Bioresource Technology 120 (2012) 70–77

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Improved composting of poultry feces via supplementation with ammonia oxidizing archaea

Kaizhi Xie [a,b], Xiaoshan Jia [b], Peizhi Xu [a,*], Xu Huang [a], Wenjie Gu [a], Fabao Zhang [a], Shaohai Yang [a], Shuanhu Tang [a]

[a] Soil and Fertilizer Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[b] Department of Environmental Science, School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China

HIGHLIGHTS

- The seeding of poultry feces-based compost with AOA accelerated composting.
- The retention of nitrogen in the compost was improved by AOA supplementation.
- A spectrum of AOA species was present after the composting process was completed.

ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 10 June 2012
Accepted 13 June 2012
Available online 21 June 2012

Keywords:
Ammonia oxidizing archaea
Aerobic composting
PCR-DGGE
Nitrogen transformation
Redundancy analysis

ABSTRACT

Ammonia-oxidizing archaea (AOA) play an important role in the oxidation of ammonia. However, the participation of AOA in the composting process has not been established. The addition of AOA to a compost mix was able to speed up both the onset of the hyperthermic phase and the composting time. The composition of the microflora and the relative abundance were determined by using denaturing gradient gel electrophoresis and quantitative real-time PCR, based on the presence of the archaeal *amoA* genes. The amplicon profiles allowed some of the major AOA species present in the final compost to be identified, and their relative abundance to be estimated from their amplification intensity. The lower pH during the lower temperature phase of compost served to enhance the nitrogen content of the final compost. The addition of AOA resulted in the expanding diversity of microflora species than that of the natural colonization.

1. Introduction

Aerobic composting is an effective and productive means of treating solid organic waste such as poultry feces (Wei et al., 2000), but the process involves a significant loss of nitrogen (Barrington et al., 2002; Eklind and Kirchmann, 2000; Gu et al., 2011). Since the organic nitrogen content of feces is readily converted by microorganisms into ammonia and the nitrate decomposed into the greenhouse gas nitrous oxide, much of the nitrogen loss can be explained by the escape of gaseous ammonia and the denitrification of nitrate (Martins and Dewes, 1992). These losses not only reduce the industrial value of the compost, but are also environmentally detrimental (Martins and Dewes, 1992; Myles et al., 2000; Wigley and Raper, 2001). The microbial ammoxidation process and its contribution to the global nitrogen cycle has received a good deal of research attention (Oved et al., 2001), and it is now widely accepted that the Alphaproteobacteria ammonia oxidizing bacteria (AOB) are the major agents of nitrification (Purkhold et al., 2000). The application of DNA-based analytical methods which do not rely on *in vitro* cell culture has, however, uncovered other microorganisms with the ability to oxidize ammonia, in particular the ammonia-oxidizing archaea (AOA) (Könneke et al., 2005). AOA species are characterized by the presence of a gene encoding ammonia monooxygenase (Venter et al., 2004) in a form which is evolutionarily distinct from the gene carried by AOB species.

AOA species have been identified in many ecological niches, including the oceans (Francis et al., 2007), soils (Leininger et al., 2006), freshwater lakes (Herrmann et al., 2009) and thermal springs (Gerhard et al., 2007). Analysis of the nitrogen cycle in freshwater lakes has indicated that AOA are 8000 times more abundant than AOB (Herrmann et al., 2008), while the use of *amoA* gene as a species group diagnostic led Leininger et al. (2006) to suggest that AOA were up to 3000 times more abundant in the soil than AOB. The same analytical technique applied to the oceanic

* Corresponding author. Tel.: +86 020 85161405; fax: +86 020 85161437.
E-mail address: pzxu007@163.com (P. Xu).

0960-8524/$ - see front matter
http://dx.doi.org/10.1016/j.biortech.2012.06.029

environment showed that the population of AOA was between one and three orders of magnitude higher than that of AOB (Mincer et al., 2007). AOA species are present in organic compost, perhaps even more abundantly than AOB ones (Yamamoto et al., 2010).

The oxidation of ammonia is an important step in the composting process because for plants, nitrate is their most easily accessible form of nitrogen (Bernal et al., 2009). They may therefore well be an important agent of ammonia oxidation in the composting process (Yamamoto et al., 2011). Much of the research focus to date regarding AOA species has concentrated on their distribution in nature and on their suitability as a model for bacterial metabolism (Park et al., 2006). As yet there has been little effort made to document their contribution to the oxidation of ammonia in compost. Here, a range of DNA-based analytical techniques was applied to study the effect of deliberate supplementation of poultry feces with AOA. The aim was to follow the process of nitrogen transformation, and to characterize the abundance and population structure of AOA developed during the composting process. The data are particularly relevant for efforts to accelerate the composting process and to enhance the retention of nitrogen.

2. Methods

2.1. AOA enrichment and quantification

High levels of AOA have been identified in recycled sludge treated using the A2/O technique (Park et al., 2006; Zhang et al., 2009). The AOA population was enriched by incubating 6.1 g active sludge in 500 mL of enrichment culture medium (0.025 g/L KH_2PO_4, 1.25 g/L $KHCO_3$, 7.5 g/L casamino acids, 10 g/L yeast extract, 3 g/L Na_3-citrate, 2 g/L KCl, 0.1 g/L $MgSO_4.7H_2O$, 10 g/L Na_2CO_3, pH 7.2). The culture was maintained for ten days at 30 °C in a thermostatic shaker rotating at 140 rpm, centrifuged (5200*g* for 20 min) and the sediment transferred to 800 mL enrichment culture medium for a further 20 days, sufficient to allow for five generations of AOA growth. The number of AOA per ml of culture was estimated by the "most probable number" PCR (MPN-PCR) method (Degrange et al., 1998), using as template a serial dilution (10^{-1}, 10^{-2}, $l0^{-3}$, 10^{-4} and 10^{-5}) of DNA extracted from the culture with a ZR Fecal DNA MidiPrep kit (Zymo Research, USA) and a nucleotide extraction Fast Prep FP220 instrument (BIO 101 Systems, USA). Prior to its dilution, the DNA was purified using a DNA purification kit (MonoFas, GL Sciences, Japan) following the manufacturer's instructions, and quantified using a Gene Quant Pro S (Amersham, Japan) device. The Arch-amoAR (5′-GCGGCCATCCATCTGTATGT) and Arch-amoAF (5′-STAATGGTCTGGCTTAGACG) primer pair (Francis et al., 2007) was used to amplify the *amoA* gene, with the addition of a GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC) to the 5′ end of Arch-amoAF to improve amplicon separation (Maeda et al., 2010). Each 50 μL PCR comprised 5 μL 10 × PCR buffer (Mg^{2+} plus), 4 μL 2.5 mM dNTP, 0.5 μL Ex Taq HS polymerase (5 U/μL, Takara Biotechnology, Japan), 1 μL of each primer(25 μM) and 2 μL DNA. The amplification regime consisted of a 95 °C/4 min denaturation, followed by 35 cycles of 95/30, 58/20, 72 °C/35 s, and a final extension step of 72 °C/5 min.

2.2. Composting process

Chicken feces were obtained from a farm located at the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, mushroom residue from a Xinghe edible fungus factory in Dongguang City, Guangdong province, and rice husk and bran from the Institute of Rice, Guangdong Academy of Agricultural Sciences. The poultry feces, rice husks, rice bran and mushroom residue were mixed in a 5:1:1:3 ratio by volume and maintained at 55–60% relative humidity (other characteristics of these materials are given in Table 1). This experiment was run from 12th May 2011 to 25th June 2011 at the Soil & Fertilizer Research Institute, Guangdong Academy of Agricultural Sciences. The forced ventilation aerobic composting process was performed in composting trough of length 60 m, width 2 m and height 1.5 m. A forced air ventilator, installed at the base of the composting trough, delivered 3000 L/min continually for 17 min every half hour.

Windrows (1.8 m width, 1.6 m height) were turned every 2–3 days using a self-propelled Sandberger (Neuson Hydraulic, Linz, Austria) turner, and immediately prior to sampling, the windrows were turned at least once. Humidity was manually controlled before each turning operation, and water was added where necessary (See Table 2).

2.3. Experimental design and sample collection

Two contrasting treatments were applied to the compost. The first involved supplementation with 5% w/v enriched AOA (treatment "T") and the other supplementation with 5% w/v heat-sterilized enriched AOA (treatment "CK"). The composting trough was divided into six compartments at intervals of 3 m and was

Table 1
Physiochemical characteristics of the raw materials used for composting (dry weight basis determined from triplicate samples).

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

Table 2
The development of AOA populations during composting.

Methods	Treatment	12d	25d	30d	45d
MPN-PCR		CFU/g dry sample			
	CK	$1.25 \pm 0.01 \times 10^6$	$6.00 \pm 1.44 \times 10^6$	$5.75 \pm 0.21 \times 10^6$	$1.25 \pm 0.75 \times 10^4$
	T	$7.00 \pm 0.50 \times 10^8$	$1.50 \pm 0.54 \times 10^9$	$1.75 \pm 0.41 \times 10^8$	$2.50 \pm 0.75 \times 10^4$
Q-PCR		*amoA* gene copy numbers/g dry sample			
	CK	$1.76 \pm 0.09 \times 10^5$	$2.12 \pm 0.44 \times 10^6$	$4.25 \pm 1.33 \times 10^6$	$2.87 \pm 0.53 \times 10^4$
	T	$9.64 \pm 0.06 \times 10^7$	$8.95 \pm 0.26 \times 10^8$	$1.30 \pm 0.32 \times 10^8$	$3.08 \pm \times 0.5710^4$

Note: data given as means ± SED.