Nitrosospira lacus sp. nov., a psychrotolerant, ammonia-oxidizing bacterium from sandy lake sediment

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A Gram-negative, spiral-shaped, chemolithotrophic, ammonia-oxidizing bacterium, designated APG3T, was isolated into pure culture from sandy lake sediment collected from Green Lake, Seattle, WA, USA. Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain APG3T belongs to cluster 0 of the genus Nitrosospira, which is presently not represented by described species, with Nitrosospira multiformis (cluster 3) as the closest species with a validly published name (identity of 98.6 % to the type strain). Strain APG3T grew at 4 °C but could not grow at 35 °C, indicating that this bacterium is psychrotolerant. Remarkably, the strain was able to grow over a wide range of pH (pH 5–9), which was greater than the pH range of any studied ammonia-oxidizing bacteria in pure culture. The DNA G+C content of the APG3T genome is 53.5 %, which is similar to that of Nitrosospira multiformis ATCC 25196T (53.9 %) but higher than that of Nitrosomonas europaea ATCC 19718 (50.7 %) and Nitrosomonas eutropha C71 (48.5 %). The average nucleotide identity (ANI) calculated for the genomes of strain APG3T and Nitrosospira multiformis ATCC 25196T was 75.45 %, significantly lower than the value of 95 % ANI that corresponds to the 70 % species-level cut-off based on DNA–DNA hybridization. Overall polyphasic taxonomy study indicated that strain APG3T represents a novel species in the genus Nitrosospira, for which the name Nitrosospira lacus sp. nov. is proposed (type strain APG3T=NCIMB 14869T=LMG 27536T=ATCC BAA-2542T).

Chemolithotrophic ammonia oxidizers are responsible for the biological transformation of reduced forms of nitrogen to nitrite in the global nitrogen cycle, a key biogeochemical process in nature that is severely impacted by human activities (Galloway, 1998). At present, 14 species are described as betaproteobacterial ammonia oxidizers (Koops & Pommerening-Röser, 2001), while four species are described as gammaproteobacterial ammonia oxidizers (Campbell et al., 2011). Although the number of bacterial species is

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Abbreviations: ANI, average nucleotide identity; AOB, ammonia-oxidizing bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, amoA gene and draft genome sequences of strain APG3T are KC477402, KC477403 and CAUA01000000, respectively.
constantly increasing, the last description of a novel species of ammonia-oxidizing bacteria (AOB) within the *Nitrosonomadaceae* dates back more than 20 years (Koops *et al.*, 1991). A key reason for this slower pace of discovery is probably the difficulty of isolation and maintenance of pure cultures of AOB. The five previously described genera of AOB (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio* and *Nitrosolobus*) had been defined primarily by differences in cell morphology, the ultrastructure of cytomembranes and several physiological characteristics (Watson & Mandel, 1971). Subsequent comparative studies of 16S rRNA gene sequences revealed that most were affiliated with a monophyletic branch within the class Betaproteobacteria. The consolidation of the genera *Nitrosovibrio*, *Nitrosospira* and *Nitrosolobus* into a single genus *Nitrosospira* was advocated based on high 16S rRNA gene sequence identity (>98%). Currently, the genus *Nitrosospira* is divided into five groups (clusters 0 to 4) based on 16S rRNA gene sequence analysis (Purkhold *et al.*, 2000). The genus *Nitrosospira* is presently represented by three species, *Nitrosospira briensis* (type species), *Nitrosospira* ("Nitrosovibrio") *tenuis* and *Nitrosospira* ("Nitrosolobus") *multiformis*, all of which are closely related and belong to one large cluster, cluster 3. Cluster 1 is presently based exclusively on environmental clone sequences. While the other three lineages (clusters 0, 2 and 4) include isolates in culture that have been used previously for either molecular or phenotypic characterizations (Aakra *et al.*, 2001; Purkhold *et al.*, 2003; Nugroho *et al.*, 2005), none of these isolates have been assigned to a species with a validly published name. Our knowledge of the ecophysiological features of these three lineages is still quite limited. Here, we report the isolation and characterization of a representative of cluster 0 of the genus *Nitrosospira*, which was isolated from sandy sediment collected from Green Lake, Seattle, WA, USA. Our polyphasic taxonomic study demonstrates that APG3\(^T\) is a novel psychrotolerant strain of the genus *Nitrosospira* that we assign to the species *Nitrosospira lacus* sp. nov.

Strain APG3\(^T\) was isolated from sediment of Green Lake, a 105 ha freshwater lake with a maximum depth of 9 m within Green Lake Park in North Central Seattle. Water samples were collected on 26 October 2008. Water quality monitoring data provided by King County, WA, show that the lake is clear and moderate in primary productivity (mesotrophic), with overall good water quality that has been stable since alum (aluminium potassium sulfate dodecahydrate) treatment was carried out in 2004 to control phosphorus concentrations in the water. The sampling water depth was approximately 30 cm, and a sandy sediment sample was collected from the shore in a sterilized 50 ml centrifuge tube (47.40' 52.11"N 122° 20' 24.82"W). Water temperature was 12.5 °C and total-N and total-P concentrations were 201 and 11.7 \(\mu\)g l\(^{-1}\), respectively (King County Lake Stewardship). Five grams sand was incubated at 20 °C with 10 ml autoclaved deionized water supplemented with a final concentration of 1 mM NH\(_4\)Cl. After the first indication of nitrite production, 1 ml sample was transferred into 9 ml APG medium, which has the following composition: 12 g HEPES, 0.074 g KCl, 0.049 g MgSO\(_4\), 7H\(_2\)O, 0.147 g CaCl\(_2\).2H\(_2\)O, 0.584 g NaCl and 250 \(\mu\)l phenol red solution (0.4% v/v) as basal medium adjusted to 11 with deionized water (pH 8.3 ± 0.1 at 25 °C). After autoclaving, 2 ml sodium bicarbonate (1 M), 1 ml *Nitrosomonas* trace elements, 0.1 ml ferric sodium EDTA (7.5 mM), 5 ml KH\(_2\)PO\(_4\) (0.4 g l\(^{-1}\)) and 10 ml NH\(_4\)Cl (1 M) were added (10 mM as final concentration). The *Nitrosomonas* trace element mixture included 30 mg H\(_2\)BO\(_3\), 100 mg MnCl\(_2\).4H\(_2\)O, 190 mg CoCl\(_2\).6H\(_2\)O, 24 mg NiCl\(_2\).6H\(_2\)O, 2 mg CuCl\(_2\).2H\(_2\)O, 144 mg ZnSO\(_4\).7H\(_2\)O, 36 mg Na\(_2\)MoO\(_4\).2H\(_2\)O and 12.5 ml HCl (25% = 7.7 M). The pure culture was obtained by serial dilution technique as described previously (Campbell *et al.*, 2011).

Although its ecological significance is not known, the morphological variety of species found in the genus *Nitrosospira* has earned taxonomic recognition in the past (Koops *et al.*, 2006). This morphological variety contrasts *Nitrosospira* with *Nitrosomonas*, in which all previously reported species are rod-shaped to coccoid, with rounded or pointed ends (Koops *et al.*, 1991). To observe the morphology and size of cells, approximately 20 ml culture was concentrated to a volume of 10 \(\mu\)l using a 0.22 \(\mu\)m filter. The concentrated cells were transferred onto a clean glass slide. Phase-contrast photomicrographs were taken with an Olympus BX51 microscope. Cell images were captured with a DP72 CCD camera and the cell size was determined by using DP-2-BSW microscope digital camera software (Olympus). The motility of cells was confirmed with use of a light microscope (Leitz Wetzlar). Approximately 5 \(\mu\)l sample from a mid-exponential-phase culture was placed on a haemocytometer (Hauser Scientific) and a coverslip was placed on top of the sample. Motility of single cells of strain APG3\(^T\) from liquid culture was visible microscopically at \(\times 400\) magnification. Transmission electron microscopic images were obtained using a Morgagni 268 transmission electron microscope (FEI Company) operating at 80 kV. A 10 \(\mu\)l sample, containing approximately 5 \(\times 10^7\) cells \(\mu\)l\(^{-1}\), was placed on a Formvar-/carbon-coated 300 mesh copper grid (Ted Pella Inc.). Cells were fixed with a negative stain of 2% phosphotungstic acid in water and transmission electron microscope images were captured on a CCD camera (Gatan Orius). Phase-contrast images and electron micrographs indicated that cells of strain APG3\(^T\) have a tightly coiled spiral shape (Fig. 1), a cell shape similar to that of *Nitrosospira briensis* (Winogradsky & Winogradsky, 1933; Watson, 1971) but different from *Nitrosospira multiiformis* (Watson *et al.*, 1971) and *Nitrosospira tenuis* (Harms *et al.*, 1976). Interestingly, and as discussed later, the genetic traits of APG3\(^T\) are more similar to those of *Nitrosospira multiiformis* than to those of *Nitrosospira briensis*. Thus, strain APG3\(^T\) is morphologically distinguishable from the genetically closest species, *Nitrosospira multiiformis*.
et al., 2009). Growth was tested at 4, 10, 25, 30 and 35 °C. Cultures were monitored for nitrite production every other day and up to 9 days. The temperature characteristics of strain APG3\(^T\) were different from those of known strains of the genus Nitrosospira and suggest that strain APG3\(^T\) is a psychrotolerant bacterium (Fig. 2a). This trait differentiates strain APG3\(^T\) from all other described species of the genus Nitrosospira (Table 1). Strain APG3\(^T\) grew optimally at 25 °C. It also grew at 4 and 10 °C, but no growth was observed at 35 °C. Theoretical minimum, optimum and maximum temperatures were calculated as −2, 24 and 36 °C, respectively, using the square root of growth rate model (Ratkowsky et al., 1982). The growth rate at 10 °C was greater than that at 35 °C, a temperature at which this bacterium ceased to grow within 3 days. Interestingly, this growth temperature response resembles the report of Nitrosomonas (formerly Nitrosococcus) mobilis, in which this bacterium grew at 35 °C for only a few days before it became increasingly inhibited and, at last, ceased to grow (Koops et al., 1990). Similarly, Nitrosospira tenuis has been reported to grow for only 1 day at 35 °C (Harms et al., 1976). Growth of strain APG3\(^T\) was examined under eight different pH conditions ranging from pH 3 to 10; APG3\(^T\) was able to grow over a wide range of pH (5–9) (Fig. 2b). According to the literature, growth of strain APG3\(^T\) at pH 5 represents the most acidic conditions reported for AOB (Allison & Prosser, 1993; Burton & Prosser, 2001); however, because no growth was observed at pH 3 or 4 and the growth optimum was between pH 7 and 8, APG3\(^T\) can be classified as an acid-tolerant rather than an acidophilic bacterium. Strains in the genera Nitrosomonas and Nitrosospira differ in their tolerance of ammonium; therefore, the maximum ammonium tolerance of APG3\(^T\) was investigated as an important physiological characteristic. Previous studies demonstrated that strains of the genus Nitrosospira are generally more susceptible to high ammonium concentrations, while the genus Nitrosomonas is represented by ammonium-tolerant and -susceptible species (Koops & Pomerening-Röser, 2001; Koops et al., 2006). The difference of ammonia oxidation kinetics between members of Nitrosospira and Nitrosomonas is likely to be a significant factor in habitat selection and niche differentiation (Schramm et al., 1998). Ammonium tolerance was determined by testing six ammonium concentrations ranging from 10 to 600 mM (Fig. 2c). Cultures were monitored for nitrite production every other day and up to 6 days and specific growth rates were calculated. Strain APG3\(^T\) grew at 10, 50 and 100 mM NH\(_4\)Cl, with fastest growth at 10 mM; growth ceased at concentrations above 200 mM NH\(_4\)Cl. The order of sensitivity to ammonium for described members of the genus Nitrosospira is as follows: Nitrosospira multiformis (50 mM) < Nitrosospira tenuis = strain APG3\(^T\) (100 mM) < Nitrosospira briensis (200 mM) (Koops et al., 2006). This result identified strain APG3\(^T\) as moderately ammonium-tolerant and physiologically distinguishable from the closely related species Nitrosospira multiformis and Nitrosospira briensis. Based on earlier reports on urea catabolism by strains of Nitrosospira (Koper et al., 2004), utilization of urea by strain APG3\(^T\) as an alternative energy source was tested. Urea utilization was tested in 10 ml NH\(_4\)Cl-free APG medium with or without 2 mM urea (Fig. 2d). Urea test medium was inoculated with 10% (v/v) culture grown on 10 mM NH\(_4\)Cl, setting the initial NH\(_4\)Cl concentration to approximately 1 mM. Nitrosomonas ureae Nm 10\(^T\) was used as a positive control for the test. Cultures were monitored for nitrite production every 2 days for 12 days. Student’s t-test was conducted to compare cell growth in triplicate cultures with and without urea after 12 days. Initial growth with and without urea was nearly identical, but a significant difference (P<0.001) became obvious after 6 days, when 66% of the ammonium was consumed. This growth pattern indicates that strain APG3\(^T\) preferentially catabolizes ammonia, but begins to utilize urea before ammonium depletion. The same growth pattern was observed when Nitrosomonas ureae Nm 10\(^T\) was used as a positive control (Fig. 2d). The genetic capacity for urea utilization was confirmed by identification of a complete urease-encoding gene cluster (ure operon; Koper et al., 2004) in the genome of APG3\(^T\). The general characteristics of described species of the genus Nitrosospira and Nitrosospira sp. Nsp5 are summarized in Table 1.

To determine the nucleic acid sequences of marker genes, DNA samples were prepared as described previously (Urakawa et al., 2010). PCR amplification of the 16S rRNA gene was carried out using primers GM3 and GM4 (Muyzer et al., 1995). The gene encoding ammonia monooxygenase subunit A (amoA) was partially amplified by PCR using primers amoA1F and amoA2R (Rotthauwe...
et al., 1997). The PCR conditions used for gene amplification were essentially the same as described previously (Urakawa et al., 2006). Amplicon sizes were examined by electrophoresis on a 1% agarose gel in 1× TAE buffer and visualized with ethidium bromide staining. The PCR products were purified by using spin columns (Microcon YM-50; Millipore) following the manufacturer’s instructions. Direct sequence determination of the purified 16S rRNA and amoA genes was performed with an Applied Biosystems automatic sequencer at the University of Washington DNA sequencing facility, and the results supported the purity of the culture. Sequences similar to those of the isolate were searched for by using BLAST (Altschul et al., 1990) with the option to exclude environmental clone sequences. Sequences were aligned manually with representative sequences of the genus Nitrosospira and related environmental isolates. For the 16S rRNA gene sequence, evolutionary history within the genus Nitrosospira was inferred by using the maximum-likelihood method based on the Tamura–Nei model (Tamura & Nei, 1993). The maximum-likelihood tree with the highest log-likelihood (−2714.0484) was selected. All positions containing gaps and missing data were eliminated, leaving a total of 1457 positions for analysis. The BLAST search with an environmental clone exclusion option indicated that strain APG3T was most closely related to two unidentified

Fig. 2. Growth properties of strain APG3T. (a) Effects of temperature on ammonium oxidation as shown by maximum nitrite production rates at different temperatures. (b) Effects of pH on ammonium oxidation as shown by nitrite production rates under different pH conditions. (c) Effects of NH4Cl concentration on ammonium oxidation as shown by maximum nitrite production rates at different NH4Cl concentrations. (d) Urea utilization by strain APG3T and Nitrosomonas ureae Nm 10T (means ± SD, n=3). All data are means of triplicate measurements.

Table 1. Characteristics of relevant members of the genus Nitrosospira

<table>
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<th>Characteristic</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DNA G+C content (%)</td>
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<td>53.5</td>
<td>54</td>
<td>53.5</td>
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<tr>
<td>Cell shape</td>
<td>Slender curved rods</td>
<td>Pleomorphic lobate</td>
<td>Tightly coiled spirals</td>
<td>Tightly coiled spirals</td>
<td>Tightly coiled spirals</td>
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<tr>
<td>Optimum pH</td>
<td>7.3–8.0</td>
<td>7.5</td>
<td>7.5</td>
<td>7.0–8.0</td>
<td>ND</td>
</tr>
<tr>
<td>Maximum NH4Cl tolerance (mM) (pH 8.0)</td>
<td>100</td>
<td>50</td>
<td>200</td>
<td>100</td>
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<tr>
<td>Use of urea*</td>
<td>+/−</td>
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</table>

*+/−, Some strains are able to utilize urea and some are not.
strains of the genus *Nitrosospira*, Nsp5 and Nsp12, with 99.8% sequence identity. The closest sequence from a species in culture was that of *Nitrosospira multiformis* ATCC 25196T, with 98.6% identity. As shown in the phylogenetic tree (Fig. 3a), the sequences of strain APG3T and *Nitrosospira* sp. Nsp5 and Nsp12 grouped in cluster 0 of the genus *Nitrosospira* (Koops & Harms, 1985). The closest member of the genus *Nitrosomonas* was *Nitrosomonas cryotolerans* ATCC 49181, with 96.3% 16S rRNA gene sequence identity. The 16S rRNA gene sequence identity between strain APG3T and the type strains of the other species of the genus *Nitrosospira* in culture (*Nitrosospira briensis* and *Nitrosospira tenuis*) was 98.1% for both strains. These values were above the 97% species cut-off proposed by Stackebrandt & Goebel (1994). Thus, the members of the genus *Nitrosospira* share high 16S rRNA gene sequence similarity. As shown in the phylogenetic tree (Fig. 3a), all currently known species of the genus *Nitrosospira* belong to cluster 3, while strain APG3T and strains Nsp5 and Nsp12 group in cluster 0. This tree topology was also supported by analyses using neighbour-joining and maximum-parsimony methods. These results suggest that strain APG3T probably represents a novel species in the genus *Nitrosospira*, and the first cultured representative of cluster 0. For the *amoA* gene sequence, the phylogeny within the genus *Nitrosospira* was inferred by using the maximum-likelihood method based on Tamura’s 3-parameter model. The maximum-likelihood tree with the highest log-likelihood (~1905.3830) was selected. All positions containing gaps and missing data were eliminated. A total of 414 positions were compared in the analysis. The robustness of tree topologies was assessed by using bootstrap analyses based on 100 replications (for the maximum-likelihood and maximum-parsimony methods) or 1000 replications (for the neighbour-joining and minimum-evolution methods). Phylogenetic analyses were carried out using MEGA 5 (Tamura et al., 2011). The BLAST search with the environmental clone exclusion option indicated that strain APG3T was most closely related to four unidentified strains of the genus *Nitrosospira*, Nsp5,
NpAV, III2 and 40KI, with 94 % sequence identity. As shown in the phylogenetetic tree (Fig. 3b), strain APG3\textsuperscript{T} and strains Nsp5 and Nsp12 again grouped together with high bootstrap confidence, clearly distinct from cluster 3 of the genus *Nitrosospira*. This tree topology was also supported by other treeing methods (neighbour-joining, maximum-parsimony and minimum-evolution). The *amoA* gene sequence identities between APG3\textsuperscript{T} and the type strains of the three species of the genus *Nitrosospira* were 85 % (*Nitrosospira multiformis*), 87 % (*Nitrosospira briensis*) and 85 % (*Nitrosospira tenuis*).

To determine the genome sequence of strain APG3\textsuperscript{T}, genomic DNA was prepared as described previously (Urakawa et al., 2010). A draft genome sequence of strain APG3\textsuperscript{T} was obtained using the Illumina HiSeq 2000 platform with 2 × 150 bp paired-end reads with a 50 bp overlap (1 310 942 146 reads, total 272-fold coverage) (Garcia et al., 2013). Reads were assembled using the CLC Genomics Workbench version 5.0 (CLC bio). The created contigs were curated using CodonCode Aligner version 3.7 (CodonCode Co.) and annotated by the RAST server (Aziz et al., 2008). Additional genome prediction, annotation and checks for compliance to EMBL recommendations were performed using the EMBL validator software and curation team. In this study, the draft genome sequence was used to analyse DNA G+C content and to confirm the presence of inventory useful for phenotypic characterization such as the urease operon and motility-related genes. The draft genome comprises 3 107 181 bases, which is similar to the genome size of *Nitrosospira multiformis* ATCC 25196\textsuperscript{T} (3.2 Mbp) but larger than the genomes of *Nitrosomonas europaea* ATCC 19718 (2.8 Mbp) and *Nitrosomonas eutropha* C71 (2.8 Mbp). The assembled draft genome consists of 84 contigs with a mean size of 41 181 bp. The DNA G+C content of 53.6 % is similar to the G+C content of the genome of *Nitrosospira multiformis* ATCC 25196\textsuperscript{T} (53.9 %), but higher than that of *Nitrosomonas europaea* ATCC 19718 (50.7 %) and *Nitrosomonas eutropha* C71 (48.5 %). The draft genome contains 3 147 protein-coding DNA sequences, 44 tRNA genes and a single 16S–23S–5S rRNA operon. We used average nucleotide identity (ANI) instead of DNA–DNA hybridization to confirm species-level differentiation between strain APG3\textsuperscript{T} and the closest species, *Nitrosospira multiformis*. The ANI calculated as described by Goris et al. (2007) for the genomes of strain APG3\textsuperscript{T} and *Nitrosospira multiformis* ATCC 25196\textsuperscript{T} (GenBank accession no. NC_007614) was 75.45 % (two-way calculated ANI was 75.50 %), which is lower than the ANI of 95 % that corresponds to a 70 % species level cutoff determined by DNA–DNA hybridization (Wayne et al., 1987). The result indicates clearly that these two genomes do not represent the same species.

To examine the global distribution and habitats of strain APG3\textsuperscript{T}, the *amoA* gene sequence of APG3\textsuperscript{T} was compared with available *amoA* gene sequences in the GenBank database. The standard nucleic acid BLAST search optimized for highly similar sequences (MEGABLAST) was used with the change of maximum target sequences set to 500 within general parameter options (Altschul et al., 1990). Two deposited sequences showed a 100 % match (GenBank accession nos JF951323 and EF107844), and 114 sequences indicated a 99 % match by BLAST search, and these 116 environmental clone sequences were selected for further sequence comparison. As discussed above, the *amoA* gene sequences closest to APG3\textsuperscript{T} from cultured strains were those of four undescribed strains of the genus *Nitrosospira* (Nsp5, NpAV, III2 and 40KI), exhibiting 94 % sequence identity. Purkhold et al. (2000) compared a wide range of cultured AOB based on 16S rRNA and *amoA* gene sequences and defined species thresholds as 97 % 16S rRNA gene sequence identity and 80 % *amoA* gene sequence identity. Moreover, the genomes of betaproteobacterial AOB usually contain two or three nearly identical *amoCAB* operons (Norton et al., 2002). We therefore assume that the compared uncultured clone sequences that showed more than 99 % sequence similarity are members of the same species as this proposed species of the genus *Nitrosospira*, and our data reflect the species distribution of this novel AOB. Sequences related to APG3\textsuperscript{T} were found widely represented in samples from Asia (56 % of all sequences) and North America (43.1 %), but they were rather rare in samples from Europe (0.9 %) (Fig. 4a). The distribution of APG3\textsuperscript{T}–like AOB is probably concentrated in the temperate climate zone, and limited in the tropical and subtropical zones. However, this species may exist in high-altitude habitats in the tropical and subtropical zones. Moreover, no sequences have been reported from the permanently cold Arctic or Antarctic regions. Thus, the psychrotolerant property of APG3\textsuperscript{T} reasonably reflects the global distribution of this novel species. No APG3\textsuperscript{T}–like AOB sequences have been reported from pelagic or coastal waters, including estuarine and tidal flats, indicating that this species originates on land, from which close relatives such as *Nitrosospira* sp. strain NpAV have been isolated (Fig. 4b) (Norton et al., 1996). Moreover, there are no reports from wastewater treatment plants or associated artificial environments. Several sequences (6.1 % of the total) have been retrieved from a wide variety of soil environments (farm, forest, turf grass and acid sulfate soils), while the majority (93.9 % in total) were found in water-saturated or moisture-rich soils (e.g. freshwater sediment and paddy fields) (Fig. 4b) (Dell et al., 2008; Ke & Lu, 2012). Strain APG3\textsuperscript{T} was cultivated from sandy lake sediment, and this is congruent with our *amoA* sequence analysis. The moisture content of soil may change and control the nutrient balance and status and the availability of organic matter (Pastor & Post, 1986). It also strongly influences the oxygen level, pH and redox potential (Pezeshki, 2001; Ma et al., 2013). Paddy fields are generally neutral to acidic (pH 4–7), and freshwater sediments are generally slightly acidic to basic (pH 6–9) (Kawaguchi & Kyuma, 1974; Oliia & Reddy, 1995). Therefore, the capacity of strain APG3\textsuperscript{T} to grow over a wide range of pH possibly supports the success of this species in various water-saturated...
or highly moist soil environments. Strain APG3\textsuperscript{T} also hydrolyses urea as an alternative energy source, which increases its chances of survival in acidic soil/sediment environments (Burton & Prosser, 2001). Overall, the unique habitat selection of this novel species of \textit{Nitrosospira} may provide new insights into the microbial ecology of ammonia-oxidizing microorganisms in nature.

On the basis of its phenotypic and genotypic characteristics, it is concluded that strain APG3\textsuperscript{T} represents a novel species in the genus \textit{Nitrosospira}. It is also the sole representative in culture of cluster 0 of the genus \textit{Nitrosospira}. We propose to name this novel species \textit{Nitrosospira lacus} sp. nov.

**Description of \textit{Nitrosospira lacus} sp. nov.**

\textit{Nitrosospira lacus} (la\’cus. L. gen. n. lacus of a lake).

Cells are spiral, 0.8–1.2 \textmu m wide and 1.3–1.7 \textmu m long. Obligate chemolithotroph, and oxidizes ammonia to nitrite. Cells are motile, and multiple genes involved in flagellum synthesis and function have been identified. The optimum temperature for growth is about 25 °C, and growth has been confirmed between 4 and 30 °C. As for most AOB, the optimum pH for growth is between 7 and 8; however, growth is observed over a wide range of pH (pH 5–9). The optimum and maximum NH\textsubscript{4}Cl concentrations in the medium are 10 and 100 mM, respectively. Cells can utilize urea as an alternative energy source, and the existence of a \textit{ure} operon has been confirmed in the genome.

The type strain APG3\textsuperscript{T} (=NCIMB 14869\textsuperscript{T} =LMG 27536\textsuperscript{T} =ATCC BAA-2542\textsuperscript{T}) was isolated from sandy sediment of Green Lake, a freshwater lake in North Central Seattle, WA, USA. The DNA G+C content of the type strain calculated on the basis of the genome sequence is 53.5 % and the genome size is 3.1 Mbp.

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