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Original Research Article

Diversity and Abundance of Ammonia-Oxidizing Bacteria and Archaea in a Freshwater Recirculating Aquaculture System

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ABSTRACT

The role of ammonia-oxidizing bacteria and archaea was evaluated using *amoA* gene in a freshwater recirculating system. Broken earthen pot pieces (BEP) were used as filter bed material. Five archaeal and four bacterial operational taxonomic units were retrieved from *amoA* genes. Shannon-Weiner and Simpson indices were higher in archaeal *amoA* sequence compared with the bacteria. Subtype diversity ratio and subtype diversity variance were 0.522 and 0.008, respectively, for archaea and 0.403 and 0.015, respectively, for bacteria. In archaea, 50% *amoA* sequences showed 99%–100% similarity with the known sequences of ammonia monooxygenase subunit A of uncultured archaeon clones and thaumarchaeote. In bacteria, 84% sequences showed 99% similarity with *amoA* sequences of different uncultured bacterial clone and Nitrosomonadaceae. Absolute quantification showed that the abundance of archaea was 12-fold higher compared with bacteria. In this recirculating system, ammonia-oxidizing archaea and bacteria played a major role; BEP supported the growth of these ammonia-oxidizing microorganisms.

1. Introduction

Recirculating aquaculture system is based on the reutilization of waste water involving microorganisms as a tool that reduces the nitrogenous wastes in the culture system. In intensive aquaculture, the main problem is the accumulation of toxic nitrogenous metabolites derived from excess, uneaten feed and excreta. Removal of these nitrogen metabolites is carried out by the process of biofiltration with the help of ammonia-oxidizing microbes. Furthermore, the treatment is completed by nitrite-oxidizing bacteria; denitrifying bacteria releases the free nitrogen into the atmosphere, else absorbed by the plants as nutrients. It is a great challenge for the aquaculturists to remove these toxic metabolites from the culture systems. Ammonia-oxidizing bacteria (AOB), two narrow clades of beta- and gamma-proteobacteria are considered to be the only members that play major role in the global nitrogen cycle (Prosser 1989; Prosser and Nicol 2008). The molecular study shows that members of the Crenarchaeota (within the archaeal domain) also play an important role in nitrification (Hansel *et al.* 2008; Könneke *et al.* 2005; Tourna *et al.* 2008). *Nitrosopumilus maritimus*, a strain of marine ammonia-oxidizing archaea (AOA), has been isolated from nitrifying filtration unit of the Shedd Aquarium (Könneke *et al.* 2005). AOA play an important role in nitrification and their *amoA* genes serve as marker for diversity and abundance (Pester *et al.* 2012). The *amoA* gene is a function-specific target for the detection of AOB (Kowalchuk and Stephen 2001).

The success of recirculating system is determined by stable and reliable performance of its biofilter. The maintenance of biofilter is most important for a start-up recirculating system as well as for a functional one (Badiola *et al.* 2012). The selection of proper substrate for biofilter is the next critical step as it influences the efficiency of water treatment and operational cost (Summerfelt 2006). A perfect biofilter should be easily available, non-poisonous, non-reactive and cost-effective. It should remove all the nitrogenous metabolites from the effluent and support the growth of dense populations of nitrifying microbes. Study shows that no biofilter has all these characteristics; each has its own advantages and disadvantages (Michaud 2007; Rusten *et al.* 2006). Various biological filters like trickling filters, rotating biological contactors, granular substrate biofilters, submerged fixed substrate biofilters (static bed), mobile substrate biofilters (moving bed) are used in recirculating system. The static bed filter has been used in the present study. This filter supports excellent volumes of water with good purification rate. A wide variety of substrates viz. rocks, shells, sand, corals, ceramic, expanded clay, plastic bio balls, etc. are commercially available as biofilter substrates (Malone and Pfeiffer, 2006).

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Replacement of such commercial products with traditional materials will significantly reduce the operational cost of the recirculating system. In the present study, broken earthen pot pieces (BEP) are used as filter bed material. The capability of these pieces to support the growth of ammonia-oxidizing microbes has been evaluated using *amoA* gene as marker.

2. Materials and Methods

2.1. Experimental set-up

The recirculating system consisted of three rectangular fish culture tanks (500 L, each) and a filtration unit (600 L). This recirculating system was kept inside the Aquahouse and the temperature was uniformly maintained inside this house. In the filtration unit, a 20-cm thick layer of BEP (7 × 3.5 cm) were used as substratum for the growth of microorganisms. These BEP are easily available, indigenous, cheaper and non-toxic to fish. These are porous in nature with large surface area which facilitates the growth of microbes. Indian major carp catla *Catla catla* fingerlings (55 ± 5 g) were cultured in this recirculating system. The stocking density was 20 fish/tank. The duration of circulation was 6 h/d. The water temperature in the filter bed was 25°C ± 1°C during the study period. Dissolved oxygen was monitored using portable oxygen meter (Intellical LDO101, Hach, USA) and the level was maintained at 5 mg/L with the help of aerator. The water quality of the fish culture tanks after treatment in the filtration unit were as follows: temperature 25.2°C ± 1°C, dissolved oxygen 6.17 ± 0.20 mg/L. pH was measured with probe (Intellical PHC101) and ranged from 7.8 to 8.25. Ammonia, nitrite and nitrate levels were 0.15 ± 0.01, 0.55 ± 0.02 and 0.11 ± 0.04 mg/L, respectively, during the culture period. Ammonia was measured with Orion Ion Analyzer (Thermo Scientific, Massachusetts, USA). Nitrite and nitrate were analysed following the standard methods (APHA 1998). The whole experiment was conducted following the guidelines of the Institutional Animal Ethics Committee (565/GO/ReBi/S/02/CPCSEA).

2.2. Batch activity test

Twelve pieces of BEP were collected from the filter bed of the recirculating system and were equally distributed in three beakers (1 L) containing 500 mL of synthetic wastewater (Table 1). The synthetic wastewater was prepared following the method of Munz *et al.* (2011). The beakers were partially closed with aluminium foil. Water sample from each beaker was collected daily and ammonia, nitrite and nitrate levels were measured. The rate of change in concentration of nitrogenous materials confirmed the nitrification process.

2.3. DNA extraction, quantification of DNA and PCR amplification

For the recovery of microbial cells, the BEP were collected from the filter bed. The outer layer of broken earthen pot was scrapped, ground to powder and used for DNA extraction. DNA was isolated from 10 g of BEP powder using PowerMax DNA Isolation Kit (MO BIO Inc., Carlsbad, CA, USA) with slight modifications as per the manufacturer's recommendations. A 1.2 mL of the solution containing sodium dodecyl sulfate (SDS) and other disrupting agents were added to the tube and vortex for 30 s to lyses the cell membrane by breaking down the associated fatty acids and lipids. These tubes were placed to the half-filled plastic bottles and kept at the incubator shaker, shaking at the maximum speed (300 rpm) for 45 min at 60°C and the rest of the protocol was followed as per the kit's manual.

Table 1. Composition of synthetic wastewater used for the batch activity study

Composition	Quantity (mg/L)
Beef extract	90
Yeast extract	90
$MnSO_4$	1.22
$FeSo_4$	10.1
KCl	3.125
K_2HPO_4	87.6
$NaHCO_3$	163.5
$CaCl_2$	1055
$MgSO_4$	10.88
NH_4Cl	1055

The isolated DNA was checked through 0.8% agarose gel. The concentration was measured using NanoDrop 1000 (Thermo Scientific). Amplification of DNA was performed with peqSTAR 2X Double block thermocycler, peqlab, using the following conditions. A total of 50 μL reaction mixture was used with 25 μL 2X Master Mix (Thermo Scientific Lithuania, California, USA), 25 ng DNA templates, 2.5 μL forward (FW) and 2.5 μL reverse (RV) primers of bacterial *amoA*—1F: 5′GGGGTTTCTACTGGTGGT 3′and 2R: 3′CCCCTCKGSAAAGCCTTCTTC 5′ (Rotthauwe *et al.* 1997) and archaeal *amoA* A—5′ STAATGGTCTGGCTTAGACG 3′ and R: 3′ GCGGCCATCCATCTGTATGT 5′ (Francis *et al.* 2005). The rest volume was made up with nuclease free water. The amplification programmes of archaeal *amoA* was 95°C for 4 min; 30 cycles consisting of 94°C for 45 s, 53°C for 45 s and 72°C for 60 s; and 72°C for 15 min. The amplification programme of bacterial *amoA* was little modified from the method of Rotthauwe *et al.* (1997). Initial denaturation was at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 30 s and extension at 72°C for 30 s; final extension was at 72°C for 10 min. All polymerase chain reaction (PCR) results were confirmed with 1.2% (w/v) agarose gel electrophoresis.

2.3.1. Cloning, sequencing and phylogenetic analysis

The triplicate PCR products were pooled and purified using NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The purified product was cloned to construct library using PBase-TA Cloning Kit (The Gemini, Singapore). The recombinant plasmids were transformed and positive colonies were randomly selected and sequenced (ABI PRISM 3730xl Genetic Analyzer, Applied Biosystems, Foster City, USA) with the help of 1st BASE (The Gemini, Singapore). The raw sequences obtained from the sequencer were screened for vector contamination using NCBI's VecScreen (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen/VecScreen.htmL). Whereas, operational taxonomic units (OTUs) were extracted by clustering sequences, using CD-HIT Suite (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=cd-hit-est). Phylogenetic analysis was performed by aligning the sequences with *Clustal X* (version 2.1) (Conway Institute, University College Dublin, Ireland, UK), and the neighbour-joining tree was constructed (Jukes and Cantor 1969) with the reference sequences of known/previously reported sequences of archaeal and bacterial *amoA* and clone sequences derived from BEP biofilter using MEGA 7 (The Pennsylvania State University, Philadelphia, USA) (Pester *et al.* 2012; Purkhold *et al.* 2000; Zhou *et al.* 2016). The ecological indices like Shannon-Weiner index (H′; Shannon 1948) and Simpson index (D; Simpson 1949) were calculated using the number of OTUs.

The *amoA* gene sequences were submitted to the NCBI. These are the accession numbers: KP272121—KP272128 and KP259843—KP259869.

2.4. Absolute quantification of *amoA* genes

Copy numbers of archaeal *amoA* and bacterial *amoA* genes were determined in triplicate for both samples by quantitative real-time PCR (ViiA 7 Real-Time PCR System, Applied Biosystems). The assay was performed in MicroAmp Fast Optical 96-Well Reaction Plates (Applied Biosystem, USA) with optical adhesive cover. New sets of primers of archaeal *amoA* (F: 5CATCCTAGAGCGGCAAAGGT3′ and R:

3′ACCCCAAGTGGGCAAATTCT5′) and bacterial *amoA* (F: 5′TGGCTCGTGACAGCGTTAAT3′ and R: 3′TACGATTGGCAAGTGGGTCG5′ (with smaller product sizes of 106 and 95 bp) were designed from the sequences of KP272121 and KP259843 using Primer3 software (BLST – NCBI, Maryland, USA) (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The product size of the previous primer was not suitable for use in real-time PCR. In real-time PCR, amplifications were carried out together with standards, constructed on the dilutions of plasmids of archaeal *amoA* (1.01×10^8 to 1.01×10^4) and bacterial *amoA* (9.5×10^{10} to 9.5×10^3). The total reaction volume (10 μL) consisted of 1 μL of genomic DNA, 0.5 μL of FW and RV primers (2.5 μM each), 5 μL of 2X Power SYBR Green PCR Master Mix (Applied Biosystem, USA) and 3 μL of PCR-grade H_2O (Merck, Darmstadt, Germany). The PCR amplification was performed under the following conditions: 50°C for 2 min, initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C, at 60°C for 1 min. The reaction carried out without DNA, served as a negative control. The amplification efficiencies of archaeal and bacterial *amoA* were 93.8% and 104.9%, respectively. To confirm the single target fragment of the PCR-amplified products, dissociation curves were analysed and plotted at the end of every quantitative real-time PCR reaction.

2.5. Statistical analysis

Student's t test ($p < 0.001$) was used to compare the quantity of *amoA* gene of archaea and bacteria.

3. Results

3.1. Batch activity test

A change in concentrations of ammonia, nitrite and nitrate confirmed the nitrification in the selected filter bed materials. The initial concentration of ammonia was 131.5 mg/L. The concentration of ammonia (Figure 1A) gradually decreased, whereas the concentrations of nitrite (Figure 1B) and nitrate (Figure 1C) gradually increased during the study period. After 5 days of incubation, the final concentrations of ammonia, nitrite and nitrate were 31.08 ± 2.94, 0.082 ± 0.014 and 7.23 ± 0.62 mg/L, respectively.

3.2. Phylogenetic analysis of sequences

A total of 55 (23 and 32 sequences of archaeal and bacterial *amoA*) clones were screened and sequenced to study the community structures of AOA and AOB in this BEP biofilter of recirculating aquaculture system. After the confirmation of their belonging to the ammonia-oxidizing microbes, the sequences were clustered into five OTUs for AOA and four OTUs for AOB, based on 97% similarity. Based on the phylogenetic tree constructed on clones derived from biofilter along with reference sequences, it was concluded that in the present study, four clones (1, 3, 6 and 7) were resembled to the cluster *Nitrososphaera* sp. The remaining clones represented different clades (Figure 2A).

Similarly, AOB sequences showed 99% similarity with alpha subunit of uncultured clones of Nitrosomonasdacea as well as the clones derived from other wastewater treatment plants and soil. Construction of phylogenetic tree revealed that 27th clone was similar with *Nitrosomonas oligotropha*, whereas 16 clones (1, 2, 7, 10, 11, 15, 21, 23, 24, 25, 27, 28, 29, 30, 31 and 32) of bacterial *amoA* were closely related to each other and *Nitrosomonas* sp. The rest of the clones belonged to different clades (Figure 2B). Shannon-Weiner index value was higher in archaeal *amoA* compared with the bacterial *amoA* (Table 2).

3.3. Absolute quantification of *amoA* genes

Copy numbers were calculated based on the standard constructed for both the genes. The values were 25.299×10^5 and 20.761×10^4 for archaea and bacteria, respectively. It was 12-fold higher in archaea compared with bacteria (Figure 3).

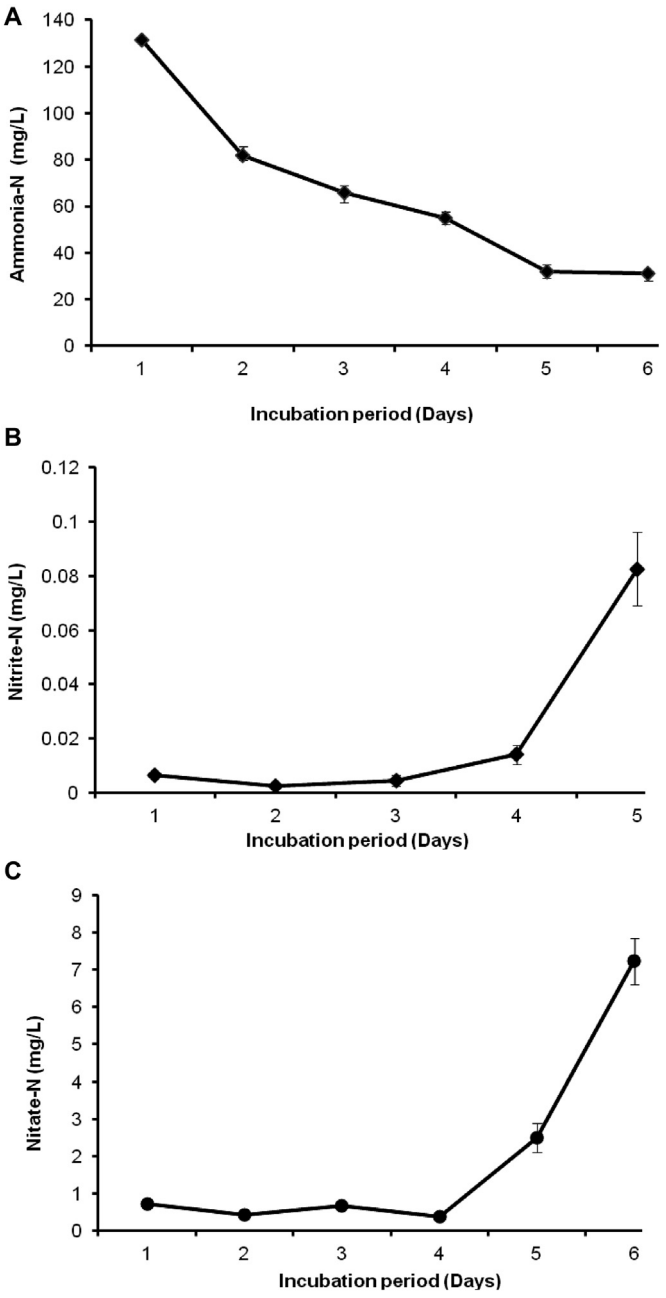

Figure 1. Rate of change of (A) ammonia-N, (B) nitrite-N and (C) nitrate-N during 5 days batch activity test. Values are given as mean ± SE ($n = 3$). SE = standard error.

4. Discussion

The first step of nitrification, the conversion of ammonia to nitrite was confirmed in the batch activity test of the present study. This nitrite subsequently transformed into nitrate. Ammonia level decreased, whereas the nitrate and nitrite levels increased because of the activity of microorganisms. Furthermore, the sequence analysis of *amoA* showed the presence of AOA and AOB in the BEP that were used in the filter bed of the recirculating system.

Shannon-Wiener and Simpson indices were higher in archaeal *amoA* genes compared with the bacteria. These indices indicated that the clone library of archaea had higher diversity of AOA

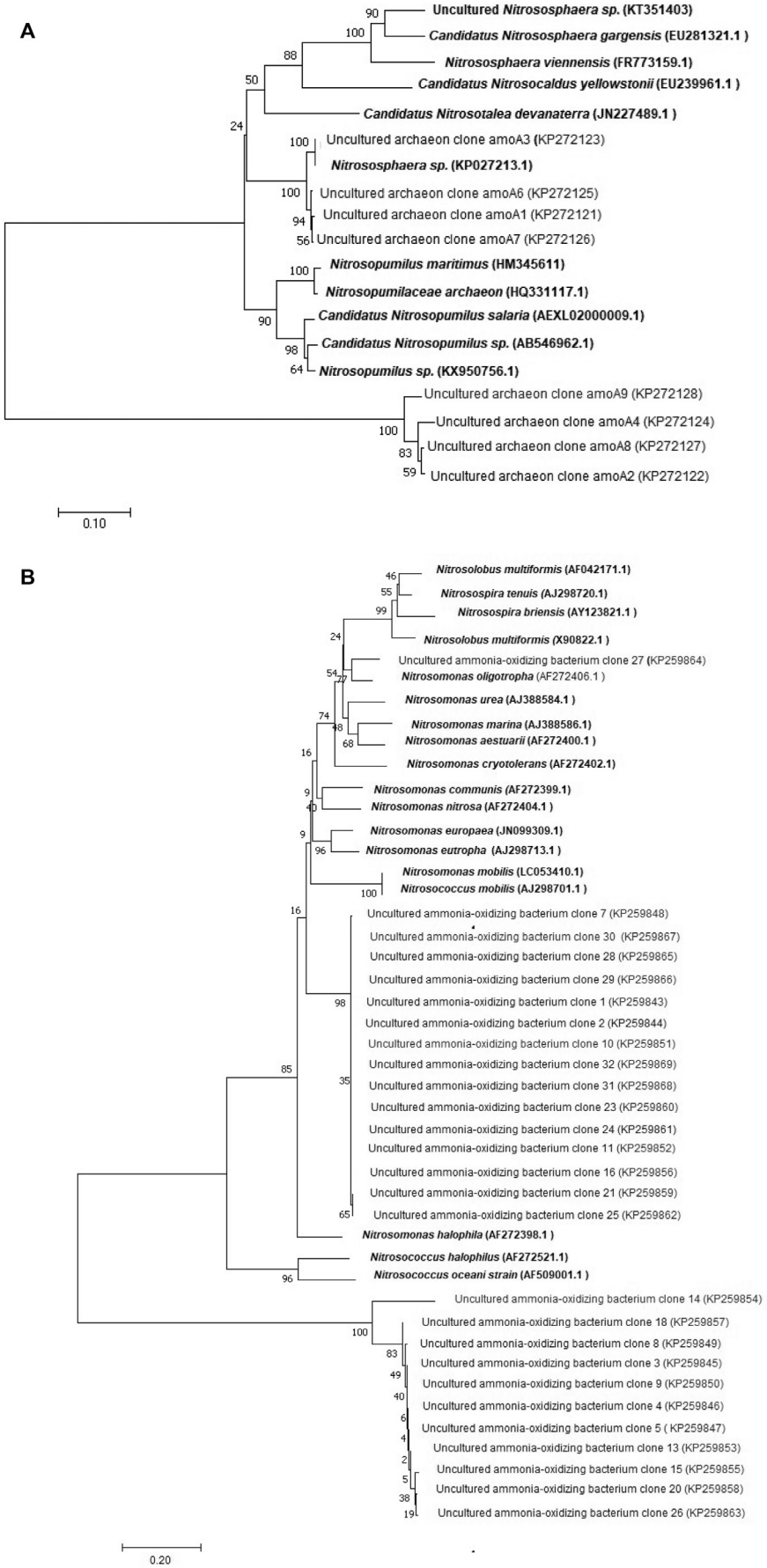

Figure 2. A phylogenetic tree (rectangular cladogram) constructed based on the sequences of (A) archaeal and (B) bacterial *amoA* gene, as determined by the neighbour-joining method. The number in the branches indicated the bootstrap value (1000 replicates).

Table 2. Shannon-Weiner and Simpson indices values in archaeal and bacterial *amoA*

Indices	Samples	
	Archaeal *amoA*	Bacterial *amoA*
Shannon-Weiner index (H′)	0.60	0.41
Simpson index (D)	0.99	0.57

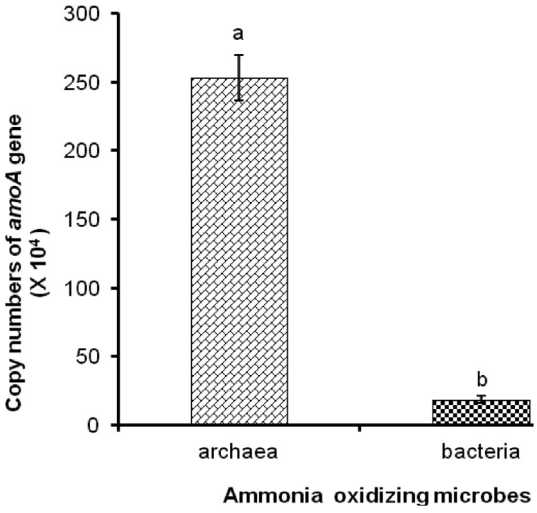

Figure 3. Abundance of *amoA* genes in archaea and bacteria.

compared with the bacteria. The condition of archaeal dominancy was reported from various habitats, like soil, marine sediments, aquarium filters, etc. Molecular surveys in the oligotrophic open and coastal ocean (based on the *amoA*) indicated that marine group I Crenarchaeota (AOA) was numerically dominant ammonia oxidizers compared with the AOB (Agogue *et al.* 2008; Beman *et al.* 2010; Church *et al.* 2010; Kalanetra *et al.* 2009; Mincer *et al.* 2007; Newell *et al.* 2011; Sintes *et al.* 2013). The dominance of archaeal ammonia oxidizers over bacteria was recorded in oceans (Lam *et al.* 2007; Wuchter *et al.* 2006), freshwater lakes (Herrmann *et al.* 2009) and soils (Martenes-Habbena *et al.* 2009). This shifting of diversity and abundance of AOA population were related to the concentration of ammonium and nitrite and pH of the culture system (Auguet *et al.* 2011; Verhamme *et al.* 2011).

The results of phylogenetic analysis revealed the presence of uncultured AOA clones of thaumarchaeote. Archaeal *amoA* gene sequences showed 99%–100% similarity with the known sequences of ammonia monooxygenase subunit A of uncultured archaeon and thaumarchaeote clones from biofilter of freshwater aquaria (Sauder *et al.* 2012). They also found that freshwater aquarium biofiltration was dominated by thaumarchaeote. In the present study, uncultured *Nitrosomonas* sp. might be the dominant community among the AOB. The pyrosequencing of *amoA* amplicons from 16 soils sampled in Austria, Costa Rica, Greenland and Namibia revealed that most of the soils were dominated by *Nitrososphaera* cluster (Pester *et al.* 2012). In the present study, the *amoA* sequences showed 99% similarity with the available *amoA* sequences of different uncultured bacterial clone and uncultured Nitrosomonadaceae. Similar result was found in other wastewater treatment plants (Bai *et al.* 2012). Amann *et al.* (1995) suggested that almost all microbial populations in filter materials seemed to be unculturable. These findings supported the results of the present investigation of uncultured clones of archaea and bacteria. The present study showed that AOA was not only more diverse compared with the AOB, but the abundance ratio was also many folds higher compared with the latter. The abundance of the gene encoding subunit of the key enzyme ammonia monooxygenase (*amoA*) in archaea (Crenarchaeota) were up to 3000-fold higher compared with the bacterial *amoA* genes (Leininger *et al.* 2006). In Hood Canal, the copy number of *amoA* gene of AOA was 2.3 to 60-fold higher compared with the AOB at all depths where ammonia oxidation occurred (Horak *et al.* 2013). High ratio of AOA/AOB *amoA* gene copy was also found in marine water, viz. the Gulf of California (Beman *et al.* 2008), the California Current (Santoro *et al.* 2010) and North Pacific Subtropical Gyre (Mincer *et al.* 2007). These results suggested that AOA were the dominant organisms involved in active ammonia oxidation. The abundance of archaeal *amoA* genes showed that archaea also played significant role in nitrification in the present study. Whereas Zhou *et al.* (2016) reported that in riparian sediment of Zhenjiang ancient canal, nitrification was more related to AOB compared with the AOA. Pester *et al.* (2012) suggested that dominance of the AOA and AOB were related with the geographical location and environmental conditions. Aquatic plants influenced the abundances and community structures of ammonia-oxidizing archaea and bacteria (Zhang *et al.* 2015). Both pure culture-based and environmental studies showed that at least some AOA had high substrate affinity for ammonia and were able to grow under extremely oligotrophic conditions (Schleper and Nicol 2010). AOA showed better adaptation with reference to substrate concentration and temperature variation (Horak *et al.* 2013). In the present study, the filter bed substrate, BEP provided optimum conditions for the diversity of the AOA.

In conclusion, the phylogenetic analysis and quantification of *amoA* genes of both archaea and bacteria confirmed that BEP supported the growth of microorganisms involved in nitrification. This is an efficient filter bed material for recirculating aquaculture system and can replace other costly substrates. In this system, AOA dominates AOB numerically, but both contributed in the functioning of the system, i.e. helped in the process of nitrification. This information may be useful to understand the molecular mechanism of ammonia oxidation in freshwater recirculating system.

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References

Agogué H, Brink M, Dinasquet J, Herndl GJ. 2008. Major gradients in putatively nitrifying and non-nitrifying archaea in the deep North Atlantic. *Nature* 456: 788–91.

Amann RI, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59: 143–69.

American Public Health Association, APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th edition. Washington, DC: American Public Health Association.

Auguet J-C, Nomokonova N, Camarero L, Casamayor EO. 2011. Seasonal changes of freshwater ammonia-oxidizing archaeal assemblages and nitrogen species in oligotrophic alpine lakes. *Appl Environ Microbiol* 77:1937–45.

Badiola M, Mendiola D, Bostock J. 2012. Recirculating aquaculture systems (RAS) analysis: main issues on management and future challenges. *Aquacult Eng* 51: 26–35. https://doi.org/10.1016/j.aquaeng.2012.07.004.

Bai Y, Sun Q, Wen D, Tang X. 2012. Abundance of ammonia-oxidizing bacteria and archaea in industrial and domestic wastewater treatment systems. *FEMS Microbiol Ecol* 80:323–30.

Beman JM, Popp BN, Francis CA. 2008. Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. *ISME J* 2: 429–41.

Beman JM, Sachdeva R, Fuhrman JA. 2010. Population ecology of nitrifying archaea and bacteria in the Southern California Bight. *Environ Microbiol* 12: 1282–92.
Church MJ, Wai B, Karl DM, DeLong EF. 2010. Abundances of crenarchaeal *amoA* genes and transcripts in the Pacific Ocean. *Environ Microbiol* 12:679–88.
Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci USA* 102:14683–8.
Hansel CM, Fendorf S, Jardine PM, Francis CA. 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl Environ Microbiol* 74:1620–33.
Herrmann M, Saunders AM, Schramm A. 2009. Effect of lake trophic status and rooted macrophytes on community composition and abundance of ammonia-oxidizing prokaryotes in freshwater sediments. *Appl Environ Microbiol* 75: 3127–36.
Horak REA, Qin W, Schauer AJ, Armbrust EV, Ingalls AE, Moffett JW, Stahl DA, Devol AH. 2013. Ammonia oxidation kinetics and temperature sensitivity of a natural marine community dominated by archaea. *ISME J* 7:2023–33.
Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HN (Ed.). *Mammalian Protein Metabolism*, Vol 13. New York: Academic Press, New York. 2 1-1 32.
Kalanetra KM, Bano N, Hollibaugh JT. 2009. Ammonia-oxidizing archaea in the Arctic Ocean and Antarctic coastal waters. *Environ Microbiol* 11:2434–45.
Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437: 543–6.
Kowalchuk GA, Stephen JR. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annu Rev Microbiol* 55:485–529.
Lam P, Jensen MM, Lavik G, McGinnis DF, Muler B, Schubert CJ, Amann R, Thamdrup B, Kuypers MMM. 2007. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proc Natl Acad Sci USA* 104: 7104–9.
Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442:806–9.
Malone RF, Pfeiffer TJ. 2006. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquacult Eng* 34:389–402.
Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. 2009. Ammonia oxidation kinetics determines niche separation of nitrifying archaea and bacteria. *Nature* 461:976–9.
Michaud L. 2007. Microbial communities of recirculating aquaculture facilities: interaction between heterotrophic and autotrophic bacteria and the system itself [PhD Dissertation]. Scienze Ambientali: Ambiente Marino e Risorse" (XVIII CICLO): University of Messina.
Mincer TJ, Church MJ, Taylor LT, Preston C, DeLong Karl DM, EF. 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environ Microbiol* 9: 1162–75.
Munz G, Lubello C, Oleszkiewicz JA. 2011. Modelling the decay of ammonium oxidizing bacteria. *Water Res* 45:557–64.
Newell SE, Babbin AR, Jayakumar A, Ward BB. 2011. Ammonia oxidation rates and nitrification in the Arabian Sea. *Global Biogeochem Cycles* 25:GB4016.
Pester M, Rattei T, Flechl S, Gröngröft A, Richter A, Overmann J, Reinhold-Hurek B, Loy A, Wagner M. 2012. *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of *amoA* genes from soils of four different geographic regions. *Environ Microbiol* 14:525–39.
Prosser JI, Nicol GW. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environ Microbiol* 10:2931–41.
Prosser JI. 1989. Autotrophic nitrification in bacteria. In: Rose AH, Tempest DW (Eds.). *Advances in Microbial Physiology*. New York: Academic Press, New York. pp. 125–81.
Purkhold U, Pommerening-Röser A, Juretschko S, Schmid MC, Koops HP, Wagner M. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. *Appl Environ Microbiol* 66:5368–82.
Rotthauwe JH, Witzel KP, Liesack W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* 63:4704–12.
Rusten B, Eikebrokk B, Ulgenes Y, Lygren E. 2006. Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacult Eng* 34:322–31.
Santoro AE, Casciotti KL, Francis CA. 2010. Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. *Environ Microbiol* 12:1989–2006.
Sauder LA, Peterse F, Schouten S, Neufeld JD. 2012. Low-ammonia niche of ammonia-oxidizing archaea in rotating biological contactors of a municipal wastewater treatment plant. *Environ Microbiol* 14:2589–600.
Schleper C, Nicol GW. 2010. Ammonia-oxidising archaea–physiology, ecology and evolution. *Adv Microb Physiol* 57:1–41.
Shannon CE. 1948. A mathematical theory of communication. *Bell Sys Tech J* 27: 379–423. and 623–656.
Simpson EH. 1949. Measurement of diversity. *Nature* 163:688.
Sintes E, Bergauer K, De Corte D, Yokokawa T, Herndl GJ. 2013. Archaeal *amoA* gene diversity points to distinct biogeography of ammonia-oxidizing Crenarchaeota in the ocean. *Environ Microbiol* 15:1647–58.
Summerfelt ST. 2006. Design and management of conventional fluidized-sand biofilters. *Aquacult Eng* 34:275–302. https://doi.org/10.1016/j.aquaeng.2005.08.010.
Tourna M, Freitag TE, Nicol GW, Prosser JI. 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environ Microbiol* 10:1357–64.
Verhamme DT, Prosser JI, Nicol GW. 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME J* 5:1067–71.
Wuchter C, Abbas B, Coolen MJL, Herfort L, Bleijswijk J, van Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Damsté JSS. 2006. Archaeal nitrification in the ocean. *Proc Natl Acad Sci USA* 103:12317–22.
Zhang J, Liu B, Zhou X, Chu J, Li Y, Wang M. 2015. Effects of emergent aquatic plants on abundance and community structure of ammonia-oxidising microorganisms. *Ecol Eng* 81:504–13.
Zhou X, Li Y, Zhang J, Liu B, Wang M, Zhou Y, Lin Z, He Z. 2016. Diversity, abundance and community structure of ammonia-oxidizing archaea and bacteria in riparian sediment of Zhenjiang ancient canal. *Ecol Eng* 90:447–58. https://doi.org/10.1016/j.ecoleng.2016.01.068.