**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
constantly increasing, the last description of a novel species of ammonia-oxidizing bacteria (AOB) within the *Nitrosomonadaceae* 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 APG3T is a novel psychrotolerant strain of the genus *Nitrosospira* that we assign to the species *Nitrosospira lacus* sp. nov.

Strain APG3T 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, WA. 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 µg 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 NH₄Cl. 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 MgSO₄, 0.147 g CaCl₂, 2H₂O, 0.584 g NaCl and 250 µl 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 KH₂PO₄ (0.4 g l⁻¹) and 10 ml NH₄Cl (1 M) were added (10 mM as final concentration). The *Nitrosomonas* trace element mixture included 30 mg H₂BO₃, 100 mg MnCl₂.4H₂O, 190 mg CoCl₂.6H₂O, 24 mg NiCl₂.6H₂O, 2 mg CuCl₂.2H₂O, 144 mg ZnSO₄.7H₂O, 36 mg Na₂MoO₄.2H₂O 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 µl using a 0.22 µm filter. The concentrated cells were transferred onto a clean slide glass. 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-B5W microscope digital camera software (Olympus). The motility of cells was confirmed with use of a light microscope (Leitz Wetzlar). Approximately 5 µl 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 APG3T from liquid culture was visible microscopically at × 400 magnification. Transmission electron microscopic images were obtained using a Morgagni 268 transmission electron microscope (FEI Company) operating at 80 kV. A 10 µl sample, containing approximately 5 × 10⁷ cells ml⁻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 APG3T 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 multiformis* (Watson et al., 1971) and *Nitrosospira tenuis* (Harms et al., 1976). Interestingly, and as discussed later, the genetic traits of APG3T are more similar to those of *Nitrosospira multiformis* than to those of *Nitrosospira briensis*. Thus, strain APG3T is morphologically distinguishable from the genetically closest species, *Nitrosospira multiformis*.

All cultures were grown and tested in 10 ml medium using 100 ml medium bottles without agitation under dark conditions. Cell growth was routinely monitored by nitrite production as described previously (Martens-Habbena...
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 APG3T were different from those of known strains of the genus Nitrosospira and suggest that strain APG3T is a psychrotolerant bacterium (Fig. 2a). This trait differentiates strain APG3T from all other described species of the genus Nitrosospira (Table 1). Strain APG3T 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 Nitrosooccus) 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, Nitrososphaera tenuis has been reported to grow for only 1 day at 35 °C (Harms et al., 1976). Growth of strain APG3T was examined under eight different pH conditions ranging from pH 3 to 10; APG3T was able to grow over a wide range of pH (5–9) (Fig. 2b). According to the literature, growth of strain APG3T 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, APG3T 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 APG3T 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 APG3T grew at 10, 50 and 100 mM NH₄Cl, with fastest growth at 10 mM; growth ceased at concentrations above 200 mM NH₄Cl. The order of sensitivity to ammonium for described members of the genus Nitrosospira is as follows: Nitrosospira multiformis (50 mM) < Nitrosospira tenuis = strain APG3T (100 mM) < Nitrosospira briensis (200 mM) (Koops et al., 2006). This result identified strain APG3T 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 APG3T as an alternative energy source was tested. Urea utilization was tested in 10 ml NH₄Cl-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 NH₄Cl, setting the initial NH₄Cl concentration to approximately 1 mM. Nitrosomonas ureae Nm 10T 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 APG3T preferentially catabolizes ammonia, but begins to utilize urea before ammonium depletion. The same growth pattern was observed when Nitrosomonas ureae Nm 10T 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 APG3T. 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 mono-oxygenase subunit A (amoA) was partially amplified by PCR using primers amoA1F and amoA2R (Rothhauwe

Fig. 1. Phase-contrast and transmission electron micrographs of cells of strain APG3T. (a) Phase-contrast photomicrograph. Bar, 5 μm. (b) Transmission electron micrograph of negatively stained cell. Bar, 200 nm.
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 1x 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 strains.

Table 1. Characteristics of relevant members of the genus Nitrosospira

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>16S rRNA cluster</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DNA G+C content (%)</td>
<td>53.9</td>
<td>53.5</td>
<td>54</td>
<td>53.5</td>
<td>53.5</td>
</tr>
<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>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
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<td>15–30</td>
<td>15–30</td>
<td>4–30</td>
<td>4–37</td>
</tr>
<tr>
<td>Optimum</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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<td>Use of urea*</td>
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<td>+/−</td>
<td>+/−</td>
<td>+</td>
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</tr>
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*+/−, 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 phylogenetic tree (Fig. 3b), strain APG3T 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 APG3T 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 APG3T, genomic DNA was prepared as described previously (Urukawa et al., 2010). A draft genome sequence of strain APG3T was obtained using the Illumina HiSeq 2000 platform with 2×150 bp paired-end reads with a 50 bp overlap (1310942146 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 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 sequence comprises 3107181 bases, which is similar to the genome size of *Nitrosospira multiformis* ATCC 25196 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 41181 bp. The DNA G+C content of 53.6% is similar to the G+C content of the genome of *Nitrosospira multiformis* ATCC 25196 T (53.9%), but higher than that of *Nitrosomonas europaea* ATCC 19718 (50.7%) and *Nitrosomonas eutropha* C71 (48.5%). The draft genome contains 3147 protein-coding DNA sequences, 44 tRNA genes and a single 16S–23S–55 tRNA operon. We used average nucleotide identity (ANI) instead of DNA–DNA hybridization to confirm species-level differentiation between strain APG3T and the closest species, *Nitrosospira multiformis*. The ANI calculated as described by Goris et al. (2007) for the genomes of strain APG3T and *Nitrosospira multiformis* ATCC 25196 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 APG3T, the *amoA* gene sequence of APG3T 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 APG3T 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 APG3T 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 APG3T-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 APG3T reasonably reflects the global distribution of this novel species. No APG3T-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 APG3T 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 & Kiyuma, 1974; Oliš & Reddy, 1995). Therefore, the capacity of strain APG3T to grow over a wide range of pH possibly supports the success of this species in various water-saturated environments.
or highly moist soil environments. Strain APG3<sup>T</sup> 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 *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<sup>T</sup> represents a novel species in the genus *Nitrosospira*. It is also the sole representative in culture of cluster 0 of the genus *Nitrosospira*. We propose to name this novel species *Nitrosospira lacus* sp. nov.

**Description of *Nitrosospira lacus* sp. nov.**

*Nitrosospira lacus* (lacus. L. gen. n. lacus of a lake).

Cells are spiral, 0.8–1.2 μm wide and 1.3–1.7 μm 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<sub>4</sub>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 *u*re operon has been confirmed in the genome.

The type strain APG3<sup>T</sup> (=NCIMB 14869<sup>T</sup>=LMG 27536<sup>T</sup>=ATCC BAA-2542<sup>T</sup>) 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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