cellulose degradation at the later thermophilic stage of composting. In summary, even GH1 bacteria families β-glucosidase genes showing low abundance in DNA may be functionally important in the later of thermophilic phase in composting. The results indicate that a complex community of bacteria and fungi expresses β-glucosidases in compost. Several β-glucosidase-producing bacteria and fungi identified in this study may represent potential indicators of composting in cellulose degradation.

Keywords: β-Glucosidase; Aerobic composting; Cellulose degradation; Functional microbial community

Introduction

Composting is a process involving a complex ecosystem with many interacting factors, in which biodegradable organic wastes are stabilized and converted by the action of some microorganisms under controlled conditions (Tang 2004; Khalil 2001). Microorganisms are the essential factors for the successful operation of composting. In order to effectively control the composting process, it's necessary to understand the microbial community structure and its change, especially its special role in decomposition of organic matters (Beffa 1996). Cellulosic biomass has been most abundant biomass that widely distributed on earth. Cellulose is degraded into monomeric sugar by cellulases, and is appropriate source of biofuels and biochemicals production, and can be used as a carbon

source during composting. However, high crystallinity and insolubility of cellulose makes difficult to degrade to soluble sugar, such as glucose.

Enzymatic hydrolysis of cellulose typically requires the synergic action of three groups of hydrolytic enzymes: endoglucanases (E.C. 3.2.1.4), cellobiohydrolases (E.C. 3.2.1.91), and β-glucosidases (BGLs, E.C. 3.2.1.21, Woodward 1991). Among them, β-glucosidase completes the final step of hydrolysis by converting the cellobiose to glucose (Alef and Nannipieri 1995). β-glucosidases are often the rate-limiting role in cellulose degradation. Their activity thus plays a vital role in the global C cycle (Knight and Dick 2004). β-glucosidases have also attracted considerable attention in recent years due to their important role in diverse biotechnological processes such as bioethanol production, hydrolysis of isoflavone glucoside, detoxification of cassava, elimination of bitter components from citrus products etc. (Singhania et al. 2012; Li et al. 2013).

β-glucosidases are a heterogeneous group of phylogenetically conserved, hydrolytic enzymes widely distributed in the living world. This enzyme family plays a pivotal role in several biological processes: in cellulolytic microorganisms, for example, β-glucosidase is involved in cellulase induction (due to its transglycosylation activities) and cellulose hydrolysis (Mach, et al. 1995; Shewale 1982). Though there is no single, well-defined method to classify these versatile enzymes, the most accepted method is to use a nucleotide sequence identity scheme. The β-glucosidases are mostly placed in either family 1 or family 3 of the glycosyl hydrolases, although these enzymes are also found in families 5, 9 and 30 of the glycosyl hydrolases (Henrissat 1991; Cantarel 2009; Opassiri et al. 2008). Family 1 comprises nearly 62 β-glucosidases from archaebacteria, plants and mammals, and includes 6-phosphoglycosidases

and thioglucosidases. Most family 1 enzymes also show significant β-galactosidase activity (Singhania 2013). Family 3 of the glycosyl hydrolases contains nearly 44 β-glucosidases and hexosaminidases expressed by bacteria, molds, and yeasts. Most of the studied fungal β-glucosidases belong to family 3 of the glycosyl hydrolases (Singhania 2013).

Large microbial diversity in soils implies various carbon hydrolyzing activities (Nannipieri et al. 2012). In spite of the fundamental role of glycoside hydrolases in nature, their diversity is still poorly understood (Pathan et al. 2015). Recently, Bao et al. (2012) obtained several β-glucosidases from yak rumen metagenome. Several sets of degenerate primers have been designed to analyze β-glucosidase gene diversity in soils (Kellner and Vandenbol 2010; Canizares et al. 2011; Li et al. 2013). Unfortunately, none of these studies used sufficient sequencing depth and only Li et al. (2013) covered both fungi and bacteria using a PCR-DGGE approach to analyze potential diversity of β-glucosidase genes in DNA and Shamina et al.(2017) analyze the genetic potential and expression of β-glucosidase genes were studied in the topsoil of a Picea abies forest. Li et al.(2015) performed Illumina MiSeq sequencing combined with Q-PCR to investigate microbiota, coupled with clone library construction to trace cellulolytic communities in traditional Chinese solid-state fermented stages. However, these approaches were still not very fruitful for increasing our understanding of the relationship between β-glucosidase activity and the degradation of cellulose in composite environmental samples.

The aim of this study was to demonstrate how the β-glucosidase genes abundance levels of bacteria and fungi differ among horizons with different cellulose content, characterize the dynamics of enzymatic

activity, cellulose degradation and the relationship with the β-glucosidase-producing microbial diversity during composting. These facts demonstrate that both bacteria and fungi likely participate in the last step of enzymatic cellulose hydrolysis, but it is unclear, what is the relative contribution of the various taxa to this process. This research can provide a ecological functional map of microbial involved in carbon metabolism in cattle manure-rice straw composting.

Methods

Composting process and compost samples

The aerobic composting of cow manure and straw was performed by adopt the small-scale laboratory compost reactors (0.5×0.5×1.1 m) at College of Resources and Environmental Sciences of northeast agricultural university in China. The cattle manure was moistened with water to achieve 67% moisture content. Rice straw and cattle mature at the ratio of 3.5:1 (w/w) were mixed with an initial C/N ratio of about 30:1. The composting process lasted approximately one month and the pile was turned manually once a week. Samples were random collected from three locations of stacks (35 cm, 65 cm and 85 cm from the top surface of the pile, respectively), mixed, and then stored at -80℃ on day 0, 1, 4, 17, 13, 23 and 29. In this experiment, the main ingredients of compost materials are shown in Table 1.

Enzymatic activities, degradation of cellobiose and lignocellulose, content of glucose and cellobiose analysis

Ten grams of sample were transferred to a flask containing 50 mL acetate buffer (0.1 M, pH5.0). The flask was shaken at 200 r/min for 1 h. The homogenate was centrifuged (1315 g) at 4 °C for 20 min, and the supernatant was filtered through filter papers (Whatman No. 1) and then used for measurement of enzymatic activity. The assay of CMCase

activity was carried out by measuring the reducing sugars by the method as described by Zeng (2010). The activity of β-glucosidase was measured by using p-nitrophenyl β-D-glucoside (PNPG) as described by Herr (1979). The glucose and cellobiose relative contents (the content of cellulose or hemicellulose in 1 g of sample) in the samples were determined with highperformance liquid chromatography (HPLC), using the Bio-Rad Aminex HPX-87H chromatographic column (Bio-Rad, America) with 0.005 M H_2SO_4 as the mobile phase, a column temperature of 60°C, and a velocity of 0.5 mL/min, as assessed by a refractive index detector. The cellulose and hemicellulose contents were determined with an ANKOM220 Fiber Analyzer using the method described by Van (1991). Briefly, the hemicellulose content was estimated as the difference between the neutral-detergent fiber (NDF) and the acid-detergent fiber (ADF); the cellulose content was estimated as the difference between the ADF and the acid-detergent lignin (ADL).

DNA purification and construction of functional gene library

DNA was extracted from compost samples (3g) using a procedure described previously (Liu et al. 2011). After DNA extraction,the crude DNA was purified using the E.Z.N.A. Gel Extraction Kit(Omega Bio-Tek, Inc., Georgia, USA). Clone libraries targeting β-glucosidase gene from all the samples were constructed to analyze the cellulolytic communities in each sample. The family 1 β-glucosidase genes from bacteria, family 3 β-glucosidase genes from bacteria and family 3 β-glucosidase genes from fungi were amplified using the above-mentioned primer pairs GH1F/GH1R, GH3BF/GH3BR and GH3EF/GH3ER, respectively (Table.1). PCR reactions were performed in triplicate 50 μL volumes containing 25 μL Premix Ex Taq (Takara, USA), 0.2 μM of each primer and 1 μL DNA templates. The PCR consisted of an initial denaturation at

94 °C for 5 min, 35 cycles of 94 °C for 10 s, 50.0 °C, 54.0 °C and 56.0 °C annealing for 34 s for family 1 and family 3 β-glucosidase genes, respectively, 72 °C for 30 s and a final elongation at 72 °C for 5 min. Pooled PCR products were visualized on 1% agarose gel, then purified using Gel Extraction Kit (Omega). The purified products were ligated to pMD^{TM}18-T Vector (Takara, USA) and transformed into Escherichia coli DH5α competent cells using the heatshock method.

Successful transformants were selected using blue/white screening and verified for correct insertion of DNA fragment by PCR amplification. The selected recombinant *E.coli* clones were routinely grown in liquid Luria–Bertani (LB) medium at 37 ℃, supplemented with 100 g ml^{-1} ampicillin (LBAmp), if needed. Then the organism suspensions were sent to Beijing Huada Gene Company (Beijing, PR China) for sequencing. Sequences were compared with each other and those that were ≥97% similarity were grouped into operational taxonomic units (OTUs) and relative abundance of each OTU was calculated. The representative sequences obtained and the closest matched GenBank reference sequences were used for multiple sequence alignment with Mothur and subsequently the phylogenetic tree was constructed by neighbor-joining (NJ) method with bootstrap value of 1, 000 in order to determine the reliability of the trees (Saitou 1987). Rarefaction and diversity analyses on OTUs were performed on resampled datasets with the same number of sequences randomly selected from all samples. Richness, diversity indices and Chao-1 estimates were calculated using SEED 1.2.1 (Vetrovský and Baldrian 2013).

Real-Time PCR quantification (qPCR) of β-glucosidase genes

The β-glucosidase gene copy numbers of GH1 family of bacteria, GH3 family of bacteria and GH3 family of fungal were determined using

real-time PCR. Real-time PCR was conducted using primer sets listed in Table 2 with SYBR® Premix Ex Taq™ II (Perfect Real Time)(TaKaRa, USA) in a typical 20 µl PCR mixture and the reaction conditions as listed in in the supplemental material. Then samples were run on the Applied Biosystems 7500/7500 Fast Real-Time PCR Systems (Applied Biosystem, America) as described previously. qPCR of β-glucosidase genes was performed in triplicate as described in the supplemental material. Melting curve analysis and agarose gel electrophoresis confirmed the specificity of the amplification.

Statistical Analysis

SPSS 13.0 for windows was used for the statistical analysis. One-way repeated measures ANOVA was used to test differences in the measured parameters during composting, and post hoc Tukey test was used to further investigate these differences ($P < 0.05$).

Results

Dynamics of physicochemical and enzymatic activities during composting.

To better characterize the physicochemical properties and enzymatic activities throughout the process of composting, the changes in temperature, cellulose and hemicellulose content, glucose and cellobiose contents, and dynamics in β-glucosidase activity and CMCase activity during composting were measured compost processes.

Temperature Evolution During Composting

In all samples taken from the compost, the temperature reached thermophilic phase (>45°C) on the second day and enter the cooling phase after 11 days of composting. Thereafter, temperature decreased gradually, and after 21 days of composting it gradually stabilized and approached the ambient temperature (Fig. 1). Compost is considered

mature when the temperature of the compost approaches ambient temperature (Wang 2011).

Dynamic of Lignocellulose During Composting

Cellulose and hemicellulose are the main components of cellulose-based organic matter in cow manure and straw, and their relative contents are measured in each period of composting. During the initial stage of composting, both cellulose and hemicellulose degraded slowly, with the relative content of cellulose and hemicellulose were 18.32% and 23.16%, respectively. In the 4th day, the relative content of cellulose had a slight rebound, subsequently, cellulose and hemicellulose were gradually degraded until the end of the composting, the remaining cellulose and hemicellulose relative contents were 13.09% and 15.12%, respectively. It is worth noting that degradation rate of cellulose and hemicellulose fastest in the later stage of the thermophilic phase (Fig. 2).

Dynamic of Cellobiose and Glucose During Composting

During the first 7 days of the composting, the concentrations of glucose and cellobiose at low level. In the interval, the contents of glucose significantly increased and appeared the first peak (1.85 mmol/kg) during the thermophilic phase of the composting. Subsequently, the concentrations of glucose appeared the highest peak (approximately 5.35 mmol/kg) on day 23. With the compost entering the cooling the cellobiose content remained accumulation to a final value of approximately 1.29 mmol/kg. With compost maturity, glucose content gradually decreased to approximately 1.83 mmol/kg on day 29 (Fig. 3).

Dynamic of β-glucosidase Activity and CMCase Activity During Composting

Fig.4 Shows the dynamic of β-glucosidase activity and CMCase activity were similar during the composting process. CMCase activities

remained between 41.830-36.500μg glucose/g dw min throughout the composting process. On the 2nd and 7th days of composting, the minimum(29.670 μg glucose/g dw min) and maximum(53.37 0μg glucose/g dw min) values were found, respectively. However, the β-glucosidase activity decreased from 0.261μmol p-Nitr/g dw min to 0.178 μmol p-Nitr/g dw min during the first day, subsequently increased significantly, appeared the highest peak (1.482μmol p-Nitr/g dw min), at the later phase of composting, β-glucosidase activity remained approximately 0.50 μmol p-Nitr/g dw min until day 29 (Fig. 4).

Cloning library and gene abundance of β-glucosidase genes

Cloning library of GH1 and GH3 β-glucosidase genes

Respectively, to define the β-glucosidase-producing microbial of GH1 family of bacteria, GH3 family of bacteria and GH3 family of fungal community dynamics during composting process, fore samples (days 1, 4, 7 and 23) were selected. Corresponding to each sample for each primer, 25-38 clones were sequenced.

For the GH1 family β-glucosidase genes from bacteria, 37 OTUs were obtained from the 126 nucleic acid sequences of 4 samples at a similarity of 97%. Taxonomic assignment of GH1 family β-glucosidase genes from bacteria, we observed that 90~95% of OTUs are belong to five major phylums, *Actinobacteria* followed by *Proteobacteria* were the most abundant phyla carrying GH1 bacterial β-glucosidase genes, but clusters belonging to *Chloroflexi*, *Deinococcus-Thermus* and *Thermotogae* were also detected (Fig. 5a). In the phylogenetic tree, a sequence with high homology was selected to analyze with OTU, we found that the main OUTs with close homology to *Bradyrhizobium*,

Thermotoga, *Micrbacterium*, *Devosia* sp., *Streptomyces* sp. and *Rhizobium*(Fig. S1a).

For the GH3 family β-glucosidase genes from bacteria, 16 OTUs were obtained from the 110 nucleic acid sequences of 4 samples at a similarity of 99%. We observed that for up to 95% of OTUs are major belong to *Proteobacteria* , *Actinobacteria*, *Firmicutes* and *Bacteroidetes*, were the most abundant phyla carrying GH3 bacterial β-glucosidase genes (Fig.5b). In the phylogenetic tree, we found that the main OUTs with close homology to *Stenotrophomonas*, *Pelosinus*, *Sphingomonas* and *Mycobacterium* (Fig. S1b). And for the GH3 family β-glucosidase genes from fungi, 26 OTUs were obtained of 3 samples at a similarity of 97%. We observed that for up to 70% of OTUs are major belong to *Ascomycota* , *Basidiomycota* and *Zygomycota* were the most abundant phyla carrying GH3 fungi β-glucosidase genes (Fig. 5c). In the phylogenetic tree, we found that the main OUTs with close homology to *Aspergillus niger*, *Thermoascus*, *Chaetomium* and *Penicillium* (Fig. S1c).

β-glucosidase genes quantification

The total abundances of GH1 and GH3 family bacterial and fungal β-glucosidase-encoding genes, and of those from individual community members, were measured by quantifying the β-glucosidase gene copy number during the aerobic composting of cow manure and straw (Fig. 6). The abundance of bacterial β-glucosidase-encoding genes(GH1) and fungal β-glucosidase-encoding genes(GH3E) showed similar trends, with a slowly decreased from 8.748 and 10.061 log copies g^{-1} to 7.413 and 9.481 log copies g^{-1}, respectively, during the first 7 days and then a decrease to 9.021 and 9.961 log copies g^{-1}, respectively, at the cooling phase of the compost process (Fig. 6a; Fig. 6c).

By constructing clone library discovery, GH1-3-8, GH1-2-5, GH1-4-24 and GH3B-3-16 genes with >90% homology to *Proteobacteria,* which have higher gene abundance during thermophilic phase, then reduced in cooling phase. The GH1-2-20 and GH3B-2-12 genes with >90% homology to *Actinobacteria,* but the gene abundances of GH1-2-20 gradually decreased from 5.318 log copies g^{-1} to 3.015 log copies g^{-1} during composting, the gene abundances of GH3B-2-12 gradually increased from 3.545 log copies g^{-1} to 4.684 log copies g^{-1} during composting. The GH3E-6-9 and GH3E-6-14 genes with >90% homology to *Ascomycota,* and the gene abundances of them have similar trends with the abundances GH3E, which have lower gene abundance during thermophilic phase, especially at the late stage of thermophilic phase. In addition, GH1-3-20 and GH3B-2-6 genes with >90% homology to *Thermotogae* and *Firmicutes*, respectively. The gene abundances of GH3B-2-12 ware relatively high during the thermophilic phase, however, the gene copies of GH3B-2-6 was approximately 3.8 log copies g^{-1} except for the value of the 7th day, which only 2.542 log copies g^{-1} (Fig. 6).

DISCUSSION

Composting is a process involving a complex ecosystem with many interacting factors, in which biodegradable organic wastes are stabilized and converted by the action of some microorganisms under controlled conditions (Tang 2004; Khalil 2001), Cellulose-degrading microbes produce cellulase enzymes that catalyze the first step of cellulose hydrolysis and release oligosaccharides, such as cellobiose; subsequently, oligosaccharides can be converted into glucose and assimilated by cellulolytic communities with the expression of β-glucosidase, which is associated with glycoside hydrolase (GH) families 1 and 3 (Singhania

2013). And β-glucosidase activity is known to increase with increasing organic matter content (Williams and Jochem 2006). Based on this idea, the cloning library and the gene abundance of GH1 and GH3 family β-glucosidase-encoding genes related to the degradation of cellulose in compost processes is identified. In this study, we found that the gene abundance of GH1 and GH3 family β-glucosidase-encoding gene showed different gene abundances and community advantage during the composting.

Here we show that cellulose degradation largely occurred during the thermophilic stage of composting (Fig. 2), and the content of glucose reached the first peak on 4th day of composting (Fig. 3). This phenomenon is due to with microbial metabolic exuberant, resulting in the activity was relatively high in the thermophilic phase, with a positive correlation between β-glucosidase and CMCase activity(Fig. 4), for degraded cellulose to produced glucose, this is consistent with Cunha-Queda (2007) and Castaldi (2008)'s statement. On the other hand, compared with the research by Li (Li et al. 2013), the dynamics of β-glucosidase activity presented quite different, this phenomenon is due to the compost in Li's study was pile-type compost. And in this experiment composting was in the tank which have the high thermal insulation, resulting in the temperature phase of composting was shortened and advanced (Fig. 1), therefore in this experiment, the peaks of β-glucosidase and CMCase activity presence earlier than that in Li's report (Li et al. 2013)(Fig. 4). Simultaneously, although the gene abundance of GH3E was higher than the gene abundance of GH1B from the overall composting process, in the thermophilic phase of composting, the abundance of fungi gene decreased significantly such as GH3E-6-9 and GH3E-6-14 (Fig 6c), while the abundance of bacterial gene showed

359 increasing trends such as GH1-3-8, GH1-2-5, GH1-3-20, GH1-4-24,
360 GH3B-3-16 and GH3B-2-12 (Fig 6a, b). A similar phenomenon was
361 found by Simmons et al. (2014) where it was shown that several GH
362 family 1 proteins (i.e., bacterial genes) were significantly overexpressed
363 in the thermophilic community, whereas GH family 3 (predominantly
364 fungal) genes were significantly overexpressed in the mesophilic
365 community. Therefore, we found in our experiment that the GH1 and
366 GH3 family bacteria community play a more important role than the GH3
367 family fungi community in producting β-glucosidase and degradation
368 cellulose at the later thermophilic stage of composting. Meanwhile,
369 vigorous microbial metabolic needs sufficient energy supply, so that
370 glucose to be used as the energy required for microbial metabolism is
371 consumed, at this no large accumulation of glucose (Fig. 4).

372 Within the fungal GH3, most gene sequences belonged to *Ascomycota*,
373 up to 40% of β-glucosidase sequences of the GH3 fungal family were not
374 assignable due to the lack of sequence information in public databases.
375 Previous studies have proved that *Aspergillus* and *Penicillium* are
376 dominant culturable micro-organisms in composting (Lacey 1991;
377 Fischer et al. 1999; Hryhorczuk et al. 2001; Kampfer et al. 2002;
378 Ryckeboer et al. 2003). At the later stage of thermophilic phase of
379 compost, gene sequences of *Ascomycota* were obviously decrease, and
380 the GH3E-6-9 and GH3E-6-14 gene abundance were significantly
381 reduced (Fig.6c), which were belong to *Ascomycota.* The results
382 demonstrates that the *Ascomycota* were not the main microbial
383 community constituents of degraded cellulose during the later stage of
384 thermophilic phase of composting.

385 Among bacterial phyla, *Actinobacteria*, *Proteobacteria* and
386 *Deinococcus-Thermus* contributed most β-glucosidase sequences of the

GH1 family (Fig.5a). In the GH3 bacterial family, most gene sequences belonged to *Firmicutes* followed by *Actinobacteria* and *Proteobacteria* (Fig. 5b). Similar situations also appeared in Pathan's study (2016). In Berlemont and Martini's report (2013), β-glucosidase and cellulase genes are present in nearly all bacterial phyla, which shows the importance of bacteria in cellulose degradation. In GH3 bacterial family, *Firmicutes* and *Proteobacteria* were even more dominant than *Actinobacteria*, *Firmicutes* as the major bacterial community components at all stages of composting (Fig. 5b), these results were consistent with previously recorded (Goff 2009; Jurado 2014; de Gannes et al. 2013). Dentified cellulolytic bacterial taxa from this study were also previously recorded as such in agricultural and forest soils (Ulrich et al. 2008; Schellenberger et al. 2010; Stursova et al. 2012). In Partanen (2010) and Chandna (2013) reported that the *Proteobacteria* were minor microbial community constituents in compost, in this experiment, however *Proteobacteria* were even more dominant during heating phase and thermophilic phase of composting (Fig. 5a,b). On the other hand, GH1-3-8, GH1-2-5, GH1-4-24 and GH3B-3-16 gene sequences belonged to *Proteobacteria*, which with high gene abundance during heating phase and thermophilic phase of composting（Fig. 6a,b）. Actinobacteria are considered to play an important role in decomposition of organic materials in composts, particularly for degradation of macromolecules such as cellulose, hemicellulose, lignin and chitin (Lacey 1973; Ryckeboer 2003). In our study, *Actinobacteria* were more dominant in cooling phase than thermophilic phase of composting (Fig. 5a,b), the GH1-2-20 and GH3B-2-12 in later stage of thermophilic phase with lower gene abundance than in cooling phase of composting, which belonged to *Actinobacteria* (Fig. 6a,b). In previous studies of compost (Klamer 1998; Ryckeboer 2003) also found reduced populations of *Actinobacteria* during the high

temperature phase. Therefore, this group of microorganisms may be a potential indicator of compost maturity(Steger 2007).

In our composting process, the metabolic activity of the microorganism was positively correlated with the β-glucosidase and CMCase activity , because the product glucose was used as an energy source, glucose was not necessarily accumulated when the microbial metabolic activity was enhanced, At the end of the composting, the microbial metabolic activity decreased and there are fewer degradable substances in the compost, so that the energy of the microorganism mainly relied on the absorption of glucose in the cooling phase of compost.

Notably, in our experiment the gene abundance of GH3E was higher than the gene abundance of GH1B from the overall composting process, however, GH1 and GH3 bacterial family provided greater contribution than the fungal GH3 family in produced β-glucosidase and involved degradation cellulose during the later stage of thermophilic phase in composting. This is exactly corroborated Pathan's repored that some genes with low abundance in DNA showed high transcriptional activity, which indicates the importance of these low abundant taxa in cellobiose utilization(Pathan et al. 2017). Simultaneously, through the taxonomic assignment analysis of GH1, GH3 family β-glucosidase genes from bacteria and fungi, showed different microbial community advantage in the various phases of composting. Indicated that through whole composting process, the β-glucosidase producing microbial community could occured community succession according to the changes of composting environmental factors, so as to achieve the microecological balance of composting environment.

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Conflict of Interest

The authors declare no conflict of interest.

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Tables and Figures

Table 1 Properties of raw materials for the composting

Materials	Water content（%）	TOC（%）	TN（%）	C/N
Cattle manure	28.77	35.22	2.53	13.92
Rice straw	12.17	40.93	0.83	49.31

568 Table 2 Primers were used for the PCR.

Primer	Sequences(5'-3')	Primer	Sequences(5'-3')
GH1 family gene (bacteria)		**GH3 family gene (Bacteria)**	
GH1BF	CCT ACC AGA TYC ARG G	GH3BF	TTC FFC GAA GAY CC
GH1BR	GAG GAA GRT CCC ART G	GH3BR	ACG CCT TYR WAR CC
KY922867		KY882360	
GH1-3-8F	ACGAGCCGGTTGTAGAAGTC	GH3B-3-16F	ACAACCGCCATGTGTTCGAT
GH1-3-8R	ACACGCCCGGAAAGATCAAG	GH3B-3-16R	CTCACCGTTGACGCGGTTAT
KY471077		KY859729	
GH1-2-5F	CGTCCATCTGGGACACCTTC	GH3B-2-6 F	GTAGCCCCACGCACCCTTGA
GH1-2-5R	CGTTCAAGCATGCCGTCAAT	GH3B-2-6 R	AGTGCGATGTATCCCCATTG
KY471086		KY859728	
GH1-3-20F	ATTACCACCGTTACCGCGAG	GH3B-2-12 F	GTTCACCGTTTGTCCTGTTGT
GH1-3-20R	ATAAAGCGTAACCCAGGGCG	GH3B-2-12 R	GAAGTTCCATACCTGACAGGC
KY471069		**GH3 family gene (fungi)**	
GH1-4-24F	ACGCGTTCTCAAAAATGCCC	GH3EF	GGT GGT CGC RRY TGG GA
GH1-4-24R	CGATGAGCCGGTCGTAGAAA	GH3ER	CCA GGC ATC GGW CAT RTC
KY922868		KY942103	
GH1-2-20F	ATCGAAAACCGGTAGGCACC	GH3E-6-9F	TTTAGTGACAAGGGTGCCGA
GH1-2-20R	CTACCAGATCGAAGGAGCGG	GH3E-6-9R	TGCTCTTGCTCGTAGGCAAT
		KY942101	
		GH3E-6-14F	GAACTACCCCCGCATCATGT
		GH3E-6-14R	GAGGAACCTGTGGCCAGAAA

569

570 Fig. 1 Changes in temperature during the composting process, showing the overall
571 trend of compost temperature, including the mesophilic phase, the initial stage of the
572 thermophilic phase, the later stage of the thermophilic phase and the cooling phase.

573 Fig. 2 Change in the relative content of cellulose and hemicellulose during
574 composting.

575 Fig. 3. Change in content of glucose and cellobiose during composting .

576 Fig. 4. Dynamics of CMCase activity and β-glucosidase activity during composting.

577 Fig. 5. Taxonomic assignment of β-glucosidase genes of bacterial and fungal β-
578 glucosidase genes from GH1 and GH3 family in composting. Unassigned sequences
579 belong to bacteria or fungi, but their identification at a phylum level is unclear.

Fig. 6. The abundance of β-glucosldase gene during composting. The abundance of the GH1 bacterial family were shown as (a); the abundance of the GH3 bacterial family were shown as (b); and the abundance of the GH3 fungal family were shown as (c).

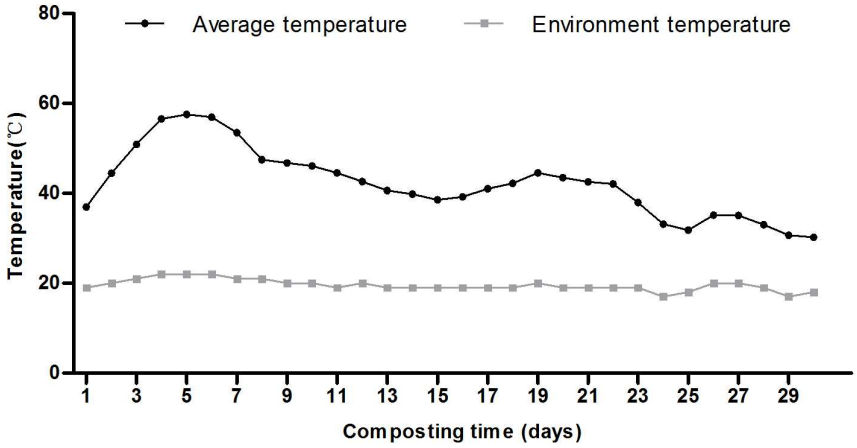

Fig. 1 Changes in temperature during the composting process, showing the overall trend of compost temperature, including the mesophilic phase, the initial stage of the thermophilic phase, the later stage of the thermophilic phase and the cooling phase.

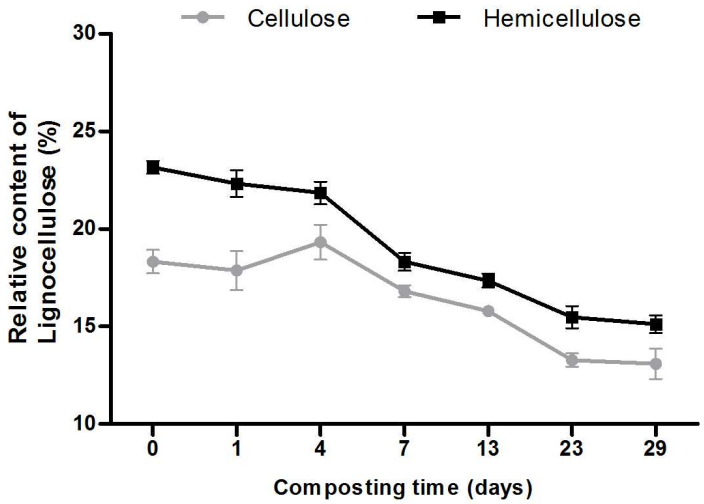

Fig. 2 Change in the relative content of cellulose and hemicellulose during composting.

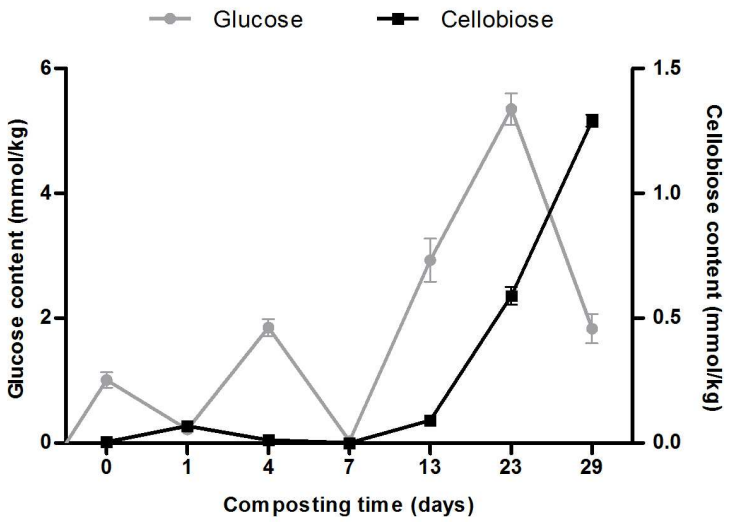

Fig. 3. Change in content of glucose and cellobiose during composting .

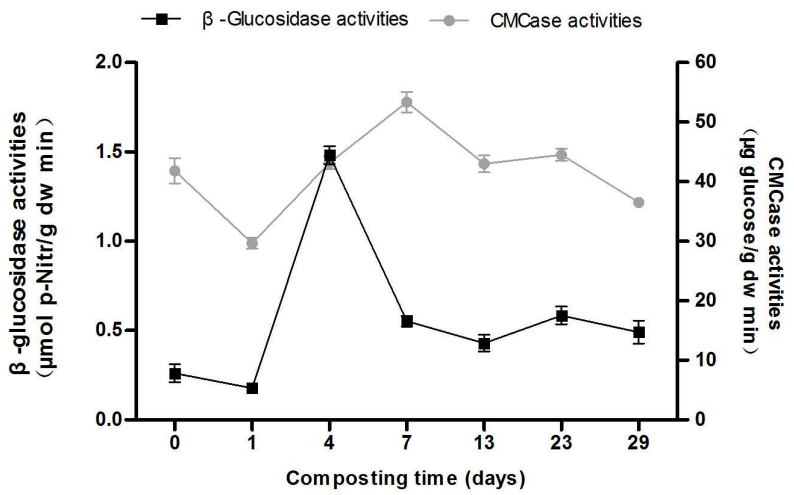

Fig. 4. Dynamics of CMCase activity and β-glucosidase activity during composting.

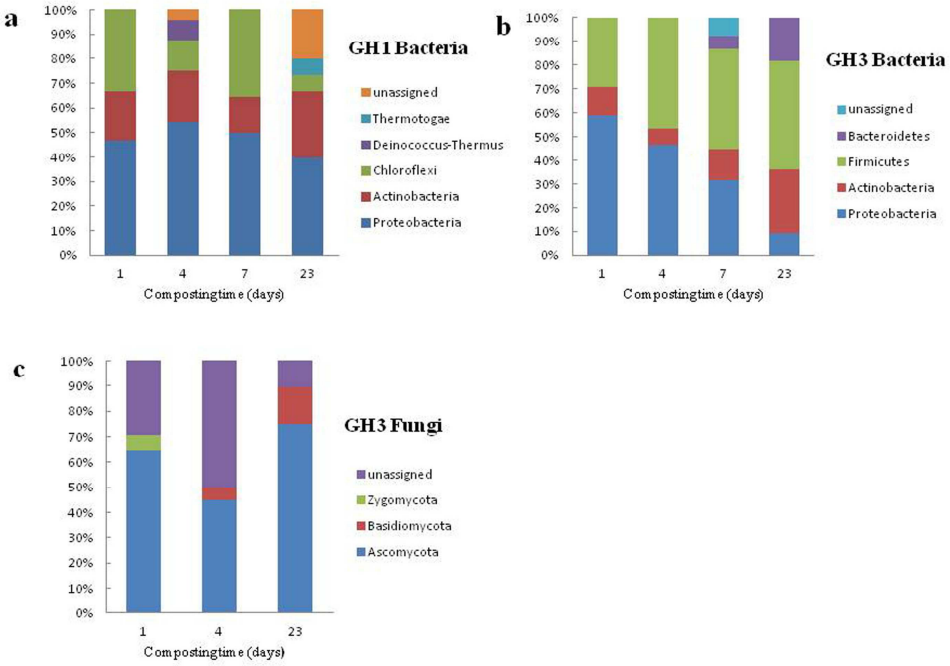

Fig. 5. Taxonomic assignment of β-glucosidase genes of bacterial and fungal β-glucosidase genes from GH1 and GH3 family in composting. Unassigned sequences belong to bacteria or fungi, but their identification at a phylum level is unclear.

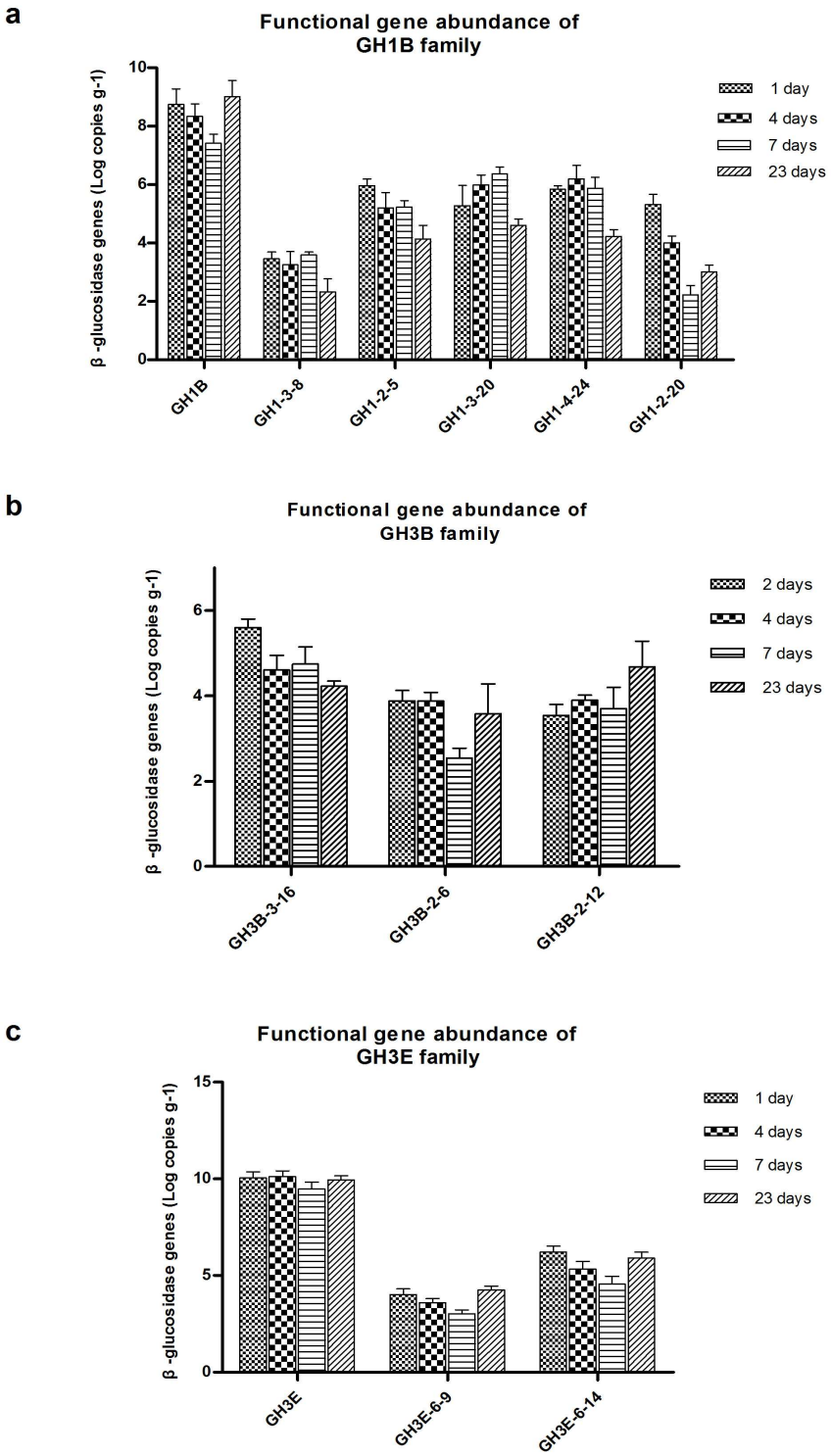

Fig. 6. The abundance of β-glucosldase gene during composting. The abundance of the GH1 bacterial family were shown as (a); the abundance of the GH3 bacterial family were shown as (b); and the abundance of the GH3 fungal family were shown as (c).

The structural and functional contributions of β-glucosidase-producing microbial communities to cellulose degradation in composting

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Supplementary Materials and Methods

Real-Time PCR quantification (qPCR) of β-glucosidase genes

qPCR of β-glucosidase genes was performed in triplicate using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China) and an ABI 7500 Fast Real Time PCR System (Applied Biosystems). Primers GH1BF/GH1BR were designed to amplify partial conserved fragments of the family 1 β-glucosidase genes from bacteria and fungi, and primers GH3EF/GH3ER were designed to amplify partial conserved fragments of the family 3 β-glucosidase genes from fungi as previously described (Li, 2013), a tenfold serial dilutions of the linearized clone KY922867 containing the family1 partial β-glucosidase gene fragments with a linear range of 3.19×10^2 to 3.19×10^9 copies μL^{-1} and linearized clone KY882360 containing the family 3 partial β-glucosidase gene fragments with a linear range of 2.22×10^2 to 2.22×10^8 copies μL^{-1} were used as a standard with R^2 of 0.998 (efficiency=77.31%) and 0.998 (efficiency=76.97%), respectively. The internal reference gene and its standard curve range of GH1 family and GH3 family β-glucosidase specific gene quantitative analysis shown in Table S1.

Each reaction was performed in a 20 μL volume containing 10 μL SYBR Premix Ex Taq (Takara, Dalian, China), 0.8 μM of each primer and 2 μL of 10-fold dilution DNA or RNA template (1–10 ng). The qPCR thermocycling steps were as follows: 95 °C for 30 s, 45 cycles of 5s at 95 °C, 34 s at 60 °C for family 1 and family 3 β-glucosidase genes. Melting curve analysis and agarose gel electrophoresis confirmed the specificity of the amplification.

31 Li, H., Xu, X., Chen, H., Zhang, Y., Xu, J., & Wang, J., et al. (2013). Molecular
32 analyses of the functional microbial community in composting by pcr-dgge targeting
33 the genes of the β-glucosidase. *Bioresource Technology, 134C*, 51-58.

Table S1 The internal reference gene and its standard curve range of GH1 family and GH3 family β-glucosidase specific gene quantitative analysis

Primers of β-glucosidase gene	Clone fragment GenBank No.	Standard linear range（log copies g-1）	R^2	Amplification efficiency
GH1-3-8F / GH1-3-8R	KY922867	3.07×10^3～3.07×10^{10}	0.984	104.45%
GH1-2-5F / GH1-2-5R	KY471077	2.97×10^3～2.97×10^{10}	0.984	104.13%
GH1-3-20F / GH1-3-20R	KY471086	3.12×10^3～3.12×10^{10}	0.980	105.93%
GH1-4-24F / GH1-4-24R	KY471069	3.16×10^3~3.16×10^{10}	0.982	105.13%
GH1-2-20F / GH1-2-20R	KY922868	3.47×10^3～3.47×10^{10}	0.983	104.74%
GH3B-3-16F / GH3B-3-16R	KY882360	2.57×10^3～2.57×10^9	0.993	82.51%
GH3B-2-6 F/ GH3B-2-6 R	KY859729	2.96×10^3～2.96×10^9	0.994	94.20%
GH3B-2-12 F/ GH3B-2-12 R	KY859728	3.09×10^3～3.09×10^{10}	0.984	104.46%
GH3E-6-9F/ GH3E-6-9R	KY942103	3.65×10^2～3.65×10^9	0.994	81.025%
GH3E-6-14F/ GH3E-6-14F	KY942101	3.49×10^2～3.49×10^9	0.994	81.454%

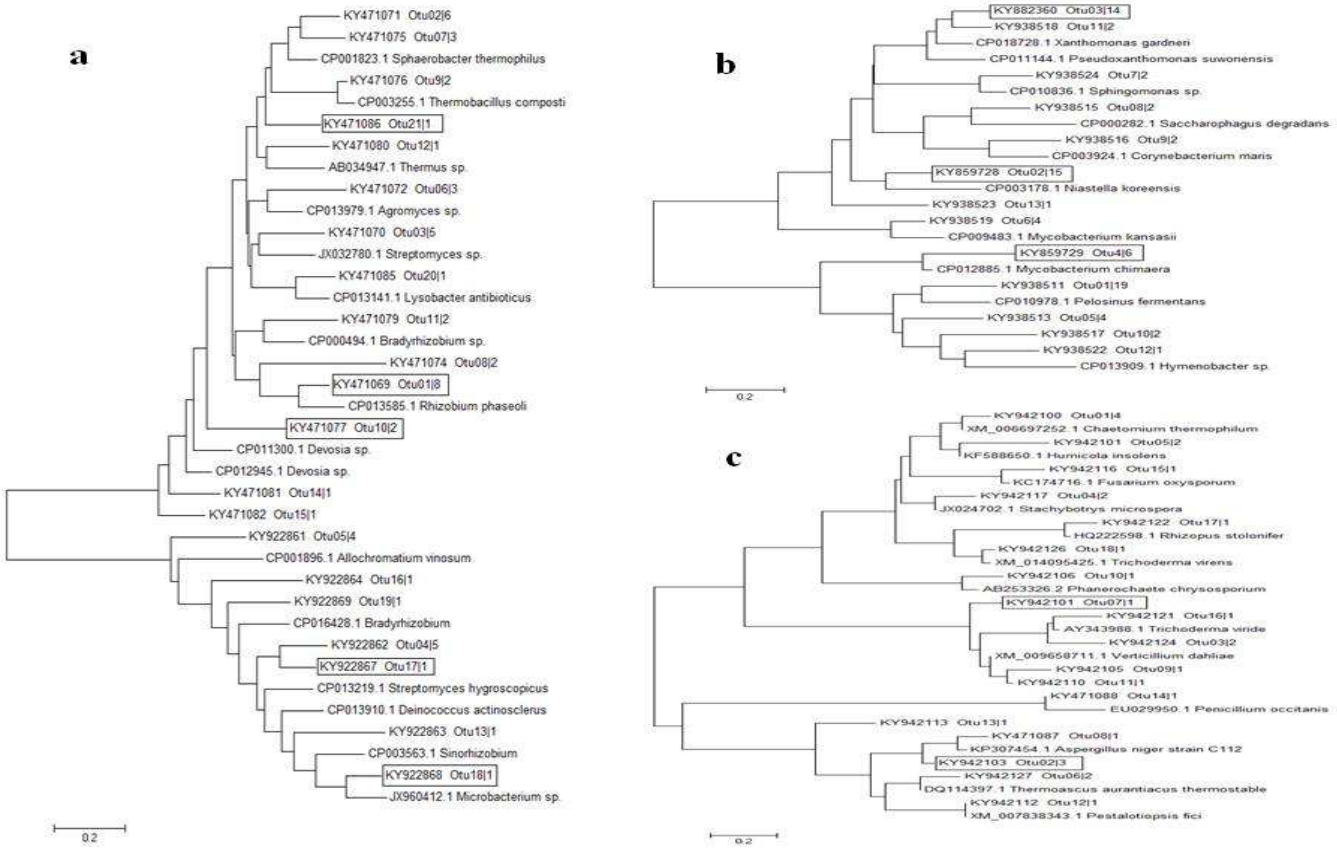

Fig. S1. Phylogenetic relationship among reference bacterial β-glucosidase encoded protein sequences and environmental sequences from this study. GH1 bacterial β-glucosidase sequences (a) , GH3 bacterial β-glucosidase sequences (b) and GH3 fungal β-glucosidase sequences (c).

Translated amino acid sequences of OTUs with abundance over > 0.2%. Sequences were aligned using Mothur to all homologous sequences retrieved from GeneBank database (https://www.ncbi.nlm.nih.gov/).