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Enrichment and Characterization of an Autotrophic Ammonia-Oxidizing Archaeon of Mesophilic Crenarchaeal Group I.1a from an Agricultural Soil▽†

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Soil nitrification is an important process for agricultural productivity and environmental pollution. Though one cultivated representative of ammonia-oxidizing *Archaea* from soil has been described, additional representatives warrant characterization. We describe an ammonia-oxidizing archaeon (strain MY1) in a highly enriched culture derived from agricultural soil. Fluorescence *in situ* hybridization microscopy showed that, after 2 years of enrichment, the culture was composed of >90% archaeal cells. Clone libraries of both 16S rRNA and archaeal *amoA* genes featured a single sequence each. No bacterial *amoA* genes could be detected by PCR. A [^{13}C]bicarbonate assimilation assay showed stoichiometric incorporation of ^{13}C into *Archaea*-specific glycerol dialkyl glycerol tetraethers. Strain MY1 falls phylogenetically within crenarchaeal group I.1a; sequence comparisons to "*Candidatus* Nitrosopumilus maritimus" revealed 96.9% 16S rRNA and 89.2% *amoA* gene similarities. Completed growth assays showed strain MY1 to be chemoautotrophic, mesophilic (optimum at 25°C), neutrophilic (optimum at pH 6.5 to 7.0), and nonhalophilic (optimum at 0.2 to 0.4% salinity). Kinetic respirometry assays showed that strain MY1's affinities for ammonia and oxygen were much higher than those of ammonia-oxidizing bacteria (AOB). The yield of the greenhouse gas N_2O in the strain MY1 culture was lower but comparable to that of soil AOB. We propose that this new soil ammonia-oxidizing archaeon be designated "*Candidatus* Nitrosoarchaeum koreensis."

Eutrophication of terrestrial and aquatic systems, caused by industrial production and use of artificial nitrogen fertilizers worldwide, has led to a host of environmental problems (13). Autotrophic nitrification is a microbially mediated process that converts ammonia to nitrate and thus plays an essential role in soil nitrogen cycles. In agricultural systems, nitrification results in substantial loss of soil fertilizer nitrogen (50 to 70%) (75) due to metabolic coupling with denitrification (13) and anaerobic ammonia oxidation (33) that discharges nitrogen as dinitrogen gas. Soil nitrification from increased agricultural activities contributes significantly to global warming, since nitrification is a major source of the strong greenhouse gas nitrous oxide (N_2O), which has a ca. 300-times-higher warming impact than CO_2 (66). Nitrous oxide emissions are also responsible for ozone depletion in the stratosphere (19).

The first step of nitrification, oxidation of ammonia, long thought to be exclusive to the domain *Bacteria* (10), was recently tied to the archaeal domain: metagenomic analysis showed that a soil fosmid clone harbored both archaeal 16S rRNA and *amoA*-like genes (86). In an independent study, archaeal *amoA*-like genes were also recovered from surface waters of the Sargasso Sea (90). There is growing evidence that these archaeal *amoA* genes occur widely in a variety of environments, including soils and marine habitats (49, 97). Further, critical evidence for autotrophic ammonia-oxidizing archaea (AOA) was obtained by the characterization of the cultivated mesophilic *Crenarchaea* (recently proposed as *Thaumarchaea* [12]) from marine (group I.1a) (40, 59, 97) and hot spring (group I.1b and thermophilic AOA lineage) environments (21, 28).

The contribution of AOA to the nitrification of soil is still controversial. Quantitative analysis of *amoA* gene copies has indicated that AOA can predominate over ammonia-oxidizing bacteria (AOB) in various soils (16, 49). Also, copy numbers of archaeal *amoA* genes were found to increase and $^{13}CO_2$ was incorporated into genomic DNA of the AOA during ammonia oxidation (99). Additionally, expression of archaeal *amoA* was elevated in ammonia-amended soils (49, 86). In contrast, there have been several recent reports demonstrating that growth of AOB (not AOA) can be coupled with ammonia oxidation in soils. For example, $^{13}CO_2$ was incorporated mainly into the

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DNA of AOB, and AOB abundance was correlated with ammonia oxidation activity (35). In other reports (23, 24), inorganic-fertilizer amendment increased the copy numbers of *amoA* of AOB, and this increase was eliminated by added dicyanodiamide (DCD) (an ammonia oxidation inhibitor), while AOA abundance did not respond to the same fertilizer amendment. The study by Schauss et al. (72) showed that both AOA and AOB were active ammonia oxidizers in soil amended with organic fertilizer. Clearly, both domains have the potential to carry out ammonia oxidation.

To date, only a limited number of AOA have been isolated and/or enriched in laboratory culture. They have predominantly been from nonterrestrial (nonsoil) habitats: seawater aquarium filters, hot springs, and marine sediment (21, 28, 40, 59, 97). Despite the common retrieval of archaeal 16S rRNA gene and *amoA* gene sequences from various terrestrial environments, we are aware of only two soil-derived AOA, both members of the crenarchaeal group I.1b lineage: "*Candidatus* Nitrososphaera viennensis" strain EN76 was isolated from garden soil (85). This narrow field of characterized AOA has impaired our ability to advance our understanding of AOA in soil environments. In the present study, we were able to obtain a highly enriched culture of an ammonia-oxidizing archaeon from an agricultural soil and to compare the ammonia oxidation properties of this AOA to those of AOB and, hence, to obtain clues about the role of AOA in the nitrogen cycle for soil environments.

MATERIALS AND METHODS

Soil sampling site. We collected soil samples from plots planted with *Caragana sinica* at the experimental agricultural station of Chungbuk National University of South Korea (36°37′N, 127°27′E). The soils were loamy sand (sand, 82.4%; silt, 6.1%; clay, 11.5%) and did not receive nitrogen fertilizers due to *C. sinica*'s ability to carry out nitrogen fixation. Bulk and rhizosphere soils collected at depths of 0 to 30 cm, respectively, were combined into a single 100-g sample and transported to the laboratory to be stored at 4°C before being used for inoculation.

Cultivation of AOA. One gram of soil was inoculated into an artificial freshwater medium (AFM), and the cultures were grown aerobically in the dark without shaking at 25°C. The AFM contained the following components per liter of culture medium: 0.4 g $MgCl_2 \cdot 6H_2O$, 0.5 g KCl, 0.2 g KH_2PO_4, 1.0 g NaCl, and 0.1 g $CaCl_2 \cdot 2H_2O$. After autoclaving 1 ml nonchelated trace element solution (95), 1 ml NaFeEDTA solution (7.5 mM) and 3 ml $NaHCO_3$ (1 M) were added aseptically per liter of media. Unless stated otherwise, each starting batch culture was supplemented with 1 mM ammonium chloride as a sole energy source. After the oxidation of ammonia (typically after 3 weeks), 1% of the culture volume was transferred to fresh AFM. The pH of the medium remained almost constant (6.8 to 7.0) during the culture cycle. The concentrations of nitrite and nitrate were determined with an ion chromatograph (ICS-1500; Dionex, Sunnyvale, CA) with an OnGuard II Ag cartridge (Dionex). The ammonia concentration was determined colorimetrically (77). After 2 years of triweekly transfers, the enrichment culture was 10-fold serially diluted in Hungate tubes and incubated under the same conditions used for the enrichment culture. The Hungate tube showing nitrification activity in most diluted series was checked for culture purity using microscopy and molecular techniques. The nitrification activity was assessed spectrophotometrically by determining the decrease in the ammonia concentration in the Hungate tube.

The activity of ammonia oxidation by *Archaea* was analyzed in the presence of various antibiotics: streptomycin (100 μg/ml), kanamycin (50 μg/ml), ampicillin (50 μg/ml), penicillin-G (50 μg/ml), gentamicin (20 μg/ml), mitomycin C (20 μg/ml), and lincomycin (50 μg/ml). The cultures were incubated for 3 weeks, as described for enrichment cultures. Ammonia oxidation activity and growth of *Archaea* were determined by spectrophotometry and by PCR amplification of archaeal 16S rRNA genes, respectively.

To compare the ammonia oxidation traits of strain MY1 to those of *Nitrosomonas europaea* ATCC 19718, the AFM medium and cultivation conditions described above were used. Inocula (mid-log-phase cultures) were at initial cell densities of $1.2 \times 10^6 \pm 0.1 \times 10^6$ and $3.3 \times 10^6 \pm 0.3 \times 10^6$ cells/ml for strain MY1 and *N. europaea*, respectively. No nitrite was formed in the control that was not inoculated.

Quantification of gene copy numbers using real-time PCR. In order to determine AOA and AOB in the cultures, the copy numbers of the *amoA* and 16S rRNA genes were determined using primers described in Table 1. Cells were harvested from a 50-ml culture by centrifugation and immediately frozen and stored at −70°C until further analysis. DNA was isolated from frozen cells using the Genomic DNA Prep Kit (Solgent, South Korea) according to the manufacturer's instructions. The concentration of DNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

All quantitative real-time PCR experiments were carried out using a Mini-Opticon real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA) and Opticon Monitor software version 3.1 (Bio-Rad Laboratories, Hercules, CA). For the amplification of all genes, the following thermal-cycling parameters were used: 15 min at 95°C and 40 cycles of 20 s at 95°C, 20 s at 55°C, and 20 s at 72°C. Readings were taken between cycles. Standard curves were generated to obtain the relationship between the known copy numbers of reference genes and cycle threshold (C_T) values as previously described (61). Standard curves were prepared in each run using standards of reference genes (archaeal 16S rRNA gene, DQ831586; bacterial 16S rRNA gene, FJ656473; archaeal *amoA*, EF534487; archaeal *nirK*, HQ631404; bacterial *nirK* and *norB*, JF737970 and JF737971, respectively) at abundances ranging from 0 to 10^9 gene copies per reaction. These curves were used to estimate gene abundance in the enrichment samples. The specificity of real-time PCRs was tested by analyzing melting curves, checking the sizes of reaction products using gel electrophoresis, and sequencing of the reaction products. Information about the PCR amplification primers for each target gene is provided in Table 1.

Phylogenetic analysis of 16S rRNA, *amoA*, and *nirK* genes. Archaeal 16S rRNA and archaeal *amoA* and *nirK* genes were amplified by PCR using the primers listed in Table 1. The PCR conditions for both the 16S rRNA and *amoA* genes were as follows: 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C; and 7 min at 72°C. The PCR conditions for the *nirK* gene amplification were 5 min 94°C; 35 cycles of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C; and 7 min at 72°C. Clone libraries were constructed using the PCR products, which were purified using the PCR Purification System Kit (Solgent, South Korea), ligated using the T&A Cloning Vector Kit (Real Biotech Corporation, Taiwan), and then transformed into *Escherichia coli* DH5α cells according to the manufacturer's instructions. Both strands of the gene from the positive clone were sequenced using corresponding PCR primers (Table 1). For phylogenetic analysis, the gene sequences of related taxa were obtained from the GenBank database. Multiple alignments of 16S rRNA gene sequences were performed using SILVA (http://www.arb-silva.de/aligner) (64) and considering the secondary structure of the rRNA gene. Shared regions of the *amoA* (560 bp) and *nirK* (460 bp) gene sequences were aligned using Clustal X (83). Phylogenetic analyses were conducted using MEGA version 4.0 (43), and the neighbor-joining tree was constructed using Kimura 2-parameter correction (39) with 1,000 replicates to produce bootstrap values.

Bicarbonate incorporation analysis. In order to determine bicarbonate incorporation by AOA during ammonia oxidation, a known amount of [^{13}C]bicarbonate (99% ^{13}C; Cambridge Isotope Laboratories, Andover, MA) was applied, resulting in a 6.0% labeling of total bicarbonate. A control culture was incubated under the same conditions without the addition of [^{13}C]bicarbonate.

Total cells were harvested by centrifugation and freeze-dried for archaeal glycerol dialkyl glycerol tetraether (GDGT) membrane lipid analysis using the following procedures described by Pitcher et al. (62). Briefly, the freeze-dried cells were extracted by a modified Bligh-Dyer extraction, and the intact polar lipids were acid hydrolyzed to release the core GDGTs. To measure the degree of ^{13}C incorporation in archaeal GDGTs, they were analyzed on an Agilent (Palo-Alto, CA) 1100 series LC/MSD SL using selective ion monitoring as described in detail elsewhere (73). *m*/*z* 1302 to 1312 and *m*/*z* 1292 to 1302 were measured in selected time intervals.

Determination of the kinetics of ammonia oxidation and oxygen uptake. Kinetic studies of oxygen uptake and ammonia oxidation were performed with the strain MY1 culture as described elsewhere (52) with appropriate modifications. Oxygen uptake was measured in an Oxygraph system (Hansatech Instruments Ltd., England) equipped with an S1 Clark-type polarographic oxygen electrode disc, a 2-ml DW1 borosilicate glass reaction/sample vessel, magnetic stir bars, and the supplied Oxygraph Plus software. Oxygen microsensors were polarized continuously for >3 h before use. All measurements were done in a recirculated water bath at 25°C. Activity measurements were carried out with late-exponential or early-stationary-phase cells by monitoring ammonia oxidation and nitrite

TABLE 1. Primers used for PCR amplification for library construction and real-time quantification

Target gene and primer	Application	Sequence (5′ to 3′)	Position	Note	Reference
16S rRNA					
519F	Archaeal quantification	CAGCMGCCGCGGTAA	519–533[a]		59, 94
727R		GCTTTCRTCCCTCACCGT	712–727[a]		
Bac518F	Bacterial quantification	CCAGCAGCCGCGGTAAT	518–534[a]		6, 55
Bac786R		CTACCAGGGTATCTAATC	786–803[a]		
20F	Archaeal clone library	TTCCGGTTGATCCYGCCRG	2–20[a]		22
1492R		TACGGYTACCTTGTTACGACTT	1510–1492[a]		94
27F	Bacterial clone library	AGAGTTTGATCMTGGCTCAG	8–27[a]	With 1492R	94
NIT2R	*Nitrobacter* spp.	CGGGTTAGCGCACCGCCT	1433–1450[a]	With 27F	91
NIT3R		CCTGTGCTCCATGCTCCG	1035–1048[a]		
Ntspa 662R	*Nitrospira* spp.	GGAATTCCGCGCTCCTCT	662–679[a]	With 27F	20
Ntspa 712R		CGCCTTCGCCACCGGCCTTCC	712–732[a]		
βAMOF	PCR (β-AOB)	TGGGGRATAACGCAYCGAAAG	143–163[a]		53
βAMOR		AGACTCCGATCCGGACTACG	1296–1315[a]		
amoA					
AamoAF	Archaeal clone library	STAATGGTCTGGCTTAGACG	19–38[b]		25
AamoAR		ACATACAGATGGATGGCCGC	582–601[b]		
I.1a-amoAF	Archaeal quantification	TGTACWCACTACTTRTTCATA	239–270[c]		This study
I.1a-amoAR		GARTGYTTRTTYTTCTTTGT	590–610[c]		
amoA1F	PCR (β-AOB)	GGGGTTTCTACTGGTGGT	332–349[d]		69
amoA2R		CCCCTCKGSAAAGCCTTCTTC	820–822[d]		
A189F	PCR (γ-AOB)	GGNGACTGGGACTTCTGG	172–189[e]		57
A682R		GAASGCNGAGAAGAASGC	665–682[e]		
nirK					
March_nirKF	PCR (archaeal *nirK*)	TCTGGTGTTAAACTAATTGGT	46–66[f]		This study
March_nirKR		GTTSCTGCGGATTGTACT	540–557[f]		
nirK1F	PCR (bacterial *nirK*)	GGMATGGTKCCSTGGCA	526–542[g]		11
nirK5R		GCCTCGATCAGRTTRTGG	1021–1040[g]		
norB					
norB1F	PCR (bacterial *norB*)	CGNGARTTYCTSGARCARCC	400–419[h]		15
norB8R		CRTADGCVCCRWAGAAVGC	1051–1069[h]		

[a] Numbering is based on the 16S rRNA gene of *E. coli*.
[b] The numbering of the gene is based on the gene of a metagenomic clone from the Sargasso Sea (AACY01435967).
[c] Numbering of each gene is based on the gene of "*Ca*. Nitrosopumilus maritimus" (NC 010085).
[d] Numbering is based on the bacterial *amoA* of *N. europaea* (L08050).
[e] Numbering is based on the bacterial *amoA* of *Nitrosococcus oceani* (AF047705).
[f] Numbering is based on the archaeal *nirK*-like gene of "*Ca*. Nitrosopumilus maritimus" (NC 010085).
[g] Numbering is based on the bacterial *nirK* gene of *Alcaligenes faecalis* (D13155).
[h] Numbering is based on the bacterial *norB* gene of *Pseudomonas stutzeri* (Z28384).

production by strain MY1 cells. Aliquots (10 ml) were removed from cultures and immediately transferred to prewarmed 25-ml glass tubes in a 25°C water bath. Subsamples were then used to fill the 2-ml DW1 electrode glass vessel assembled with an oxygen electrode disc and plunger assembly before being carefully sealed. Oxygen uptake was monitored continuously after an initial equilibration of at least 40 min. Ammonia was added as necessary from a stock solution using a Hamilton syringe. The apparent half-saturation constant (K_m) for oxygen and ammonia and the maximum velocity (V_{max}) were determined from plots of oxygen uptake rates.

Determination of N_2O yield. Production of N_2O was measured in serum bottles (120 ml with a 70-ml headspace; sealed with a butyl rubber stopper) that contained 50 ml of AFM inoculated with strain MY1 or *N. europaea*. We used two different oxygen tensions for cultivation; one was fully aerated, and the other was oxygen limited. For the oxygen-limited condition, we removed oxygen from the medium by purging with N_2 gas and then added only 2 ml of air to the headspaces of bottles. The cultures were incubated at 25°C for 2 weeks under dark conditions. Triplicate samples were prepared and incubated for determination of the N_2O yield. The concentration of N_2O gas in the headspace was measured with a gas chromatograph with an electron capture detector (GC/ECD) (Agilent 6890A). Sample gas was taken from the headspaces of bottles using a 5-ml gas-tight syringe. The GC was fitted with a 1-ml sample loop and a 4-m stainless steel column packed with Porapak Q (Restek). The oven was isothermal (50°C), and N_2O concentrations were determined three times for each sample. We used six certified reference gas mixtures of N_2O in nitrogen—0.198, 0.331, 0.550, 0.798, 12.0, and 94.9 μmol N_2O/mol—to calibrate the GC/ECD. These reference gas mixtures were gravimetrically prepared by the Korea Research Institute for Standards and Science and verified by international comparisons, CCQM-K68, in 2010 (46). The uncertainty in the N_2O concentration was ca. 7%, caused by the nonlinearity of the ECD and the sampling procedure. Oxygen in the headspace was determined by using GC/thermal conductivity detector (TCD) as described previously (47).

FISH and electron microscopy analysis. Fluorescence *in situ* hybridization (FISH) was carried out on paraformaldehyde-fixed samples as described by Amann et al. (4). Cells from a 10-ml culture volume were harvested and resuspended in phosphate-buffered saline (PBS) (130 mM NaCl, 10 mM sodium phosphate, pH 7.5), fixed by the addition of 3 volumes of cold paraformaldehyde solution (4% in PBS), and stored at 4°C for 16 h. Cells were harvested and washed 3 times with PBS solution to remove residual fixation solution and concentrated 10-fold by centrifugation in PBS solution. The fixed cell suspension was mixed with an equal volume of cold absolute ethanol, and the mixture was stored at −20°C. For the FISH analysis of *Bacteria* and *Archaea*, paraformaldehyde-fixed samples were hybridized with Cy3-labeled *Archaea*-specific probe (Arc915) (2) and 6-carboxyfluorescein (FAM)-labeled *Bacteria*-specific probe (EUB338) (3). DAPI (4′,6-diamidino-2-phenylindole) was used to visualize total cells. Samples were observed with a Nikon 80i fluorescence microscope (Nikon, Tokyo) with an oil immersion objective.

For scanning electron microscopy (SEM), cells were harvested and immersed in 4% (vol/vol) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for

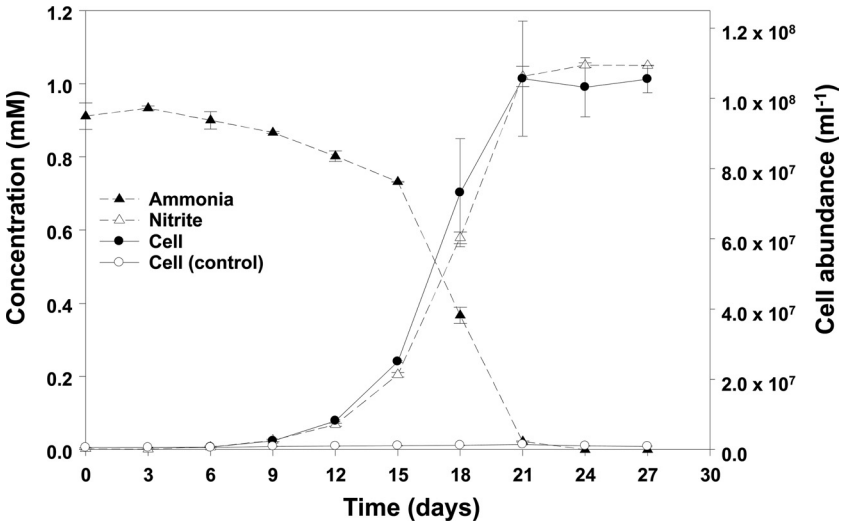

FIG. 1. Ammonia oxidation by the enrichment culture. The concentrations of ammonia and nitrite and the cell abundance of *Archaea* are indicated. FISH of filtered culture samples was used for determination of archaeal cell numbers. The control culture contained nitrate instead of ammonia. The error bars represent the standard deviations from triplicate experiments.

24 h at 4°C and dehydrated through a graded ethanol series (70 to 100%). The specimens were examined with a Zeiss DSM 940 electron microscope (Carl Zeiss). To enable transmission electron microscopy (TEM) analyses of AOA from the enrichment cultures, the cells from a 10-ml culture were fixed in a 2.5% paraformaldehyde-1.5% glutaraldehyde mixture buffered with 0.1 M phosphate (pH 7.2) for 2 h at 4°C, postfixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in a graded ethanol series (70% to 100%), transferred to propylene oxide, and embedded in Epon-812 (TAAB, England) (50). Ultrathin sections generated with an Ultracute (Leica, Austria) ultramicrotome were stained with uranyl acetate and lead citrate and examined with a CM 20 electron microscope (Philips, Netherlands) (68).

Nucleotide sequence accession numbers. The sequences obtained in this study have been deposited in GenBank (NCBI) under accession numbers HQ331116 to HQ331117, HQ631403 to HQ631405, and JF737930 to JF37971.

RESULTS

Establishment of an ammonia-oxidizing enrichment culture. Soil samples were gathered from field plots where the leguminous plant *C. sinica* was growing and were used for enrichment cultivation of ammonia-oxidizing microorganisms. The properties of the soil were as follows: sandy loam texture; pH, 5.6; organic C, 16 g/kg; total N, 0.5 g/kg; ammonia N, 3 mg/kg; nitrate N, 6 mg/kg; and total P, 150 mg/kg. After the initial soil inoculum (1 g in 100 ml AFM) catalyzed conversion of ammonia to nitrate, the nitrifying culture was repeatedly transferred (1% [vol/vol]) to fresh AFM triweekly. Early signs that the culture carried active *Archaea* were derived from sequences of PCR-amplified archaeal 16S rRNA (primer set 20F-1492R) and *amoA* (primer set AamoAF-AamoAR) genes (data not shown). The series of 1% enrichment transfers continued for 2 years. Finally, the culture was serially diluted to extinction in 10-fold steps, and the highest dilution showing nitrifying activity was selected for further characterization.

Characterization of the microbial community showed that the initially complex archaeal community became refined and eventually seemed uniarchaeal, as revealed by a single band in denaturing gradient gel electrophoresis (DGGE) analysis (data not shown). Ammonia oxidation by the final (serially diluted) culture is shown in Fig. 1. After inoculation (initial cell concentration, ca. 10^6 cells/ml), ammonia was stoichiometrically converted to nitrite over a period of about 3 weeks. Concomitantly, growth of archaeal cells (determined by *Archaea*-specific FISH of filtered culture samples) occurred only in the inoculated treatment (Fig. 1). In medium without ammonia as an electron donor, no archaeal growth was observed.

Bacterial 16S rRNA gene sequence analysis revealed that all sequences of *Bacteria* cooccurring in the *Archaea*-dominated enrichment culture belonged to the *Proteobacteria* and *Bacteroidetes*/*Chlorobi* groups (see Fig. S1 in the supplemental material). Three clone sequences of *Proteobacteria* were dominant (82.5%). The closest relatives to the cloned sequences were *Acinetobacter calcoaceticus*, *Pseudomonas knackmussii*, and *Variovorax boronicumulans*, which are catabolically diverse heterotrophs generally viewed as contributing to mineralization of organic compounds in typical soils (see Fig. S1 in the supplemental material).

Quantitative purity of the ammonia-oxidizing archaeal enrichment and partial characterization of its key autotrophic member, "strain MY1," using a $^{13}CO_2$ incorporation assay. The degree to which the ammonia-oxidizing culture was enriched in *Archaea* was determined by microscopy of cell preparations, using domain-specific FISH probes. Ninety-one percent of the cells were archaeal, as determined by the ratio of cell counts/ml ($1.4 \times 10^8 \pm 1.1 \times 10^7$ *Archaea* versus $1.3 \times 10^7 \pm 2.1 \times 10^6$ *Bacteria*). This was confirmed with real-time PCR of domain-specific 16S rRNA genes: *Archaea* represented 90% of the rRNA genes (archaeal [$1.1 \times 10^8 \pm 0.4 \times 10^8$ copies/ml] versus bacterial [$1.0 \times 10^7 \pm 0.2 \times 10^7$ copies/ml]). The yield of *Archaea* from ammonia oxidation was estimated at 1.1×10^5 cells/ml/μM ammonia consumed; this is lower than the yield reported for an acidophilic AOA, "*Candidatus* Nitrosotalea devanaterra" (4.5×10^5 cells/ml/μM ammonia consumed) (48) but higher than that of the marine AOA "*Candidatus* Nitrosopumilus maritimus" (5.0×10^4 cells/ml/μM ammonia consumed) (40).

The bacterial cells present in the ammonia-oxidizing enrich-

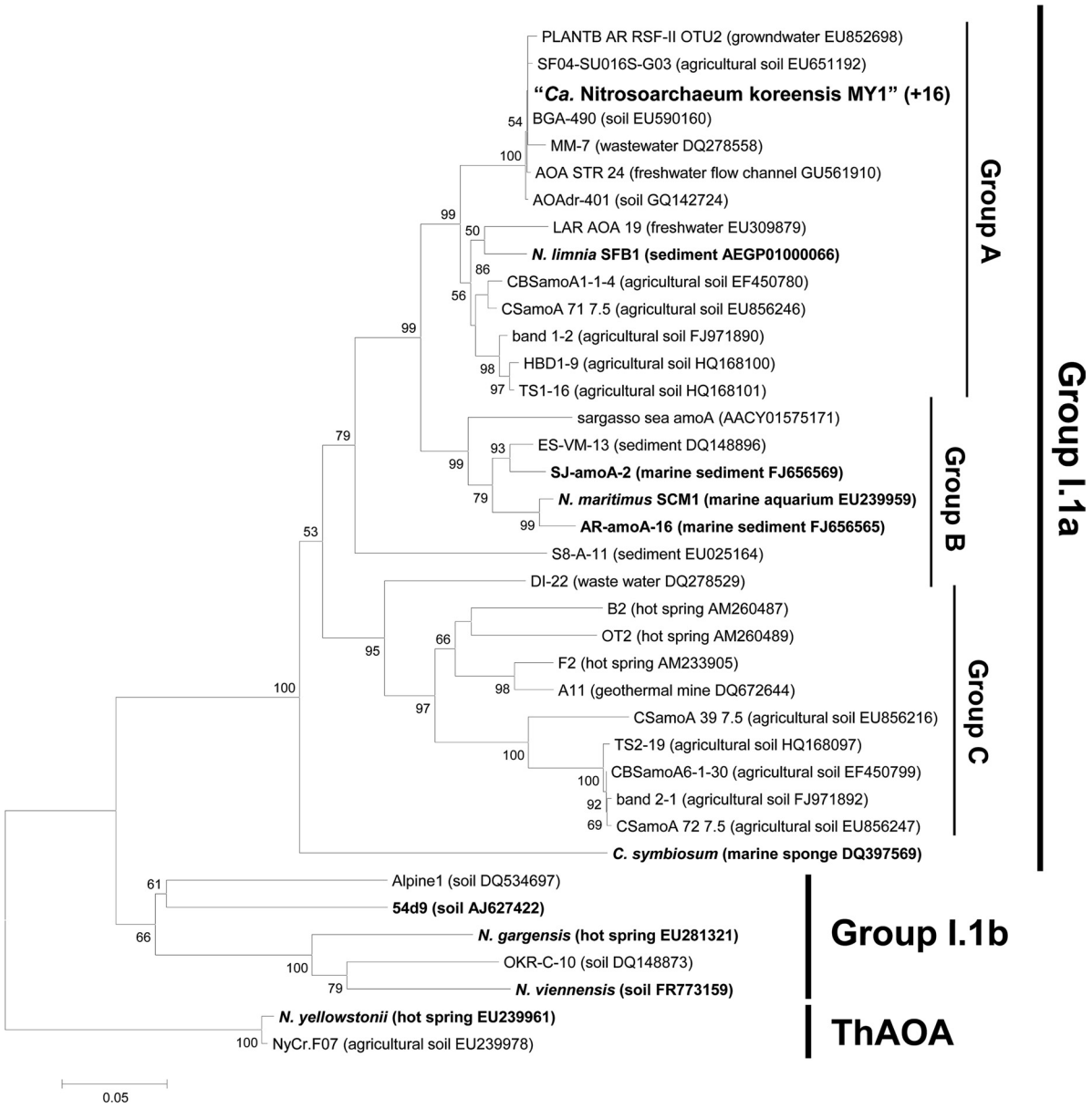

FIG. 2. Phylogenetic analysis of the archaeal *amoA* gene sequence (ca. 560 bp) obtained from strain MY1. Archaeal *amoA* genes were amplified using primers AamoAF and AamoAR. The *amoA* gene sequences from 16 clones randomly selected from the library were identical. Branching patterns supported by more than 50% bootstrap values (1,000 iterations) by means of neighbor joining are denoted by their respective bootstrap values. Cluster groups are denoted at the right based on the origins of reference sequences. "ThAOA" indicates thermophilic AOA lineage. The scale bar represents 5% estimated sequence divergence. Enriched or isolated AOA among the reference sequences are indicated in boldface.

ment could not be eliminated by treatment with various antibiotics (streptomycin, kanamycin, ampicillin, penicillin G, gentamicin, mitomycin C, or lincomycin); this was because the antibiotics inhibited both the *Bacteria* and the *Archaea* (see Fig. S2 in the supplemental material).

After DNA extraction, attempts to amplify bacterial *amoA* genes and 16S rRNA genes of gamma- and betaproteobacterial AOB using specific primers (bacterial *amoA*, amoA1F-amoA2R and A189F-A682R; bacterial 16S rRNA gene, βAMOF-βAMOR) failed. However, nitrite-oxidizing bacteria (NOB) related to *Nitrobacter vulgaris* DSM 10236^T (97.9% similarity [89]) and *Nitrospira* sp. (95.7% similarity [32]) were initially detected by PCR assays in the culture. NOB were eventually eliminated from the culture by using 10 μM chlorate, a NOB inhibitor. In contrast to the bacterial *amoA* genes, archaeal *amoA* genes could be amplified using specific primers (Table 1). Archaeal *amoA* gene amplicons, all sharing identical sequences, were obtained from 16 randomly selected library clones from the enrichment culture; they fell within "group A" of known archaeal *amoA* genes (Fig. 2). The uniformity of the archaeal *amoA* gene library indicated a high-purity enrichment.

PCR amplification and cloning of the archaeal 16S rRNA genes from the highly purified enrichment culture yielded a

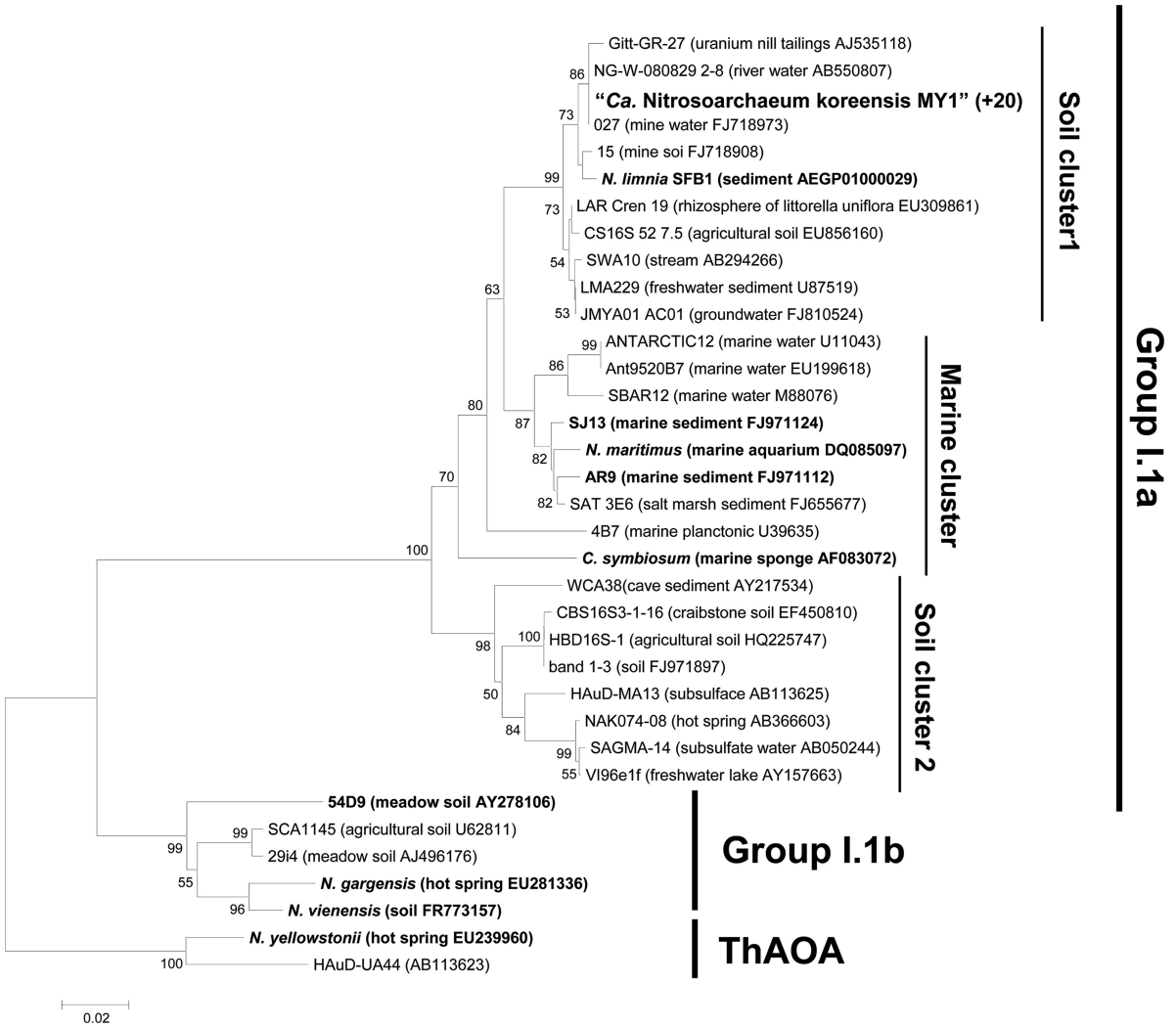

FIG. 3. Phylogenetic analysis of the archaeal 16S rRNA gene sequence (ca. 1.3 kbp) obtained from strain MY1. Archaeal 16S rRNA genes were amplified using primers 20F and 1492R. The 16S rRNA gene sequences from 20 randomly selected clones from the library were identical. Branching patterns supported by more than 50% bootstrap values (1,000 iterations) by means of neighbor joining are denoted by their respective bootstrap values. Cluster groups are denoted at the right based on the origins of reference sequences. "ThAOA" indicates thermophilic AOA lineage. The scale bar represents 2% estimated sequence divergence. Enriched or isolated AOA among the reference sequences are indicated in boldface.

library of 20 identical sequences. Analysis of the 16S rRNA gene showed a sequence, here referred to as "strain MY1," that is phylogenetically affiliated with group I.1a within *Crenarchaea*, composed mostly of sequences from soil (Fig. 3). The 16S rRNA gene tree (Fig. 3) shows two soil clusters in the I.1a group that are distinct from the marine cluster; the latter contains mostly marine sequences, including the first cultivated mesophilic ammonia-oxidizing archaeon, "*Ca.* Nitrosopumilus maritimus" (40). "Soil cluster 1" and "soil cluster 2" contain mostly sequences from terrestrial environments. The similarities between the 16S rRNA gene sequence of strain MY1 and those of "*Ca*. Nitrosopumilus maritimus," "*Ca*. Nitrososphaera viennensis," and "*Ca.* Nitrosoarchaeum limnia" (9) were 96.9%, 84.8%, and 99.1%, respectively, while the 16S rRNA gene sequence similarity between strains MY1 and 54d9, a fosmid clone of crenarchaeal group I.1b previously found in soil (86), was only 83.1% (Fig. 3).

The phylogeny of strain MY1's *amoA* sequence was congruent with that of strain MY1's16S rRNA gene sequence (compare Fig. 2 and 3); this is consistent with MY1's affiliation with the soil cluster of crenarchaeal group I.1a. The similarities of the *amoA* (AmoA) sequences of strain MY1 to those of "*Ca*. Nitrosopumilus maritimus," "*Ca*. Nitrososphaera viennensis," and "*Ca.* Nitrosoarchaeum limnia" were 89.2% (96.7%), 70.0% (82.0%), and 94.5% (99%), respectively. The similarity of the *amoA* (AmoA) sequence of strain MY1 with that of 54d9 was 74.8% (83.7%) (Fig. 2).

To further confirm that the active agent (strain MY1) in our highly purified enrichment culture was, in fact, an autotrophic archaeon, we investigated its carbon fixation capability with a labeling experiment. We completed ammonia oxidation assays in [^{13}C]bicarbonate-amended treatments (6.0% ^{13}C enriched) and subsequently extracted glycerol dialkyl glycerol tetraethers (GDGTs), known to be characteristic of *Archaea* (62, 76). As

determined by liquid chromatography/mass spectrometry (LC/MS), the major core GDGTs of strain MY1 contained no rings (GDGT-0) or crenarchaeol; this is the characteristic lipid distribution of AOA (59, 62, 76). The shift in molecular mass of the GDGTs by ~6 Da for GDGT-0 (see Fig. S3A and B in the supplemental material) and crenarchaeol (see Fig. S3C and D in the supplemental material) indicated complete incorporation of the ^{13}C label into these AOA membrane lipids, providing evidence for autotrophic growth of strain MY1.

The influence of environmental variables on ammonia oxidation by strain MY1. In an attempt to understand the physiological capabilities that may have allowed strain MY1 to function in terrestrial environments, the traits of strain MY1 were compared to those of an ammonia-oxidizing bacterium in soil, *N. europaea* ATCC 19718. Treatment with known ammonia oxidation inhibitors (10 μM nitrapyrin, 50 μM chlorite, or 500 μM DCD) strongly inhibited ammonia oxidation by both strain MY1 and *N. europaea*. However, allylthiourea (up to 500 μM) did not inhibit archaeal ammonia oxidation by strain MY1, while *N. europaea* was very sensitive to allylthiourea (<10 μM) (see Fig. S4 in the supplemental material). The typical MIC of allylthiourea for ammonia oxidation by AOB is ca. 1 to 10 μM (8).

The abilities of strain MY1 and *N. europaea* ATCC 19718 to convert ammonia to nitrite were also compared. Under our culture conditions, oxidation of ammonia by strain MY1 only started after 9 to 12 days of inoculation, while no lag time was detected in *N. europaea*. Ammonia oxidation by strain MY1 was not significantly inhibited in the presence of up to 5 mM ammonia and was still observed at 10 mM (Fig. 4A). In the presence of 20 mM ammonia, ammonia oxidation by strain MY1 was completely inhibited, while no inhibition of ammonia oxidation was observed at this concentration for *N. europaea*. This result indicates that, like other AOA (51, 59), strain MY1 has a lower tolerance for ammonia than AOB.

Nitrite produced by ammonia oxidation is toxic and may suppress the growth of ammonia oxidizers (79, 81). Strain MY1 was more sensitive to nitrite than *N. europaea* (Fig. 4B). Above 2 mM nitrite, ammonia oxidation by strain MY1 was inhibited. Although ammonia oxidation by strain MY1 was still observed with up to 5 mM nitrite, it was completely inhibited with 10 mM nitrite. However, ammonia oxidation by *N. europaea* was not affected by 10 mM nitrite and possibly even higher nitrite concentrations (Fig. 4B).

The responses to temperature were similar for strain MY1 and *N. europaea* (Fig. 4C). Strain MY1 showed maximum ammonia oxidation at 25°C, with a slightly decreased rate at 20°C. However, the ammonia oxidation rate for strain MY1 was significantly reduced at 15°C; it took 6 months for complete oxidation of 1 mM ammonia (data not shown).

Strain MY1 showed optimal ammonia oxidation at pH 7.0. At pH 6 and 8, the lag time was slightly increased. *N. europaea* showed an optimum pH range of 7 to 9 (Fig. 4D). At pH 6, the growth of *N. europaea* was depressed, and oxidation of 1 mM ammonia was not complete after prolonged incubation. This result indicates that the optimal pHs for ammonia oxidation were slightly basic and neutral for *N. europaea* and strain MY1, respectively.

Growth of strain MY1 was in the range of 0.1 to 0.4% salinity and was severely inhibited at <0.1 and >0.4% salinity (Fig. 4E). *N. europaea* was not sensitive to salinity up to 2%, although the strain was isolated from a soil environment. The properties demonstrated here indicate that strain MY1 is mesophilic, neutrophilic, and nonhalophilic.

Kinetics of ammonia oxidation and oxygen uptake. The ammonia oxidation rate was determined during batch growth experiments with strain MY1 (Fig. 1). The specific oxidation rate of ammonia was 2.5 fmol cell^{-1} day^{-1}, which is much lower than that of AOB (36). Strain MY1's rate was only slightly lower than that reported for "*Ca*. Nitrosopumilus maritimus" (4.0 fmol cell^{-1} day^{-1} [40]) and than for AOA enrichment cultures from marine sediments originating in the East Sea, South Korea (2.8 fmol cell^{-1} day^{-1} [59]), the Arctic Sea (3.0 fmol cell^{-1} day^{-1} [59]), and the North Sea (4.3 fmol cell^{-1} day^{-1} [97]). Ammonia oxidation by strain MY1 was significantly inhibited by shaking, which was also observed in "*Ca*. Nitrosopumilus maritimus" (51) and in AOA enriched from marine sediments (59).

Oxygen uptake coupled with ammonia oxidation was respirometrically measured in order to determine the affinities (K_m) for oxygen and ammonia of strain MY1. The kinetics of ammonia oxidation and oxygen consumption by strain MY1 followed Michaelis-Menten-type kinetics (Table 2). The affinities for oxygen and ammonia (and maximum rates of oxygen uptake and ammonia oxidation) for strain MY1 were slightly lower than those of "*Ca*. Nitrosopumilus maritimus" (51) and AOA enriched from marine sediments (59). However, the affinities for oxygen and ammonia of strain MY1 were much higher than those of AOB, and the maximum rates of oxygen uptake and ammonia oxidation in strain MY1 were much lower than those of AOB (Table 2). The K_m range of AOB is broad, from ca. 30 to 2,000 μM (42, 45, 63, 78). These results indicate that, as reported for marine AOA (51, 59), strain MY1 is physiologically adapted to low levels of ammonia and oxygen.

N_2O production. Microbial N_2O production arises during nitrification (via chemical decomposition of hydroxylamine and nitrite) and during denitrification via the direct action of nitric oxide reductase (NorB) on NO (18). We monitored N_2O production by strain MY1 and *N. europaea* in batch cultures with various ammonia and oxygen concentrations (Table 3). The total amount of N_2O accumulated by strain MY1 was substantial but far lower than that produced by *N. europaea*: at 2 mM ammonia, strain MY1's N_2O production was 18% of that of *N. europaea* (Table 3, column 2); when normalized to the nitrite yield (Table 3, column 4), the yield of N_2O by strain MY1 was ca. 15% of that of *N. europaea*. In tests at 4 mM ammonia, the trend toward relatively low (~14 to 16%) N_2O production by strain MY1 recurred (Table 3). As expected for an aerobic process, reduction of the oxygen tension drastically reduced the proportion of ammonia oxidized (0.3 to 0.4 mM in the 2 mM ammonia treatments) for both strain MY1 and *N. europaea*; corresponding yields of N_2O when normalized to nitrite increased about 2-fold for both cultures (Table 3).

The *nirK* gene (encoding nitrite reductase) confers nitrite tolerance in nitrifiers and is involved in nitrifier denitrification, reducing nitrite to NO, which can then be further reduced to N_2O (96). *nirK* was successfully PCR amplified from strain MY1; the gene showed 85.8% and 40.4% nucleotide sequence similarities with those of "*Ca*. Nitrosopumilus maritimus" and

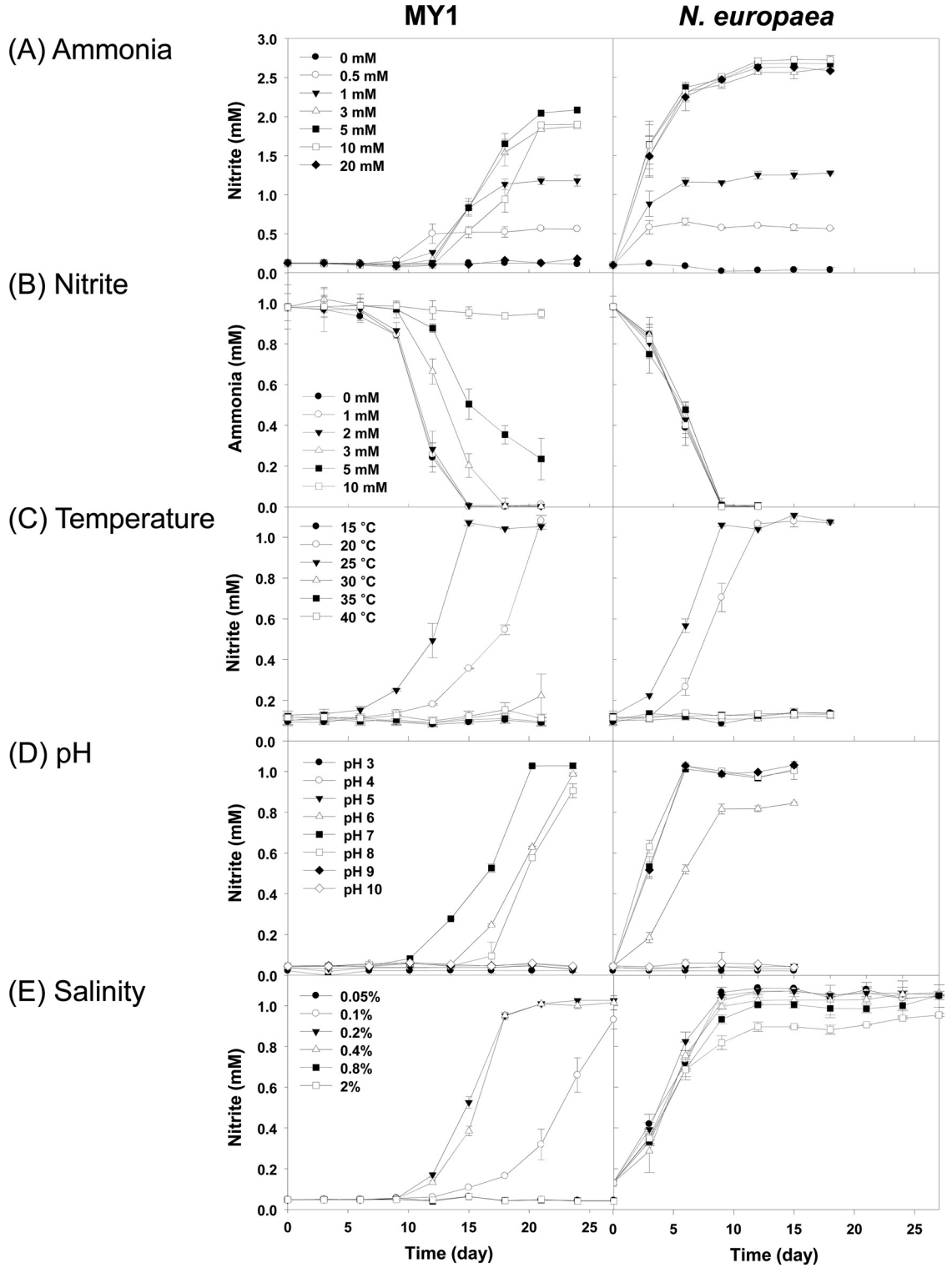

FIG. 4. Effects of ammonia (A), nitrite (B), temperature (C), pH (D), and salinity (E) on the ammonia oxidation activities of strain MY1 and *N. europaea*. The same AFM and cultivation conditions were used for strain MY1 and *N. europaea*. Ammonia oxidation activity was indicated by the amount of nitrite accumulated. In the study on nitrite inhibition, ammonia consumption was determined. The error bars represent standard deviations from triplicate experiments.

the group I.1b soil fosmid clone 54d9, respectively (see Fig. S5 in the supplemental material). We found expression of the archaeal *nirK* and archaeal *amoA* genes during oxidation of ammonia and simultaneous N_2O production (see Fig. S6 and Table S1 in the supplemental material); as expected, in the presence of the ammonia oxidation inhibitor (50 μM chlorite) or nitrite (without ammonia), neither N_2O production nor archaeal *nirK* gene expression was observed (data not shown).

TABLE 2. Comparison of kinetics of ammonia oxidation and oxygen uptake for strain MY1 and previously characterized AOA or cultures

Strain	Ammonia[a,b]		Oxygen[b]		Reference
	K_m (μM)	V_{max} (μmol N mg protein^{-1} h^{-1})	K_m (μM)	V_{max} (μmol O_2 mg protein^{-1} h^{-1})	
AOA					
MY1	0.69 (0.04)	11[c]	10.38 (1.08)	20[c]	This study
AR enrichment culture	0.61 (0.02)	14[c]	2.01 (0.45)	11[c]	59
"*Ca*. Nitrosopumilus maritimus"	0.13 (0.04)	24	3.90 (0.60)	36	51
AOB					
N. europaea C-31 (ATCC 25978)	1,300	36	186	129	59
N. europaea (ATCC 19718)	553	122	ND[d]	ND	51
Activated sludge	59	ND	15.6	ND	29

[a] The values are given for NH_4^+ and NH_3.
[b] The numbers in parentheses indicate the standard deviation from triplicate experiments.
[c] We assumed that the protein content per cell of AOA (~10 fg/cell) equals that of "*Ca*. Nitrosopumilus maritimus."
[d] ND, not determined.

To address the possibility that denitrifying *Bacteria* in the coculture might contribute to N_2O production, we simultaneously monitored bacterial *nirK* and *norB* gene expression: neither was expressed (see Fig. S6 and Table S1 in the supplemental material). We acknowledge that the high specificity of reverse transcription (RT)-PCR assays may fail to detect related mRNA targets (in this case, expressed bacterial *nirK* and *norB*); nonetheless, the assay is broadly accepted as state of the art for assessing expression of genes involved in N_2O production by denitrification pathways (11, 15). Thus, we have assembled four independent lines of evidence that argue against the possibility that minor denitrifying bacterial populations in the enrichment are responsible for N_2O production in the strain MY1 culture: (i) assays were clearly aerobic (ammonia oxidation and N_2O production were impaired at reduced O_2 [see above]), (ii) the AFM had only NH_3 as an electron donor, (iii) an ammonia oxidation inhibitor (which does not inhibit denitrification) completely inhibited the production N_2O, and (iv) neither of the two bacterial genes that might potentially participate in N_2O production by *Bacteria* present in the coculture was expressed. Thus, by weight of evidence, we conclude that the archaeal strain MY1 is the agent in N_2O production.

TABLE 3. Nitrous oxide production from strain MY1 and *N. europaea*[a]

Strain and conditions[c]	Production		
	N_2O (N_2O-N) (μmol)	NO_2^- (NO_2^--N) (μmol)	N_2O-N/NO_2^--N (%)
MY1			
2 mM NH_4^+	0.135 (0.065)	100.6 (10.2)	0.13
4 mM NH_4^+	0.231 (0.022)	105.5 (21.1)	0.22
2 mM NH_4^+ (low oxygen)[b]	0.039 (0.003)	17.0 (5.3)	0.23
N. europaea			
2 mM NH_4^+	0.750 (0.241)	81.3 (5.2)	0.92
4 mM NH_4^+	1.534 (0.717)	116.4 (11.4)	1.32
2 mM NH_4^+ (low oxygen)	0.493 (0.012)	21.2 (10.6)	2.07

[a] The data are mean values of triplicate experiments, and the numbers in parentheses indicate the standard deviations. Both cultures were incubated in AFM in sealed serum bottles for 2 weeks prior to N_2O determination in headspace gases.
[b] After the headspace was replaced by N_2 gas, 2 ml air was injected. Headspace oxygen was 0.34% (0.03%) (vol/vol) under low-oxygen conditions, which is equivalent to 0.4 mM dissolved oxygen. The control (without inoculation) showed about 372 (23.5) ppb N_2O, which corresponds to the atmospheric N_2O concentration in the laboratory.
[c] The initial archaeal and bacterial cell concentrations in the culture were 1.7×10^6 and 1.5×10^6 cells/ml, respectively.

Ultrastructural analysis. FISH analysis showed archaeal dominance over bacteria in the strain MY1 culture (Fig. 5A and B). Unlike other rod-shaped mesophilic archaeal cells (40, 48), ribosomes of strain MY1 were not largely concentrated at the poles. As observed in Fig. 5C, the chromosomes of strain MY1 were mostly localized at the sides of the cells. The cells were Gram negative. SEM analysis revealed short-rod morphology (diameter, 0.3 to 0.5 μm, and length, 0.6 to 1.0 μm) with a slightly curved peanut shape (Fig. 5D). Thin-section TEM analysis showed that strain MY1 appeared to have a significant intracellular volume with low electron density (Fig. 5E and F). Such a structure, consisting of inner membrane and periplasmic space, is unusual in the archaeal domain and has previously been described only in the thermophilic crenarchaeal genus *Ignicoccus* (44, 65). It is not clear if the electron-dense space is enveloped by a membrane.

DISCUSSION

This study revealed that an autotrophic ammonia-oxidizing archaeon affiliated with the soil cluster of crenarchaeal group I.1a was enriched from an agricultural soil. The medium composition and cultivation conditions for the enrichment were not drastically different from those for typical AOB isolated from soil. We speculate that *Archaea*, not *Bacteria*, were enriched because of a combination of the native archaeal populations in the soil inoculum and the biochemical traits (especially ammonia and oxygen affinities [Table 2]) that were fortuitously suited to our incubation conditions. We were unable to eliminate cocultured bacteria, despite efforts utilizing serial dilution cultures and/or antibiotic treatments. The vast majority of prior reports of *Archaea* carrying out ammonia oxidation have been derived from enriched cocultures (21, 28, 59, 97); the notable exceptions are the pure culture, "*Ca*. Nitrosopumilus maritimus" (40), and "*Ca*. Nitrososphaera viennensis" (85). Oxidation of ammonia by strain MY1 began after a substantial lag

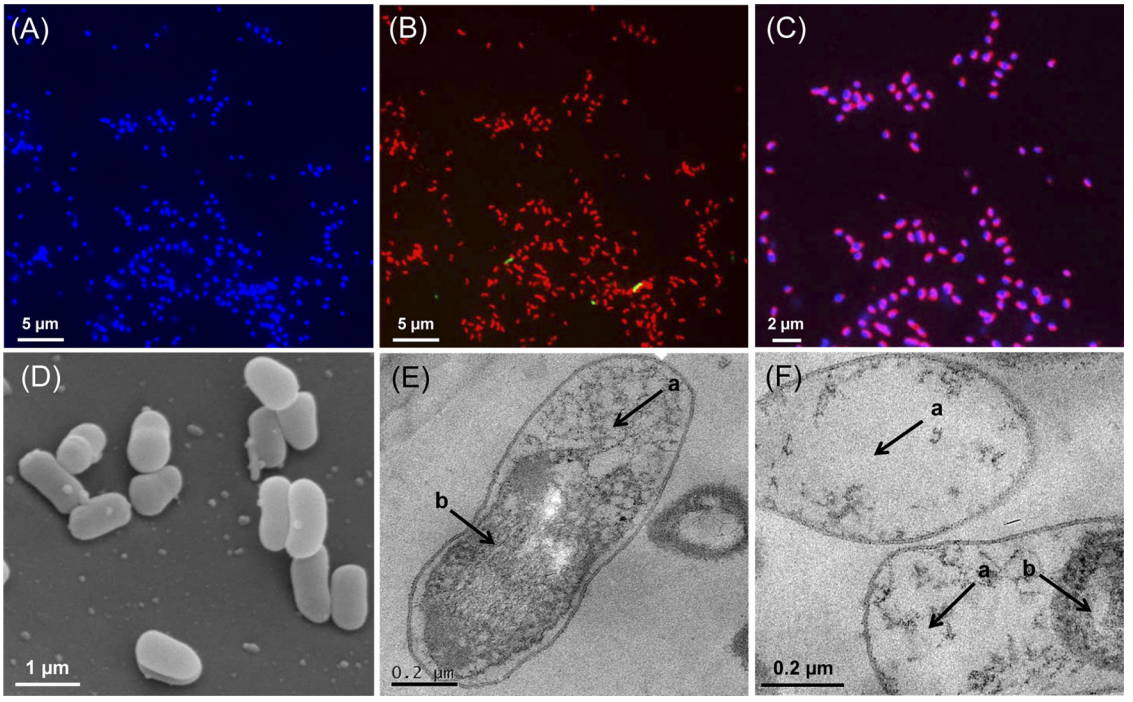

FIG. 5. Morphology of strain MY1. Shown is FISH analysis of the strain MY1 culture. (A) Epifluorescence micrographs of images from cells stained by DAPI (blue). (B) Merged image of Cy3-labeled *Archaea*-specific probe (Arc915; red) and FAM-labeled *Bacteria*-specific probe (EUB338; green). (C) Merged image of Cy3-labeled *Archaea*-specific probe (Arc915) and DAPI. Magenta indicates the archaeal cells. (D) Scanning electron micrograph of MY1 cells. (E and F) Transmission electron micrographs of ultrathin sections of MY1 cells. The arrows indicate electron-light areas (a) and electron-dense areas (b).

period, while *N. europaea* showed no observable lag period under our culture conditions (see Fig. S3 in the supplemental material). Such lag periods have been frequently observed (28, 59) before initiation of ammonia oxidation by AOA. Also, Park et al. (59) demonstrated that growth of sulfur-oxidizing bacteria was a prerequisite for the enrichment culture of AOA from marine sediment. Despite significant effort in the present study to obtain a pure archaeal culture (e.g., see Fig. S1 and S2 in the supplemental material), we found that three typical terrestrial heterotrophic bacteria among the *Proteobacteria* (*Acinetobacter*, *Pseudomonas*, and *Variovorax*) persisted in the nitrifying culture. This observation leads us to raise the possibility that interdomain population interactions may contribute to the successful cultivation of AOA; further investigation is warranted.

Ammonia concentration is known to select for distinct groups of AOB in soils (93). Tolerance of ammonia by AOB has been documented up to 600 mM (41, 80). Tolerance for ammonia toxicity by strain MY1 was much lower than that of *N. europaea* (this study) and slightly lower than that of "*Ca*. Nitrososphaera viennensis" (15 mM [85]). In contrast, strain MY1's tolerance for ammonia was slightly higher than that of other AOA, including the marine "*Ca*. Nitrosopumilus maritimus" (51), AOA enrichment culture AR (4.0 mM [59]), and "*Candidatus* Nitrososphaera gargensis" (3.1 mM [28]). This tolerance by strain MY1 is consistent with generally higher ammonia concentrations that occur in soil pore waters than in marine environments (<0.03 to 1 μM in the open ocean and <0.03 to 100 μM in coastal waters [14]).

Strain MY1 was also more sensitive to nitrite than *N. europaea* (Fig. 4B). Thus, tight metabolic coupling of AOA and NOB might be essential for the activity of AOA in soil niches. Strain MY1 showed active nitrification in a typical pH range of neutral soil (pH 6 to 8). The activity of AOB has been found to be severely repressed below pH 7 and thus is considered more favored in neutral and basic environments (1, 36). Although ammonia oxidation by AOA was optimal at 20 to 25°C, it was still observed at 15°C with severe repression. In contrast, although "*Ca*. Nitrososphaera viennensis" was isolated from temperate soil, the optimal growth temperature was 37°C (85). We also documented that strain MY1 has a relatively high affinity for ammonia and oxygen (Table 2). These traits have been shown in other AOA to provide important competitive advantages (51, 59) and likely provide similar advantages to strain MY1-like *Archaea* in soil. AOA's preference for oligotrophic environments may correspond to the general capacity of archaeal groups to dominate and outcompete *Bacteria* under environmental conditions of chronic energy stress (87). Taken together, our results imply that strain MY1 and its relatives are adapted to carry out autotrophic ammonia oxidation in temperate soils.

Inhibitors of ammonia oxidation significantly repressed ammonia oxidation by both strain MY1 and *N. europaea*. However, tolerance for inhibition of allylthiourea of strain MY1 was much higher than that of *N. europaea*. It was reported that "*Ca.* Nitrososphaera gargensis," enriched at 46°C from a hot spring, showed some degree of tolerance for 100 μM allylthiourea (28). Differential tolerance for ammonia oxidation inhibitors could be employed to assess the relative contributions

of AOA versus AOB to nitrification in soils. For example, Taylor et al. (82) recently used the absence of inhibition of ammonia oxidation by allylthiourea to argue that AOA may exhibit nitrification activity. In addition, Santoro and Casciotti (71) observed partial inhibition of marine archaeal ammonia-oxidizing activity by allylthiourea at 86 μM, a concentration known to completely inhibit cultivated AOB (31) and environmental AOB (26).

Autotrophic nitrification is known to be a significant source of N_2O in soil environments (17, 36, 37, 74). Recently, Santoro et al. (70) reported that marine AOA (crenarchaeal group I.1a) can produce N_2O. Since the predominance of archaeal over bacterial *amoA* genes in various soils was demonstrated (49, 56, 72, 99), N_2O produced by AOA has been suspected to be an overlooked but significant source of greenhouse gas emissions from soils. In this study, we observed that strain MY1 clearly produced N_2O during ammonia oxidation (Table 3); the N_2O yield was 5- to 6-fold lower than that from *N. europaea* under the same conditions. However, the N_2O production rates are expected to be variable, depending on the ammonia concentration and dissolved oxygen concentration, as reported elsewhere (27, 34). It is accepted that the values for molar yield of N_2O on the basis of nitrite produced are more robust for relative comparisons (74). The yield of N_2O from strain MY1 on the basis of nitrite produced (0.13 to 0.23%) falls in the same order of magnitude as AOB: *Nitrosospira* spp. and *Nitrosomonas* spp. (0.03 to 0.7% [74]), *N. europaea* strain 28 (0.05 to 1.95% [67]), *Nitrosomonas* sp. strain ATCC 25978 (0.2%), *Nitrosospira* sp. strains (0.05 to 1% [36]), and other AOB (0.05 to 3.3% [17]). Oxygen-limited conditions increased the yield of N_2O at 2 mM ammonia, and this type of response has typically been observed in AOB (17). Overall, these results indicate that N_2O production from soil AOA should be considered a significant source of greenhouse gases emitted from soils.

Two different physiological pathways of N_2O production by nitrifiers have been proposed: (i) during ammonia oxidation, chemical decomposition of the metabolites hydroxylamine and nitrite, and (ii) during respiratory reduction of nitrite to dinitrogen (involving *nirK* and *norB*), N_2O is a key metabolite (5, 96). The presence of the *nirK* gene was documented in strain MY1 (see Fig. S5 in the supplemental material); in "*Ca*. Nitrosopumilus maritimus" (92), and, likewise, in the AOA enrichment cultures SJ and AR; and in several environmental metagenomic studies (7, 86, 90). Here, we showed in strain MY1 that the archaeal *amoA* and *nirK* genes were highly expressed during ammonia oxidation (see Fig. S6 and Table S1 in the supplemental material). In the genome of strain MY1 (38), we have not been able to recognize the canonical hydroxylamine oxidoreductase or nitric oxide reductase (*nor*) gene. Thus, the mechanism by which strain MY1 produces N_2O is unclear. The biochemical and genetic mechanisms involved in the production of N_2O by AOA should be explored in the future.

The sequences of the 16S rRNA and *amoA* genes related to those of strain MY1 have been widely detected in previous reports examining various types of terrestrial environments, including agricultural soils (56, 58, 84, 99), lake littoral rhizosphere (30), contaminated ground water (98), and water treatment plants (60, 88) (see also the reference sequences in Fig. 2 and 3). These phylogenetic analyses imply that MY1-like AOA may be cosmopolitan ammonia-oxidizing archaea in terrestrial environments. Particularly interesting is the match between strain MY1's 16S rRNA and *amoA* gene sequences and several previously reported clones from soil microcosm studies: 16S rRNA gene clone CS16S_52_7.5 (97.6% similarity [56]) and *amoA* gene clones CSamoA_71_7.5 (95.4% [56]), CBSamoA1-1-4 (95.1% [84]), band 11-2 (94.5% [58]), and HBD1-9 and TS1-16 (94.1% and 94.3%, respectively [99]). Signals from all of these sequences showed enhanced intensity during ammonia oxidation in agricultural plots and thus suggest the likely involvement of MY1-like organisms in soil nitrification.

Strain MY1 is a new mesophilic ammonia-oxidizing archaeon cultivated from soil. Based on the results of this study, we propose, according to Murray and Stackebrandt (54), provisional classification of the novel strain as "*Candidatus* Nitrosoarchaeum koreensis."

Taxonomy. The short description of "*Canditatus* Nitrosoarchaeum koreensis" sp. nov is as follows: Ko.re.en′sis. L. masc. adj. koreensis, of or belonging to Korea. Locality. Temperate soils. AOA with nearly identical 16S rRNA and *amoA* gene sequences have been detected in various terrestrial environments, including soils, freshwater and groundwater. Diagnostics. Short rod-shaped cells with a size of 0.3 to 0.5 by 0.6 to 1.0 μm. Periplasmic space is extraordinarily wide with inner membrane-like structure. Gram negative. Aerobic chemolithotrophic metabolism with oxidation of ammonia to nitrite. Carbon dioxide is used as the sole carbon source. Nonmarine. Growth range between 15°C and 30°C with an optimum at 25°C. Growth pH range between 6 and 8 with an optimum at 7.0. Nitrite was not toxic up to 2 mM with a tolerance limit of 5 mM. Half-saturation constants (affinity) for oxygen and ammonia are 10.38 and 0.69 μM, respectively. Adapted to ammonia concentrations up to 5 mM with a tolerance limit of 10 mM. N_2O was produced. Produces characteristic GDGT membrane lipids, including crenarchaeol. Phylogeny. Phylogenetically assigned to the genus *Nitrosoarchaeum* of the phylum *Crenarchaea* based on 16S rRNA gene (HQ331116), *amoA* gene (HQ221117), and genomic sequence (AFPU00000000). Not isolated.

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REFERENCES

1. **Allison, S., and J. I. Prosser.** 1991. Survival of ammonia oxidising bacteria in air-dried soil. FEMS Microbiol. Lett. **79:**65–68.
2. **Alm, E. W., D. B. Oerther, N. Larsen, D. A. Stahl, and L. Raskin.** 1996. The oligonucleotide probe database. Appl. Environ. Microbiol. **62:**3557–3559.
3. **Amann, R. I., et al.** 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. **56:**1919–1925.
4. **Amann, R. I., W. Ludwig, and K. H. Schleifer.** 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59:**143–169.
5. **Arp, D. J., and L. Y. Stein.** 2003. Metabolism of inorganic N compounds by ammonia-oxidizing bacteria. Crit. Rev. Biochem. Mol. Biol. **38:**471–495.
6. **Baker, G. C., J. J. Smith, and D. A. Cowan.** 2003. Review and re-analysis of domain-specific 16S primers. J. Microbiol. Methods **55:**541–555.

7. **Bartossek, R., G. W. Nicol, A. Lanzen, H. P. Klenk, and C. Schleper.** 2010. Homologues of nitrite reductases in ammonia-oxidizing archaea: diversity and genomic context. Environ. Microbiol. **12:**1075–1088.
8. **Bédard, C., and R. Knowles.** 1989. Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers. Microbiol. Rev. **53:**68–84.
9. **Blainey, P. C., A. C. Mosier, A. Potanina, C. A. Francis, and S. R. Quake.** 2011. Genome of a low-salinity ammonia-oxidizing archaeon determined by single-cell and metagenomic analysis. PLoS One **6:**e16626.
10. **Bock, E., and M. Wagner.** 2006. Oxidation of inorganic nitrogen compounds as an energy source, p. 457–495. *In* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (ed.), The Prokaryotes. Springer, New York, NY.
11. **Braker, G., A. Fesefeldt, and K. P. Witzel.** 1998. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. Appl. Environ. Microbiol. **64:**3769–3775.
12. **Brochier-Armanet, C., B. Boussau, S. Gribaldo, and P. Forterre.** 2008. Mesophilic crenarchaeota: proposal for a third archaeal phylum, the *Thaumarchaeota*. Nat. Rev. Microbiol. **6:**245–252.
13. **Canfield, D. E., A. N. Glazer, and P. G. Falkowski.** 2010. The evolution and future of Earth's nitrogen cycle. Science **330:**192–196.
14. **Capone, D. G.** 2000. The marine microbial nitrogen cycle, p. 455–494. *In* D. L. Kirchman (ed.), Microbial ecology of the oceans. Wiley-Liss, New York, NY.
15. **Casciotti, K. L., and B. B. Ward.** 2005. Phylogenetic analysis of nitric oxide reductase gene homologues from aerobic ammonia-oxidizing bacteria. FEMS Microbiol. Ecol. **52:**197–205.
16. **Chen, X. P., Y. G. Zhu, Y. Xia, J. P. Shen, and J. Z. He.** 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? Environ. Microbiol. **10:**1978–1987.
17. **Colliver, B. B., and T. Stephenson.** 2000. Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. Biotechnol. Adv. **18:**219–232.
18. **Conrad, R.** 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol. Rev. **60:**609–640.
19. **Crutzen, P. J.** 1981. Atmospheric chemical processes of the oxides of nitrogen, including N_2O, p. 17–44. *In* C. C. Delwiche (ed.), Denitrification, nitrification, and atmospheric nitrous oxide. Wiley, New York, NY.
20. **Daims, H., J. L. Nielsen, P. H. Nielsen, K. H. Schleifer, and M. Wagner.** 2001. In situ characterization of *Nitrospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. Appl. Environ. Microbiol. **67:**5273–5284.
21. **de la Torre, J. R., C. B. Walker, A. E. Ingalls, M. Konneke, and D. A. Stahl.** 2008. Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. Environ. Microbiol. **10:**810–818.
22. **DeLong, E. F.** 1992. *Archaea* in coastal marine environments. Proc. Natl. Acad. Sci. U. S. A. **89:**5685–5689.
23. **Di, H. J., et al.** 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. **72:**386–394.
24. **Di, H. J., et al.** 2009. Nitrification driven by *Bacteria* and not *Archaea* in nitrogen-rich grassland soils. Nat. Geosci. **2:**621–624.
25. **Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley.** 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. U. S. A. **102:**14683–14688.
26. **Ginestet, P., J. M. Audic, V. V. Urbain, and J. C. Block.** 1998. Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. Appl. Environ. Microbiol. **64:**2266–2268.
27. **Goreau, T. J., et al.** 1980. Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol. **40:**526–532.
28. **Hatzenpichler, R., et al.** 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proc. Natl. Acad. Sci. U. S. A. **105:**2134–2139.
29. **Henze, M., W. Gujer, T. Mino, and M. van Loosdrecht.** 2000. Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA Publishing, London, United Kingdom.
30. **Herrmann, M., A. M. Saunders, and A. Schramm.** 2008. *Archaea* dominate the ammonia-oxidizing community in the rhizosphere of the freshwater macrophyte *Littorella uniflora*. Appl. Environ. Microbiol. **74:**3279–3283.
31. **Hooper, A. B., and K. R. Terry.** 1973. Specific inhibitors of ammonia oxidation in *Nitrosomonas*. J. Bacteriol. **115:**480–485.
32. **Hovanec, T. A., L. T. Taylor, A. Blakis, and E. F. Delong.** 1998. *Nitrospira*-like bacteria associated with nitrite oxidation in freshwater aquaria. Appl. Environ. Microbiol. **64:**258–264.
33. **Humbert, S., et al.** 2010. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. ISME J. **4:**450–454.
34. **Hynes, R. K., and R. Knowles.** 1984. Production of nitrous oxide by *Nitrosomonas europaea*: effects of acetylene, pH, and oxygen. Can. J. Microbiol. **30:**1397–1404.
35. **Jia, Z., and R. Conrad.** 2009. *Bacteria* rather than *Archaea* dominate microbial ammonia oxidation in an agricultural soil. Environ. Microbiol. **11:**1658–1671.
36. **Jiang, Q. Q., and L. R. Bakken.** 1999. Comparison of *Nitrosospira* strains isolated from terrestrial environments. FEMS Microbiol. Ecol. **30:**171–186.
37. **Kester, R. A., W. De Boer, and H. J. Laanbroek.** 1997. Production of NO and N_2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. Appl. Environ. Microbiol. **63:**3872–3877.
38. **Kim, B. K., et al.** 2011. Genome sequence of an ammonia-oxidizing soil archaeon, "*Candidatus* Nitrosoarchaeum koreensis" MY1. J. Bacteriol. **193:**5539–5540.
39. **Kimura, M.** 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:**111–120.
40. **Könneke, M., et al.** 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437:**543–546.
41. **Koops, H. P., B. Bottcher, U. C. Moller, A. Pommerening-Roser, and G. Stehr.** 1991. Classification of eight new species of ammonia-oxidizing bacteria: *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. J. Gen. Microbiol. **137:**1689–1699.
42. **Koops, H. P., and A. Pommerening Röser.** 2001. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiol. Ecol. **37:**1–9.
43. **Kumar, S., K. Tamura, and M. Nei.** 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. **5:**150–163.
44. **Küper, U., C. Meyer, V. Muller, R. Rachel, and H. Huber.** 2010. Energized outer membrane and spatial separation of metabolic processes in the hyperthermophilic archaeon *Ignicoccus hospitalis*. Proc. Natl. Acad. Sci. U. S. A. **107:**3152–3156.
45. **Laanbroek, H. J., P. L. E. Bodelier, and S. Gerards.** 1994. Oxygen consumption kinetics of *Nitrosomonas europaea* and *Nitrobacter hamburgensis* grown in mixed continuous cultures at different oxygen concentrations. Arch. Microbiol. **161:**156–162.
46. **Lee, J. B., et al.** 2011. Final report on international comparison CCQM-K68: nitrous oxide in synthetic air. Metrologia **48:**08004.
47. **Lee, J. S., et al.** 2010. Final report on international key comparison CCQM-K53: oxygen in nitrogen. Metrologia **47:**08005.
48. **Lehtovirta-Morley, L. E., K. Stoecker, A. Vilcinskas, J. I. Prosser, and G. W. Nicol.** 2011. Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. Proc. Natl. Acad. Sci. U. S. A. **108:**15892–15897.
49. **Leininger, S., et al.** 2006. *Archaea* predominate among ammonia-oxidizing prokaryotes in soils. Nature **442:**806–809.
50. **Luft, J. H.** 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9:**409–414.
51. **Martens-Habbena, W., P. M. Berube, H. Urakawa, J. R. de la Torre, and D. A. Stahl.** 2009. Ammonia oxidation kinetics determine niche separation of nitrifying archaea and bacteria. Nature **461:**976–979.
52. **Martens-Habbena, W., and D. A. Stahl.** 2011. Nitrogen metabolism and kinetics of ammonia-oxidizing archaea. Methods Enzymol. **496:**465–487.
53. **McCaig, A. E., T. M. Embley, and J. I. Prosser.** 1994. Molecular analysis of enrichment cultures of marine ammonia oxidisers. FEMS Microbiol. Lett. **120:**363–367.
54. **Murray, R. G., and E. Stackebrandt.** 1995. Taxonomic note: implementation of the provisional status *Candidatus* for incompletely described procaryotes. Int. J. Syst. Bacteriol. **45:**186–187.
55. **Muyzer, G., E. C. de Waal, and A. G. Uitterlinden.** 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:**695–700.
56. **Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser.** 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ. Microbiol. **10:**2966–2978.
57. **Nold, S. C., J. Zhou, A. H. Devol, and J. M. Tiedje.** 2000. Pacific Northwest marine sediments contain ammonia-oxidizing bacteria in the beta subdivision of the *Proteobacteria*. Appl. Environ. Microbiol. **66:**4532–4535.
58. **Offre, P., J. I. Prosser, and G. W. Nicol.** 2009. Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. FEMS Microbiol. Ecol. **70:**99–108.
59. **Park, B. J., et al.** 2010. Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in co-culture with sulfur-oxidizing bacteria. Appl. Environ. Microbiol. **76:**7575–7587.
60. **Park, H. D., G. F. Wells, H. Bae, C. S. Criddle, and C. A. Francis.** 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Appl. Environ. Microbiol. **72:**5643–5647.
61. **Park, S. J., B. J. Park, and S. K. Rhee.** 2008. Comparative analysis of archaeal 16S rRNA and *amoA* genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments. Extremophiles **12:**605–615.
62. **Pitcher, A., et al.** 2010. Crenarchaeol dominates the membrane lipids of *Candidatus* Nitrososphaera gargensis, a thermophilic group I.1b archaeon. ISME J. **4:**542–552.

63. **Prosser, J. I.** 1989. Autotrophic nitrification in *Bacteria*. Adv. Microb. Physiol. **30:**125–181.
64. **Pruesse, E., et al.** 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. **35:**7188–7196.
65. **Rachel, R., I. Wyschkony, S. Riehl, and H. Huber.** 2002. The ultrastructure of Ignicoccus: evidence for a novel outer membrane and for intracellular vesicle budding in an archaeon. Archaea **1:**9–18.
66. **Rahn, T., and M. Wahlen.** 1997. Stable isotope enrichment in stratospheric nitrous oxide. Science **278:**1776–1778.
67. **Remde, A., and R. Conrad.** 1990. Production of nitric oxide in *Nitrosomonas europaea* by reduction of nitrite. Arch. Microbiol. **154:**187–191.
68. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17:**208–212.
69. **Rotthauwe, J. H., K. P. Witzel, and W. Liesack.** 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. **63:**4704–4712.
70. **Santoro, A. E., C. Buchwald, M. R. McIlvin, and K. L. Casciotti.** 2011. Isotopic signature of N_2O produced by marine ammonia-oxidizing archaea. Science **333:**1282–1285.
71. **Santoro, A. E., and K. L. Casciotti.** 12 May 2011. Enrichment and characterization of ammonia-oxidizing archaea from the open ocean: phylogeny, physiology and stable isotope fractionation. ISME J. doi:10.1038/ismej.2011.58.
72. **Schauss, K., et al.** 2009. Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environ. Microbiol. **11:**446–456.
73. **Schouten, S., C. Huguet, E. C. Hopmans, M. V. Kienhuis, and J. S. Sinninghe Damsté.** 2007. Analytical methodology for TEX86 paleothermometry by high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. Anal. Chem. **79:**2940–2944.
74. **Shaw, L. J., et al.** 2006. *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ. Microbiol. **8:**214–222.
75. **Singh, S. N., and A. Verma.** 2007.The potential of nitrification inhibitors to manage the pollution effect of nitrogen fertilizers in agricultural and other soils: a review. Environ. Practice **9:**266–279.
76. **Sinninghe Damsté, J. S., et al.** 2002. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature **419:**708–712.
77. **Solorzano, L.** 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14:**799–801.
78. **Stehr, G., B. Böttcher, P. Dittberner, G. Rath, and H. P. Koops.** 1995. The ammonia-oxidizing nitrifying population of the River Elbe estuary. FEMS Microbiol. Ecol. **17:**177–186.
79. **Stein, L. Y., and D. J. Arp.** 1998. Loss of ammonia monooxygenase activity in *Nitrosomonas europaea* upon exposure to nitrite. Appl. Environ. Microbiol. **64:**4098–4102.
80. **Suwa, Y., Y. Imamura, T. Suzuki, T. Tashiro, and Y. Urushigawa.** 1994. Ammonia-oxidizing bacteria with different sensitivities to $(NH_4)_2SO_4$ in activated sludges. Water Res. **28:**1523–1532.
81. **Tan, N. C., et al.** 2008. Physiological and phylogenetic study of an ammonium-oxidizing culture at high nitrite concentrations. Syst. Appl. Microbiol. **31:**114–125.
82. **Taylor, A. E., L. H. Zeglin, S. Dooley, D. D. Myrold, and P. J. Bottomley.** 2010. Evidence for different contributions of *Archaea* and *Bacteria* to the ammonia-oxidizing potential of diverse Oregon soils. Appl. Environ. Microbiol. **76:**7691–7698.
83. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876–4882.
84. **Tourna, M., T. E. Freitag, G. W. Nicol, and J. I. Prosser.** 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environ. Microbiol. **10:**1357–1364.
85. **Tourna, M., et al.** 2011. *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. Proc. Natl. Acad. Sci. U. S. A. **108:**8420–8425.
86. **Treusch, A. H., et al.** 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ. Microbiol. **7:**1985–1995.
87. **Valentine, D. L.** 2007. Adaptations to energy stress dictate the ecology and evolution of the *Archaea*. Nat. Rev. Microbiol. **5:**316–323.
88. **van der Wielen, P. W., S. Voost, and D. van der Kooij.** 2009. Ammonia-oxidizing bacteria and archaea in groundwater treatment and drinking water distribution systems. Appl. Environ. Microbiol. **75:**4687–4695.
89. **Vanparys, B., et al.** 2007. The phylogeny of the genus *Nitrobacter* based on comparative rep-PCR, 16S rRNA and nitrite oxidoreductase gene sequence analysis. Syst. Appl. Microbiol. **30:**297–308.
90. **Venter, J. C., et al.** 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66–74.
91. **Wagner, M., G. Rath, H. P. Koops, J. Flood, and R. Amann.** 1996. In situ analysis of nitrifying bacteria in sewage treatment plants. Water Sci. Technol. **34:**237–244.
92. **Walker, C. B., et al.** 2010. *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proc. Natl. Acad. Sci. U. S. A. **107:**8818–8823.
93. **Webster, G., T. M. Embley, T. E. Freitag, Z. Smith, and J. I. Prosser.** 2005. Links between ammonia oxidizer species composition, functional diversity and nitrification kinetics in grassland soils. Environ. Microbiol. **7:**676–684.
94. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
95. **Widdel, F., and F. Bak.** 1992. Gram-negative mesophilic sulfate-reducing bacteria, p. 3352–3378. *In* A. Balows, M. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.). The prokaryotes, 2nd ed., vol. IV. Springer-Verlag, New York, NY.
96. **Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema.** 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. **33:**1723–1732.
97. **Wuchter, C., et al.** 2006. Archaeal nitrification in the ocean. Proc. Natl. Acad. Sci. U. S. A. **103:**12317–12322.
98. **Yagi, J. M., E. F. Neuhauser, J. A. Ripp, D. M. Mauro, and E. L. Madsen.** 2010. Subsurface ecosystem resilience: long-term attenuation of subsurface contaminants supports a dynamic microbial community. ISME J. **4:**131–143.
99. **Zhang, L. M., et al.** 2010. Autotrophic ammonia oxidation by soil *Thaumarchaea*. Proc. Natl. Acad. Sci. U. S. A. **107:**17240–17245.