Microbial Community Dynamics during Composting Process of Animal Manure Analyzed by Molecular Biological Methods

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Abstract

Composting is a biological process involving stabilization of animal manure and transformation into organic fertilizer. Microorganisms such as bacteria and archaea participate in the composting process. Because bacteria form huge communities in compost, they are thought to play an important role as decomposers of organic substances. However, only few studies are tracking bacterial communities throughout the composting process. The role of archaeal communities in composting has not been also elucidated. To study bacterial and archaeal community dynamics, animal manure composts were analyzed by molecular biological methods.

A clone library constructed from bacterial 16S rRNA genes showed that the bacterial community structure dynamically changed with processing time. Based on phylum-level analysis, Firmicutes and Bacteroidetes were dominant at day 0. Phylum Firmicutes kept their abundance for 20 days, indicating that they may be active under high temperatures. In the final compost, the library consisted of various genes belonging to the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria.

In contrast, the clone library of archaeal 16S rRNA genes was simply constructed by two groups, the methane-producing archaea (methanogens) and ammonia-oxidizing archaea (AOA). Thermophilic *Methanosarcina* spp.-like genes were constantly present, indicating that this methanogen may adapt to environmental changes such as high temperature. AOA-like sequences were first detected from compost and displayed a high similarity to cultured AOA originating from hot springs. Further study revealed that the abundance of AOA markedly varied because of raw material or composting process. In this study, we revealed the pattern of changes in the prokaryotic communities involved in the composting process.

Introduction

Cattle manure accounts for a large part of the total animal waste generated in Japan (MAFF, 2013) and can cause environmental problems such as soil contamination, air pollution, or offensive odor emission without appropriate treatment (Bernal et al., 2008). Composting is the most effective technique for mineralization of organic substances, microbial stabilization, removal of odors, and sanitization (Bernal et al., 2008; Haga, 1999). In addition, the final product can be applied to agricultural soil as high quality fertilizer (Haga, 1999). Throughout the process, various microorganisms such as bacteria, archaea, and fungi play an important role in degradation (Ryckeboer et al., 2003). Microbial community structure and diversity change dramatically during composting. These changes are affected by temperature, pH, moisture content, and aerobic/anaerobic conditions (Ryckeboer et al., 2003; Schloss et al., 2003). Because the bacterial population is more vast and complex than others (Ryckeboer et al., 2003), it has been considered that the bacterial community is the main decomposer in compost. To follow the dramatic change in structure and diversity of the microbial community during composting, researchers have recently used culture-independent techniques analyzing 16S ribosomal RNA genes (Chachkhiani et al., 2004; Peters et al., 2000; Tang et al., 2004). Using culture-dependent techniques, it is possible to detect only about 8.5% of total microbes (Gong et al., 2005). The changes in the bacterial community structure during the composting of animal manure have been analyzed in other studies (Cho et al., 2008; Green et al., 2004; Maeda et al.,

2010; Sasaki et al., 2009; Yamamoto et al., 2009); however, there are only few studies tracking the bacterial community in detail throughout the entire composting process. On the other hand, existing studies of the archaeal community are a minor component of the microbial community studies on composting because archaea mainly live in extreme environments such as thermophilic or anaerobic conditions (Insam and de Bertoldi, 2007). However, some reports have indicated the presence of methane-producing archaea (methanogens) in compost (Jäckel et al., 2005; Thummes et al., 2007). Moreover, a new archaeal group called ammonia-oxidizing archaea (AOA) was discovered living in moderate environments like seawater and soil and were found to be essential organisms in ammonia oxidation (Könneke et al., 2005; Leininger et al., 2006; Treusch et al., 2005). Therefore, archaea can be also considered as an essential component of the microbial community in compost. However, there is no existing study of the entire archaeal community in the composting of animal manure.

In this review, we investigated the bacterial and archaeal community structures and how they changed during the composting process.

Bacterial community structure during the composting process

It has been considered that bacteria play an important role throughout the process because of the existence of a wide variety of species and characteristics. Denaturant gradient gel electrophoresis (DGGE) has been widely used for the analysis of bacterial communities (Green et al., 2004; Maeda et al., 2010; Sasaki et al., 2009; Yamamoto et al., 2009). However, it is not sufficient to clarify the change in the dominant microbial species in bacterial communities during the composting process. The clone library method enables detection of a larger number of species within microbial communities than using DGGE analysis. However, only few studies are tracking bacterial communities throughout the composting process (Cho et al., 2008; Guo et al., 2007; Yamada et al., 2008). Here, we performed a composting experiment as a model case of field-scale composting and tracked the bacterial community using the cloning procedure. Cattle manure and sawdust were mixed and piled for about 30 days with aeration. To study the bacterial community, composting material was treated for 84 days more without daily mixing. Changes in physical and chemical parameters are shown in Fig. 1. The temperature reached 76.1 ℃ within 5 days and remained at >50 ℃ for 23 days. Moisture content decreased from about 70% to 41.8 % by the end of the process (Fig. 1).

We selected 5 samples at different time points for analysis. Total DNA was extracted and approximately 600 bp of bacterial 16S rRNA gene were amplified. PCR products with the correct DNA fragments were cloned and then selected for sequencing.

The clone library constructed from the sample collected on day 0 mainly consisted of members of the phyla Firmicutes and Bacteroidetes (Fig. 2), indicating that both members of bacteria originating from cattle manure (Ozutsumi et al., 2005). The number of clones grouped under the phylum Firmicutes, which can be active under high temperatures, maintained their relative abundance for 20 days. Especially on day 5 and day 20, the Phylum consists of the member of class Clostridiales and Bacillales, corresponding to other reports (Yamada et al., 2008). Thereafter,

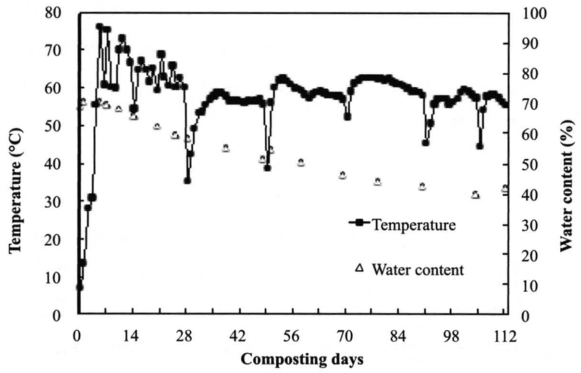

Fig. 1. Changes in temperature and water content during the composting process for analysis of bacterial community.

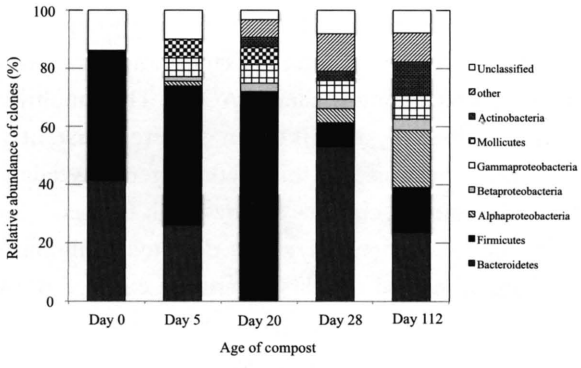

Fig. 2. Changes in bacterial community constructed from cloned 16S rRNA genes.

the ratio of the members of the phylum Firmicutes decreased, while those of the phyla Actinobacteria and Proteobacteria increased. By the end of the composting process, the library consisted of phyla Proteobacteria (32.7%), Bacteroidetes (26.5%), Firmicutes (18.4%) and Actinobacteria (12.2%), and others (10.2%), showing that various bacteria lived and forms complex community in the final compost. Bacteria sequences were classified into class Rhizobiales and Burkholderiales, that often observed in soil environment were also contained, suggesting that composting material reached stable phase.

We observed that the bacterial community reflected composting conditions and changed its structure accordingly. However, metabolic activity of bacterial community was not analyzed in this study. To determine dominant species in each stage of the composting process, RNA-based analysis is required. Some studies reported the community structure of one particular bacterial group diversity in compost with reverse-transcription PCR and DGGE (Halet et al., 2006; Kowalchuk et al., 1999). In addition, metagenomic analysis may be also the powerfull method for understanding whole microbial community in compost (Martins et al. 2013). It will cover all aspects of microbial ecology in composting in combination with PCR-based DNA analysis.

Archaeal community structure during the composting process

It has long been considered that the archaeal community has a low abundance in composting process because they are usually oligotrophic, thermophilic, or hyperthermophilic (Insam and de Bertoldi, 2007). However, the archaeal community has recently been considered as an essential component of the microbial community in compost. For instance, considerable methane emissions have been measured from compost, suggesting that methane-producing archaea (methanogens) live actively in compost (Beck-Friis et al., 2000; Fukumoto et al., 2003; Hao et al., 2001). In addition, some archaea of the phylum Crenarchaeota can oxidize ammonia under natural environments such as seawater, freshwater, various soil, and hot spring (Hatzenpichler et al., 2008; Könneke et al., 2005; Leininger et al., 2006; Treusch et al., 2005). AOA also was detected from artificial sites, like rice field (Fujii et al., 2010). However, no useful information about archaea in the compost of cattle manure was available, although methanogen-like sequences had been detected in cattle manure (Gattinger et al. 2007). Thummes et al. (2007) analyzed about 120 clone sequences using composting materials; however, no sequence belonging to AOA was detected.

Therefore, the composting experiment to analyze the archaeal community was performed using cattle manure and sawdust as described above (Yamamoto et al., 2011). Changes in physical and chemical parameters are shown in Fig. 1. The temperature reached 77.9°C and was maintained at >60°C for 18 days. The initial moisture content was about 67% (Fig. 3), and it continued to decline from day 8 and eventually reached its lowest value (~30%).

Compost samples for analysis were selected at 6 time points. In total, 14 OTUs were generated from sequenced clones, the number of which varied from 36 to 78. Almost all detected OTUs were related either to methanogens or to AOA (Fig. 4). Results indicated that both methanogens and AOA were dominant archaeal species during the composting process of cattle manure. The archaeal community

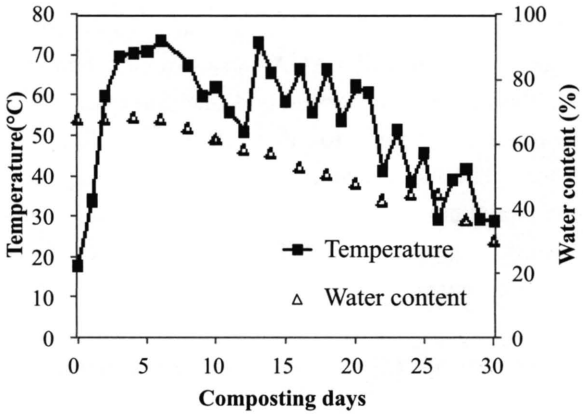

Fig. 3. Changes in temperature and water content during the composting process for analysis of archaeal community.

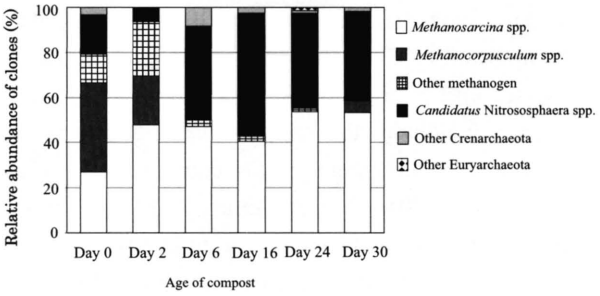

Fig. 4. Changes in archaeal community constructed from cloned 16S rRNA genes.

structure found in the present study differed in some respects from that found in previous reports analyzing composting materials. During the first two days, OTUs grouped into methanogens were dominant. We detected some OTUs, which were closely related with sequences from groundwater, animal rumen, or manure.

Another OTU formed the dominant group after day 2 and showed a high homology with uncultured thermophilic *Methanosarcina* spp. Thummes et al. (2007) also detected some clones grouped into this cluster from different composting materials. Thus, thermophilic *Methanosarcina*-like organisms appear to adapt to the composting environment and increase its detection rate after other methanogens originating from cattle manure have decreased in abundance because of high temperatures.

It is notable that AOA-like sequences were detected throughout the composting process. Our study indicated that AOA was an essential component of the archaeal community in compost, particularly from days 6 to 30. A large part of OTU was closely related to *Candidatus* Nitrososphaera gargensis with high homology (98%). This *Candidatus* Nitrososphaera gargensis-like AOA can grow under moderate thermophilic condition and may have the ability to oxidize ammonia to nitrite (Hatzenpichler et al., 2008). The AOA detected in the sample adapted to the temperature or ammonium concentration in cattle manure compost. Other OTUs were classified as soil crenarchaeota.

Diversity of AOA in various animal manure composts

Nitrification by ammonia oxidation occurs during composting process (Bernal et al., 2009; Vuorinen and Saharinen, 1997). Organic nitrogen in fresh manure is oxidized to nitrate through nitrification by ammonia oxidizing organisms. It has been reported that ammonia-oxidizing bacteria (AOB) may play an important role in nitrification during composting (Prosser and Nicol, 2008). However, previous researchers concluded that archaeal *amoA* gene was not detected from any stages of the composting process (Maeda et al., 2010; Yamada et al., 2007). Our study detected the archaeal *amoA* gene in compost for the first time (Yamamoto et al., 2010). It shows that AOA may have been more abundant than AOB during the cooling and maturation stages of composting (Yamamoto et al., 2010). In the samples collected from cattle manure compost, only one or two archaeal *amoA* bands were detected in the course of the composting process (Yamamoto et al., 2011). It was shown a useful information that the diversity of AOA community was low and AOA may have an ability of ammonia oxidation in composting. In addition, our group also revealed that an archaeon related to *Candidatus* Nitrososphaera gargensis was dominant in liquid cultures seeded with cattle manure compost (Oishi et al., 2012). However, there is no information available about AOA communities in other animal manure compost. To clearly understand the nitrification process in compost ecosystems, we researched AOA community structure in various animal composts (Yamamoto et al., 2012). For AOA community analysis, the bacterial *amoA* sequence was also amplified to compare their diversity and abundances.

Samples collected from other cattle manure compost were also able to amplify archaeal *amoA* sequences and show DGGE fingerprints. In contrast, a few *amoA* sequences were amplified using samples from fresh manure, swine manure compost, and chicken manure compost. The number of detected *amoA* sequences per sample varied from 1 to 4, except for the samples from one facility. All sequences were divided into about three groups: one is phylogenetically related to *Candidatus* Nitrososphaera gargensis (group NG), while the other two groups had *amoA* sequences from hot spring or wastewater as close relatives (Fig. 5). However, there were a few compost samples where AOA dominated over AOBs, indicating that the presence and abundance of ammonia oxidizers was determined by some unknown factors. We hypothesized that the difference of ammonia concentration between raw animal manure is the most influential factor in AOA presence and cattle manure was the best material to their growth in composting.

The number of AOA cells in comparison to ammonia-oxidizing bacteria (AOB) in the sample was estimated by real-time PCR (Fig. 6). In cattle manure compost, the abundance of archaeal *amoA* genes was clearly lower than that of bacterial genes. These results provided the finding that the abundance of AOA in compost was not influenced by the difference of animal species generating manure. Real-time PCR confirmed that archaeal *amoA* gene copy numbers were greater than bacterial gene copy numbers in the

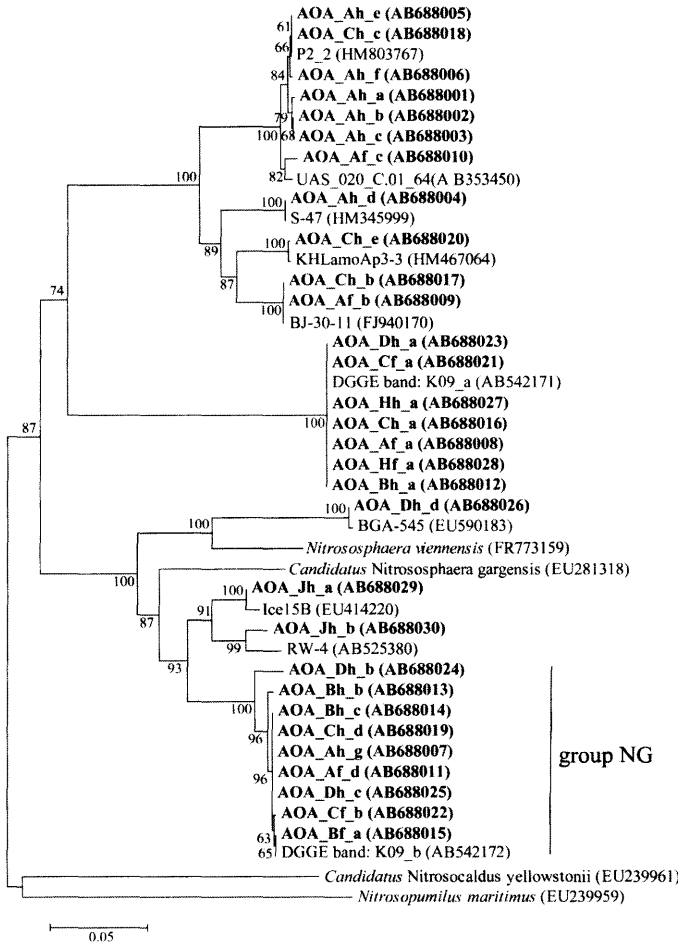

Fig. 5. Phylogenetic tree of the archaeal *amoA* sequences obtained from composting materials.

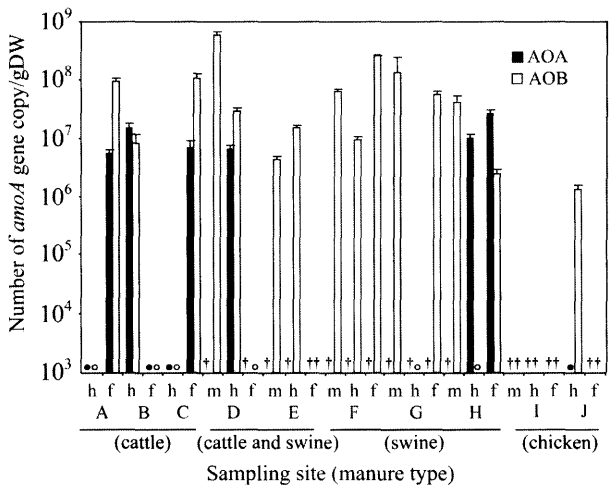

Fig. 6. *AmoA* gene copy numbers for AOA and AOB. Capital letter indicates sampling facilities. Samples were obtained from each facility at fresh manure (m), the high temperature stage (h), and the end of the composting (f). Samples with dagger did not perform real-time PCR. The sample with gene copy number below the detection limit were represented as closed circle (AOA) and opened circle (AOB).

end product at facility H. The AOA/AOB ratios varied from 0.06 to 10.54. This variation may have been caused by the operating conditions of the composting process, such as the addition of finished compost.

Our results so far suggest that the concentration of ammonium and the temperature are factors that control the AOA community. Further study is needed to show whether AOA are critical to nitrification in manure compost and influence the quality of the compost. To evaluate the contribution of AOA in nitrification, we need to collect and analyze more composting samples and measure various parameters to create meta-data for future research.

Conclusion

In this study, we revealed a part of the pattern of the changes in the bacterial community in the composting process by analyzing a large number of bacterial sequences. In addition, we detected AOA from compost for the first time. By studying various samples originating from different animal manure samples, we were also able to suggest that AOA could be involved in the nitrification of composting systems, although their abundance was lesser than that of AOBs. This study underlines the importance of investigating archaeal communities to understand the microbiology of compost.

However, metabolic activity of bacterial and archaeal community were not analyzed in this study. RNA-based analysis and metagenomics will cover all aspects of microbial ecology in composting by combining with PCR-based DNA analysis.

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