Analysis of Methane Monooxygenase Genes in Mono Lake Suggests That Increased Methane Oxidation Activity May Correlate with a Change in Methanotroph Community Structure

Ju-Ling Lin,¹ Samantha B. Joye,² Johannes C. M. Scholten,¹ Hendrik Schäfer,¹
Ian R. McDonald,³ and J. Colin Murrell^{1*}

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England¹; Department of Marine Sciences, University of Georgia, Athens, Georgia²; and Department of Biological Sciences, University of Waikato, Hamilton, New Zealand³

Received 25 March 2005/Accepted 19 May 2005

Mono Lake is an alkaline hypersaline lake that supports high methane oxidation rates. Retrieved *pmoA* sequences showed a broad diversity of aerobic methane oxidizers including the type I methanotrophs *Methylobacter* (the dominant genus), *Methylobacterium*, and *Methylothermus*, and the type II methanotroph *Methylocystis*. Stratification of Mono Lake resulted in variation of aerobic methane oxidation rates with depth. Methanotroph diversity as determined by analysis of *pmoA* using new denaturing gradient gel electrophoresis primers suggested that variations in methane oxidation activity may correlate with changes in methanotroph community composition.

Methanotrophs are a unique group of methylotrophic bacteria which utilize methane as the sole carbon and energy source (2, 7) and can be divided into two groups, type I and type II, based on their cell morphology, ultrastructure, phylogeny, and metabolic pathways. Recently novel alkaliphilic methanotrophs have been isolated, including “*Methylobacterium alcaliphilum*” (17, 27) and *Methylobacterium buryatense* (16).

Methanotrophs oxidize methane to methanol using the enzyme methane monooxygenase (MMO). There are two distinct types of MMO, a membrane-bound, particulate methane monooxygenase and a cytoplasmic, soluble methane monooxygenase. Almost all known methanotrophs possess *pmoA*, which encodes a key polypeptide (PmoA) of particulate MMO (21). The only exception is *Methylocella palustris*, which appears to have only soluble MMO (5). Since the phylogeny of *pmoA* genes is congruent with the 16S rRNA phylogeny of the methanotrophs, PCR primers targeted to *pmoA* have been used extensively to assess methanotroph diversity in the environment (4, 8, 9, 13, 20, 21).

Mono Lake is an alkaline (pH 9.8), hypersaline (84 to 94 g liter⁻¹) soda lake located east of the Sierra Nevada, approximately 160 km south of Lake Tahoe, California. It is an old (about 760,000 years) alpine lake (1,946 m elevation) that is located in a region of active volcanism. Methane concentrations in the lake are high (1 to 100 μ M), with both biogenic and thermogenic sources of methane and extensive areas of methane seepage from lake bed sediments. Molecular ecological studies of 16S rRNA genes in Mono Lake using denaturing gradient gel electrophoresis (DGGE) showed few (8 to 20) operational taxonomic units (OTUs) per sample, suggesting a low diversity of microorganisms compared

to other aquatic environments, where 20 to 40 OTUs per sample are typical (10).

Microbial diversity patterns showed distinct communities at different depths within the lake, suggesting that the microbial community has adapted to specific biogeochemical conditions (10, 14). A study of methane oxidation rates in conjunction with methanotroph abundance and diversity (based on fluorescence in situ hybridization and 16S rRNA) showed that methane oxidation rates varied significantly over space and time; while absolute numbers of methanotrophs did not vary, the relative abundance of certain methanotrophs did change over time (3). The genes involved in methanotrophy in Mono Lake have not been examined, and such data may improve our understanding of the controls on aerobic methane oxidation in this system. In this study we also compared the diversity of methanotrophs at different depths in Mono Lake using an improved *pmoA* primer set in DGGE.

Sample collection. Samples were collected after 5 years of meromixis, a condition of persistent chemical stratification, which created an ideal environment to study the response of microbial assemblages to physical and chemical gradients in relation to temporal and seasonal changes in biogeochemical parameters (14). Water samples from Mono Lake (central California) were collected from station 3 at depths of 12.5 m (J9), 16.5 m (J12), 17.5 m (J14), and 18.5 m (J16). Approximately 1 liter of water was filtered through Sterivex-GS filter cartridges (0.22- μ m pore size; Millipore) and filters were stored frozen in SET buffer (20% [wt/vol] sucrose, 0.05 M EDTA, 0.05 M Tris-HCl, pH 7.6) before DNA extraction.

Geochemistry and methane oxidation activity. Water samples from discrete depths were collected using a 5-liter Niskin bottle. The dissolved oxygen concentration was determined using an O₂ sensor (Yellow Spring Instruments) that was lowered through the water column (stopping when anoxic conditions were confirmed). Dissolved CH₄ concentrations were determined using a headspace extraction-gas chromatography

* Corresponding author. Mailing address: Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England. Phone: 44 02476 523553. Fax: 44 02476 523568. E-mail: j.c.murrell@warwick.ac.uk.

FIG. 1. A. Methane oxidation rates (◆) and methane concentration (◻). B. Methane turnover time (days; ○), methane concentration (◻), and oxygen concentration (×) for Mono Lake station 3 in July 2000.

technique (15) and concentrations were calculated by comparison with a standard curve generated using a certified standard (10% CH₄ in ultrapure He, Scott Speciality gas mix 875).

Methane oxidation rates were measured using a ¹⁴CH₄ radiotracer technique (15). Incubations (5 ml of Mono Lake water) were conducted in triplicate using a gas-tight syringe

incubation technique (15). A ¹⁴CH₄ stock solution was prepared by amending sterile filtered (0.2-μm) Mono Lake water with 1 ml of ¹⁴CH₄ in a headspace-free serum bottle; 100 μl of this tracer solution was injected into each syringe, giving a tracer activity of ~50,000 dpm/ml. Samples were incubated at in situ temperature for 48 h and then incubations were terminated by transferring the sample to a scintillation vial containing a pellet of NaOH, which halted microbial activity. Residual ¹⁴CH₄ tracer was removed by venting the vials on a shaker table (50 rpm for 60 min). Samples were then amended with scintillation cocktail (Scintiverse BD; Fisher Scientific) and ¹⁴CO₂ activity was determined using a Beckman 6500 liquid scintillation counter. Methane oxidation rates were calculated using a standard equation (15). The methane turnover time (days) was calculated by dividing the concentration of methane (μmol liter⁻¹) at a given depth by the rate of methane oxidation (nmol liter⁻¹ day⁻¹) at that depth.

DNA extraction, PCR, DGGE, and analysis of clones. The method of Somerville (25) was used for DNA extraction from environmental water samples and has previously lysed all methanotrophs in aqueous environmental samples (12). Primer A189f (11) in combination with either primer A682r (11) or mb661r (4) was used to amplify *pmoA*. DGGE primer set A189f-GC/mb661r_nd (CCGGCGCAACGTCCTTACC) was also used to amplify *pmoA*. Amplification reactions were performed as previously described (18), slightly modified for DGGE by addition of 1.3% (vol/vol) dimethyl sulfoxide-1.3 M glycine betaine. DGGE bands were separated on a 6.5% (wt/vol) acrylamide gel with a 35 to 80% gradient. PCR products were purified with the QIAGEN PCR purification kit (QIAGEN, Hilden, Germany) and ligated into the pCRII vector (Invitrogen, San Diego, CA). Plasmid DNA was extracted (23) and clones containing correct-sized inserts were grouped into OTUs based on their restriction pattern by using EcoRI/RsaI and EcoRI/PvuII/HincII.

The ARB program (19) was used for sequence alignment, and phylogenetic analyses were carried out as described previously (22). Phylogenies were reconstructed with evolutionary distance (Dayhoff PAM model), maximum parsimony (PROTPARS), and maximum likelihood (Protein_ML) analyses (19).

Methane oxidation measurement. Concentrations of reduced metabolites, e.g., methane (and ammonium and sulfide, data not shown), increased rapidly below the oxycline (Fig. 1A). In July 2000, elevated rates of methane oxidation (>20 nmol liter⁻¹ day⁻¹; hereafter, nM day⁻¹) were observed between 15- and 24-m depths. Two distinct peaks of activity were present, one (160 ± 35 nM day⁻¹) at the base of the oxycline (16.5 m) and one (110 ± 30 nM day⁻¹) in the seasonally anoxic zone (23 m) just above the chemocline (Fig. 1). The peak of aerobic methane oxidation was located at the base of the oxycline where methane (>2 μM) began to increase and oxygen concentrations (<62 μM) were low (Fig. 1A). Rates of aerobic methane oxidation were slightly higher than anaerobic rates (though the difference was not statistically significant; rates and patterns of anaerobic methane oxidation are discussed in a separate paper [S. Carini et al., unpublished data]). The methane pool turnover time was most rapid (<10 days) at the base of the oxycline (Fig. 1B), where dissolved oxygen concentrations were less than 16 μM. The turnover time of methane in surface and bottom waters was on the time scale of years.

FIG. 2. Evolutionary distance tree of the derived amino acid sequences of *pmoA* genes amplified from DNA of Mono Lake water samples. Clones obtained from Mono Lake and Russian soda lakes are prefixed with ML and SL, respectively. Clones amplified with A189f and mb661r are prefixed with MLM, and clones amplified with A189f and A682r are prefixed with MLA. The tree was constructed with a filter (142 aligned amino acid positions; this excludes ambiguities and missing data), with *AmoA* from *Nitrosomonas europaea* (AF037107) as the outgroup, and is based on a ProtDist and neighbor-joining analysis. Bootstrap values greater than 50% derived from 100 replicates are shown and were obtained using a distance matrix program neighbor-joining method within Phylip. The number of clones assigned to each OTU by restriction fragment length polymorphism analysis is shown in parentheses. The bar represents 10% sequence divergence.

Diversity of functional genes of methanotrophs in Mono Lake water. PCR products were amplified successfully from all DNA samples from 4 depths, with *pmoA* primers. Since the highest rates of aerobic methane oxidation activity were noted at around 16.5 m (J12), DNA extracted from water samples taken from this depth were used to examine methanotroph diversity in Mono Lake. Restriction fragment length polymorphism analysis of *pmoA* clones identified 13 OTUs in the A189f/mb661r library (MLM library) and seven OTUs in the A189f/A682r library (MLA library).

Representative clones from each OTU were sequenced and phylogenetically analyzed (Fig. 2) and five groups of clones were identified. *pmoA* sequences around 90% identical to *Methylobacter pmoA* sequences were the most dominant *pmoA* sequences retrieved from Mono Lake libraries, representing 100% of clones in the MLA library and 81% of clones in the MLM library. These *Methylobacter*-related OTUs subgrouped into two clades (groups I and II). One clade (group I) contained *pmoA* sequences closely related to the *pmoA* sequence of *Methylobacter psychrophilus* Z-0021^T (26), and the other (group II) was closely related to *pmoA* from the estuarine methanotroph *Methylobacter* sp. strain BB5.1 (24). In the MLM library, 8% of *pmoA* clones were 99% identical to *pmoA* of *Methylococcoides burtonensis* (group III), 8% were 98% identical to *pmoA* from *Methylothermus* strain HB (1) (group IV), and 3% were 91% identical to *pmoA* from a type II methanotroph, *Methylocystis* (11) (group V).

DGGE analysis of methanotrophs in Mono Lake with *pmoA* PCR primers. The degenerate property of primer set A189f/mb661r made it unsuitable for application in DGGE. Therefore, a nondegenerate mb661r primer (mb661r_nd, CCG GCGCAACGTCCTTACC) was designed and tested in PCR with genomic DNA from methanotroph and ammonia-oxidizing bacterium type strains. A *pmoA* band of the predicted size was amplified from all methanotroph type strains tested and no PCR amplification occurred with DNA from ammonia oxidizer type strains tested. PCR primer set A189f_GC/mb661r_nd was then used to amplify *pmoA* genes from PCR template DNA from Mono Lake water.

DGGE profiles showed similar fingerprints throughout the four depths of water sampled (four dominant bands; P1, P2, P3, and P4), with one extra band (P5) found in sample J12 (and also there is a corresponding weak band in J14) (Fig. 3). Dominant bands were excised from the gel, reamplified by PCR, and run on an identical DGGE gel to confirm their positions relative to those in the original sample. Single-band PCR products were purified and sequenced and the DNA sequences were analyzed phylogenetically (Fig. 2). This revealed that dominant *pmoA* DGGE bands from Mono Lake water were closely related to the derived PmoA sequences identified in the *pmoA* clone library from sample J12. The five DGGE *pmoA* bands represented each of the groups of clones detected (see Fig. 2); band P1 (group II), band P2 (group I), band P3 (group III), band P4 (group IV), and band P5 (group V).

Mono Lake is an extreme environment characterized by high salinity and alkalinity and contains high methane concentrations from both biogenic and thermogenic sources. In this study, the identities of aerobic methane oxidizers in Mono Lake were determined by constructing *pmoA* libraries from DNA extracted from depths with high rates of aerobic meth-

FIG. 3. *pmoA* DGGE profile from Mono Lake water DNA. The gel had a gradient of 35 to 80% denaturant. Lanes: 1, sample J18 from 18.5 m; 2, sample J14 from 17.5 m; 3, sample J12 from 16.5 m; 4, sample J9 from 12.5 m. Bands selected for sequencing (P1, P2, P3, P4, and P5) are labeled.

ane oxidation. Phylogenetic analysis showed that *pmoA* sequences of *Methylobacter* (group I and II), *Methylococcoides* (group III), *Methylothermus* (group IV), and *Methylocystis* (group V) were present (Fig. 2). The *pmoA* sequences of *Methylobacter* were the most frequently retrieved sequences from Mono Lake water DNA. In a study of *pmoA* diversity in Russian soda lakes (18), clones which were closely related to *pmoA* from a broad range of methanotrophs were identified, including *Methylobacter*, *Methylothermus*, *Methylomonas*, *Methylococcoides*, *Methylocaldum*, *Methylocystis*, *Methylosinus*, and *Methylocapsa*. Several representatives of these clones are included in the phylogenetic analysis of the Mono Lake *pmoA* clones (Fig. 2).

In the Russian soda lakes, the dominant *pmoA* clone types were related to *Methylococcoides burtonensis* and *Methylothermus* sp. strain HB (18), similarly to some of the clones seen in this study, suggesting that similar *pmoA* sequences are likely to be found in high-pH environments (Fig. 2). The A189f/A682r primer set amplifies *amoA* and *pmoA*; however, no *amoA* sequences were retrieved in this study, suggesting a low abundance of *amoA* sequences or that Mono Lake contains ammonia oxidizers containing *amoA* not amplified by primers A189f and A682r. A study based on analysis of ammonia-oxidizing bacteria from Mono Lake using nitrifier-specific 16S rRNA gene primers revealed the presence of at least four different β -subdivision ammonia oxidizers (28); however, primers specifically targeting *amoA* were not used.

DGGE profiles of *pmoA* from the four depths of water were similar (Fig. 3), with four bands identical for all four depths, the only difference being band P5 (*Methylocystis* sp., group V) detected in samples J12 and J14, where the highest rates of

aerobic methane oxidation were measured. This indicated that variations in methane oxidation activity may correlate with some changes in methanotrophic community structure (as shown by DGGE of *pmoA*). A recent study (3) of Mono Lake using fluorescence in situ hybridization probes specific for all known type I and type II methanotrophs (6) revealed relatively stable numbers of populations of both types of methanotrophs throughout the water column. Type I methanotrophs were more abundant in almost all (>95%) samples examined and accounted for 3 to 5% (5×10^5 to 9×10^5 cells ml⁻¹) of total microbial community, while type II methanotrophs (1.5×10^5 to 3.5×10^5 cells ml⁻¹) accounted for 2% of the microbial community.

The samples analyzed in the present study were collected 3 years prior to those discussed in Carini et al. (3) and represented meromictic conditions of 5 years' duration (versus 8 years for the latter study). Despite this temporal offset, both sets of samples included similar dominant methanotrophs, suggesting that the methanotroph population had become well established and was stable over time under meromictic conditions.

In summary, type I methanotrophs are the most abundant methanotrophs in Mono Lake, as seen from *pmoA* libraries in this study of Mono Lake, in the cell numbers measured by fluorescence in situ hybridization (3) and appear to be the most abundant methanotrophs in alkaline environments compared with studies on other alkaline soda lakes (18). DGGE analysis of *pmoA* genes revealed diversity similar to that found in *pmoA* clone libraries, and DGGE bands appeared to indicate some change in methanotroph community structure at the depths of highest aerobic methane oxidation, with the possibility that methanotrophs related to *Methyocystis* may be stratified around the zone of highest methane turnover.

Nucleotide sequence accession numbers. Sequences from this study have been deposited in GenBank under accession numbers AY571985 to AY572004 and DQ059819 to DQ059823/SEC

J.L. was supported by funding from the Ministry of Education, Taiwan, R.O.C. This work was supported by funding from the European Union 5th Framework Programme (Grant QLRT-1999-31528), the United Kingdom Natural Environment Research Council (Fellowship Award GT5/99/FS/11), and the U.S. National Science Foundation's Microbial Observatory Program (MCB 99-77901).

We thank members of the Mono Lake MObs program, especially Stephen Carini, for assistance with field and lab work.

REFERENCES

- Bodrossy, L., K. L. Kovacs, I. R. McDonald, and J. C. Murrell. 1999. A novel thermophilic methane-oxidizing γ -proteobacterium. *FEMS Microbiol. Lett.* **170**:335–341.
- Bowman, J. P., L. I. Sly, P. D. Nichols, and A. C. Hayward. 1993. Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *Int. J. Syst. Bacteriol.* **43**:735–753.
- Carini, S., N. Bano, G. LeCleir, and S. B. Joye. 2005. Aerobic methane oxidation and methanotroph community composition during seasonal stratification in Mono Lake, California (USA). *Environ. Microbiol.* **7**:1127–1138.
- Costello, A. M., and M. E. Lidstrom. 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl. Environ. Microbiol.* **65**:5066–5074.
- Dedysh, S. N., W. Liesack, V. N. Khmelenina, N. E. Suzina, Y. A. Trotsenko, J. D. Semrau, A. M. Bares, N. S. Panikov, and J. M. Tiedje. 2000. *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *Int. J. Syst. Evol. Microbiol.* **50**:955–969.
- Eller, G., S. Stubner, and P. Frenzel. 2001. Group-specific 16S rRNA targeted probes for the detection of type I and type II methanotrophs by fluorescence in situ hybridisation. *FEMS Microbiol. Lett.* **198**:91–97.
- Hanson, R. S., and T. E. Hanson. 1996. Methanotrophic bacteria. *Microbiol. Rev.* **60**:439–471.
- Henckel, T., M. Friedrich, and R. Conrad. 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. *Appl. Environ. Microbiol.* **65**:1801–1808.
- Henckel, T., U. Jäkel, S. Schnell, and R. Conrad. 2000. Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. *Appl. Environ. Microbiol.* **66**:1801–1808.
- Hollibaugh, J. T., P. S. Wong, N. Bano, S. K. Pak, E. M. Prager, and C. Orrego. 2001. Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. *Hydrobiologia* **466**:45–60.
- Holmes, A. J., A. M. Costello, M. E. Lidstrom, and J. C. Murrell. 1995. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiol. Lett.* **132**:203–208.
- Holmes, A. J., N. J. P. Owens, and J. C. Murrell. 1995. Detection of novel marine methanotrophs using phylogenetic and functional gene probes after methane enrichment. *Microbiology* **141**:1947–1955.
- Holmes, A. J., P. Roslev, I. R. McDonald, N. Iversen, K. Henriksen, and J. C. Murrell. 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. *Appl. Environ. Microbiol.* **65**:3312–3318.
- Humayoun, S. B., N. Bano, and J. T. Hollibaugh. 2003. Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Appl. Environ. Microbiol.* **69**:1030–1042.
- Joye, S. B., T. L. Connell, L. G. Miller, R. S. Oremland, and R. S. Jellison. 1999. Oxidation of ammonia and methane in an alkaline, saline lake. *Limnol. Oceanogr.* **44**:178–188.
- Kaluzhnyaya, M., V. N. Khmelenina, B. T. Eshinimaev, N. E. Suzina, D. I. Nikitin, A. Solonin, J. L. Lin, I. R. McDonald, J. C. Murrell, and Y. A. Trotsenko. 2001. Taxonomic characterization of new alkaliphilic and alkali-tolerant methanotrophs from soda lakes of the southeastern Transbaikalian region and description of *Methylobacterium buryatense* sp. nov. *Syst. Appl. Microbiol.* **24**:166–176.
- Khmelenina, V. N., M. G. Kalyuzhnyaya, N. G. Starostina, N. E. Suzina, and Y. A. Trotsenko. 1997. Isolation and characterisation of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. *Curr. Microbiol.* **35**:257–261.
- Lin, J. L., S. Radajewski, B. T. Eshinimaev, Y. A. Trotsenko, I. R. McDonald, and J. C. Murrell. 2004. Molecular diversity of methanotrophs in Transbaikalian soda lake sediments and identification of potential active populations by stable isotope probing. *Environ. Microbiol.* **6**:1049–1060.
- Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Förster, I. Brettke, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R. Lüßmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K.-H. Schleifer. 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**:1363–1371.
- McDonald, I. R., G. H. Hall, R. W. Pickup, and J. C. Murrell. 1996. Methane oxidation potential and preliminary analysis of methanotrophs in blanket bog peat using molecular ecology techniques. *FEMS Microbiol. Ecol.* **21**:197–211.
- Murrell, J. C., I. R. McDonald, and D. G. Bourne. 1998. Molecular methods for the study of methanotroph ecology. *FEMS Microbiol. Ecol.* **27**:103–114.
- Radajewski, S., G. Webster, D. S. Reay, S. A. Morris, P. Ineson, D. B. Nedwell, J. I. Prosser, and J. C. Murrell. 2002. Identification of active methylophilic populations in an acidic forest soil by stable-isotope probing. *Microbiology* **148**:2331–2342.
- Saunders, S. E., and J. F. Burke. 1990. Rapid isolation of miniprep DNA for double strand sequencing. *Nucleic Acids Res.* **18**:4948.
- Smith, K. S., A. M. Costello, and M. E. Lidstrom. 1997. Methane and trichloroethylene oxidation by an estuarine methanotroph, *Methylobacter* sp. strain BB5.1. *Appl. Environ. Microbiol.* **63**:4617–4620.
- Somerville, C. C., I. T. Knight, W. L. Straube, and R. R. Colwell. 1989. Simple, rapid method for direct isolation of nucleic acids from aquatic environments. *Appl. Environ. Microbiol.* **55**:548–554.
- Tourova, T. P., M. V. Omelchenko, and L. V. Vasil'eva. 1999. The phylogenetic position of *Methylobacter psychrophilus* sp. nov. *Mikrobiologiya* **68**:493–497.
- Trotsenko, Y. A., and V. N. Khmelenina. 2002. Biology of extremophilic and extremotolerant methanotrophs. *Arch. Microbiol.* **177**:123–131.
- Ward, B. B., D. P. Martino, M. C. Diaz, and S. B. Joye. 2000. Analysis of ammonia-oxidizing bacteria from hypersaline Mono Lake, California, on the basis of 16S rRNA sequences. *Appl. Environ. Microbiol.* **66**:2873–2881.