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Evolution of microbial community diversity and enzymatic activity during composting

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Abstract

The composting of organic material is dependent on microbial activity. However, the dynamics of the microbial community during the composting process remain obscure. Here, denaturing gradient gel electrophoresis of 16S rDNA amplicons in a chicken manure-based compost was applied to characterize the components of the microbial community during the composting process. In addition, the activity of key microbial enzymes was monitored. *Arcobacter* spp. and *Marinospirillum* spp. were the dominant species prior to composting, whereas *Thermotogae* spp. became more strongly represented as the composting process proceeded. *Bacillus* and *Cohnella* spp. were featured at various phases. Correlation analysis showed that the diversity of the microbial community was positively correlated with the compost pH, its total nitrogen level, its carbon-to-nitrogen ratio and the activity of protease, and negatively correlated with its organic carbon content and seed germination indices.

Keywords: Aerobic composting; Raw material; Chicken manure; DGGE; Enzymology

1. Introduction

Without proper disposal, organic waste represents a potent source of environmental pollution (Kumar, 2011), and in some cases, a human health hazard (Domingo and Nadal, 2009). However, correct treatment of this material can recycle some of the energy and nutrients, reduce relevant waste pollution and prevent specific diseases from spreading (Kumar, 2011), as well as reducing risk. Composting is a particularly attractive means of treating animal manure. During the composting process, the microbial community must adapt to a gradually reduced supply of nutrients, along with changes in the compost temperature and its content of water, carbon dioxide, oxygen and ammonium (Tuomela et al., 2000). As a result, the various biotic components of the compost undergo species succession during the composting process (Bernal et al., 2009; Collins et al., 2006; Kumar, 2011). An understanding of the dynamics of the microbial community would therefore be useful as part of an attempt to improve the efficiency of composting (Brown et al., 2008).

Studying the nature of microbial succession via in vitro cultivation of species present can lead to misinterpretation of diversity (Hugenholtz et al., 1998). A more accurate approach is represented by DNA fingerprinting. In the present study, a combination of PCR and denaturing gradient gel electrophoresis (DGGE) was employed to identify components of microbial succession during composting of chicken manure (Lee et al., 2010). The species which dominate the composting process release a range of hydrolytic enzymes (in particular, cellulases, hemicellulases, proteases, lipases, phosphatases and arlylsulphatases) (Ben-David et al., 2011; Portillo et al., 2011; Shi et al., 2011), which act to depolymerase various organic waste constituents (Kandeler et al., 1999; Marx et al., 2001). Thus, in addition to species identification, activities of the key microbial enzymes involved in composting were

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characterized to gain further information relevant to control of the process (Maeda et al., 2010; Xing et al., 2005; Yamamoto et al., 2011).

2. Materials and methods

2.1. Composting material

The raw base material for composting comprised a mixture of five parts of chicken manure, one part each of rice husk and rice bran and three parts of mushroom residue. Samples of the material prior to composting were subjected to an array of physical and chemical analyses (Table 1). A sample of ~2 g was assessed for moisture content by baking overnight at 105 °C. A suspension of 3 g in 27 ml distilled water was assayed for its pH and electric conductivity (EC) using a Sartorius LE438 electrode. Air-dried samples were analyzed for content in organic matter (OM) using the potassium dichromate method, total nitrogen (N) content using the Kjeldahl method, total phosphate using the vanadium molybdate yellow colorimetric method and total potassium using flame photometry (APHA et al., 1990; NY525-2002, China). A conversion factor of 1.724 is commonly used to convert organic carbon to organic matter: organic matter (%) = organic carbon (%) × 1.724. The ratio of carbon (C) and N content (the C/N ratio) was calculated by dividing OM content by N content. The physico-chemical properties measured for the raw material are provided in Table 1.

2.2. Composting conditions

In our study, one cycle under the following conditions constituted the complete process of compost maturity from April 23 to June 6, 2011. The composting experiment lasted 45 days. The components of the composting material were thoroughly mixed and the water content of the mixture adjusted to 55%. The material was loaded into a 25 m × 2 m × 1.5 m trough equipped at its base with a forced air ventilator able to supply 3000 l/min air at 13 min after every 17 min. The 25 m wide, 1.6 m high pile of compost was turned over every 2–3 days using a self-propelled Sandberger turner (Neuson Hydraulic, Linz, Austria), and the pile was turned at least once immediately prior to sampling. Humidity was manually controlled before each turning operation, and water was added when necessary.

2.3. Sampling strategy

A total of 4 samplings were taken from 5 different areas (center and 4 corners, Fig. 1A) in the composting near each location from 1, 2, 3 and 4 (Fig. 1B), and the sampling center of the compost pile at a depth of ~0.7 m.

Samples were taken at 10 am on days 1, 3, 5, 12, 15, 25, 30 and 45 following the initiation of the composting process. Approximately 500 g was collected at each sampling and then mixed adequately for analysis. Each sample was split into three parts: one part was stored at 4 °C for later chemical analysis (Floch et al., 2009), one was assayed immediately for the activity of five enzymes (assay procedures given in Table 2) and the third was stored at −80 °C for subsequent DNA extraction.

For dehydrogenase, a 1.5 g sample was suspended in 3 ml water and 3 ml 3% w/v 2,3,5-triphenyl-tetrazolium chloride (TTC) at 37 °C for 24 h in the dark, after which 10 ml methanol was added. The suspension was filtered through a glass fiber filter and the filter rinsed in methanol until no red staining (due to the presence of reduced TTC) remained. The volume of filtrate was finally adjusted to 100 ml with water and absorbance at 485 nm was compared to that obtained from a range of triphenyl formazan standards (Tabatabai, 1982).

For cellulose, a 1.5 g sample was suspended in 0.5% w/v carboxymethyl cellulose in acetate buffer pH 5.9 and shaken on a rotary shaker for 24 h at 30 °C. The suspension was then centrifuged (17,000 × g, 10 min) and the sugar concentration of the supernatant determined using a colorimetric anthrone method (Pancholy and Rice, 1973).

For β-glucosidase, a 1.5 g sample was suspended in 8 ml 0.1 M acetate buffer (pH 5.0) plus 2 ml *p*-nitropenyl-β-glucopyranoside. After holding for 1 h at 37 °C, 2 ml 0.5 M $CaC1_2$ and 8 ml 0.5 M NaOH were added and the suspension was filtered through a glass fiber filter. The release of *p*-nitrophenol was measured spectrophotometrically at 410 nm (Martens et al., 1992).

For protease, a sample (1.5 g) was suspended in 10.8 ml 0.1 M Tris–HCl buffer (pH 8.1) and 12 ml 2 mM benzyloxycarbonyl-phenylalanyl leucine buffer. After 1 h of agitated incubation at 40 °C, the sample was cooled to 20 °C and enzyme activity was stopped by adding 1.2 ml 5 N HC1. After centrifugation at 8000 × g for 10 min, 0.8 ml of the supernatant was neutralized with 0.2 ml 1 N NaOH and the amino acid contents determined with ninhydrin reagent (Ladd and Butler, 1972).

For urease, a sample (1.0 g) was suspended in 6 ml Tris–H_2SO_4 buffer (pH 8.5) and 3 ml 0.1 M urea and incubated at 37 °C for 2 h. The suspension was then filtered through Whatman no. 41 paper and the NH_4^+ released was measured by colorimetry (Frankenberger and Tabatabai, 1980).

Enzyme assays were performed in 4 replicates for each sample. A unit (U) of enzyme activity was defined as mmole

Table 1
Physico-chemical properties of raw materials used for composting (dry weight-based, $n = 3$).

Raw materials	Moisture content (%)	EC (ms/cm)	Organic matter (%)	Total N (%)	Total P (%)	Total K (%)	C/N ratio
Fresh chicken manure	75.35	6.15	48.58	2.45	2.88	3.96	11.50
Rice husk	10.57	2.85	36.25	0.56	0.23	1.07	37.57
Rice bran	18.8	2.40	63.19	1.02	1.69	1.52	35.96
Mushroom residue	8.93	1.55	80.54	1.23	1.60	1.44	38.00

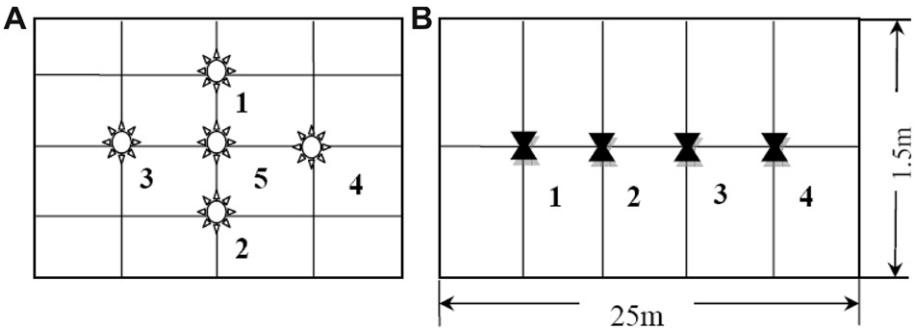

Fig. 1. The composite sampling strategy. A: Sampling locations within the composting trough, B: locations chosen for temperature measurements.

of substrate hydrolyzed or oxidized min^{-1}, and per g of sample dry weight (U/g DS).

Two aliquots of the first part were used to determine pH and E_4/E_6 (the ratio of optical densities or absorbances of dilute, aqueous humic acid solution at 465 and 665 nm) within one week of sampling. For the former, the sample was homogenized for 30 min in ten volumes of distilled water and left to stand at room temperature for 30 min; the pH of the supernatant was then measured. For the latter, the sample was homogenized in 20 volumes of distilled water for 30 min, centrifuged at 13,900 × g for 15 min and absorbance of the supernatant was then measured at both 465 nm and 665 nm. The remainder of the first part of the composite sample was then air-dried, sieved (<2 mm) and used for chemical analyses mentioned in the previous section. The temperature of the compost was taken at locations 1–4 at a depth of 70 cm (Fig. 1B) using a TES1310 thermometer (Taishi, Taiwan) equipped with a K-type thermocouple detector. The temperature was measured twice a day (at 10 am and 10 pm) over the first six days of composting, once every other day at 10 am from days 7–18 and once every fourth day at 10 am for the remainder of the period.

Seed germination indices (GIs) were determined as follows. Distilled water (50 ml) was added to 5 g of fresh compost, which was mechanically shaken for 1 h and then filtered. Compost extract (5 ml) was added to culture dishes (9 cm diameter) containing two pieces of filter paper. A total of 20 radish seeds were evenly distributed on the surface of the filter paper and then incubated in the dark at 30 °C for 48 h. The control for the GI index test was water. The procedures were replicated three times for each sample. Root lengths and germination rates were measured and GI was calculated as described previously (Gu et al., 2011).

2.4. DNA extraction, amplification and DGGE analysis

Total genomic DNA was extracted from each composite subsample using a ZR fecal DNA MidiPrep kit (ZYMO RESEARCH, USA) and a Fast Prep FP220 instrument (BIO 101 Systems, USA). The resulting DNA was purified using a MonoFas kit (GL Sciences, Japan) according to the manufacturer's instructions, and the concentration of purified DNA measured with a Gene Quant Pro S device (Amersham, Japan). The microbial 16S rDNA sequences present in these DNAs were amplified by primer pair 341f: (5′-GCCCTACGGGAGGCAG-CAG-3′) and 534r (5′-ATTACCGCGGCTGCTGG-3′) (Shenggong, Shanghai). The GC clamp 5′-$CGC_3GC_2GCGC_4G$-$CGC_3G_2C_3GC_2GC_5GC_4$-3′ was attached to the 5′ end of 341f to improve amplicon separation (Muyzer et al., 1993). Each PCR comprised a 50 ng template, 15 pM primer, 15 nM dNTP, 5 μl 10× PCR buffer (25 mM $MgCl_2$) and 5 U Taq polymerase, with the volume made up to 50 μl with water (Xie et al., 2012). The

Table 2
Methods used for enzyme assays.

Enzymes	Substrate	Incubation conditions				Product assay	
		Buffer	Temperature	Time	Reference	Product	Method[a]
Dehydrogenase	2,3,5-Triphenyltetra-zolium chloride (TTC)	–	37 °C	24 h	Tabatabai (1982)	Triphenyl formazan (TPF)	A_{485}
Cellulase	Carboxymethyl-cellulose	0.2 M acetate buffer (pH 5.9)	30 °C	24 h	Pancholy and Rice (1973)	Reducing sugars	Anthrone, Brink et al. (1960)
β-Glucosidase	*p*-Nitrophenyl-β-glucopyranoside	1 M acetate buffer (pH 5.0)	37 °C	1 h	Martens et al. (1992)	*p*-Nitrophenol	A_{410}
Protease	Benzyloxycarbonyl-phenylalanyl-leucine	0.1 M Tris–HC1 buffer (pH 8.1)	40 °C	1 h	Ladd and Butler (1972)	Amino acids	Ninhydrine, Moore and Stein (1954)
Urease	Urea	0.1 M Tris–H_2SO_4 (pH 8.5)	37 °C	2 h	Frankenberger and Tabatabai (1980)	Ammonium	Indophenol blue, Chariot (1961)

[a] Spectrophotometric measures by a lambda 5 Perkin–Elmer spectrophotometer.

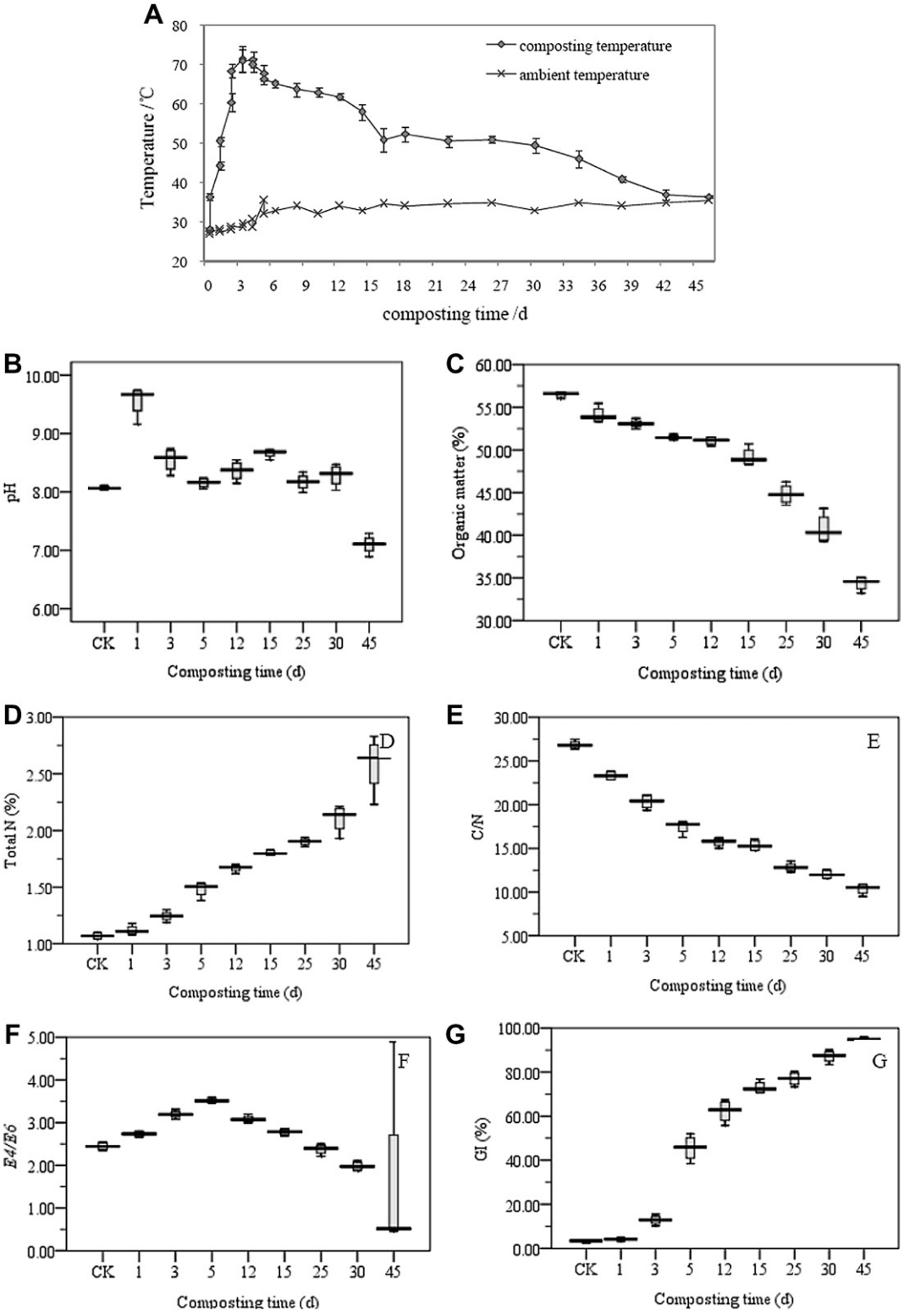

Fig. 2. Physico-chemical changes in compost samples over time. A: Temperature, B: pH, C: organic matter content, D: total nitrogen content, E: ratio between total carbon and total nitrogen contents, F: E_4/E_6, G: GI value. The rectangular box is the main part of the diagram. The three lines represent (from top to bottom) the 75, 50 and 25 percentiles of the variable and 50% of the observed value drops in this area. The vertical line in the center is a tentacle line, whereas the horizontal lines at the top and bottom represent, respectively, the maximum and minimum value of the variable.

amplification cycle consisted of initial denaturation of 95 °C/9 min, followed by 30 cycles of 95 °C/30 s, 50 °C/45 s, 72 °C/120 s, and a final elongation step of 72 °C/10 min. To check for amplification, a 4 μl aliquot of the PCR product was first separated electrophoretically through a 0.8% agarose gel. The remainder of the reaction was then subjected to DGGE

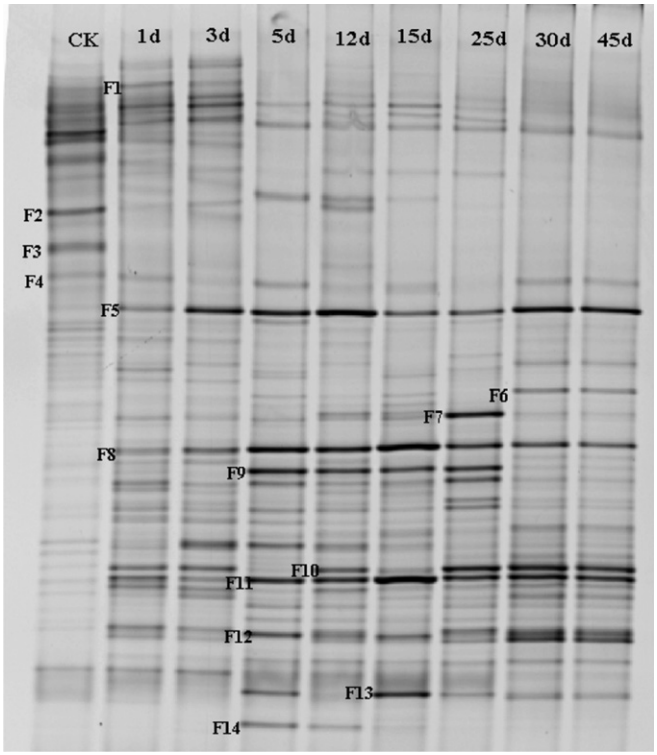

Fig. 3. DGGE profiles of microbial 16S rDNA PCR amplicons derived from DNA template extracted from compost. Fragments F1–F14 were cloned and sequenced.

following the DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Each 8% polyacrylamide gel contained a linear 30%–60% denaturing gradient formed by formamide and urea. The samples were electrophoresed at 60 °C, 100 V for 12 h and the gels were then stained for 20–30 min in Goldview (SBS Genetech Co., Ltd.). The UV-excited fluorescent signal was captured by a video camera (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed using Bio-Rad Quantity One v4.3.0 software.

2.5. Assessment of microbial diversity

The Shannon–Wiener diversity index (H), richness (S) and evenness index (E_H) formed the major basis for assessing the diversity of the microbial community (Luo et al., 2004), as shown in Eqs. (1) and (2) below:

$$H = -\sum_{i=1}^{s} \frac{n_i}{N} \ln \frac{n_i}{N} = -\sum_{i=1}^{s} P_i \ln P_i \quad (1)$$

$$E_H = \frac{H}{H_{max}} = \frac{H}{\ln S} \quad (2)$$

where P_i represented the frequency of the ith DGGE band, n_i the amplification intensity of the ith DGGE band and $N = \sum n_i$. The value of H ranged from 0 to ln S.

2.6. Cloning and DNA sequencing

DNA fragments of interest were excised from the DGGE gels and immersed in sterilized water overnight (Hirooka et al., 2009). A 1 μl aliquot of this extract was then used as the template for further PCR based on 341f (without the GC clamp) and 534r. The resulting amplicons were purified using MagExtractor-PCR and Gel Clean Up kits (Toyobo, Osaka, Japan) and cloned using a Novagen® Perfectly Blunt™ cloning kit (EMD Chemicals, San Diego, CA, USA). A PCR was used to validate the cloning step (primers T7 and U19, EMD Chemicals, USA) and the size of the amplicon was checked by electrophoresis through a 1.2% agarose gel. The PCR product was then purified using an ExoSAP®-IT kit (USB, Cleveland, OH, USA), and submitted for sequencing. Sequence data were aligned using MEGA software and phylogeny was constructed based on maximum parsimony (Tamura et al., 2007). The resulting sequences were deposited in the GenBank database as accession numbers JQ268734 to JQ268736.

2.7. Statistics

Correlation analyses and Duncan's multiple comparisons were performed using SPSS v15.0 (IBM, USA), assigning a significance threshold of $P < 0.05$.

3. Results

3.1. Physico-chemical changes

The temperature rose from 30 °C to 40 °C over the first two days of the 45-day composting period, reached 60–70 °C over the period from days 3–14 and then fell back to below 50 °C by day 34 (Fig. 2A). The temperature remained above 55 °C for 11 consecutive days, a temperature high enough to successfully prevent multiplication of pathogenic microorganisms and to kill any *Ascaris* eggs present (this measure is in line with national standards for the non-hazardous treatment of chicken manure) (GB7959-87, China). The pH increased from 8.07 to 9.56 within the first days and then decreased to a near-neutral level by the end of the experiment (Fig. 2B). The OM content fell monotonically from an initial level of 55% to ~30% (Fig. 2C), while the N content increased from 1% to over 2.5% (Fig. 2D). As a consequence, C/N decreased

Table 3
Diversity indices of the bacterial community in compost samples.

Diversity index	CK	1d	3d	5d	12d	15d	25d	30d	45d
H	2.475 b	2.761 a	2.680 a	2.066 d	2.373 b	2.209 c	2.414 b	1.861 e	1.923 e
E_H	0.876 b	0.978 a	0.949 a	0.732 d	0.841 b	0.783 c	0.855 b	0.659 e	0.681 e
S	0.585 b	0.652 a	0.633 a	0.488 d	0.560 b	0.522 c	0.570 b	0.440 e	0.454 e

Note: different lower cases in the same row indicate statistically significant differences in the same diversity index ($P < 0.05$).

throughout the composting period, decreasing from 25 at the outset to ~10 by the conclusion (Fig. 2E). E_4/E_6 increased from >2 to 3.5, peaking within the first week (Fig. 2F), thereafter it fell to <2 by the end of the experiment. The GI rose throughout the composting process, starting at <20%, increasing to 50% after one week and exceeding 85% by the end of the experiment (Fig. 2G).

3.2. DGGE profiles

The variation in DGGE profiles showed that the composition of the microbial community shifted as the composting process progressed (Fig. 3). Fragments F2–F4 were represented in the profile prior to composting, while F5, F6, F8 and F10 appeared later in the experiment. Of particular note was the appearance of F9 by day 5 and its disappearance after day 30, a period which corresponded to the bulk of the composting process, suggesting that the source of this fragment was important for composting. F13 grew in abundance around day 15, while this occurred for F7 some ten days later. The appearance of F6 only after day 30 suggested that its source was active exclusively in the later phase of composting. The behavior of the three diversity indices H, S and E_H was similar, with each rising over the first three days, then falling thereafter (Table 3).

3.3. The species origin of differentially represented 16S rRNA sequences

Sequence alignment indicated that F9 was likely amplified from the DNA of *Bacillus* sp. TP-84 (99% sequence identity, SI). The F4 sequence shared 90% SI with F9. The appearance of both F6 and F13 (100% SI with *Bacillus* sp. SCSSS08 and 96% SI with *Bacillus* sp. MSP06G, respectively) probably signaled the presence of *Bacillus* spp. in the compost microflora. However, the F2 sequence was related to the 16S rRNA of *Arcobacter* sp. (97% SI), while that of F3 was highly similar (99% SI) to the homolog of *Marinospirillum minutulum*. The F10 and F12 sequences both shared a strong level of SI with homologs from, respectively, *Cohnella fontinalis* (99%) and *Cohnella* sp. HIO-4 (99%), whereas the F5 sequence was 95% similar to the

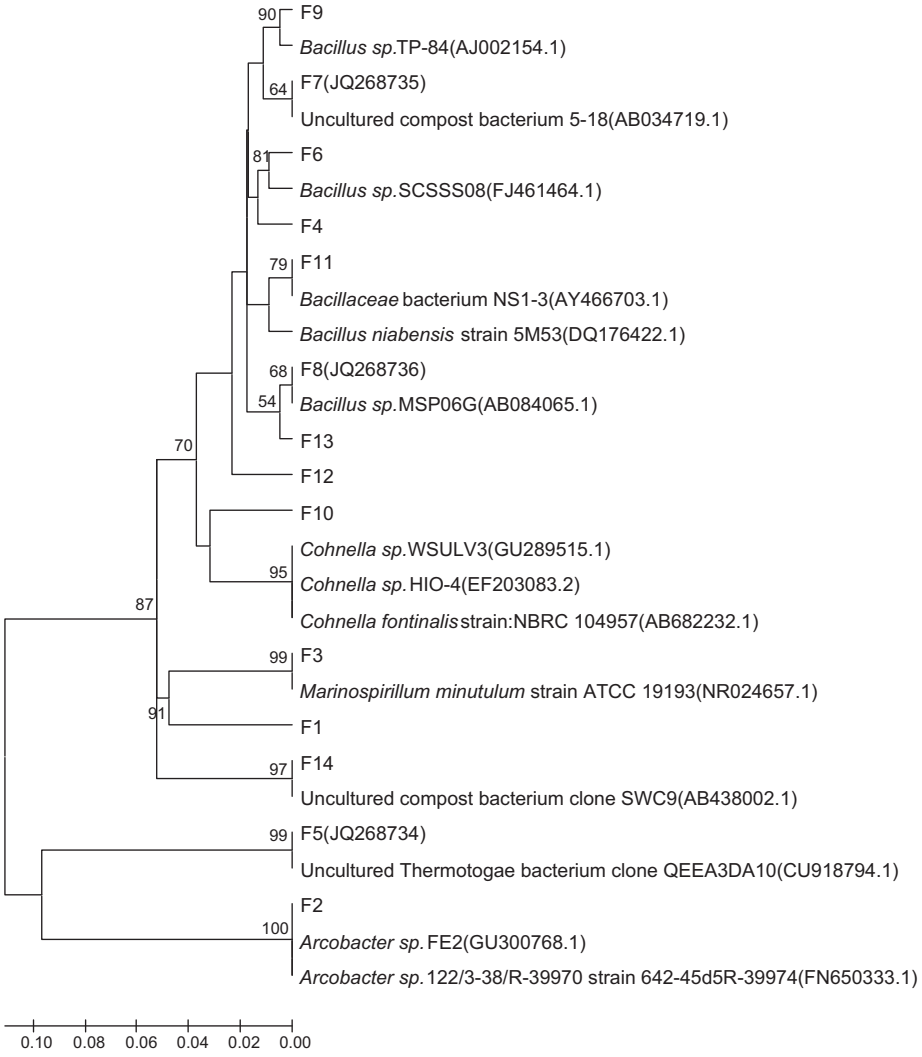

Fig. 4. Phylogeny of 16S rDNA PCR amplicons. Bootstrap values are given for each branch.

homolog from *Thermotogae bacterium* SulfLac1. The sequences of the other fragments isolated shared <80% SI with known microbial 16S rRNA sequences.

3.4. Enzyme activities

Dehydrogenase, cellulase and β-glucosidase activity varied in a similar fashion during the composting process, namely, decreasing at the outset, then increasing and finally tailing off (Fig. 5). Activity peaked for all three enzymes on day 12, with 60.84 mU/g DS, 1.32 U/g DS and 3.60 mU/g DS, respectively. In contrast, protease activity increased markedly within the first three days and then decreased thereafter. Urease activity fell substantially at the outset, then fluctuated around a peak at day 15.

3.5. The relationship between physico-chemical and biological variations

The pH in the compost was negatively correlated with its N content and GI, and positively with its OM content, C/N ratio and protease activity; at the same time, N content was negatively correlated with both OM content and C/N ratio, and positively with GI (Table 4). Other inter-variable correlations are presented in the same table.

4. Discussion

Here, a 45-day composting experiment was conducted to elucidate species succession of the microbial community in the compost. The species composition changed markedly as composting progressed, and particular correlations could be drawn between the biological and physico-chemical states of the material. The temperature rose rapidly and remained relatively high for the first two weeks (Fig. 2A). A similar phenomenon has previously been reported on raw materials (Brown et al., 2008). Temperatures above 50 °C are known to promote the rate of composting and thereby accelerate the reduction in OM content (Fig. 2C). Meanwhile, the pH of the compost gradually fell, presumably reflecting the production of organic acids as a byproduct of the decomposition of OM. The increasing level of the N content and the fall in the C/N point to a steady rise in the utility of the compost as plant

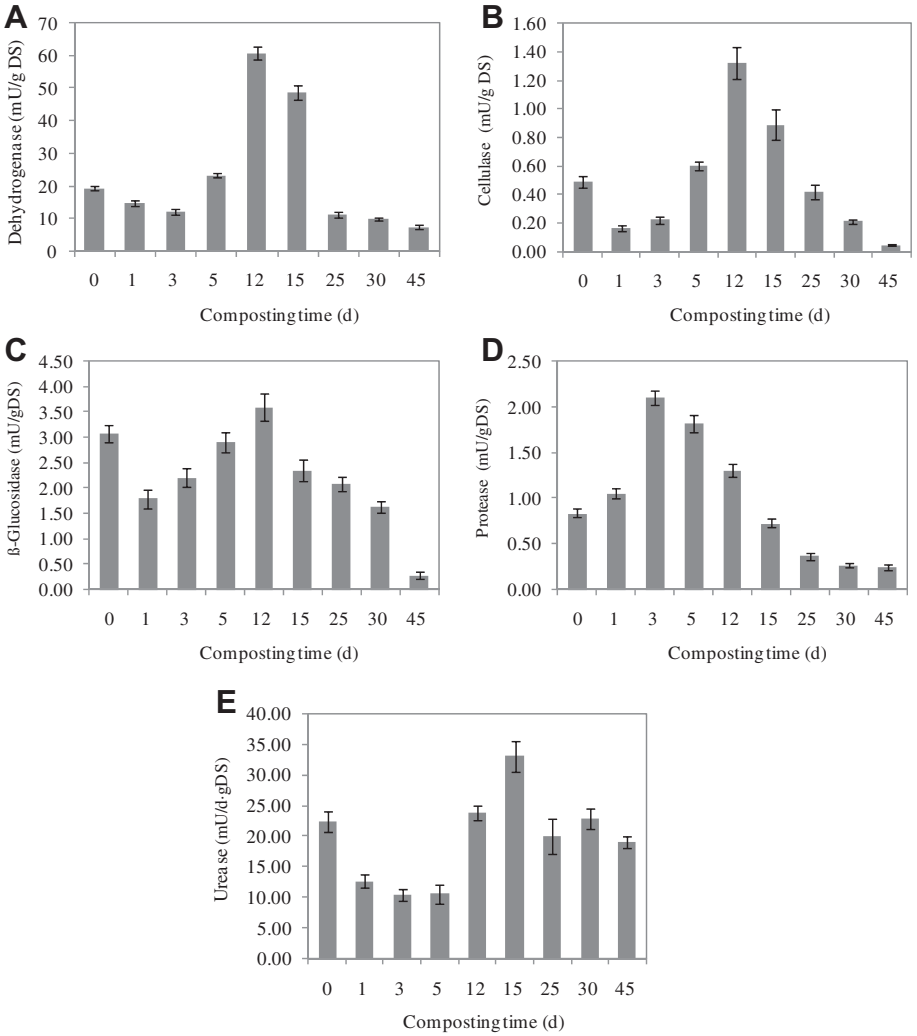

Fig. 5. Enzymatic activities in compost. A: Dehydrogenase, B: cellulase, C: β-glucosidase, D: protease, E: urease.

Table 4
Correlation coefficients of major physico-chemical properties and relevant enzymatic activities in compost samples.

	pH	TN	OM	C/N	E_4/E_6	GI	Dehydrogenase	Cellulase	ß-Glucosidase	Protease	Urease	*S*	*H*
TN	−0.646**												
OM	0.633**	−0.957**											
C/N	0.502**	−0.909**	0.871**										
E_4/E_6	0.272	−0.369*	0.479**	0.285									
GI	−0.546**	0.926**	−0.858**	−0.961**	−0.335*								
Dehydrogenase	0.195	−0.108	0.328	0.001	0.310	0.092							
Cellulase	0.035	−0.063	0.290*	−0.054	0.294	0.157	0.954**						
β-Glucosidase	0.032	−0.453**	0.617	0.385*	0.507**	−0.302	0.698**	0.793					
Protease	0.329*	−0.645**	0.669**	0.484**	0.587**	−0.617**	0.217	0.194	0.487**				
Urease	−0.102	0.334*	−0.192	−0.278	−0.251	0.476**	0.537**	0.514**	0.124	−0.564			
S	0.660**	0.731**	−0.764**	0.719**	0.290	−0.795**	0.039	−0.034	0.203	0.464**	−0.316		
H	0.660**	0.731**	−0.763**	0.718**	0.290	−0.794**	0.039	−0.033	0.203	0.464**	−0.316	1.000**	
E_H	0.661**	0.731**	−0.763**	0.717**	0.290	−0.794**	0.041	−0.032	0.203	0.465**	−0.316	1.000**	1.000**

Note: TN, total nitrogen; OM, organic matter; C/N, ratio of carbon to nitrogen; E_4/E_6, the ratio of optical densities or absorbances of dilute, aqueous humic acid solution at 465 and 665 nm; GI, seed germination index; *S*, abundance; *H*, Shannon–Wiener index; E_H, evenness index; $n = 36$, $F = 0.329$ for $P < 0.05$, $F = 0.424$ for $P < 0.01$; ** and * indicate $P < 0.01$ and $P < 0.05$, respectively.

fertilizer. Reflecting this, the GI, which represents a measure of how readily the material can support seed germination, rose over the course of the experiment (Fig. 2D–G) to a level which was no longer harmful for plant growth (Tiquia, 2005; Wu and Ma, 2001). Presumably, these alterations in the physico-chemical state of the compost were largely brought about by microbial activity.

The diversity indices *H*, E_H and *S* all rose markedly over the first few days of composting (Table 3), consistent with the rapid proliferation of a range of microbial species. It has been established that thermophilic microbes dominate composts with a strict thermogenic phase (Alfreider et al., 2002; Miyatake and Iwabuchi, 2005). The diversity of fatty acid methyl esters has been reported to increase in the secondarily-produced compost from the onset of composting (Kato and Miura, 2008). The diversity indices then fell, demonstrating that some of these species began to dominate the community. It has been suggested that successful composting requires a level of interaction between microbial species (Randazzo et al., 2002; Yamamoto et al., 2011). The identification of distinctive species enabled by DGGE separation of 16S rRNA amplicons, combined with the sequencing of these products, has led to a detailed picture of the dynamics of the microbial community to be obtained. The microbial composition indeed shifted with time: for example, *Arcobacter* and *Marinospirillum* spp. were present in the compost at the outset, but disappeared once the composting process was under way (Figs. 3 and 4). *Arcobacter* spp. are known to be pathogenic to both humans and domestic animals, so their removal is a particularly positive outcome of composting.

At a temperature of 50–55 °C, members of the genus *Bacillus* begin to dominate the microbial community in composts (Claus and Berkeley, 1986). The 16S rRNA gene sequences represented in this taxon displayed a high level of heterogeneity (Ash et al., 1993). Recently, a phylogenetic analysis based on 16S rRNA sequences and certain biochemical traits identified the presence of four distinct species in this group of micro-organisms, namely *Geobacillus thermodenitrificans*, *Bacillus smithii*, *Ureibacillus suwonensis* and *Aneurinibacillus thermoaerophilus* (Charbonneau et al., 2012). The present study has highlighted the fact that both *Bacillus* and *Cohnella* species were retained in the compost samples throughout the experiment, whereas *Bacillus* sp. TP-84 died out about half way through the process (Figs. 3 and 4). Overall, therefore, our experiments support the observation that *Bacillus* species represent the major bacterial components of the microflora present in degrading organic matter. Blanc et al. (1997) noted that *Bacillus* spp. prefer warm environments (55–65 °C) and, according to Peter et al. (Dürrschmidt et al., 2001), those adapted to grow best in the temperature range 50–74 °C enhance organic decomposition and utilization during the high temperature stage. Thus, the appearance of *Bacillus* sp. TP-84 (as indicated by DGGE fragment F9) in the compost is likely attributable to the elevation in temperature during the early phase of the composting process. Note that certain thermophilic anaerobic cellulose-degrading bacteria have also been identified in high-temperature composts based on cow manure.

Microorganism-induced degradation of organic matter relies on the activity of various hydrolytic enzymes (Raut et al., 2008), and in the composting process, the most important of these are depolymerase, cellulase, β-glucosidase and protease (Mondini et al., 2004). With respect to enzyme activity, protease was highly expressed on day 3, whereas the activities of dehydrogenase, cellulase and β-glucosidase all peaked on day 12, and that of urease not until day 15 (Fig. 5). Similar temporal trends of protease activity have been detailed by both de la Horra et al. (2005) and Liu et al. (2011). This pattern of expression probably reflected microbial succession during the composting process. Both cellulase and protease activities reported here were lower than those reported in composts based on either pig slurry/straw, horse manure/cardboard production residue or vegetable matter/cardboard production residue/straw. This discrepancy in measured enzyme activities may well have arisen from differences associated with the use of alternative raw materials (Queda et al., 2002). The monitoring of enzyme activities throughout the compost process is informative with respect to

both carbon and nitrogen dynamics, as well as aiding in understanding transformations that occur during composting (Vargas-García et al., 2010). The strong correlation between N content and the activity of β-glucosidase, protease and urease (Table 4) suggests that the nitrogenous material in the compost derived mainly from decomposition of glucosidase and protease during the early phase of composting, and of urea during its later stage. Both OM content and C/N were correlated with β-glucosidase and protease activity, which would be expected if the amount of OM became limiting (Floch et al., 2009; Souza et al., 2012). Furthermore, cellulase and β-glucosidase are known to be important in degradation of cellulose, and their levels of activity appear to be correlated with one another (Liu et al., 2011). The strong correlation between the GI and the activities of protease and urease suggested that incomplete transformation of organic nitrogenous compounds to nitrate probably contributed to the phytotoxicity of the immature compost. The activities of the five enzymes analyzed were intercorrelated (Table 4), confirming the presence of interactions among microbial species. Despite the lack of a correlation between H, E_H or S and any of the enzyme activities, we cannot rule out the possibility that the abundance of specific microbe species underlies observed variations in enzyme activity.

In conclusion, this study reports a composting experiment based on chicken manure, rice husk and bran, and mushroom residue. Dynamic changes in the diversity of the microbial community were observed, as well as in a number of key physico-chemical properties and enzymatic activity. *Bacillus*, *Cohnella* and *Thermotogae* species proved to be important components of the composting process. The data provide a handle for improving composting maturity and should contribute to a better understanding of the biodegradation processes exploited in waste management.

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