Appl Microbiol Biotechnol (2015) 99:9191–9201
DOI 10.1007/s00253-015-6754-3

ENVIRONMENTAL BIOTECHNOLOGY

Environmental factors shaping the abundance and distribution of laccase-encoding bacterial community with potential phenolic oxidase capacity during composting

Lunhui Lu[1,2,3] · **Guangming Zeng**[1,2] · **Changzheng Fan**[1,2] · **Jinsong Guo**[3] · **Jiachao Zhang**[4] · **Ming Chen**[1,2] · **Haipeng Wu**[1,2] · **Yujie Yuan**[1,2] · **Xiaoxiao He**[1,2] · **Yan He**[1,2]

Received: 8 March 2015 / Revised: 26 May 2015 / Accepted: 30 May 2015 / Published online: 24 June 2015

Abstract Increasing molecular evidence points to a wide occurrence of laccase-like multicopper oxidase (LMCO)-encoding genes in bacteria. Most researches mainly focused on the bacterial LMCO diversity, whereas the processes and the environmental factors responsible for structuring bacterial LMCO communities remain relatively unknown in a composting system. Six gene libraries were constructed from samples in representative stages during composting. A total of 185 sequences obtained from sample DNA extracts were classified to 59 operational taxonomic units (OTUs) based on 10 % cutoff. The distribution profile of bacterial LMCO genes showed that proteobacterial- and actinobacterial-associated species were the dominant communities during composting. Pearson correlation analysis indicated that the pile temperature and water-soluble carbon (WSC) content were significantly positively correlated with bacterial LMCO gene OTU numbers, Chao1 and Shannon index, whereas the humic acid (HA)-like carbon content had the most significant effect on the distribution of the bacterial LMCO genes during composting by redundancy analysis. These findings will improve the understanding of the mutual relationship between environmental factors and bacterial LMCO community compositions in composting.

Keywords Composting · Bacteria · Laccase-like multicopper oxidases · Environmental factors

✉ Guangming Zeng
zgming@hnu.edu.cn

✉ Changzheng Fan
fancz@hnu.edu.cn

[1] College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China

[2] Ministry of Education, Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Changsha 410082, People's Republic of China

[3] Key Laboratory of Reservoir Aquatic Environment of CAS, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

[4] College of Resources and Environment, Hunan Agricultural University, Changsha 410128, People's Republic of China

Introduction

Composting is an effective and productive process of decomposing solid organic waste into simpler nutrients by mixed microorganisms (Lu et al. 2014; Zhang et al. 2013). As the quickest way to produce high-quality compost, aerobic composting is a widely used way of stabilizing organic wastes and converting them to a final product that can be used as a soil conditioner or good-quality fertilizer (Xiao et al. 2011a; Zeng et al. 2011; Zhang et al. 2011). Numerous indigenous microorganisms in compost play a major role in the decomposition and transformation of degradable organic compounds (Belyaeva and Haynes 2009). In nature, lignocelluloses account for the major part of biomass; consequently, the degradation process is essential for the global carbon cycle. Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation, and it also slows biodegradation of cellulose and hemicellulose because it is closely associated with the cellulose component of plant cell walls (Huang et al. 2010).

As the vital functional enzymes for lignocelluloses degradation, laccases (benzenediol/oxygen oxidoreductases, EC1.10.3.2) or laccase-like multicopper oxidases (LMCOs), members of the multicopper oxidoreductase family, can

catalyze the oxidation of a wide range of aromatic substrates, by coupling with the reduction of molecular oxygen to water (Kellner et al. 2009; Majeau et al. 2010). Traditional fungal LMCOs were most widely studied for their significance in various biotechnological applications (Couto and Herrera 2006), such as degradation of xenobiotics (Johannes and Majcherczyk 2000), decolourization of dyes (Peralta-Zamora et al. 2003), the development of biosensors (Jarosz-Wilkołazka et al. 2005), and the effluent treatment (Jaouani et al. 2005). However, based on bioinformatics searches, increasing evidence suggests that LMCO-encoding genes may be widespread in bacteria (Ausec et al. 2011b). Strong laccase activities have been detected in diverse bacteria like *Stenotrophomonas maltophilia* (Ryan et al. 2009), *Sinorhizobium meliloti* (Rosconi et al. 2005), *Streptomyces* sp. C1 (Lu et al. 2013) and *Pseudomonas desmolyticum* (Kalme et al. 2009). The possibility that bacterial laccases play a role in the breakdown of recalcitrant biopolymers has been presented recently (Ahmad et al. 2010; Bugg et al. 2011), and bacterial laccases may have several properties that are not characteristic of fungal enzymes, such as stability at higher temperatures and alkaline pH values. Laccase of *Streptomyces* sp. C1 isolated from composting systems showed high thermo and pH resistance and the CotA laccase from *Thermus thermophiles* had a half-life of inactivation at 80 °C over 14 h (Lu et al. 2013; Miyazaki 2005). These properties probably make bacterial laccases preponderant during composting.

Instead of monitoring bacterial species based on their ribosomal RNA genes, studies analyzing functional genes encoding laccases could monitor changes in bacterial LMCO communities and provide information on their potential role in lignocelluloses degradation processes during composting. Changes in microbial community composition are often correlated with the dynamics of environmental factors. Most researches focused on analyzing the laccase gene diversity in ecosystems (Ausec et al. 2011a; Kellner et al. 2009), whereas the corresponding studies about changing environmental factors responsible for driving the diversity and distribution of bacterial LMCO-encoding communities during composting were still missing in the previous researches (Freedman and Zak 2014; Luo et al. 2015; Van der Gucht et al. 2007). The compositions of bacterial LMCO genes are likely to be influenced by several physicochemical parameters in composting systems. It is of interest to conduct such research to correlate the environmental factors and the bacterial LMCO gene abundance for identifying the factors that are important in structuring bacterial LMCO gene assemblages during composting. The potential response of environmental factors on bacterial LMCO community would highlight the susceptibility of composting microbes to environmental change (Dumbrell et al. 2010).

This study aims to identify the possible environmental factors shaping the distribution, diversity, and abundance of bacterial LMCO genes in the composting system. Establishing the interactions between the environmental factors and bacterial LMCO genes would broaden our knowledge of driver mechanism of bacterial LMCO community dynamics and lignocellulose biodegradation process during composting.

Materials and methods

Composting and sample collection

The source materials for composting consisted mainly of corn stalks, soil, cattle manure, grass clippings, and sawdust. The physicochemical properties of composting materials used in this study were displayed in Table 1. The composting experiment system was designed as described previously (Lu et al. 2013) and included packing 100 kg of source materials into a pilot-scale reactor. Air from a compressor was supplied at the bottom at a constant flow rate of 0.25 L min^{-1} kg^{-1} (wet weight) to maintain aerobic conditions throughout the experimental run. Three subsamples of 50 g on days 0, 4, 8, 15, 30, and 50 (defined as D0, D4, D8, D15, D30, and D50) were taken from the top, middle, and bottom layers at each sampling occasion. Three subsamples for evaluating bacterial LMCO gene diversity were mixed thoroughly and stored immediately at −20 °C before use at each sampling time. Moisture content was adjusted to about 50–60 % during composting by adding sterile deionized water after sampling. The mixture was turned twice a week during the first 2 weeks and once a week afterwards.

Physicochemical parameters and humic-like fraction determination

The pile temperature during composting was determined daily. The pH, moisture content, C/N ratio, and water-soluble carbon (WSC) of the selected samples during composting were analyzed according to Zhang et al. (2011).

The extraction and fractionation of humic-like fraction were carried out according to the method described by Lopez et al. (2006). Humic extract (HE) was obtained by shaking a 1-g sample with pyrophosphate-NaOH solution for 24 h. The dark-colored extract was filtered through a 0.45-μm Millipore filter until transparent solution was obtained for HE carbon analysis. The insoluble fraction that contained humic acid-like carbon (HA) was separated by centrifugation. The fulvic acid (FA)-like carbon was calculated as subtracting the HA from HE. Results are presented as carbon content per unit dry weight in each fraction.

Table 1 The common characteristics of the materials used in the composting

Materials	pH	TOC[a] (g kg^{-1})	TKN[b] (g kg^{-1})	C/N[c]	Moisture (%)
Stalks	ND[d]	501.18 (10.33)	10.22 (0.38)	49.04 (2.55)	12.45 (0.69)
Cattle manure	ND	84.25 (2.80)	2.15 (0.15)	39.19 (0.22)	82.04 (2.47)
Grass clippings	ND	438.77 (11.1)	42.23 (1.39)	10.39 (0.35)	14.36 (0.51)
Soil	4.96 (0.86)	62.13 (3.36)	2.51 (0.24)	24.75 (1.36)	40.68 (0.41)
Sawdust	ND	45.66 (2.23)	0.39 (0.02)	117.08 (1.36)	10.33 (0.22)

Means are shown with standard deviation in parentheses

[a] Total organic carbon

[b] Total Kjeldahl nitrogen

[c] Total organic carbon to total Kjeldahl nitrogen

[d] ND Sample not determined

Analysis of bacterial laccase activity

Phenol oxidase activity was analyzed according to the method described by Lu et al. (2014) using L-dihydroxyphenylalanine (L-DOPA) as substrate. Total 5 g compost samples were shaken with 95 mL of sterile 0.9 % NaCl solution at 25 °C for 1 h. The resulting suspension was used for measuring the phenol oxidase activity. Each reaction contained 50 μL of compost suspension, 150 μL of citrate phosphate buffer (pH 8.0, 50 mM), and 50 μL of substrate (25 mM). The mixtures were centrifuged at 3500 rpm for 4 min and then the supernatant was transferred to a 96-well microplate. Negative control samples were autoclaved beforehand and prepared in the same way. The absorbance was measured at 475 nm by a Multiskan Spectrum spectrophotometer (Thermo, Vantaa, Finland). The enzymatic activity was calculated with extinction coefficient of 3600 M^{-1} cm^{-1} (Sinsabaugh 2010). Enzyme activity was defined as micromoles per hour per gram dry matter.

Genomic DNA extraction, PCR, cloning, and sequencing

Total genomic DNA was extracted in triplicate with a FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) from approximately 0.5 g of freeze-dried compost samples according to the instructions. The DNA extracts were pooled for each sample to reduce sample variability for the following research.

Fragments of the bacterial LMCO genes were amplified by the degenerate primer pair Cu1AF/Cu2R (Ausec et al. 2011a). PCR mixtures contained 25 μL of 2× Power Taq PCR MasterMix (Bioteke, Beijing, China), 1 μL of each primer (25 μM), 10 ng of the DNA extracts in a total volume of 50 μL. PCR amplification was run on a MyCycler (Bio-Rad, Hercules, CA, USA) with an initial cycle of denaturation (5 min at 94 °C) followed by 35 cycles (45 s at 94 °C, 30 s at 58 °C, and 80 s at 72 °C) and a final elongation step (7 min at 72 °C). The PCR products were cloned into pGEM®-T Easy Vector System I (Promega, Madison, USA) after gel purification with TIANgel Midi Purification Kit (TianGen, Beijing, China) as described by the manufacturers. A total of 240 clones obtained from PCR products of compost DNA extracts were selected from the plates (six samples × 40 clones). Finally, the nucleotide sequences obtained were deposited in the European Molecular Biology Laboratory (EMBL, http://www.ebi.ac.uk/) with an accession number of LN559483-LN559616.

Real-time quantitative PCR

Real-time PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, Hercules, CA, USA) to determine the abundance of bacterial LMCO genes in the samples. The 25 μL qPCR mix contained 2 ng of compost microbial DNA, 0.5 μL of each primer (25 μM, Cu1AF/Cu2R), 10 μL of 2.5× RealMasterMix (TianGen, Beijing, China), and 1.5 μL of 20× SYBR solution (TianGen, Beijing, China). The protocol was as follows: 2 min at 94 °C; 40 cycles consisting of 10 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C; followed by a final extension of 7 min at 72 °C; end at 4 °C. The fluorescent signal was measured at the end of each extension step. A negative control without the template DNA was included in every qPCR assay. The standard curve of bacterial LMCO genes was linear over six orders of magnitude from 1.0×10^3 to 1.0×10^8 copies. The presence of PCR inhibitors in DNA extracted from composting samples was examined by diluting DNA extract and mixing a known amount of standard DNA to a DNA extract before qPCR (Kramer et al. 2009). In none of the cases, inhibition was detected.

Phylogenetic and statistical analyses

The operational taxonomic units (OTUs)-based analyses (the Shannon-Wiener index (H), Chao1 and ACE richness estimators) were performed using the Mothur program (http://www.mothur.org/) (Schloss et al. 2009). The representative sequences based on 10 % cutoff, and sequences with high identity were retrieved from National Center for

Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) for phylogenetic analysis. A neighbor-joining (NJ) tree was constructed using the Kimura two-parameter model as implemented in MEGA 5 program (Tamura et al. 2011). Branch support was assessed using 1000 bootstrap replicates. Three replicates were performed on each sample day for assaying physicochemical parameters, humic-like fractions, phenol oxidase activity, and bacterial LMCO gene abundance. The mean values of three replicates were compared by one-way analysis of variance (ANOVA) using post hoc Tukey's test to assess the significant differences ($P<0.05$). All univariate data were analyzed using the software package SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA).

The correlation between the distributions of bacterial LMCO communities and environmental factors during composting was assayed by redundancy analysis (RDA) using CANOCO software V4.5 (Biometris, Wageningen, The Netherlands) (Lepš and Šmilauer 2003). Forward selections were used to find out the parameters that had significant influences on the microbial community compositions. Moreover, to differentiate the sole influence of each significant parameter, partial RDA was performed following the forward selection. Additionally, a Pearson correlation analysis (significance level [$\alpha=0.05$]) was used to test univariate correlations between the bacterial LMCO gene diversity, abundance, and environmental factors by SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA).

Results

Physicochemical parameters and humic-like substances

The pile temperature higher than 50 °C was kept for at least 8 days, in which the pathogens from original materials were thoroughly disinfected. The six representative samples from the mesophilic stage (day 0), the thermophilic stage (day 4), the earlier cooling stage (days 8 and 15), and the maturation stage (days 30 and 50) were selected for the following experiments. The physicochemical properties of composting samples used in this study were shown in Table 2.

The pH value increased mainly due to the mineralization of organic nitrogen and release of ammonium. The WSC that represents the most easily biodegradable carbon had slightly increased firstly and then decreased rapidly in the maturation phase of composting. Transformation of humic substances in the compost could be assessed by the humification index (HI), which increased rapidly in our study. Jouraiphy et al. (2005) attributed such an increase to the formation of humic substances resulting from polymerization of simple molecules.

Phenol oxidase activity and abundance of bacterial laccase-like copper oxidases genes during composting

Generally, the total bacterial LMCO activity was always at a high level in the composting process. The L-DOPA-oxidizing activity showed a rapid increase in enzyme activity in the first 4 days and reached a peak on the fourth day (4.26 μmol h^{-1} g^{-1}), followed by a slight decrease in the maturation phase of composting. The changing trend of bacterial LMCO gene abundance is according with that of phenol oxidase activity during composting. Bacterial LMCO gene copy numbers increased rapidly in the thermophilic phase, which might be ascribed to the high substrate availability in the pile and the thermostability of the bacterial laccase-producing species (Drenovsky et al. 2004). During the whole composting, a significant relationship between bacterial LMCO gene abundance and laccase activity was observed ($R^2=0.895$, $P=0.004$), suggesting a potential link between bacterial LMCO community and phenol activities (Fig. 1).

Distribution of laccase OTU types and phylogenetic analysis

Six small gene libraries were constructed from three replicates of composting samples D0, D4, D8, D15, D30, and D50, and 40 clones of each were analyzed. A total of 185 laccase gene sequences were retrieved from the six selected composting samples, the 51 loss being due to nontarget fragments, another four due to repetition. Based on the 10 % cutoff, the obtained sequences of bacterial LMCO genes were assigned to 59 OTUs. The representative sequences of 59 OTUs from this study and the ones from NCBI resulted in an unrooted NJ tree of 102 gene sequences (Fig. 2). As suggested by Kellner et al. (2008), a cysteine residue (position 25 in their amino acid sequence alignment) is characteristic of fungal laccases. The cysteine residue was absent from sequences obtained in this study, indicating these sequences were indeed genes from bacterial origin. The aligned sequences were assigned to 14 different lineages: *Proteobacteria* clades (clades I~VIII and X~XII), *Actinobacteria*/*Firmicutes* clade IX, *Chlamydiae* clade XIII, and *Bacteroidetes* clade XV. As within the clade IX, these sequences were assigned to either of these two phyla, indicating that laccase genes of *Actinobacteria* and *Firmicutes* are not well separated. A laccase-like sequence of *Phenylobacterium zucineum* (*α-proteobacteria*) was clustered in clade VII with those of *Burkholderiales* (*ß-proteobacteria*), thus OTU14, OTU15, OTU23, OTU24, OTU26, OTU29, and OTU32 in clade VII were uncertainly defined as of *α-proteobacteria/ß-proteobacteria* origin.

Total 11 distinct proteobacterial clades were identified, each with referenced sequences from a different order of

Table 2 Physicochemical parameters of the samples selected

Sampling days	T[a]	pH	C/N[b]	Moisture (%)	WSC[c] (mg L^{-1})	HA[d]	FA[e]	HI[f]
0	35.6	6.85	29.5	58.8	170.2	35.7	55.2	0.65
4	64.5	6.32	25.9	53.6	242.5	39.6	46.8	0.85
8	52.3	7.28	22.0	52.2	150.3	45.2	40.6	1.11
15	32.8	7.85	18.8	48.5	76.2	60.8	35.2	1.73
30	32.3	7.77	16.6	49.8	68.2	72.5	30.6	2.37
50	31.9	7.80	14.9	48.9	62.2	76.5	28.2	2.71

[a] Temperature

[b] Total organic carbon to total Kjeldahl nitrogen

[c] Water-soluble carbon

[d] Humic acid-like carbon

[e] Fulvic acid-like carbon

[f] Humification index

Proteobacteria. Nucleotide sequences within these groups were >70 % identical to the reference sequences from the databases, which mostly resembled like genera such as *Rhizobium*, *Sinorhizobium*, *Caulobacter*, and *Tistrella* (*α-proteobacteria*), *Phenylobacterium*, and *Burkholderia* (*ß-proteobacteria*), *Pseudomonas*, *Escherichia*, *Xanthomonas*, and *Stenotrophomonas* (*γ-proteobacteria*), *Sorangium* (*δ-proteobacteria*). Sequences referred to *Enterobacteriales* (clade I) were only present in samples D0 and D4 (Fig. 2), suggesting most of the pathogens from the source materials had been killed by the heat generated in the thermophilic phase. The sequences of actinobacterial-associated group (clade XI) mainly resembled *Thermobacillus composti*, *Streptosporangium roseum*, *Kytococcus sedentarius*, and *Micrococcus luteus*, suggesting the high resistance to high temperature and alkaline condition of this group.

Temporal variation of bacterial laccase-like genes during composting

The temporal variations in bacterial laccase-like genes were analyzed. The total 185 sequences were distributed in the libraries as follows: D0 (32 sequences), D4 (35 sequences), D8 (33 sequences), D15 (31 sequences), D30 (27 sequences), and D50 (27 sequences). Shannon-Wiener indexes (*H*), NP-Shannon index, Chao1 and ACE richness estimators, and Simpson index of the each clone library were listed in Table 3. The Shannon diversity indices, including the individual sequence types and OTU numbers at each sample, varied basically consistently with the richness of bacterial LMCO gene population. The Shannon diversity of the laccase gene population reached the peak in D4 (*H* value 3.48 at the genetic distance of 0.02), the lowest value in D30 (*H* value 2.99). An obvious increase of LMCO gene richness was observed in the

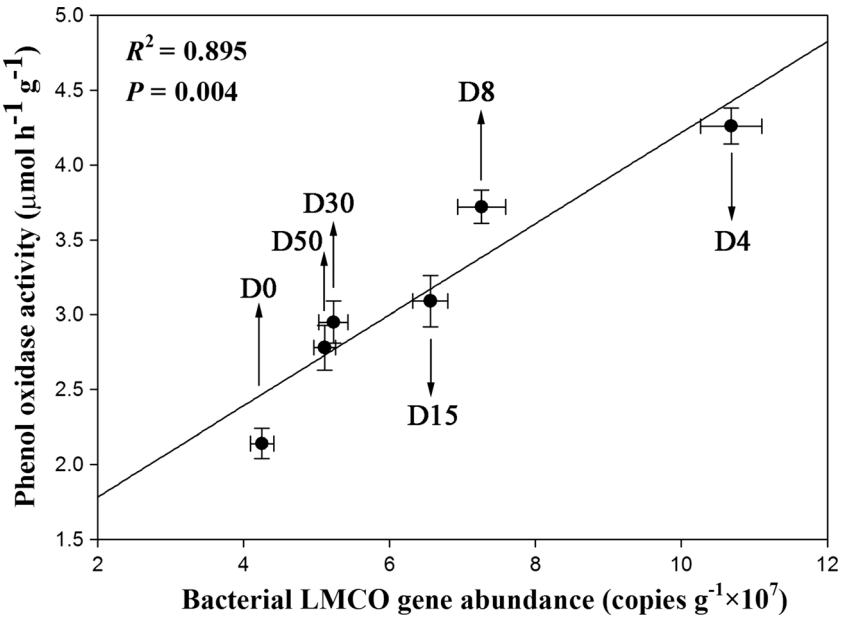
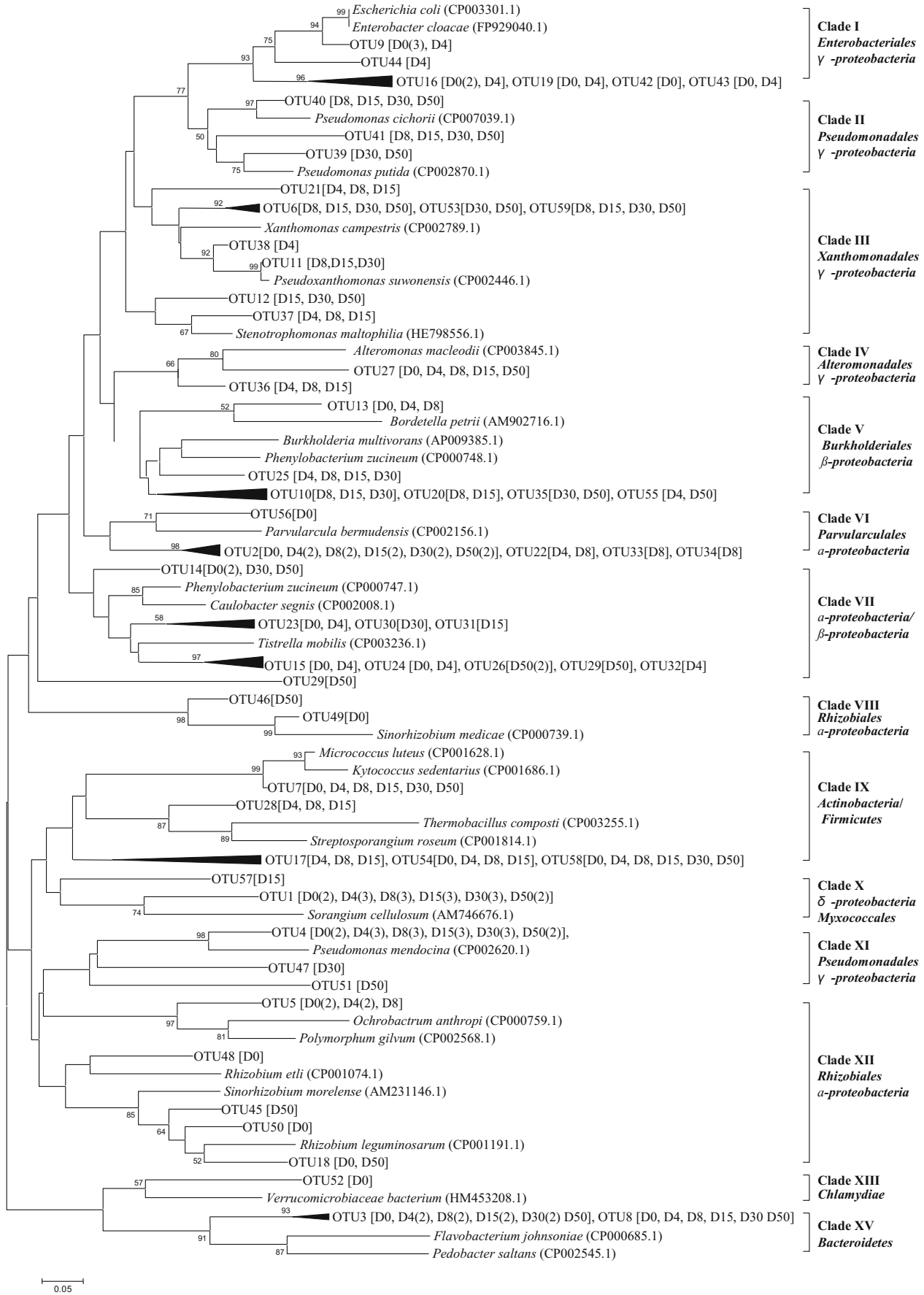


Fig. 1 Linear regression analysis between phenol oxidase activity and bacterial LMCO gene abundance during composting. D0, D4, D8, D15, D30, and D50 indicated the sampling time, and the *R* square (R^2) and probability (*P*) value of the linear regression analysis were shown


Escherichia coli (CP003301.1)
Enterobacter cloacae (FP929040.1)
OTU9 [D0(3), D4]
OTU44 [D4]
OTU16 [D0(2), D4], OTU19 [D0, D4], OTU42 [D0], OTU43 [D0, D4]
Clade I
Enterobacteriales
γ -proteobacteria
OTU40 [D8, D15, D30, D50]
Pseudomonas cichorii (CP007039.1)
OTU41 [D8, D15, D30, D50]
OTU39 [D30, D50]
Pseudomonas putida (CP002870.1)
Clade II
Pseudomonadales
γ -proteobacteria
OTU21[D4, D8, D15]
OTU6[D8, D15, D30, D50], OTU53[D30, D50], OTU59[D8, D15, D30, D50]
Xanthomonas campestris (CP002789.1)
OTU38 [D4]
OTU11 [D8,D15,D30]
Pseudoxanthomonas suwonensis (CP002446.1)
OTU12 [D15, D30, D50]
OTU37 [D4, D8, D15]
Stenotrophomonas maltophilia (HE798556.1)
Clade III
Xanthomonadales
γ -proteobacteria
Alteromonas macleodii (CP003845.1)
OTU27 [D0, D4, D8, D15, D50]
OTU36 [D4, D8, D15]
Clade IV
Alteromonadales
γ -proteobacteria
OTU13 [D0, D4, D8]
Bordetella petrii (AM902716.1)
Burkholderia multivorans (AP009385.1)
Phenylobacterium zucineum (CP000748.1)
OTU25 [D4, D8, D15, D30]
OTU10[D8, D15, D30], OTU20[D8, D15], OTU35[D30, D50], OTU55 [D4, D50]
Clade V
Burkholderiales
β-proteobacteria
OTU56[D0]
Parvularcula bermudensis (CP002156.1)
OTU2[D0, D4(2), D8(2), D15(2), D30(2), D50(2)], OTU22[D4, D8], OTU33[D8], OTU34[D8]
Clade VI
Parvularculales
a-proteobacteria
OTU14[D0(2), D30, D50]
Phenylobacterium zucineum (CP000747.1)
Caulobacter segnis (CP002008.1)
OTU23[D0, D4], OTU30[D30], OTU31[D15]
Tistrella mobilis (CP003236.1)
OTU15 [D0, D4], OTU24 [D0, D4], OTU26[D50(2)], OTU29[D50], OTU32[D4]
OTU29[D50]
Clade VII
a-proteobacteria/
β-proteobacteria
OTU46[D50]
OTU49[D0]
Sinorhizobium medicae (CP000739.1)
Clade VIII
Rhizobiales
a-proteobacteria
Micrococcus luteus (CP001628.1)
Kytococcus sedentarius (CP001686.1)
OTU7[D0, D4, D8, D15, D30, D50]
OTU28[D4, D8, D15]
Thermobacillus composti (CP003255.1)
Streptosporangium roseum (CP001814.1)
OTU17[D4, D8, D15], OTU54[D0, D4, D8, D15], OTU58[D0, D4, D8, D15, D30, D50]
Clade IX
Actinobacteria/
Firmicutes
OTU57[D15]
OTU1 [D0(2), D4(3), D8(3), D15(3), D30(3), D50(2)]
Sorangium cellulosum (AM746676.1)
Clade X
δ -proteobacteria
Myxococcales
OTU4 [D0(2), D4(3), D8(3), D15(3), D30(3), D50(2)],
Pseudomonas mendocina (CP002620.1)
OTU47 [D30]
OTU51 [D50]
Clade XI
Pseudomonadales
γ -proteobacteria
OTU5 [D0(2), D4(2), D8]
Ochrobactrum anthropi (CP000759.1)
Polymorphum gilvum (CP002568.1)
OTU48 [D0]
Rhizobium etli (CP001074.1)
Sinorhizobium morelense (AM231146.1)
OTU45 [D50]
OTU50 [D0]
Rhizobium leguminosarum (CP001191.1)
OTU18 [D0, D50]
Clade XII
Rhizobiales
a-proteobacteria
OTU52 [D0]
Verrucomicrobiaceae bacterium (HM453208.1)
Clade XIII
Chlamydiae
OTU3 [D0, D4(2), D8(2), D15(2), D30(2) D50], OTU8 [D0, D4, D8, D15, D30 D50]
Flavobacterium johnsoniae (CP000685.1)
Pedobacter saltans (CP002545.1)
Clade XV
Bacteroidetes
0.05

◀ **Fig. 2** Neighbor-joining tree of representative bacterial LMCO gene fragments obtained from composting samples together with the reference sequences retrieved from GenBank. Branch support was assessed using 1000 bootstrap replicates. The occurrence times of each obtained laccase OTU is given in brackets, if >1. D0(3), for example, indicating the OTU appear for three times in the sample on day 0

initial 4 days, which was followed by a steady decrease in the following days.

The distribution profiles of laccase genes suggested that 138 (74.59), 17 (9.19 %), 29 (15.68 %), and 1 (0.54 %) of the 185 laccase genes were clustered in *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*/*Firmicutes*, and *Chlamydiae*, respectively. The dominance structure of LMCO genes in composting samples was also analyzed in this research. Of the 59 different OTUs obtained, 12 OTUs were detected at least four times in all samples, which were considered as dominant in this study. Especially sequences resembled laccase-like genes of *Myxococcales* (clade X, *δ-proteobacteria*), *Xanthomonadales* (clade III, *γ-proteobacteria*), *Actinobacteria/Firmicutes* (clade IX), *Burkholderiales* (clade V, *ß-proteobacteria*), and clade VII (*α/ß-proteobacteria*) were present in all six libraries, indicating the potential important role of those groups in organic compound decomposition in the whole process of composting. Among them, 12 sequences (6.49 %) affiliated with *Sorangium cellulosum* in *δ-proteobacteria* were only classified to OTU1 and found in all six samples (clade X). OTU7, OTU54, and OTU58 were related to sequences in *Actinobacteria*/*Firmicutes* (clade IX) and found in all six samples. Thus, proteobacterial- and actinobacterial-associated species were considered as the dominant communities during composting.

Environmental factors shaping the bacterial laccase-like multicopper oxidases microorganisms during composting

The linear relationships between different environmental factors and bacterial LMCO gene OTU numbers, abundance, Shannon index, and Chao1 index were characterized by the Pearson correlation coefficient, as shown in Table 4. Interestingly, both the pile temperature and WSC content were significantly positively correlated with bacterial LMCO gene OTU numbers, abundance, Shannon index, and Chao1 index ($P<0.05$), indicating that those two factors are the most two important ones influencing the bacterial LMCO gene diversity and richness. The laccase-producing bacteria grew rapidly when the concentration of easily available WSC was high in the warming period of composting. The pH value was negatively correlated with all the variations of bacterial LMCO genes. Additionally, the HA content was significantly negatively correlated with the Shannon index ($P<0.05$).

To identify the potential relationship between the community structure of bacterial LMCO communities and environmental factors during composting, RDA was conducted based on the bacterial LMCO gene community structures and the variation of the environmental factors. The first two canonical

Table 3 OTU-based analyses of NP-Shannon, Shannon diversity index (*H*), Simpson index, and richness estimators (Chao1 and ACE) of bacterial LMCO genes from each composting sample

Days	No. of gene sequences	Distance	OTU	NP-Shannon	*H*	Simpson	Chao1	ACE
D0	32	0.01	30	5.49	3.38	0.004	156 (72–412)	240 (43–3305)
		0.02	29	5.07	3.34	0.006	110 (56–270)	154 (44–1102)
		0.10	25	4.14	3.15	0.016	54 (34–113)	62 (40–112)
D4	35	0.02	33	5.68	3.48	0.003	188 (85–494)	289 (48–4306)
		0.03	32	5.36	3.42	0.007	249 (102–704)	330 (107–1211)
		0.10	27	4.19	3.21	0.017	69 (41–155)	75 (43–170)
D8	33	0.01	31	5.56	3.41	0.004	166 (76–439)	256 (45–3620)
		0.03	28	4.61	3.27	0.009	97 (51–237)	117 (56–316)
		0.10	26	4.25	3.14	0.021	103 (50–273)	119 (55–328)
D15	31	0.01	28	5.10	3.28	0.009	191 (79–544)	253 (84–934)
		0.03	26	4.48	3.19	0.013	84 (45–205)	101 (49–273)
		0.08	23	3.92	3.03	0.024	74 (38–197)	68 (37–167)
D30	27	0.01	27	4.69	3.09	0.004	114 (62–259)	153 (52–776)
		0.03	25	4.14	3.01	0.006	109 (52–293)	169 (36–1978)
		0.09	19	3.60	2.83	0.031	49 (27–131)	46 (27–115)
D50	27	0.01	27	4.76	3.22	0.003	85 (50–183)	111 (50–367)
		0.03	26	4.24	3.12	0.008	176 (73–507)	351 (36–10787)
		0.07	22	3.74	2.83	0.014	45 (29–95)	41 (36–48)

The numbers in brackets represent 95 % confidence intervals of the indices

Table 4 Correlation analyses of environmental factors and bacterial LMCO gene OTU numbers, relative abundance, Shannon index, and Chao1 estimator

Environmental factors	Pearson correlation coefficient						
	Temperature	pH	C/N[a]	WSC[b]	Moisture	HA[c]	FA[d]
No. of OTUs[e]	0.926*	−0.879*	0.668	0.927*	0.021	−0.836	0.658
Gene abundance	0.945*	−0.848	0.599	0.916*	−0.088	−0.603	0.481
Shannon index	0.901*	−0.912*	0.782	0.923*	0.247	−0.944*	0.802
Chao1	0.886*	−0.82	0.548	0.883*	−0.044	−0.722	0.501

*P=0.05, significant level

[a] Total organic carbon to total Kjeldahl nitrogen

[b] Water-soluble carbon

[c] Humic acid-like carbon

[d] Fulvic acid-like carbon

[e] Operational taxonomic units

axes explained 43.2 and 28.2 % of the variation in bacterial LMCO species data (Fig. 3). Of all the environmental factors investigated, the FA content appeared to make the most significant influence on the community distributions of bacterial LMCO genes during composting (P=0.005), following by C/N ratio, moisture, and HA content. FA content, C/N ratio, moisture, and HA content solely explained 10.9, 9.7, 8.3, and 7.9 % of the variation in the bacterial LMCO gene community, respectively. The RDA model statistically explained up to 39.6 % of the variation (P<0.05) in the bacterial LMCO gene composition (Table 5). The variation shared by FA content, C/N ratio, moisture, and HA content was 7.5 %. The pH, WSC content, and temperature also contributed to the bacterial LMCO gene composition-environment relationship during composting, although the significance level was lower (P<0.10). The distributions of bacterial LMCO genes in D0, D4, and D15 were positively correlated with biodegradable WSC content and pile temperature, and distributions in the maturation phase (D30 and D50) corresponded to the formation of HA.

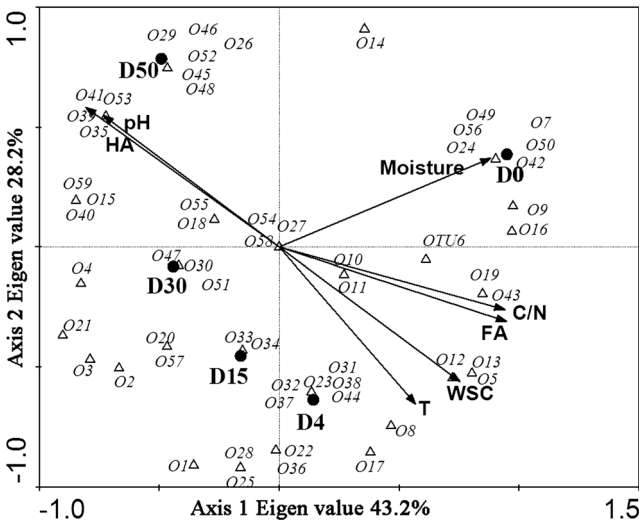


Fig. 3 Redundancy analysis of bacterial LMCO gene composition and environmental factors during composting. Environmental factors are indicated by a *continuous line*; Numbers refer to sampling days by *solid circles*; The OTUs were represented by *open triangles*

Discussion

Very few studies have focus on the bacterial LMCO genes in agricultural waste composting ecosystem. This study is the first investigation and evaluation of the diversity, population compositions, and impact factors on bacterial LMCO community distribution during agricultural waste composting. Bacterial LMCO genes were detected in 14 clades representing a wide phylogenetic range. This broad distribution of LMCO genes among prokaryotes might be nuanced because some bacteria species have at least two different LMCO genes in their genome. Interestingly, sequences with known bacterial LMCO genes retrieved from totally sequenced genomes of *Phenylobacterium zucineum* (from plasmid and chromosome, respectively) were absolutely different and formed several mixed clusters (clades V and VII). This possibility is also supported by the relatively high genetic distance found between the two LMCO gene types in *Sinorhizobium morelense* (Kellner et al. 2008). Therefore, distinct clades could reflect phylogenetic distance between different bacteria with LMCO genes or genetic distance between distinct genes within one species with isoenzymes (Kellner et al. 2008). Horizontal gene transfer possibly could give some explanations as to why laccases from the same organism can be so diverse (Ausec et al. 2011b).

The lignin degradation ability of bacteria is, at present, not well understood compared with the fungi, but the observations provide important clues that bacteria also contain similar types of extracellular lignin-degrading laccase enzymes, and laccase-producing bacterial microorganisms were potentially involved in lignocellulose breakdown during agricultural

Table 5 Partial redundancy analysis results of the influence of each significant parameter on the bacterial LMCO gene community

Parameters included in the model	Eigen value	Variation explained solely (%)	*P* value	Pseudo-*F* value
FA[a]	0.098	9.8	0.005	3.22
C/N[b]	0.083	8.3	0.012	2.94
Moisture	0.074	7.4	0.036	2.78
HA[c]	0.066	6.6	0.048	2.75
All the above parameters together	0.396	39.6	0.024	2.64

Partial RDAs were based on the Monte Carlo test (n=499), kept only the significant factors in the models. For each partial model, the other significant factors were used as covariables

[a] Fulvic acid-like carbon

[b] Total organic carbon to total Kjeldahl nitrogen

[c] Humic acid-like carbon

waste composting. Bacterial laccases could offer advantages over their fungal counterparts, such as stability at higher temperatures or at an alkaline pH, which will make them keep activity under the extreme conditions of composting environments (Ausec et al. 2011b; Kirby 2006). It was described that the bacterial strains identified to have lignin degradation capacity mainly divide into three classes: *Actinobacteria*, *α-proteobacteria*, and *γ-proteobacteria* (Bugg et al. 2011). In the present study, 15.68 % of the 185 laccase genes (results not shown) were clustered in *Actinobacteria/Firmicutes*, which is consistent with the results of Xiao et al. (2011b) that a diverse actinomycetal community was detected in the continuous thermophilic composting. Sequences classified to OTU7 were found to be affiliated with *Micrococcus* spp. (*Actinobacteria/Firmicutes*) in the clade IX. Sequences in clades II, III, and XI appeared to be affiliated with *Pseudomonas* spp. in *Pseudomonadales* and *Stenotrophomonas* in *Xanthomonadales*. Extracellular laccases from *Stenotrophomonas* and *Pseudomonas* spp. were extensively studied for their significance in degrading various industrial dyes (Galai et al. 2009; Kalme et al. 2009; Kalyani et al. 2008). Additionally, Ryan et al. (2009) have summarized that *S. maltophilia* have an outstanding range of activities, including the breakdown of natural and artificial pollutants that are vital to bioremediation. Polyphenol oxidase from *S. meliloti* also showed strong in vitro enzyme activity and great alkaline and thermal stability (Rosconi et al. 2005). Due to their stability at an elevated temperature, enzyme reactions are faster and less susceptible in composting environment, suggesting a potential vital role of bacterial LMCO community in decomposing lignocelluloses.

The temperature and the WSC were significantly positively correlated with bacterial LMCO gene OTU numbers, abundance, Shannon index, and Chao1 index in Pearson correlation analysis. The reason for this phenomenon might be that the changing trends of temperature, the WSC content were consistent with the bacterial LMCO gene abundance, diversity, and richness. The results obtained in this study were basically in agreement with the obvious findings. Cahyani et al. (2003) indicated that pile temperature and substrates available to bacteria were considered as the main factors determining the bacterial community. Zhang et al. (2011) commented that WSC showed a predominant effect on the bacterial community composition during composting. The reason for the significant effect of WSC might be that the biodegradable WSC can rapidly promote microbial activity and then alter microbial communities by selecting for the most competitive populations during decomposition (Said-Pullicino et al. 2007). The abundance of bacterial LMCO genes reached the peak on day 4 when the temperature were the highest whereas the pH value was the lowest, which indicated the appropriate high temperature was favorable for the propagation of the bacterial LMCO community.

RDA ordination clearly indicated three phases: the mesophilic stage (D0), the thermophilic and the earlier cooling stage (D4 and D15), and the maturation stage (D30 and D50). Generally, in this study, the HA content appeared to be the most significant factor on the bacterial LMCO gene distributions among these chosen factors in multivariate analysis. The other significant factors were C/N ratio, moisture content, and FA content. As suggested by Caricasole et al. (2010), the significance of HA content may be because the degraded lignin fragments are building units for humic substances, and the mineralization of lignin by compost microorganisms could probably provide an evaluation of the humification process in compost (Caricasole et al. 2011). Kögel-Knabner (2002) also explained that lignin undergoes a gradual oxidative transformation process during biodegradation, thus introducing carboxyl groups into the molecule, which is found in the HA fraction extractable by NaOH. This might be indications that the bacterial LMCO community played a vital important role in the lignocellulose degradation during composting.

The discrepancy of the two-analysis method was that the temperature was not the significant factor in the multivariate analysis. This is probably due to the multicollinearity interactions between environmental factors, and it is difficult to

distinguish between the separate effects of these factors by studying each of them as an independent variable (Zhang et al. 2011). Thus, the method of RDA was more appropriate in identifying the most significant factors that influence the distribution of bacterial LMCO gene compositions among large sets of environmental factors. In conclusion, the HA substances had the most significant effect on the distribution of the bacterial LMCO genes during composting, whereas pile temperature and WSC content were significantly positively correlated with bacterial LMCO gene OTU numbers, abundance, Shannon index, and Chao1. The environmental factors were shaping the distributions and abundance of bacterial LMCO community in the composting process.

In summary, the present study demonstrates that direct amplification of laccase gene sequences from compost DNA extracts allows tracing the distribution and diversity of bacterial specific groups potentially involved in lignocellulose degradation. The results showed proteobacterial- and actinobacterial-associated species were the dominant communities in the composting system. This research also gives some clues of identifying the significant environmental factors determining the abundance and distribution of bacterial LMCO genes during composting. However, the responses of environmental conditions to bacterial LMCO gene distributions and the ecological role of bacterial LMCO community still remain poorly understood. The challenge at this point is to strengthen investigations on detection of clearly verifiable extracellular laccases (e.g., by screening of ecologically relevant bacteria for all potential laccases and linking their genetic potential to produce laccase exoenzymes under laboratory and natural conditions), with a combined analysis of gene expression and protein synthesis in order to clear which genes correspond to which functions (Bugg et al. 2011; Theuerl and Buscot 2010), and next-generation sequencing to obtain high-throughput sequencing results for more comprehensive analyses of bacterial LMCO diversity (Luo et al. 2015).

Acknowledgments The authors thank the editors and reviewers for very helpful comments and suggestions. This study was financially supported by the National Natural Science Foundation of China (50908079, 50978088 and 51039001), the Hunan Provincial Natural Science Foundation of China (10JJ7005), and the Hunan Provincial Innovation Foundation for Postgraduate (CX2012B137).

Conflict of interest The authors declare that they have no competing interests.

References

Ahmad M, Taylor CR, Pink D, Burton K, Eastwood D, Bending GD, Bugg TDH (2010) Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Mol Biosyst 6(5):815–821

Ausec L, van Elsas JD, Mandic-Mulec I (2011a) Two- and three-domain bacterial laccase-like genes are present in drained peat soils. Soil Biol Biochem 43(5):975–983

Ausec L, Zakrzewski M, Goesmann A, Schluter A, Mandic-Mulec I (2011b) Bioinformatic analysis reveals high diversity of bacterial genes for laccase-like enzymes. PLoS ONE 6(10), e25724

Belyaeva ON, Haynes RJ (2009) Chemical, microbial and physical properties of manufactured soils produced by co-composting municipal green waste with coal fly ash. Biores Technol 100(21):5203–5209

Bugg TDH, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22(3):394–400

Cahyani VR, Matsuya K, Asakawa S, Kimura M (2003) Succession and phylogenetic composition of bacterial communities responsible for the composting process of rice straw estimated by PCR-DGGE analysis. Soil Sci Plant Nutr 49(4):619–630

Caricasole P, Provenzano MR, Hatcher PG, Senesi N (2010) Chemical characteristics of dissolved organic matter during composting of different organic wastes assessed by C^{13} CPMAS NMR spectroscopy. Bioresour Technol 101(21):8232–8236

Caricasole P, Provenzano MR, Hatcher PG, Senesi N (2011) Evolution of organic matter during composting of different organic wastes assessed by CPMAS C^{13} NMR spectroscopy. Waste Manag 31(3): 411–415

Couto SR, Herrera JLT (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24(5):500–513

Drenovsky RE, Vo D, Graham KJ, Scow KM (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb Ecol 48(3):424–430

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J 4(3):337–345

Freedman Z, Zak DR (2014) Atmospheric N deposition increases bacterial laccase-like multicopper oxidases: implications for organic matter decay. Appl Environ Microbiol 80(14):4460–4468

Galai S, Limam F, Marzouki MN (2009) A new *Stenotrophomonas maltophilia* strain producing laccase use in decolorization of synthetic dyes. Appl Biochem Biotechnol 158(2):416–431

Huang DL, Zeng GM, Feng CL, Hu S, Lai C, Zhao MH, Su FF, Tang L, Liu HL (2010) Changes of microbial population structure related to lignin degradation during lignocellulosic waste composting. Bioresour Technol 101(11):4062–4067

Jaouani A, Guillén F, Penninckx MJ, Martínez AT, Martínez MJ (2005) Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzym Microb Tech 36(4):478–486

Jarosz-Wilkołazka A, Ruzgas T, Gorton L (2005) Amperometric detection of mono- and diphenols at *Cerrena unicolor* laccase-modified graphite electrode: correlation between sensitivity and substrate structure. Talanta 66(5):1219–1224

Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol 66(2):524–528

Jouraiphy A, Amir S, El Gharous M, Revel JC, Hafidi M (2005) Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. Int Biodeter Biodegrad 56(2):101–108

Kalme S, Jadhav S, Jadhav M, Govindwar S (2009) Textile dye degrading laccase from *Pseudomonas desmolyticum* NCIM 2112. Enzym Microb Tech 44(2):65–71

Kalyani DC, Patil PS, Jadhav JP, Govindwar SP (2008) Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas* sp SUK1. Bioresour Technol 99(11):4635–4641

Kellner H, Luis P, Zimdars B, Kiesel B, Buscot F (2008) Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. Soil Biol Biochem 40(3):638–648

Kellner H, Luis P, Schlitt B, Buscot F (2009) Temporal changes in diversity and expression patterns of fungal laccase genes within the organic horizon of a brown forest soil. Soil Biol Biochem 41(7):1380–1389

Kirby R (2006) Actinomycetes and lignin degradation. Adv Appl Microbiol 58:125–168

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34(2):139–162

Kramer M, Obermajer N, Matijašić BB, Rogelj I, Kmetec V (2009) Quantification of live and dead probiotic bacteria in lyophilised product by real-time PCR and by flow cytometry. Appl Microbiol Biotechnol 84(6):1137–1147

Lepš J, Šmilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge

Lopez MJ, Vargas-Garcia MDC, Suarez-Estrella F, Moreno J (2006) Biodelignification and humification of horticultural plant residues by fungi. Int Biodeter Biodegrad 57(1):24–30

Lu LH, Zeng GM, Fan CZ, Ren XJ, Wang C, Zhao QR, Zhang JC, Chen M, Chen AW, Jiang M (2013) Characterization of a laccase-like multicopper oxidase from newly isolated *Streptomyces* sp. C1 in agricultural waste compost and enzymatic decolorization of azo dyes. Biochem Eng J 72:70–76

Lu LH, Zeng GM, Fan CZ, Zhang JC, Chen AW, Chen M, Jiang M, Yuan YJ, Wu HP, Lai MY, He YB (2014) Diversity of two-domain laccase-like multicopper oxidase genes in *Streptomyces* spp.: identification of genes potentially involved in extracellular activities and lignocellulose degradation during composting of agricultural waste. Appl Environ Microbiol 80(11):3305–3314

Luo L, Zhou Z-C, Gu J-D (2015) Distribution, diversity and abundance of bacterial laccase-like genes in different particle size fractions of sediments in a subtropical mangrove ecosystem. Ecotoxicology. doi:10.1007/s10646-015-1452-6

Majeau JA, Brar SK, Tyagi RD (2010) Laccases for removal of recalcitrant and emerging pollutants. Bioresour Technol 101(7):2331–2350

Miyazaki K (2005) A hyperthermophilic laccase from *Thermus thermophilus* HB27. Extremophiles 9(6):415–425

Peralta-Zamora P, Pereira CM, Tiburtius ER, Moraes SG, Rosa MA, Minussi RC, Durán N (2003) Decolorization of reactive dyes by immobilized laccase. Appl Catal B Environ 42(2):131–144

Rosconi F, Fraguas LF, Martinez-Drets G, Castro-Sowinski S (2005) Purification and characterization of a periplasmic laccase produced by *Sinorhizobium meliloti*. Enzym Microb Technol 36(5–6):800–807

Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat Rev Microbiol 7(7):514–525

Said-Pullicino D, Kaiser K, Guggenberger G, Gigliotti G (2007) Changes in the chemical composition of water-extractable organic matter during composting: distribution between stable and labile organic matter pools. Chemosphere 66(11):2166–2176

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537–7541

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42(3):391–404

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Theuerl S, Buscot F (2010) Laccases: toward disentangling their diversity and functions in relation to soil organic matter cycling. Biol Fertil Soils 46(3):215–225

Van der Gucht K, Cottenie K, Muylaert K, Vloemans N, Cousin S, Declerck S, Jeppesen E, Conde-Porcuna JM, Schwenk K, Zwart G, Degans H, Vyverman W, De Meester L (2007) The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc Natl Acad Sci U S A 104(51):20404–20409

Xiao Y, Zeng GM, Yang ZH, Ma YH, Huang C, Shi WJ, Xu ZY, Huang J, Fan CZ (2011a) Effects of continuous thermophilic composting (CTC) on bacterial community in the active composting process. Microb Ecol 62(3):599–608

Xiao Y, Zeng GM, Yang ZH, Ma YH, Huang C, Xu ZY, Huang J, Fan CZ (2011b) Changes in the actinomycetal communities during continuous thermophilic composting as revealed by denaturing gradient gel electrophoresis and quantitative PCR. Bioresour Technol 102(2):1383–1388

Zeng GM, Zhang JC, Chen YN, Yu Z, Yu M, Li H, Liu ZF, Chen M, Lu LH, Hu CX (2011) Relative contributions of archaea and bacteria to microbial ammonia oxidation differ under different conditions during agricultural waste composting. Bioresour Technol 102(19):9026–9032

Zhang JC, Zeng GM, Chen YN, Yu M, Yu Z, Li H, Yu Y, Huang HL (2011) Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting. Bioresour Technol 102(3):2950–2956

Zhang JC, Zeng GM, Chen YN, Yu M, Huang HL, Fan CZ, Zhu Y, Li H, Liu ZF, Chen M, Jiang M (2013) Impact of *Phanerochaete chrysosporium* inoculation on indigenous bacterial communities during agricultural waste composting. Appl Microbiol Biotechnol 97(7):3159–3169