Archaeal Community during Cattle Manure Composting Process in Field-scale Facility

Archaeal Community during Cattle Manure Composting Process in Field-scale Facility

Nozomi YAMAMOTO*, Chika TADA and Yutaka NAKAI

Laboratory of Sustainable Environmental Biology, Graduate School of Agricultural Science, Tohoku University
232-3, Yomogida, Naruko-Onsen, Osaki, Miyagi, 989-6711, Japan
***e-Mail: nozomy@bios.tohoku.ac.jp**

Keywords: 16S rRNA gene, ammonia-oxidizing archaea (AOA), cloning, compost, methane-producing archaea (methanogen)

Received 24 December 2010; accepted 31 January 2011

Abstract

Composting process is a useful technique to transform cattle manure into organic fertilizer. During the process, complex microbial communities consisting of bacteria, archaea, and fungi carried out biodegradation of substrates. Because it has been considered that bacteria play an important role in composting, their community structure was studied well. However, archaeal one has not been identified clearly. To understand their community structure and abundance, cattle manure was composted in field-scale facility and composting materials were analyzed by culture-independent approaches.

Clone library constructed from archaeal 16S rRNA genes showed that archaeal community in compost was mainly consisted of methane-producing archaea (methanogen) and ammonia-oxidizing archaea (AOA). During first 2 days, clones which were related to methanogens in the animal rumen or manure were detected, suggesting that fecal methanogen could survive in the early stage of composting. Other methanogen, which grouped into thermophilic *Methanosarcina* spp. were present thoroughout the process, indicated that they might adapt the environmental changes such as high temperature. AOA-like sequences were detected from all investigated samples. They showed high identity with cultured AOA originated from hot spring. In this study, we revealed the changes in archael community in the composting process. It was also suggested that AOA could actively involve in nitrification of composting systems.

Introduction

Livestock manure accounts for a large part of the total waste generated in the livestock industry (Haga, 1999). Animal manure should be treated properly because huge amount of manure can cause environmental problems such as air, water, and soil pollution (Bernal et al., 2008). Composting of animal manure is one of the most effective techniques in terms of mineralization of organic components, microbial stabilization, and removal of odors and so on (Bernal et al., 2008). In addition, final product can apply to agricultural soil as high quality fertilizer (Bernal et al., 2008). Composting is a biological process involving various microorganisms (Insam and de Bertoldi, 2007). To follow microbial community whose structure and diversity change dramatically during composting, researchers recently use culture-independent techniques like DNA analysis. It can detect both uncultured and cultured organisms from compost while culture dependent one can only detect about 8.5% of total microbes (Gong et al., 2005). By using this approach, the changes in the bacterial community structure during the composting of animal manure were analyzed well (Guo et al., 2007; Yamada et al., 2008; Yamamoto et al., 2009) because it has been considered that bacteria play an important role with various characteristics. On the other hand, archaea had been recognized as minor components of the microbial community in compost because they mainly live in thermophilic or anaerobic environments (Insam and de Bertoldi, 2007). But some reports showed considerable methane production from cattle manure compost (Hao et al., 2001). Others indicated the pres-

ence of methane-producing archaea (methanogen) from composting of organic waste (Thummes et al., 2007) or rice straw (Cahyani et al., 2004). Moreover, new archaeal species called ammonia-oxidizing archaea (AOA) was discovered living in moderate environments like seawater and soil and shown as essential actor in nitrogen cycle in various environments (Prosser and Nicol, 2008). Therefore, archaea can be also considered as an essential component of the microbial community in compost. Indeed, we detected archaeal *amoA* gene encoding ammonia monooxygenase subunit A from cattle manure compost (Yamamoto et al., 2010). However, there is no study about whole archaeal community in composting of animal manure.

In this study, we analyzed the archaeal community structure during composting process of cattle manure using culture-independent techniques to evaluate its composition and how it changed.

Materials and methods

Composting process and sampling

The composting experiment was performed at a field-scale facility in Field Science Center, Graduate School of Agricultural Science, Tohoku University (Miyagi, Japan). For composting experiment, 1,140 kg of dairy cattle manure and 230 kg of sawdust were used. The mixture was then piled and stirred with a shovel loader 3 or 5 times per week for 30 days.

Analysis of chemical and physical parameters

The temperature was automatically measured with a temperature/humidity data logger (TR-71S; T&D Corporation, Nagano, Japan) at a depth of 30 cm from the surface. Moisture content was determined by measuring weight of samples after these were placed in drying oven at 105˚C overnight. PH was measured using a pH meter (WM-22EP; DKK-TOA Corporation, Tokyo, Japan).

DNA extraction

We selected 6 samples (day 0, 2, 6, 16, 24, and 30) for DNA extraction. Compost samples were collected at a depth of 30 cm from the surface before stirring and transferred to the laboratory on ice. All samples were freeze-dried overnight using a freeze dryer (FDU-830; Tokyo Rikakikai Co. Ltd., Tokyo, Japan) to maintain the water content at a low level and prevent loss of DNA during storage (Miller et al., 1999; Reuter et al., 2009). Total DNA was extracted from 0.025 g freeze-dried compost using PowerSoilê DNA Isolation kit (MO Bio Labs, Inc., Carlsbad, CA, USA). The extracted DNA was then dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR, cloning analysis, and sequencing

Approximately 1,400 bp of archaeal 16S rRNA gene was amplified using a primer set listed in Table 1. PCR was performed using Ex Taq (Takara Bio Inc., Shiga, Japan) with an iCycler (Bio-Rad Labs Inc., Hercules, CA, USA). PCR products with correct DNA fragments were cloned using Novagen Perfectly Blunt Cloning kits (EMD Chemicals Inc., San Diego CA, USA), according to the manufacturer's instructions. Clones with objective DNA fragments were then selected and amplified using the primer set that targets the cloned plasmid (pT7Blue vector, EMD Chemicals Inc.) (Table 1). PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA). The purified products were sequenced using the BigDye Terminator Cycle Sequencing kit v.1.1 (Applied Biosystems, Foster City, CA, USA). Obtained products were analyzed using an ABI PRISM 3100-Avant Autosequencer (Applied Biosystems).

Table 1. Primer sets used in this study

Name	Sequence (5'-3')	Targets	Reference
Arch21f	TTCCGGTTGATCCY[1]GCCGGA	Archaeal 16S rRNA gene	DeLong (1992)
1492r	ACGGY[1]TACCTTGTTACGACTT	16S rRNA gene	DeLong (1992)
T7	TAATACGACTCACTATAGGG	Plasmid DNA	EMD Chemicals Inc. (WI, USA)
U19	GTTTTCCCAGTCACGACGT	Plasmid DNA	EMD Chemicals Inc. (WI, USA)

[1]C or T

Construction of clone library

The sequences were assembled using Seqscape software (Applied Biosystems). After chimeric sequences were removed from the library using the Pintail program, the analyzed sequences were compared to sequences registered in the database of the DNA Databank of Japan (DDBJ) using the BLAST www system (http://blast.ddbj.nig.ac.jp/top-j.html). They were divided into operational taxonomic units (OTUs) for sequences with >97% homology to each other (McCaig et al., 1999).

Results

Changes in chemical and physical parameters during the composting process

Changes in temperature and moisture content are shown in Fig. 1. The temperature was about 14˚C at the beginning of the composting process (Fig. 1a). After the examination was started, it increased rapidly within 2 days. The highest temperature was recorded on day 5 (77.9˚C) and kept >60˚C for 18 days. From day 21, the temperature began to decrease and reached 29.3˚C at the end. The initial moisture content was about 67% (Fig. 1b). It continued to decline from day 8 and reached low value (about 30%). The pH was mildly alkaline (8.2–8.9) during the composting process except for on day 0.

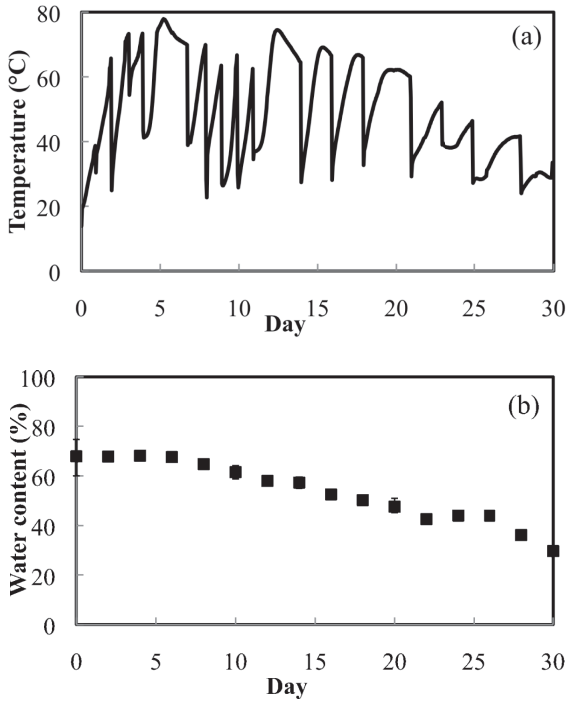

Fig. 1. Changes in (a) temperature and (b) water content during the composting process.

Archaeal community structure during the composting process

Number of sequenced clones was from 36 (day 6) to 78 (day 24). In total, 14 OTUs were generated and almost OTUs had most related species grouped into either the methanogens or AOA (Fig. 2). OTU1 was detected on day 0 and day 2 and nearly identical to the sequence originating from the groundwater. Other OTUs (i.e. OTU7) detected from only day 0 and day 2 were mainly related to uncultured methanogens originated from animal rumen or manure with relatively high homology. *Methanomicrococcus*-like sequences were obtained from only day 2 (OTU4). OTU2 was the large part of total clones during composting process. It was close to uncultured thermophilic *Methanosarcina* spp. with high homology while OTU1 was absent or observed at low abundance. OTU12 was detected from all investigated samples. It was closely related to '*Candidatus* Nitrososphaera gargensis' with high homology (98%). OTU13 and OTU14 had uncultured sequences as most relative sequences but closet sequences for both OTUs were obtained from same soil environment (Bintrim et al., 1997).

Discussion

Clone library indicated that both methanogen and AOA were the dominant archaeal species during the composting process of cattle manure. The archaeal community structure found in the present study had some differences from previous reports analyzing

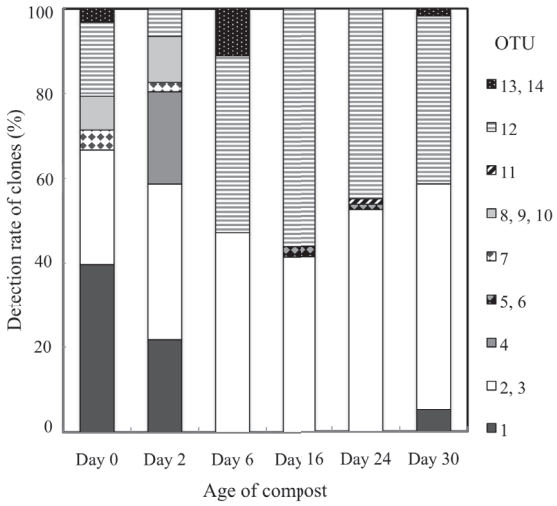

Fig. 2. Relative abundance of the OTUs in the composting materials. An OTU consisted of clones with more than 97% homology.

composting materials. For example, we detected some OTUs with sequences from animal waste and rumen as most relatives. Four OTUs (OTU4, 8–10) were closely related to uncultured methanogens found in animal rumen, intestine, and in anaerobic digesters (Snell-Castro et al., 2004).

OTU2 became the most dominant OTU in the clone libraries on days 6, 16, 24, and 30. These sequences related an uncultured clone from a thermophilic anaerobic waste digester (Tang et al., 2004) and grouped into themophilic *Methanosarcina* spp. Thummes et al. (2007) also detected some clones grouped into this cluster from different composting materials. Gattinger et al. (2007) reported themophilic *Methanosarcina*-like sequences were detected in fertilized soil. Thus, *M. thermophila* appears to adapt to the composting environment and increase its detection rate after other methanogens originated from cattle manure have decreased in abundance due to high temperature.

He et al. (2000) reported an anaerobic microsite inside composting food waste particles. Our results indicate that the methanogenic community was present within anaerobic sites of composting material under aerobic condition. In addition, methanogens might transit from compost to soil when compost is applied to soil (Cahyani et al. 2004; Gattinger et al. 2007).

It's notable that AOA-like sequences were detected throughout composting process. Thummes et al. (2007) analyzed about 120 clone sequences using composting materials, however, no sequence belonging AOA was detected. Our study indicated that AOA was an essential component of the archaeal community in compost, especially from days 6 to 30. OTU12 had high similarity to those of *Candidatus* Nitrososphaera gargensis, which obtained from hot springs (Hatzenpichler et al. 2008). One possible assumption is that the member of OTU12 might derive from finished composting materials produced in the same facility. It is highly possible that AOA usually present and is critical to nitrification in cattle manure compost since our previous study showed the existence of archaeal *amoA* gene encoding ammonia monooxygenase, the key enzyme responsible for ammonia oxidation (Yamamoto et al., 2010).

In the present study, archaeal community structure during composting process using cattle manure was displayed for the first time. Archaeal community was mainly consisted of methanogen and AOA. At the initial stage of composting, some methanogens originated from animal manure or rumen was dominated. After reaching high temperature, thermophilic *Methanosarcina*-like species were the most dominant methanogens because they could adapt to increasing temperatures. In addition, we detected AOA from compost for the first time and found that they existed throughout the composting process. It was also suggested that AOA could actively involve in nitrification of composting systems. This study provides the importance for studying archaeal community to understand microbiology of compost.

Acknowledgements

This work was supported, in part, by the Foundation of the Ministry of Education, Culture, Sports, Science and Technology, Japan, as a "Project of Integrated Compost Science" and by a grant from the Livestock Technology Association, Japan.

References

Bernal, M. P., J. A. Alburquerque, and R. Moral (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour Technol, 100: 5444-5453.

Bintrim, S. B., T. J. Donohue, J. Handelsman, G. P. Roberts and R. M. Goodman (1997) Molecular phylogeny of Archaea from soil. Proc. Natl. Acad. Sci. USA, 94: 277-282.

Cahyani, V. R., K. Matsuya, S. Asakawa and M. Kimura (2004) Succession and phylogenetic profile of methanogenic archaeal communities during the composting process of rice straw estimated by PCR-DGGE. Soil. Sci. Plant. Nutr., 50: 555-563.

DeLong, E. F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci.USA, 89: 5685-5689.

Gattinger, A., M. G. Höfle, M. Schloter, A. Embacher, F. Böhme, J. C. Munch and M. Labrenz (2007) Traditional cattle manure application determines abundance, diversity and activity of methanogenic Archaea in arable European soil. Environ. Microbiol., 9: 612-624.

Gong, C. H., J. Koshida, K. Inoue and T. Someya (2005) Fluorescence direct count of bacteria in various manures and composts as compared with plate count. Jpn J Soil Sci Plant Nutr, 76: 401-410.

Guo, Y., N. Zhu, S. Zhu, and C. Deng (2007) Molecular phylogenetic diversity of bacteria and its spa-

tial distribution in composts. J. Appl. Microbiol., 103:1344-1354.

Haga, K. (1999) Development of composting technology in animal waste treatment. Asian-Aus. J. Ani. Sci., 12: 604-606.

Hao, X., C. Chang, F. J. Larney and G. Travis (2001) Greenhouse gas emissions during cattle feedlot manure composting. J. Environ. Qual., 30: 376-386.

Hatzenpichler, R., E. Lebedeva, E. Spieck, K. Stoecker, A. Richter, H. Daims and M. Wagner (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proc. Natl. Acad. Sci. USA, 105: 2134-2139.

He, Y., Y. Inamori, M. Mizuochi, H. Kong, N. Iwami and T. Sun (2000) Measurements of N_2O and CH_4 from the aerated composting of food waste. Sci. Total Environ., 254: 65-74.

Insam, H. and M. de Bertoldi (2007) Microbiology of the composting process. L. Diaz, M. de Bertoldi, W. Bidlingmaier and E. Stentiford (Eds.), Compost Science and Technology, Elsevier Ltd., Oxford, UK, 26-48.

McCaig, A., L. Glover and J. Prosser (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol., 65: 1721-1730.

Miller, D. N., J. E. Bryant, E. L. Madsen and W. C. Ghiorse (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl. Environ. Microbiol., 65: 4715-4724.

Prosser, J. I. and G. W. Nicol (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ. Microbiol., 10: 2931-2941.

Reuter T., W. Xu, T. Alexander, K. Stanford, Y. Xu and T. McAllister (2009) Purification of polymerase chain reaction (PCR)-amplifiable DNA from compost piles containing bovine mortalities. Bioresour. Technol., 100: 3343-3349.

Snell-Castro, R., J. Godon, J. Delgenès and P. Dabert (2005) Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. FEMS Microbiol. Ecol., 52: 229-242.

Tang, J., T. Kanamori, Y. Inoue, T. Yasuta, S. Yoshida and A. Katayama (2004) Changes in the microbial community structure during thermophilic composting of manure as detected by the quinone profile method. Process Biochem., 39: 1999-2006.

Thummes, K., J. Schäfer, P. Kämpfer and U. Jäckel (2007) Thermophilic methanogenic Archaea in compost material: occurrence, persistence and possible mechanisms for their distribution to other environments. Syst. Appl. Microbiol., 30: 634-643.

Yamada, T., A. Suzuki, H. Ueda, Y. Ueda, K. Miyauchi and G. Endo (2008) Successions of bacterial community in composting cow dung wastes with or without hyperthermophilic pre-treatment. Appl. Microbiol. Biotechnol., 81: 71-81.

Yamamoto, N., K. Otawa and Y. Nakai (2009) Bacterial communities developing during composting processes in animal manure treatment facilities. Asian-Aus. J. Ani. Sci., 22: 900-905.

Yamamoto, N., K. Otawa and Y. Nakai (2010) Diversity and abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea during cattle manure composting. Microb. Ecol., 60: 807-815.