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Dynamic Changes in Bacterial Communities During Compost and Earthworm Treatment of Low-Grade Potassium Ore

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Both composting and earthworm treatment can degrade potassium rock powders and bacteria play a key role during the bio-processings. To understand the dominant bacteria and bacterial profile in biological conversion of the ore including compost and earthworm treatment, the bacterial communities in the compost were studied by using the method of denaturing gradient gel electrophoresis (DGGE) after ore powder was co-composted with organic wastes and then treated with earthworms. Results showed bacterial community structure changed very quickly during the early stages of solid-state fermentation, but relatively stable in the later stages of fermentation and during earthworm treatment. The dominant species of bacteria largely varied in the earlier stage of composting, but they were stable in the latter stage and during earthworm treatment. Two classes of bacteria, represented by band 12 (likely *Alteromonas*) and band 14 (likely *Firmicutes*) in DGGE profile, were found to be dominant species over the entire solid-state fermentation period. No special dominant bacterial species appeared during earthworm treatment. Phylogenetic studies of the bacteria based on 16S rRNA sequences indicate that major 13 bands came from phyla *Proteobacteria* and *Firmicutes*, suggesting that bacteria in these phyla played an important role during the compost treatment.

Keywords: bacterial communities, compost, earthworm, low-grade potassium ore

Introduction

Soluble potassium resources, as a major source of fertilizer potassium, are very rare in some countries such as China, India and Brazil, but there are plenty of potassium-bearing rocks and sand paticles in the form of silicates in these countries (Basak and Biswas 2009; Sheng and He 2006). These potassium cannot be absorbed directly by crops (Basak and Biswas 2009; Ghiri et al. 2010; Peterburgsky and Yanishevsky 1961; Sugumaran and Janarthanam 2007).

Studies have shown that compost treatment can release potassium from low-grade potassium ore, or convert potassium to bioavailable form, which can increase crop yield (Biswas 2011; Nishanth and Biswas 2008). Compost treatment requires large amounts of cellulosic materials, such as crop stems, rice hulls, and cotton seed hulls. China and India are large agricultural countries where cellulosic resources are abundant. In the past, farmers usually compost or chop these agricultural byproducts into smaller pieces and return them to field.

Because these treatments are time consuming and offer very limited benefits, farmers no longer compost, but directly burn them. This method not only wastes resource, but also pollutes environment. It had been proven that composting technology is efficient in releasing potassium from potassium-bearing rock (Nishanth and Biswas 2008). Thus, if these crop byproducts are composted with low grade potassium ore, it will not only ease the shortage of potassium resources, but also change the waste into valuable organic fertilizer and promote the recycling of resources.

Compost fermentation is a dynamic process in which bacterial community composition changes rapidly and multiple microorganisms work together (Hultman et al. 2008). There have been many researchs on the microbial succession during composting process. For example, Ishii et al. (2000) analyzed the microbial succession during a laboratory-scale composting process of garbage by DGGE and they found that in the mesophilic phase some fermenting bacteria, such as lactobacillus, were present with the existing organic acids;

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Table 1. Mineralogy of the PBR used in this study

Minerals	K-Feldspar	Quartz	Hematite	Montmorillonite	Illite	Hornblende
Percent	70.99	23.45	3.62	1.66	0.15	0.13

in the thermophilic phase thermophilic bacillus appeared and, after the cooling phase, bacterial populations were more complex than in previous phases and the phylogenetic positions of those populations were relatively distant from strains so far in the DNA database.

Peters et al. (2000) characterized the diversity and succession of microbial communities during composting of an organic agricultural substrate using PCR–single-strand-conformation polymorphism-pased genetic profiles of SSU rRNA genes and they found that DNA sequencing of those molecular isolates, isolated from the profiles by PCR reamplification and cloning, showed similarities in the range of 92.3 to 100% to known gram-positive bacteria with a low or high G+C DNA content and to the SSU rDNA of γ-Proteobacteria. Microorganisms interact with their environment in compost to degrade efficiently organic materials, convert organic materials into humus (Tuomela et al. 2000). At the same time, mineral powder inside the compost is also effectively weathered (Nishanth and Biswas 2008). Microorganisms are the most critical, most active factor in compost, among which bacteria are most abundant and most important (Khalil et al. 2001; Wang et al. 2006). Many studies indicated earthworms can accelerate degradation of potassium ore (Carpenter et al. 2007; Liu et al. 2011; Suzuki et al. 2003), its gut microbes may play an important role in increasing rates of mineral weathering (Carpenter et al. 2007; Liu et al. 2011).

Although the succession of microbial communities during composting of organic substrate has been well studied by many researchers, the dynamic changes in bacterial communities during compost and earthworm treatment of low grade potassium ore are unknown. It is essential to monitor the dynamic microbial communities so that material conversion during compost process and earthworms treatment can be discerned. Denaturing gradient gel electrophoresis (DGGE) is one of culture-independent fingerprinting techniques and usually employed to assess the structure of microbial communities in environmental samples without cultivation, and to determine the community dynamics in response to environmental variations (Ercolini 2004). In this study, low grade potassium ore powder was co-composted with organic wastes and then treated with earthworms, and then we characterized the dominant bacterial flora and communities of the compost using DGGE of 16S rRNA gene amplicons in combination with sequence analysis.

Materials and Methods

Materials

The potassium-bearing rock (PBR) was collected from Luoyang, Henan Province, China. The rock was crushed and sieved to pass through 0.074 mm mesh size. The mineral composition and the elemental composition of the rock, shown by Tables 1 and 2, were determined using the K-value method of X-ray diffraction (Rigaku, D/MAx-2200) (Li et al. 2003) and X-ray fluorescence (Axios, PW4400), respectively, in the Institute of Geochemistry, Chinese Academy of Sciences.

The earthworms *Eisenia foetida* and effective microorganisms (EM) preparation were purchased from Henan Academy of Agricultural Sciences and Beijing Baolvyuan Bio-tech Co. Ltd., respectively. The dominant bacteria in the EM preparation are the members of *Bacillus* and *Lactobacillus* according to its product manual. Inosine fermentation waste and waste of oyster mushroom culture were collected from Xinxiang Pharmaceutical Factory of Henan Province and Henan Institute of Science and Technology, respectively.

Methods

Compost and earthworm treatment of PBR powders and sample collection.

Solid-state fermentation. Potassium containing rocks were pulverized to 200 micron. Waste of oyster mushroom culture (24%) was grounded, dried and mixed with rock powder (60%), wheat bran (8%), sugar cane dregs (4%), and inosine fermentation waste (4%). EM preparation (0.1%) was added to a fermentation media to increase compost bacteria and speed up the composting process. Water content was adjusted to 60–65%. After mixing, a sample numbered 1 was collected from the fresh compost. Then, the mixture was piled for fermentation. During this period, the compost pile was turned over every 4 d. Each time when the pile was mixed thoroughly, a sample was gained from the five subsamples, which were collected from 5 locations evenly spaced on the pile. Each subsample was about 20 g, taken from 1–2 feet depth below pile surface. Subsamples were mixed thoroughly to obtain a homogenous sample and stored in an ultralow temperature freezer. A sample was collected every time and a total of 8 samples, numbered 2–9, were collected during fermentation.

Table 2. Element composition of the PBR

Composition	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	LOI	Others
Percent	70.36	11.52	0.53	0.06	0.38	2.53	6.36	8.02	0.24

Earthworm treatment. After fermentation, the compost was transferred to plastic boxes (41 × 30 × 14.5 cm) and loaded to 8–10 cm depth. Each box is seeded with 700 earthworms (730 worms/cubic meter). The compost was treated with worm at 60% humidity for 28 days at 20°C. Samples were taken regularly (with the worms in sample removed) and saved in an ultralow freezer. A sample was collected every 8 d, and 4 samples were obtained, numbered 10–13.

DNA extraction. DNA was extracted using a Soil Extraction Kit (Omega, USA) according to the manufacturer's instructions. The crude DNA was purified through a minicolumn purification method (Yin et al. 2008) and analyzed by ethidium bromide-UV detection on an agarose gel.

PCR amplification. The V3 regions of bacterial 16S rRNA gene from the compost DNA extracts were amplified by PCR from the compost DNA extracts with the primer pair 338f (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG -3′) and 518r (5′-ATT ACC GCG GCT GCT GG -3′) (Chen et al. 2008; Muyzer et al. 1993; Van der Gucht et al. 2007). For the purpose of separating these 16S rRNA gene of bacterial communities in a DGGE gel, a GC clamp was added at the end of primer 338f. PCR reaction mix was composed of 5 μl of 10× PCR buffer, 4 μL of Mg^{2+} (25 mmol/L), Then, 1 μL of dNTP (10 mmol/L).

Then, 2 μL of each primer (10 pmol/L), 0.5 μL template DNA (25 to 50 ng) and sterile ddH_2O to a final volume of 50 μL. Amplification was performed in a thermocycler (Bio-rad, France) using the following program: initial denaturation: 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 60 s at 52°C and 30 s at 72°C, with a final extension for 10 min at 72°C. After amplification, PCR products were analyzed by electrophoresis in 1% (w/v) agarose gels.

Denaturing gradient gel electrophoresis (DGGE). DGGE was conducted with the Decode system (Bio-Rad laboratories, Hercules, CA, USA). Acrylamide gel concentration was 8%. The denaturing gradient was 35–65%, where the 100% denaturant was defined as 7 mol/L urea plus 40% formamide. The gel slab was prewarmed to 60°C and loaded with 50 μl sample per lane. Electrophoresis was conducted at 180 V for 5 h. Upon completion, gel was stained with 0.04% Goldview, photographed with GDS-8000 (Gene Inc) and analyzed.

Analysis of DGGE gels. Digitized images of DGGE fingerprints were used to quantify diversity as enabled by Quantity One software (Bio-Rad), which detects bands and quantifies relative concentrations of DNA from cumulative pixel intensities within a given lane. Similarity among fingerprints was assessed by cluster analysis using the unweighted pair-group average method (UWPGA) in the "phylogenetic analysis" mode of the Quantity One software. Richness estimates were simply the number of bands detected in each lane, and the Shannon-Weiner index (H') and Simpson index (D), used to summarize the diversity of a bacterial community, were calculated by using the number and intensity of bands in each DGGE profile as representations of the number and relative abundance of different phylotypes in each compost sample.

The Shannon-Weiner index (H') and Simpson index (D) were represented, respectively, by $H\prime = -\sum_{i=1}^{S} P_i \ln P_i$ and $D = 1 - \sum_{i=1}^{s} P_i^2$, where S is the observed number of bands in a lane and P_i is the importance probability of the bands in a lane. H' was calculated on the basis of the bands on the gel tracks that were applied for the generation of the dendrograms by using the intensities of the bands as judged by peak height in the densitometric curves. P_i was calculated as follows: $P_i = n_i/n''$, where n_i is the height of a peak and n'' is the sum of all peak heights in the densitometric curve. Evenness index (E) derived from Shannon-Weiner index was calculated with the equation $E = \frac{H'}{H_{\max}}$, where $H_{\max} = \ln S$, which occurs when all bands are present in equal intensity (Bates and Garcia-Pichel 2009; Konstantinov et al. 2004).

Sequencing of DGGE bands. Representative bands were excised from DGGE gels with a sterile scalpel, placed in 1.5 mL vials and purified using a Gel extraction Kit following manufacturer's instructions (Omega, USA). After reamplification using the original 338f and 518r primer set, PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

Phylogenetic analysis. The obtained 16S rRNA gene sequences were compared to the National Center for Biotechnology Information database using the BLAST to find very similar sequences in NCBI's databases (Altschul et al. 1997). Those similar sequences were downloaded from Genbank, and aligned using ClustalW2 (Larkin et al. 2007) with parameters set to default. Chimeric sequences were removed based on the BLAST, ClustalW2 and Chimera Check program results (Cole et al. 2005). The data sets without the chimeric sequences were aligned using ClustalW2 again and used to construct molecular phylogenetic trees. Minimum evolution (ME) criteria were used to determine the relationships among sequences. For the ME analysis, the Tamura-Nei's nucleotide substitution model was selected with pairwise deletion of gaps. Meanwhile, bootstrap reassembling analysis based on 1000 replicates was used to assess the confidence values attached to the individual nodes.

Results and Analysis

DGGE Analysis of Bacteria During Compost

Thirteen representative samples of compost, including 1 sample from the fresh mixture of compost, 8 samples collected during the composting process and 4 samples obtained during earthworm treatment, were analyzed with DGGE. Figure 1 shows a DGGE photograph. Bands were automatically recognized with Quantity One program and clustered using Unweighted Pair Group Method with Arithmetic mean (UPGMA). Results were shown in Figure 2. Diversity indices were calculated from the number of bands and intensities of DGGE photographs (Table 3).

Accordingly the UPGMA cluster analysis based on DGGE band profiles, all lanes were divided into four clusters. Lane

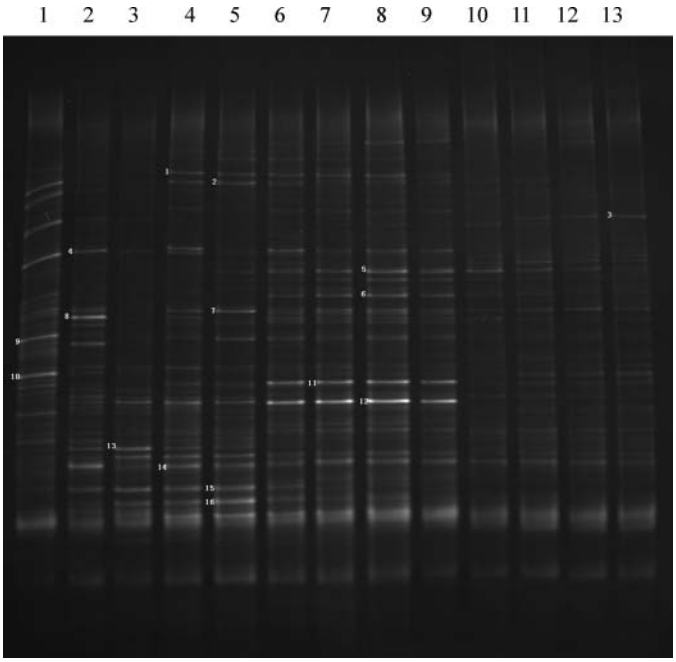

Fig. 1. DGGE profiles of bacterial 16S rRNA gene fragments in the compost enriched by PBR powders. Lane 1 was from the sample of fresh mixture of compost, lanes 2–9 were from those collected during composting and lanes 10–13 were from those obtained during earthworm treatment.

1 was an independent cluster, which indicates that its band profile was very different from those of other lanes and the bacterial community in sample 1 differed greatly from those of other samples taken later. Lanes 2–5, corresponding to the samples 2–5, which were collected from the compost of the early stage of fermentation, was the second cluster. The third cluster contained lanes 6–9, which were correlated with samples 6–9 collected from the compost of the late stage of fermentation. The last cluster contained lanes 10–13, that correlated with the samples from earthworm treatment (Samples 10–13). Similar bacterial community structures were observed within a cluster. The band patterns in the same cluster are more similar to each other than to those in other clusters, indicating that there are more similarities in bacterial communities within a cluster than between clusters.

Figures 1 and 2 indicate that bacterial communities were most similar among the four samples from the later stage of fermentation, followed by the four samples from earthworm treatment. There were more differences among bacterial communities in early fermentation stages.

Bacterial diversity indices were high for all samples (Table 3). Among these, the eighth day sample (Sample 3) had the lowest diversity index. DGGE has a specific limitation that has limited sensitivity of detection for some rare community members (only predominant species in a community are displayed). After being piled up for fermentation, the temperature of compost was increasingly higher. The compost temperature

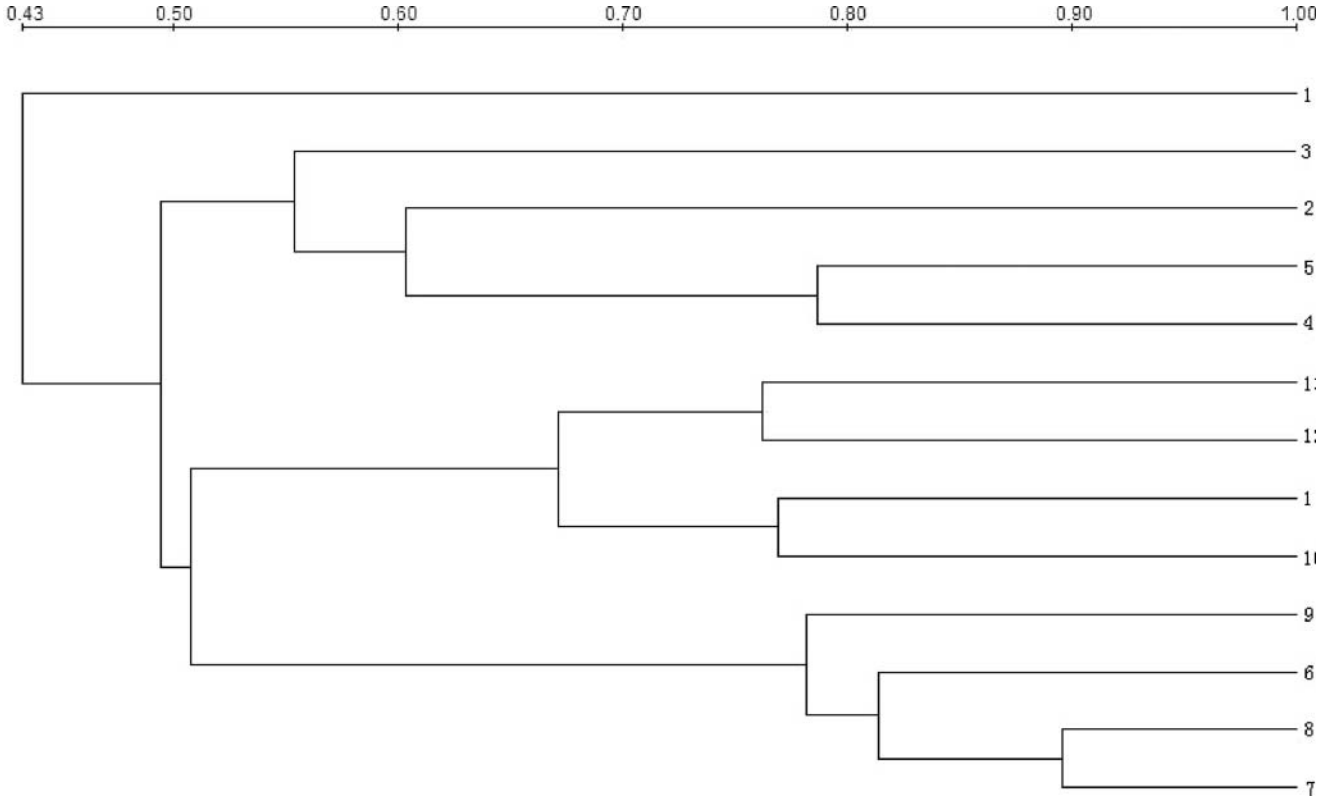

Fig. 2. Bacterial community fingerprint comparison between the compost samples with cluster analysis using the unweighted average pair-group method (UPGMA). The sample correlating with lane 1 was collected from the fresh compost. The samples associated with lanes 2–9 were obtained during composting. The samples associated with 10–13 were collected during earthworm treatment.

Table 3. Microbial diversity indexes from the 13 lanes

Diversity index	Sample number												
	1	2	3	4	5	6	7	8	9	14	18	20	22
Richness *S*	25	24	18	25	22	23	28	25	26	21	21	17	19
Shannon-Wiener *H′*	3.04	2.86	2.46	3.03	2.88	2.98	3.14	3.03	3.07	3.00	3.01	2.79	2.90
Simpson *D*	0.95	0.93	0.89	0.95	0.94	0.94	0.94	0.94	0.94	0.95	0.95	0.94	0.95
Evenness *E*	0.94	0.90	0.85	0.94	0.93	0.95	0.94	0.94	0.94	0.98	0.99	0.99	0.99

was higher than 50°C after 4 d of composting. The number of some bacteria which could not tolerate high temperature was increasingly less, and their DGGE bands would not be detected when the number was less than a certain amount. As the fermentation continues, bacteria tolerating high temperature increased, which lead to the increase of diversity indices during the later stage.

Molecular Identification of Major Band in DGGE Graph

To further study the bacterial community in the compost, 16 major bands were recovered from DGGE gel, and sequenced. Results indicate that bands 1, 2, and 15 were chimerical. The sequences in the remaining 13 bands have been submitted to European Nucleotide Archive (ENA) with accession number of HE802275 to HE802287. They were compared to the National Center for Biotechnology Information database using the BLAST to find very similar sequences in NCBI's databases (Altschul et al. 1997).

The results of the partial sequence analysis of these bacterial species and their tentative phylogenetic affiliation are shown in Table 4. All bacterial species representing the 13 DGGE bands belong to either phylum *Proteobacteria* or phylum *Firmicutes*. The bacteria, which correlate with bands 3–6 and 11–12, were closest to *Gammaproteobacteria.* The bacterial species that correlates with band 16 was closest to *Betaproteobacteria.* The microbes, which correlate with bands 7–10, 13 and 14, were closest to *Firmicutes.*

To further precisely classify the relationship, molecular phylogenetic trees were constructed using minimum evolution method (Figure 3). The phylogenetic tree was consistent with the results from BLAST. The bacteria representing bands 3–6, 11, 12 and 16 were *Proteobacteria* species. Among these microbes, except the bacterium representing band 16, other bacteria of *Proteobacteria* were from *Gammaproteobacteria* species. The bacterium correlating with band 16 could not be confidently assigned to *Gammaproteobacteria* or *Betaproteobacteria*, based on phylogenetic tree. According BLAST results, it is more likely from *Betaproteobacteria*.

Succession of Prevalent Species During Compost

The temperature of compost changed during the composting process. The initial internal temperature of compost was 27°C and then rose to 50–55°C with the development of composting. Thereafter, the temperature returned to the mesophilic range until the composting finished. The temperature would affect microbial community and dominant bacteria of the compost. Prevalence of bacterial species was determined according to band intensities in DGGE. In the initial mixture for

Table 4. Assignment of taxonomic groups to band sequences extracted from a DGGE gel based on ~150 bp and the closest sequence match of known phylogenetic affiliation

Band[a]	Identity[b]	Taxonomic group	Similarity	Accession number
3	*Pseudomonas peli* (HM371423)	*Gammaproteobacteria*	100%	HE802275
4	*Pseudomonas syringae* (GQ870341)	*Gammaproteobacteria*	94%	HE802276
5	*Marinobacter sp.* (GQ131626)	*Gammaproteobacteria*	95%	HE802277
6	*Cellvibrio japonicus* (NR_028836)	*Gammaproteobacteria*	97%	HE802278
7	*Bacillus licheniformis* (GQ392053)	*Firmicutes*	97%	HE802279
8	*Clostridium symbiosum* (EF025909)	*Firmicutes*	100%	HE802280
9	*Ureibacillus thermosphaericus* (AB300774)	*Firmicutes*	96%	HE802281
10	*Lactobacillus fermentum* (GQ231445)	*Firmicutes*	96%	HE802282
11	*Alteromonas* sp.(EU714266)	*Gammaproteobacteria*	96%	HE802283
12	*Alteromonas* sp.(EU714267)	*Gammaproteobacteria*	96%	HE802284
13	*Thermobacillus* sp. (GU183629)	*Firmicutes*	87%	HE802285
14	*Bacillus* sp. (GQ889490)	*Firmicutes*	91%	HE802286
16	*Rhodocyclus tenuis* (NR_025839)	*Betaproteobacteria*	87%	HE802287

[a]Band number as indicated in Figure 1.
[b]Closest match to band sequence obtained by searching using BLAST program. Exclude uncultured/environmental sample sequences were excluded during searching. Numbers in parentheses indicate the GenBank accession number.

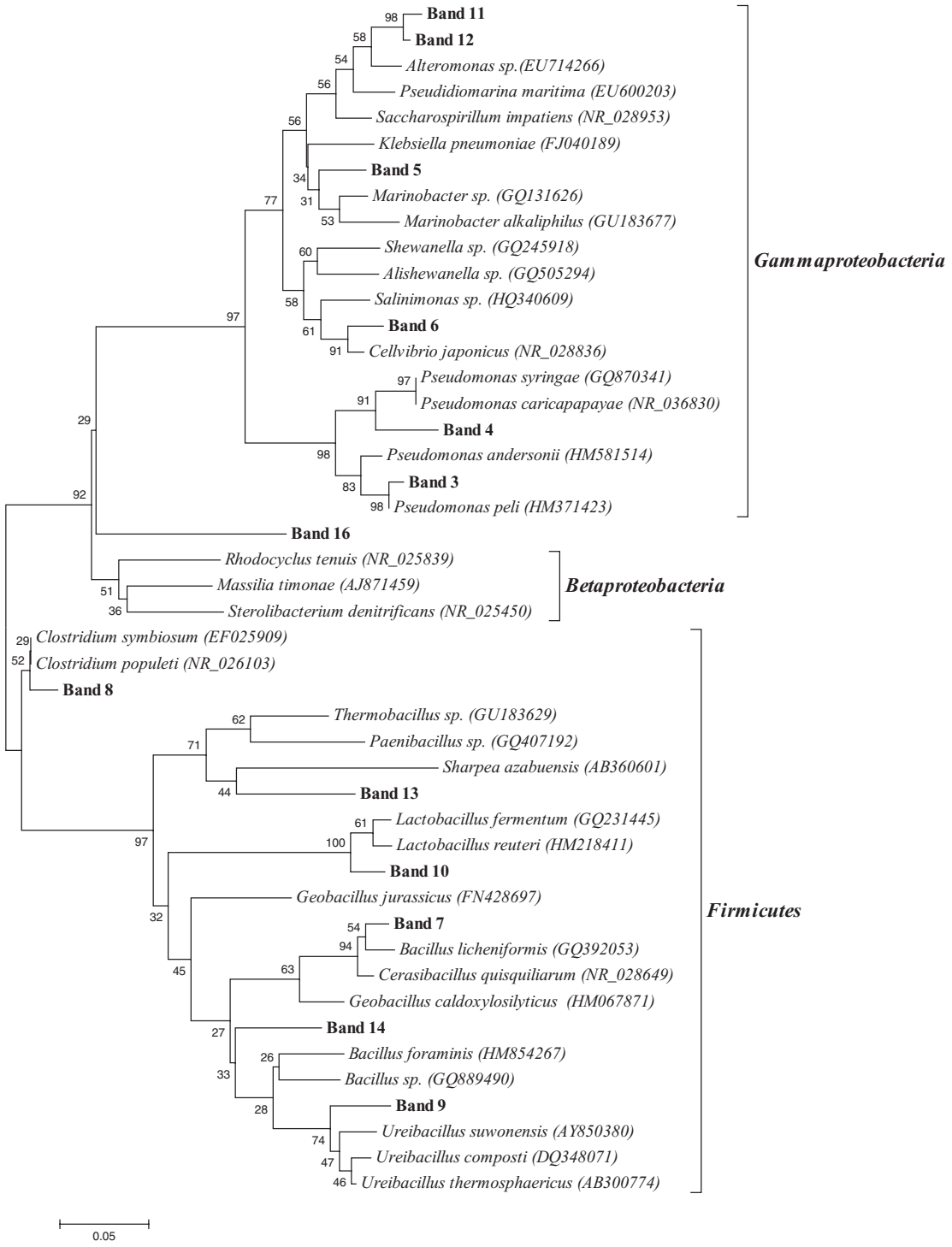

Fig. 3. A minimum evolution tree showing phylogeny of 16S rRNA gene sequences from the predominant bands in the DGGE profile. Sequences were aligned with ClustalW, and distances were calculated with the Tamura-Nei algorithm. Bar indicates 5% sequence divergence. Numbers on nodes correspond to percentage bootstrap values for 1000 replicates.

fermentation, dominant bacteria were represented by *Pseudomonas* (associated with bands 3 and 4), *Ureibacillus* (associated with band 9) and *Lactobacillus* (associated with band 10). As the fermentation proceeds, dominant bacteria were represented by only *Pseudomonas* (associated with band 4). In earthworm-treated compost, the *Pseudomonas*, associated with band 3, returned to be the dominant type.

During the early stage of fermentation, bacterial community structure changed dynamically. Dominant populations include *Pseudomonas* (band 4), *Bacillus* (band 7), *Clostridium*

(band 8), *Alteromonas* (band 12), *Formicutes* (bands 13, 14), *Proteobacteria* (band 16). But none of these were dominant all the time during this period. This might be due to the rapid elevation in compost temperature during this period. Nutrition ingredient also changed along with the temperature increase, which may also contribute to the dynamic change in bacterial communities.

During the late stage of fermentation, bacterial communities were stable in the compost. The dominant populations include *Pseudomonas* (band 4), *Marinobacter* (band 5), *Cellvibrio* (band 6), *Alteromonas* (band 11), *Alteromonas* (band 12), *Firmicutes* (band 14). It is noteworthy that bands 11 and 12 were much brighter than others in the four samples during the late stage of fermentation, suggesting that they were absolutely dominant. Most of dominant species belong to *Gammaproteobacteria* during late stage of fermentation.

Only *Alteromonas* (band 12) and *Firmicutes* (band 14) were dominant during the entire solid-state fermentation. The dominance of *Firmicutes* extended to earthworm treatment stage, suggesting the adaptability of this species.

During earthworm treatment, no band was extremely bright as seen in previous stages, although the number of bands was not decreased. The evenness indices were higher than those of samples from early stages. These observations suggest bacterial species were uniform during earthworm treatment, without species absolutely dominating the community. In addition, the DGGE profile had little changes, suggesting earthworm treatment had little influence on the prevalence of bacterial species.

Discussion

Bacterial Diversity During Compost Process

Compost treatment effectively converts potassium from unavailable state in minerals to bioavailable state (Biswas 2011; Nishanth and Biswas 2008). Identification of effective bacterial species that act in this process would allow the manufacture of highly efficient preparation to be used in solid-state fermentation. Results from DGGE indicate that compost treatment involves a diverse community of bacteria. The bacterial community changes continuously, especially during the early stages. These indicate that there were many bacterial populations in rapid succession during compost (Hultman et al. 2008).

Due to the continuous change in temperature, pH and organic material, microbial community changes also in the compost, leading to more efficient degradation and utilization of organic substances. It is also likely that high temperature, acids and other chemical components also played a role in the release of potassium from mineral (Vassilev and Vassileva 2003). In future research, some bacteria, which can adapt to the different stages of composting, need to be isolated to develop highly efficient microbial agent of compost to release potassium from potassium-bearing rock. The microbial agent should be compound microorganisms, which will ensure that there are microbes to weather potassium-bearing rock powders in all stages of composting.

The Dominant Microbial Group in the Compost

The dominant bacteria in the compost enriched by potassium rock powders are mostly species from *Firmicute* and *Proteobacteria*. The result was similar to the bacterial species reported earlier in some types of composts. Numerous studies indicated that some members of *Firmicute* and *Proteobacteria* are dominant in many types of compost. For example, after inoculating exogenous microbial agent into fresh pig manure, *Clostridium stercorarium* subsp. *thermolacticum* sp. was the dominant group in the whole composting process. Uncultured bacteria *Bacillus coagulans* sp. and *Clostridium thermocellum* sp. became the main groups on the 10th and 16th days after microbial agent inoculation, while uncultured *Firmicutes* sp. and *delta proteobacterium* became the dominant groups on the 5th and 16th days in the treatment non-inoculation, respectively (Xie et al. 2009). All of these dominant bacteria in the compost belong to *Firmicute* and *Proteobacteria.* Among the bacterial communities of the organic municipal waste composts, the members of the phylum *Firmicutes* were by far the largest group in the full-scale process and in the pilot-scale process at different composting stages (Partanen et al. 2010).

Although there was a large difference between the composts and ours at the species and strain level, all of the members of *Firmicute* in these composts belonged to the classes *Bacillales*, *Clostridia* and *Lactobacillales.* In the process of composting, these microorganisms of the two phyla play an important role, such as breaking down organic matter and producing carbon dioxide, water, heat, and humus (Zheng et al. 2007). Many microbes excrete organic acids into compost, which may be the main agent leading the weathering of the potassium-bearing rock powders in the compost.

The Effect of Earthworm Treatment on the Bacterial Communities of Compost

Many studies showed that earthworms accelerate weathering of rocks (Carpenter et al. 2007, 2008; Edwards and Bohlen 1996; Needham et al. 2004; Suzuki et al. 2003), the microbes from its gut may be important weathering agents of rocks (Carpenter et al. 2007; Liu et al. 2011). In this research, potassium ore powder was treated with earthworms after composting with organic wastes. The UPGMA cluster analysis showed that the bacterial community of earthworm-treated samples has differences from other samples.

This indicates that earthworm treatment had some effect on the bacterial communities of the compost. Earthworm-treated samples still showed high bacterial diversity, but none of new dominant bacteria was found in them. Earthworms might have brought some bacteria to compost, but these bacteria were nondominant in compost. Although DGGE technology may resolve DNA fragment difference as small as 1 base pair theoretically if right conditions were selected, it is not practical to recover all fragments from the gel. When DNA from a species is less than 1.5% of the total DNA, it may not be detectable with DGGE (Muyzer et al. 1993). To further detect the microorganisms from earthworm treated compost, other methods are needed, such as 16s rRNA library, DNA microarray and environmental metagenomics.

Conclusion

There are abundant bacteria during compost and earthworm treatment of PBR powders. The bacterial community changed rapidly during early stages. In contrast, the differences were small between bacterial communities in samples from late stage of composting, so were differences between samples from earthworm treatment. At the same time, dominant bacteria changed largely during the early composting stage, and remained stable in the late stages. There were no specific dominant bacteria in the earthworm treatment stage. The dominant bacteria are mostly from *Proteobacteria* and *Firmicute*, suggesting these two groups of bacteria played a major role in compost treatment of PBR powders.

Acknowledgments

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