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ORIGINAL PAPER

Variations of culturable thermophilic microbe numbers and bacterial communities during the thermophilic phase of composting

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Abstract Composting is a process of stabilizing organic wastes through the degradation of biodegradable components by microbial communities under controlled conditions. In the present study, genera and species diversities, amylohydrolysis, protein and cellulose degradation abilities of culturable bacteria in the thermophilic phase of composting of cattle manure with plant ash and rice bran were investigated. The number of culturable thermophilic bacteria and actinomyces decreased with the increasing temperature. At the initiation and end of the thermophilic phase, genera and specie diversities and number of bacteria possessing degradation abilities were higher than during the middle phase. During the thermophilic composting phase, *Bacillus*, *Geobacillus* and *Ureibacillus* were the dominant genera, and *Geobacillus thermodenitrificans* was the dominant species. In later thermophilic phases, *Geobacillus toebii* and *Ureibacillus terrenus* were dominant. *Bacillus*, at the initiation, and *Ureibacillus* and *Geobacillus*, at the later phase, contributed the multiple degradation abilities. These data will facilitate the control of composting in the future.

Keywords Thermophilic composting phase · Genera and species diversities · Culturable bacterial community · Culturable thermophilic microbe number · Degradation abilities

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Introduction

Waste production has been increased in recent years due to increased agricultural and industrial production (Bhatia et al. 2012). The modernization of farms is increasing with the development of animal husbandry, and animal dung has become one of the major sources of pollution. However, there is a great deal of organic matter in animal dung, such as nitrogen and phosphorus; thus, the value of animal dung as a fertilizer has always been recognized. Currently, composting is seen as an alternative method of recycling farm manure where farms have insufficient agricultural land for direct use of the fertilizer (Liu et al. 2011) and is one of the most favoured options for municipal solid waste recycling for waste streams with high content of biodegradable materials. Application of compost can reduce the severity of plant disease, improve soil fertility, and suppress certain soil-born plant pathogens (Takaku et al. 2006; Ofosu-Budu et al. 2010).

Composting is a process involving a complex ecosystem of many interacting factors, in which biodegradable organic wastes are stabilized and converted by some microorganisms under controlled conditions (Wang et al. 2011). The high organic carbon content and biological activity of compost make it effective for applications including erosion control and revegetation (Anastasi et al. 2005). The composting process involves three phases and uses diverse microflora, such as mesophilic and thermophilic bacteria, fungi and actinomycetes to eventually convert organic waste to humus (Zeng et al. 2001). Conditions during the various phases, such as oxygen and nutrient availability, determine the development of microbial populations in compost. The temperature increases by up to 65–80 °C and results in a rapid transition from a mesophilic to a thermophilic microbial community (Blanc

et al. 1999). This thermogenic phase is followed by a slow decrease in temperature where the diversity of microorganisms increases, fungi and mesophilic bacteria reestablish themselves; additionally, biotransformations of the organic matter occur (de Bertoldi et al. 1983). The increase in temperature is important for public health because pathogens in the compost are destroyed during the thermophilic composting process (Tang et al. 2004). The structure of the microbial community changes during this process along with a progressive breakdown of complex compounds to simpler molecules (Tiquia et al. 2002). Thus, the most critical parameter influencing the rate of composting and the quality of the product is temperature. A current method for understanding the composting mechanism is the investigation of changes in microbial communities during the thermogenic phase. These changes may play key roles in these ecosystems, and a deeper knowledge of these microbial communities may uncover common composting mechanisms (Ishii and Takii 2003).

Compost microbes are tremendously diverse, and their ecologies are extremely complex. Culture-independent methods have been wide used to study variations in the structure of microbiological communities of composting samples. However, culture-independent methods could not distinguish the ecological functions of active microbes from dormant microbes. In particular, the ecological contribution of dead microbes could be overestimated due to DNA was extracted from both living and dead microbes (He et al. 2008; Mijangos et al. 2009). In addition, the amount of extractable DNA is very small in thermogenic composting samples, and many dormant microbes remain in the sample. Since the ecological role of bacteria in natural environments can be estimated only when they are successfully cultivated and characterized, cultivation-based study still remains important (Tamaki et al., 2005). Moreover, the readily culturable bacteria of compost microbial communities may be most important in terms of both biomass and activity (Ellis et al. 2004; Tamaki et al. 2005). Thus, culture-dependent approaches have been used previously to study microbes that are active during the composting process (Takaku et al. 2006). However, in the majority of these papers, the culturing temperature for thermophilic microbes was and 50–55 °C (Coelho et al. 2013) or plates were incubated at either 60 or 75 °C (Blanc et al. 1999). All results were limited to describe the ecological functions of culturable microbes because the culturing temperatures used were not the temperatures in the compost at the time of sampling. Much less is known of the interrelationship between the succession of culturable microbial communities in the thermophilic phase of composting and the degradation of complex compounds, particularly during the composting of agricultural wastes.

During agricultural waste composting, lignocellulose, starch and protein account for the major part of biomass, and consequently, their degradation is essential for composting to occur (Yu et al. 2007). The main objective of this study was to obtain more useful information on the succession of culturable microbial communities during the thermophilic phase and their abilities to degrade complex compounds, which will assist in identifying the members of the culturable microbial community responsible for the degradation of each complex compound, to elucidate the relationship between the culturable microbial community and the composting process and to facilitate the control of composting in the future.

Materials and methods

Composting process and compost samples

The composting was performed in Jiangyin Lianye Co. Ltd. (Wuxi, China) and was made from cattle manure, plant ash and rice bran (95:3:2, w/w/w). Mixtures of raw materials with initial moisture content of about 67 % were arranged in 3 large, strip-shaped windrows (50 m long, approximately 112 ton each) with cross section as a isosceles triangle (3 m wide and 1.5 m high). Temperature was monitored throughout the process every 2 days at different locations within the stacks from 3 windrows (surface 10 cm, core 50 cm and bottom 90 cm).

All samples were collected during the thermophilic phase (temperature $\geq$50 °C). Once the temperature was $>$ 50 °C, the sample collection was initiated. One kilogram of fresh composted sample was collected from three locations of each windrow in the stack every 5 days from the beginning to the end with temperature less than 50 °C, and were placed in sterilized bags and stored at 4 °C. The water content of all samples was measured after drying at 105 °C for 24 h.

Quantification of the number of culturable microbes

The culturable microbes were enumerated using a standard tenfold dilution method. Diluted sample suspensions (bacteria: 10^{-5}, 10^{-6}, 10^{-7}; actinomycetes: 10^{-3}, 10^{-4}, 10^{-5}; fungi: 10^{-1}, 10^{-2}, 10^{-3}) were inoculated onto plates containing suitable agar media for bacteria, actinomycetes, and fungi including LB medium (tryptone 10 g, yeast extract 5 g, NaCl 10 g, agar 20 g, distilled water 1,000 ml), Gause NO.1 medium (soluble starch 20 g, KNO_3 1 g, NaCl 0.5 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g, $K_2Cr_2O_7$ 0.1 g, agar 20 g, distilled water 1,000 ml, pH 7.2) and Martin's Bengal rose agar (glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, Bengal rose 30 mg, streptomycin 30 mg, agar 20 g, distilled water 1,000 ml), respectively. Plates for the determination of bacteria, actinomycetes or fungi were incubated for 3–4,

6–7 or 4–5 days, respectively. The culture temperatures for each sample were the same as the real-time values detected during the composting at the time of collection. The results are expressed in log form (CFU g^{-1} dry compost). Morphological, growth, and odor properties of colonies from the plates of each sample were used to identify different strains. Similar colonies were enumerated, and one was selected. All selected culturable microbes were then purified for further study.

Identification of the bacterial strains

The isolated strains were identified using morphological, physiological and biochemical properties and 16S rRNA sequence analysis. Genomic DNA was extracted, and the 16S rRNA gene was amplified using polymerase chain reaction (PCR) using the following primers: 5′-AGAGTTT GATCCTGGCTCAG-3′ as forward and 5′-TACGGTTAC CTTGTTACGACTT-3′ as the reverse. Conditions for PCR were: 5 min of denaturation at 94 °C, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The 16S rRNA gene sequence of all strains was sequenced and analyzed using BLAST searches. Data are expressed as the highest identity for each strain returned by the BLAST searches based on comparisons to known species in the NCBI database (Li et al. 2011).

Quantification of amylohydrolysis capability of all culturable bacteria

Each strain was incubated at 50 °C for 48 h on LB agar plates containing 1 % (w/v) starch. Colonies on the original plates were flooded with iodine solution to visualize the hydrolysis of starch around the colonies because the production of extracellular amylase forms a zone of clearance around the colony (Agrawal et al. 2005).

Quantification of protein degradation capability of all culturable bacteria

Skim-milk medium (10 g defatted milk powder, 10 g peptone, 5 g yeast extract powder, 5 g NaCl, 20 g agar per liter) described by Brown and Foster (1970) was used to detect the ability to degrade protein. Strains were allowed to grow on the plates at 50 °C for 2 days. The clearance zone and the colony diameter were then measured.

Quantification of cellulose degradation capability of all the culturable bacteria

The ability to degrade cellulose in all strains was evaluated according to Kasana et al. (2008). Strains were allowed to grow on cellulose-containing plates (0.2 % $NaNO_3$, 0.1 % K_2HPO_4, 0.05 % $MgSO_4$, 0.05 % KCl, 0.2 % carboxymethylcellulose (CMC) sodium salt, 0.02 % peptone, and 2 % agar). CMC plates were then flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 min. The clearance zones and colony diameters were then measured.

Results

Changes in temperature and water content

Temperature in the compost piles rose immediately after the onset of composting (Fig. 1A). The temperature reached 50 °C by the third day and remained above 50 °C between days 3 and 33 of composting, and this period was defined as the thermophilic phase. The maximum temperature was greater than 70 °C. Seven samples were collected at 3, 8, 13, 18, 23, 28 and 33 days. The water content of each sample decreased after the onset of composting. After 33 days of composting, the water content was less than 55 % (Fig. 1B).

Enumeration of composting bacteria, fungi and actinomyces

The number of bacteria, fungi and actinomyces in all samples was counted in nutrient agar plates under different thermophilic conditions. The number of thermophilic bacteria incubated at 51 °C was more than 7.0 lg CFU g^{-1} of dry weight at day 3 whereas the lowest population of thermophilic bacteria was incubated at 68 °C at day 23. After 23 days of composting, the number increased again to more than 7.0 lg CFU g^{-1} of dry weight at day 38 when cultured at 47 °C (Fig. 1C). For actinomyces, the number was 6.76 lg CFU g^{-1} of dry weight at day 3 when cultured at 51 °C, and it decreased to its lowest level (4.56 lg CFU g^{-1} of dry weight) by day 18 when incubated at 68 °C and, thereafter, reached 6.05 lg CFU g^{-1} of dry weight at day 38 (Fig. 1C). Several attempts were made to cultivate fungi on the diluted heterotrophic growth medium incubated at different thermophilic conditions, however, all were unsuccessful.

Diversity in genera of culturable thermophilic bacteria

Microbial activity during the thermophilic phase of composting was primarily due to the thermophilic bacterial community. At the beginning of the thermophilic phase, the bacterial genera diversity was higher than in latter phase (Fig. 2a). A total of 243 × 10^6 strains belonging to thirteen genera including *Actinotalea*, *Bacillus*,

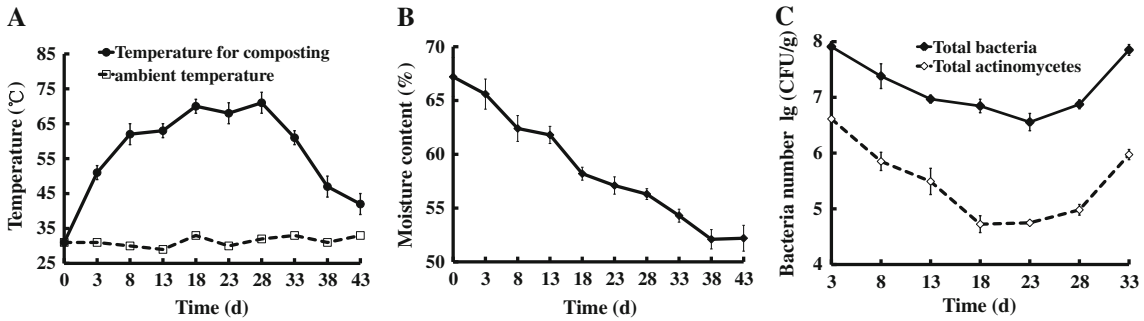

Fig. 1 Changes in temperature, water content, number of culturable thermophilic bacteria and actinomyces in the compost piles. **a** Temperature, **b** water content, **c** number of culturable thermophilic bacteria

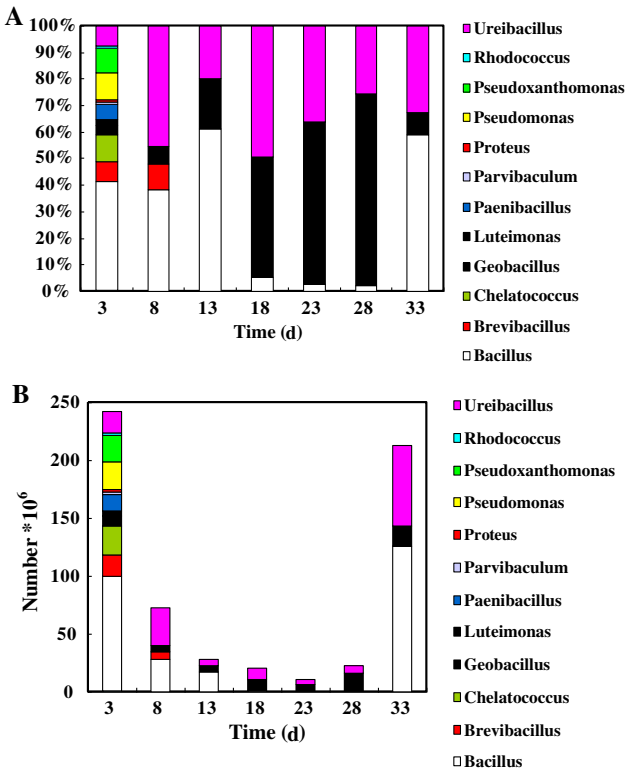

Fig. 2 Relative abundances and number of culturable thermophilic bacteria taxa (genera) at each timepoint and variation in the genera represented. **a** Relative abundances of culturable thermophilic bacteria taxa (genera); **b** number of culturable thermophilic bacteria taxa (genera)

Brevibacillus, *Chelatococcus*, *Geobacillus*, *Luteimonas*, *Paenibacillus*, *Parvibaculum*, *Proteus*, *Pseudomonas*, *Pseudoxanthomonas*, *Rhodococcus* and *Ureibacillus*, were isolated. Of these genera, *Bacillus*, *Pseudomonas*, *Chelatococcus*, *Pseudoxanthomonas* and *Ureibacillus* accounting for 40.7, 9.9, 9.9, 9.5 and 7.4 % of the total population, respectively, were considered dominant (over 7.0 %). After 8 days of composting, just four genera were found: *Bacillus*, with 28×10^6 strains, accounted for 38.9 %, *Brevibacillus*, with 7×10^6 strains, accounted for 9.7 %, *Geobacillus*, with 5×10^6 strains, accounted for 6.9 % and *Ureibacillus*, with 33×10^6 strains, accounted for 45.8 % of the total population. The number of genera then decreased to three during the latter phase (days 13 to 33) of composting and comprised *Bacillus*, *Geobacillus* and *Ureibacillus*. However, *Geobacillus* and *Ureibacillus* were the only two dominant genera in the samples collected on days 18, 23 and 28 of composting. Then, the temperature decreased to less than 70 °C (approximately 60 °C) on day 33, and the number of strains increased to 213×10^6, and *Geobacillus* and *Ureibacillus* were again the two dominant genera (Fig. 2a, b). Through the thermophilic phase of composting, *Bacillus*, *Geobacillus* and *Ureibacillus* were isolated from all samples. The number of *Bacillus*, being the dominant genus during days 3–13, varied during composting (Fig. 2a). The number of *Geobacillus* strains increased during composting, considered as the dominant genus during the high-temperature phase (temperatures greater than 60 °C) (Fig. 2a). *Ureibacillus* maintained consistency as the dominant genus during the thermophilic phase of composting (Fig. 2a).

Species diversity of the culturable thermophilic bacteria

The abundance and diversity of the composting culturable thermophilic bacteria decreased between day 3 and day 28 and increased again thereafter to day 33. The richness of species in the sample from day 3 exceeded that of days 8, 13, 18, 23, 28 and 33 by 237.5, 298.8, 308.3, 316.2, 306.2 and 41.7 %, respectively. A total of 27 species were found in the sample from day 3 whereas only 8, 8, 6 and 5 species were found in samples from days 13, 18, 23 and 28, respectively (Fig. 3a). The abundance of composting culturable thermophilic bacteria in the sample from day 3 exceeded that of days 8, 13, 18, 23, 28 and 33 by 70.4, 70.4, 77.8, 81.5, 77.8 and 51.9 %, respectively (Fig. 3b). The species diversity (Sannon-Wiener index and Simpson index) of the composting culturable thermophilic bacteria

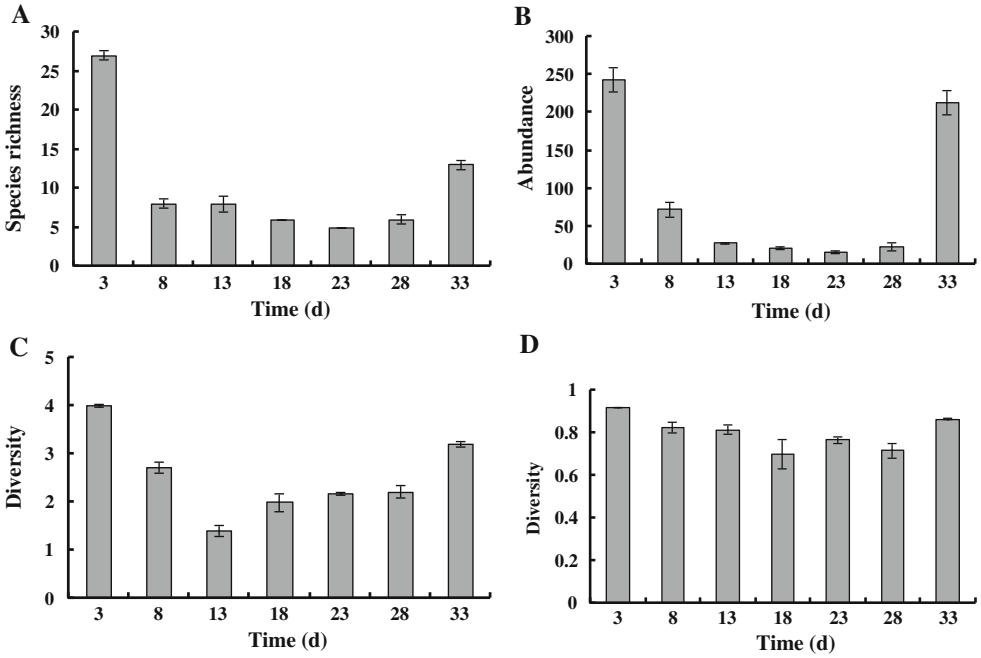

Fig. 3 Variation in species richness (number of species g^{-1} dry weight compost sample), abundance (10^3 CFU g^{-1} dry weight compost sample) and diversity (Sannon-Wiener index and Simpson index) during composting. **a** Species richness, **b** abundance, **c** Sannon-Wiener index, **d** Simpson index

reached a maximum in the sample from day 3, decreased by day 28 and then increased in the sample from day 33 (Fig. 3c, d).

The species composition pattern (the distribution of relative abundances of the different species) varied significantly among the samples (Fig. 4A). The abundance of the dominant and less-dominant species was affected by the composting. In the sample from day 3, species showing an abundance of >5.0 % were considered dominant: *Bacillus licheniformis* accounted for 7.0 %, *B. thermodenitrificans* accounted for 11.1 %, *Chelatococcus daeguensis* accounted for 9.88 %, *Pseudomonas tuomuerensis* accounted for 9.88 %, *P. taiwanensi* accounted for 9.47 %, and *Ureibacillus suwonensis* accounted for 7.41 % of the total population. On day 8 of composting, *B. aerius*, *B. licheniformis*, *Brevibacillus limnophilus*, *Geobacillus thermodenitrificans*, *Ureibacillus thermosphaericus*, and *Ureibacillus suwonensis* were only found. However, *B. sonorensis* was observed for the first time (it was not observed in the sample from day 3). *B. licheniformis*, *U. thermosphaericus* and *U. suwonensis* were considered the dominant species. By day 13, *B. sonorensis* disappeared, and *B. thermoamylovorans* and *B. amyloliquefaciens* appeared. With the exception of *B. amyloliquefaciens*, all other species were dominant. In the sample from day 18, *G. thermodenitrificans*, *U. terrenus*, *G. pallidus*, and *G. toebii* only were observed. With the exception of *G. thermodenitrificans*, this timepoint marked the first appearance of these species. *G. thermodenitrificans*, *U. terrenus* and *G. toebii* were the dominant species. In the sample from day 23, *G. pallidus* disappeared, and *U. suwonensis* again appeared. All species observed were considered dominant. *B. aerophilus*, *G. caldoxylosilyticus*, *G. toebii*, *G. thermodenitrificans* and *U. terrenus* were observed in the sample from day 28. The abundance of *G. toebii*, *G. thermodenitrificans* and *U. terrenus* was >10 %, and they were considered dominant: *G. toebii* accounted for 19.1 %, *G. thermodenitrificans* accounted for 34.7 %, and *U. terrenus* accounted for 19.1 % of the total population. Many species were observed in the final sample, and an abundance of >5.0 % was considered dominant: *B. licheniformis* accounted for 13.1 %, *B. thermoamylovorans* accounted for 19.2 %, *G. toebii* accounted for 7.0 %, *U. terrenus* accounted for 15.5 %, *U. thermosphaericus* accounted for 6.1 %, and *U. uwonensis* accounted for 8.5 % (Fig. 4a, b).

Geobacillus thermodenitrificans was isolated from all samples, and its number varied during composting. It decreased between days 3 and 18, increased to 7.8×10^6 CFU g^{-1} in the sample from day 28 and then decreased to 3×10^6 CFU g^{-1} in the final sample. It was the dominant specie during days 8–28 (Fig. 4a). *G. toebii* and *U. terrenus* was not detected in the initial three samples, and their numbers increased to a maximum in the final sample. These two species were dominant during the later thermophilic composting phase from 18 to 33 days (Fig. 4a).

Amylohydrolysis, protein degradation and cellulose degradation abilities of all the culturable bacteria

The number of bacteria possessing amylohydrolysis, protein and cellulose degradation abilities is shown in Fig 5a–c, respectively. The number of bacteria that could hydrolyze starch decreased from 36×10^6 CFU g^{-1} in the

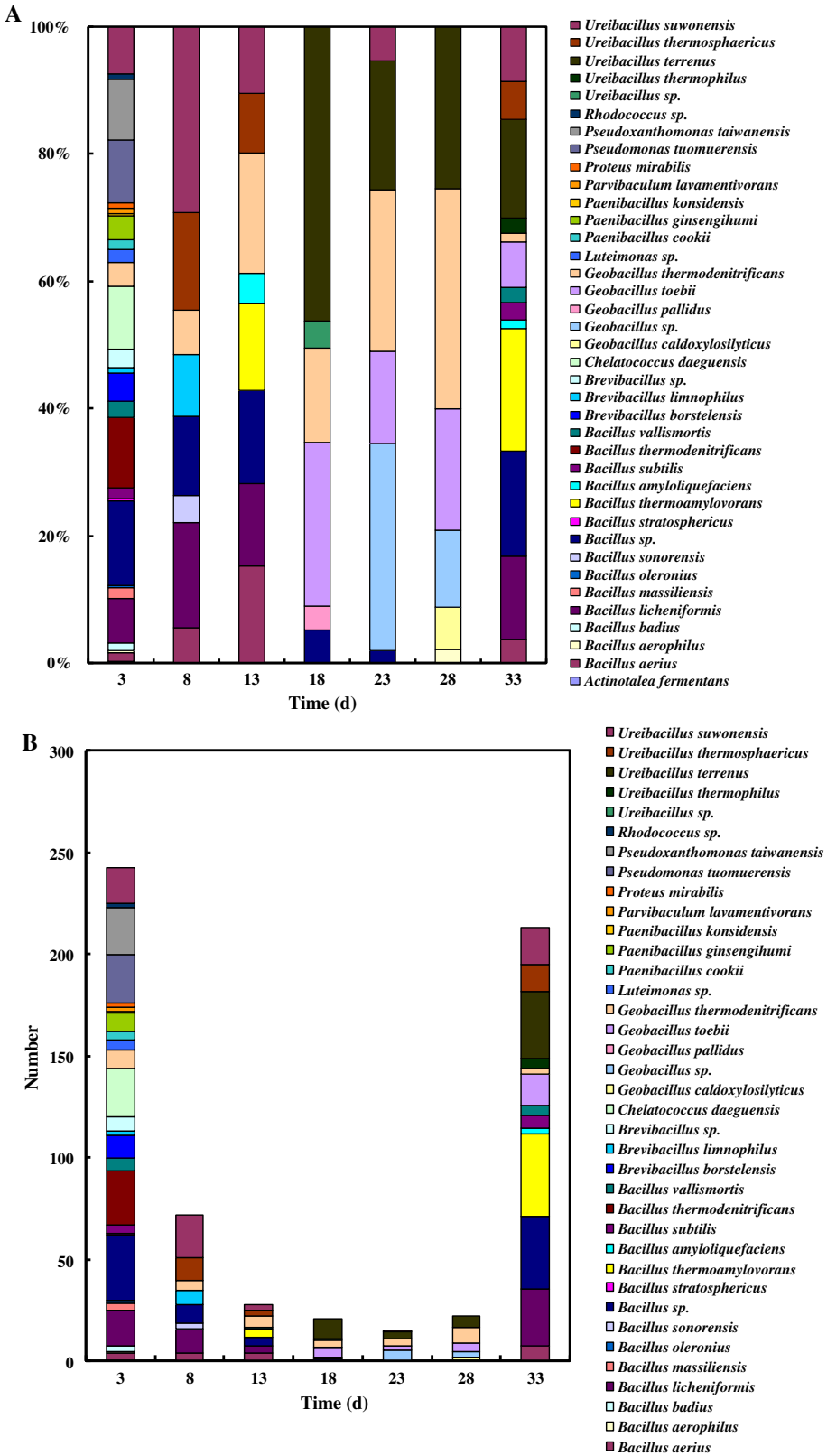

Fig. 4 Relative abundances and number of culturable thermophilic bacteria taxa (species) at each timepoint and variation in the species represented. **a** Relative abundances of culturable thermophilic bacteria taxa (species), **b** number of culturable thermophilic bacteria taxa (species)

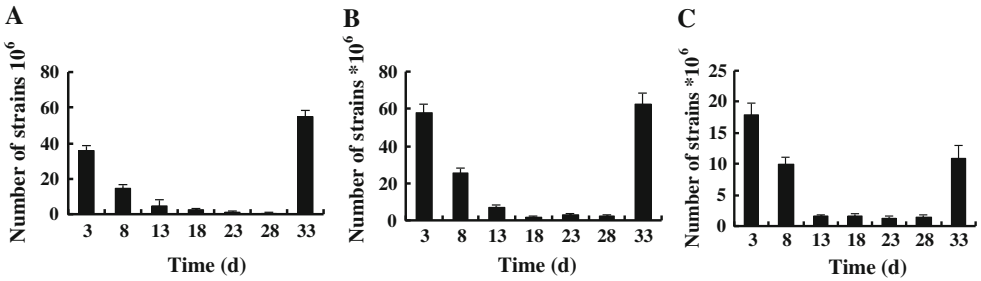

Fig. 5 Number of strains possessing starch, protein and cellulose degradation ability. **a** Number of strains that can degrade starch, **b** number of strains that can degrade protein; **c** number of strains that can degrade cellulose

sample from day 3 to 1×10^6 CFU g^{-1} by day 28; however, the number increased to 55×10^6 CFU g^{-1} by the final sampling. The number of bacteria with ability to degrade protein decreased from 58×10^6 CFU g^{-1} at day 3 to approximately 2×10^6 CFU g^{-1} by day 28. The number then increased to 63×10^6 CFU g^{-1} by the final sampling. The number of bacteria that could degrade cellulose decreased from 18×10^6 CFU g^{-1} at day 3 to approximately 1.5×10^6 CFU g^{-1} by day 28; however, the number increased to 11×10^6 CFU g^{-1} at the final sampling.

Multiple degradation abilities of all the culturable bacteria

Many culturable bacteria isolated from the thermophilic phase of composting possessed multiple degradation abilities. Bacteria that could degrade more than two substrates are listed in Table 1 and comprised *B. subtilis*, *B. tequilensis*, *B. licheniformis* and *Paenibacillus konsidensis* at day 3, *B. licheniformis*, *U. suwonensis* and *B. methanolicus* at day 8, *B. licheniformis* and *B. methanolicus* at day 13, *U. terrenus* and *G. toebii* at day 18, *U. terrenus*, *G. thermodenitrificans* and *U. suwonensis* at day 23, *U. terrenus* at day 28 and *B. tequilensis*, *B. licheniformis* and *B. subtilis* at day 33.

Discussion

Aerobic, thermophilic composting is a widely practiced method used for the disposal of organic wastes (Droffner and Brinton 1995). The goals of composting in solid-waste management are to rapidly reduce pathogens, odors, "putrescible" organic matter, moisture, and bulk, thereby producing a biologically "stabilized" material. It is generally agreed that given reasonable initial environmental moisture, air space, and C/N ratio conditions could influence the temperature and rate of composting and the quality of the product (McKinley and Vestal 1984). The temperature typically rises to above 50 °C during the process, and the maximum desired composting temperature is considered to be 60 °C based on the diversity of the microbial species and the rate of decomposition (Strom 1985). In the present study, the temperature reached greater than 50 °C in just 3 days during composting of cattle manure with plant ash and rice bran (95:3:2, w/w/w), indicating that the composting process was successful.

Changes in the number of the culturable microbial community were observed during the composting process in the present study. Many reports have discussed the abundance of culturable microbes but the culturing temperatures used were not based on the compost temperature. Indeed, the culturing temperatures used in the majority of the papers was approximately 25 °C for mesophilic microbes and approximately 55 or 70 °C for thermophilic microbes (Blanc et al. 1999; Takaku et al. 2006). In this study, we used the temperatures in the compost at the time of sampling for incubating the microbes. Microbes that were capable of growing at this temperature could be involved in the composting. Bacteria were observed during thermophilic compost phase. Decomposition of organic wastes during the composting process is performed by a succession of microbial communities including fungi, which are critical for the utilization of complex substrates, such as hemicellulose, cellulose and lignin (Marshall et al. 2003). However, despite using several methods, no fungi were detected in our study.

Different bacterial populations were found during the thermophilic composting phases in the previous researches. However, using molecular analysis, the only bacteria isolated were *bacillus* in the peak-heating phase of fresh wastes (Danon et al. 2008). Using cultivation-independent methods, Cahyani et al. (2003) reported that *Bacillus* and *actinomycetes* were the dominant genera during the thermophilic stage of rice straw. Steger et al. (2007) revealed changes within the Actinobacteria community in a full-scale composting process of organic household waste over a period of 57 weeks. *Bacillus* and *actinomycetes* were also observed in our study. In addition to these genera, we

Table 1 Ability of the strains to degrade two or three types of organic matter (starch, protein and cellulose)

Sample	Strains	Species	Starch	Protein	Cellulose
3 days	BA5	*Bacillus subtilis*	+	+	−
	BA12	*Bacillus subtilis*	+	+	−
	BA21	*Bacillus tequilensis*	+	+	−
	BA22	*Bacillus subtilis*	+	+	−
	BA23	*Bacillus subtilis*	+	+	−
	BA32	*Bacillus tequilensis*	+	+	−
	BA42	*Bacillus licheniformis*	+	+	−
	BA77	*Bacillus licheniformis*	−	+	+
	BA95	*Paenibacillus konsidensis*	+	−	+
	BA101	*Bacillus licheniformis*	+	+	−
8 days	BB2	*Bacillus licheniformis*	+	+	−
	BB11	*Ureibacillus suwonensis*	+	+	−
	BB20	*Bacillus licheniformis*	+	+	+
	BB22	*Bacillus licheniformis*	+	+	−
	BB29	*Bacillus licheniformis*	−	+	+
	BB30	*Bacillus licheniformis*	+	+	+
	BB32	*Bacillus licheniformis*	+	+	−
	BB33	*Bacillus licheniformis*	−	+	+
	BB38	*Bacillus licheniformis*	+	+	−
	BB45	*Bacillus licheniformis*	+	+	+
	BB47	*Bacillus licheniformis*	−	+	+
	BB51	*Bacillus methanolicus*	+	+	−
13 days	BC19	*Bacillus licheniformis*	+	+	−
	BC25	*Bacillus licheniformis*	+	+	−
	BC31	*Bacillus methylotrophicus*	+	+	−
	BC40	*Bacillus sp*	+	+	−
	BC41	*Bacillus licheniformis*	+	+	−
18 days	BD19	*Ureibacillus terrenus*	−	+	+
	BD23	*Ureibacillus terrenus*	−	+	+
	BD68	*Geobacillus toebii*	+	+	+
23 days	BE8	*Ureibacillus terrenus*	−	+	+
	BE18	*Geobacillus thermodenitrificans*	−	+	+
	BE35	*Ureibacillus suwonensis*	+	+	+
	BE36	*Geobacillus thermodenitrificans*	+	+	−
28 days	BF22	*Ureibacillus terrenus*	+	+	+
	BF44	*Ureibacillus terrenus*	+	+	+
33 days	BG7	*Bacillus tequilensis*	+	+	+
	BG9	*Bacillus tequilensis*	+	+	−
	BG36	*Bacillus licheniformis*	+	+	−
	BG43	*Bacillus subtilis*	+	+	+

+ Organic matter was degraded; −Organic matter was not degraded

frequently detected *Geobacillus* and *Ureibacillus* which may have been the dominant genera during the thermophilic phase of composting.

During the thermophilic composting phase, thermophilic microbes are responsible for the process of degradation, and both the growth and activity of non-thermo-tolerant microbes are inhibited. Optimization of compost quality correlates directly to the composition and succession of the microbial community during the composting process (Danon et al. 2008). Thus, monitoring the successive waves of thermophilic microbial communities during the thermophilic composting phase is important for effective

management of composting. Thermogenic composts are known to host a variety of thermophilic micro-organisms that were recently investigated using culturing methods and identified as *Thermus thermophilus*, *Bacillus* spp. and *Hydrogenobacter* spp. (Blanc et al. 1999). In our study, *G. thermodenitrificans* was observed in all samples, and *G. toebii* and *U. terrenus* may have been the dominant species during the later thermophilic composting phase. *B. licheniformis*, *B. aerius* and *U. thermosphaericus* were detected in initial and final samples of the thermophilic composting phase. During temperature increases, these species could form spores and may not grow, whereas when the temperature decreased to <60 °C, they could germinate again.

To control the composting process effectively, it is necessary to understand the particular role of microbes in the decomposition of organic matter (Yu et al. 2007). Our study showed that the number of bacteria possessing amylohydrolysis, protein and cellulose degradation abilities were higher at the beginning and end of the high-temperature composting phase. Temperatures exceeding 60 °C inhibited microbial activity severely. Incubating samples at these temperatures revealed that samples taken from high-temperature periods of composting (60–75 °C) typically showed lower number of bacteria with degradation activities than samples taken from the compost while it was at temperatures of <60 °C. However, the numbers of these mesophiles were $>10^6$ CFU g^{-1}, and several of which possessed multiple degradation abilities, such as *B. licheniformis*, *B. methanolicus*, *U. terrenus*, *G. toebii*, *G. thermodenitrificans* and *U. suwonensis*. Of these species, *U. terrenus*, *G. toebii, G. thermodenitrificans* and *U. suwonensis* were detected when temperatures >70 °C, and these populations remained stable during this high temperature phase. It is well known that *Bacillus* spp. are frequently detected in compost samples and that these species may contribute to the vigorous degradation of organic compounds (Hanajima et al. 2009). *Ureibacillus*, which belongs to the phylum Firmicutes, was described as an aerobic and thermophilic bacterial genus (Fortina et al. 2011), Furthermore, it was found in a microbial population capable of degrading lignocellulose (Wang et al. 2011) and also in the anaerobic thermophilic composting of sludge (Steger et al. 2005). Vargas-García et al. (2007) also revealed that inoculating *Ureibacillus* spp. presenting a great diversity of enzymatic activities capable of lignocellulose biodegradation in compost was effective in increasing the decomposition of organic matter. Charbonneau et al. (2012) reported that several isolates screened for thermotolerant hydrolytic activities at high temperature related to *G. thermodenitrificans* exhibited high thermotolerant extracellular protease and α-amylase activities. Therefore, the existence of *Ureibacillus*, *Geobacillus* and *Bacillus* in our composting suggested that these organisms may contribute significantly to the degradation of organic compounds, in particular to the degradation of cellulosic material during the thermophilic composting phase. These findings suggest that mesophiles and facultative thermophiles play important roles during the composting process. In addition, calculating microbial activities may provide a useful means of following functional changes in populations and successive communities with respect to temperature and perhaps other environmental factors.

Conclusions

The number and diversity of culturable thermophilic bacteria decreased in the thermophilic phase of composting. Genera of *Bacillus*, *Geobacillus* and *Ureibacillus* were the dominant genera and *G. thermodenitrificans* was the dominant specie. *G. toebii* and *U. terrenus* were dominant species in later stage. The number of bacteria possessed the starch, protein and cellulose degradation abilities were higher in the beginning and end of thermophilic phase. Bacteria which possessed the multiple degradation abilities mainly were *Bacillus* in the prior stage, and *Ureibacillus* and *Geobacillus* in the later stage of thermophilic composting phase.

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