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TAXONOMIC DESCRIPTION

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# *Lichenicola cladoniae* gen. nov., sp. nov., a member of the family *Acetobacteraceae* isolated from an Antarctic lichen

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**Abstract**

Two Gram-stain-negative, facultative anaerobic, chemoheterotrophic, pink-coloured, rod-shaped and non-motile bacterial strains, PAMC 26568 and PAMC 26569$^T$, were isolated from an Antarctic lichen. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains PAMC 26568 and PAMC 26569$^T$ belong to the family *Acetobacteraceae* and the most closely related species are *Gluconacetobacter takamatsuzukensis* (96.1%), *Gluconacetobacter tumulisoli* (95.9%) and *Gluconacetobacter sacchari* (95.7%). Phylogenomic and genomic relatedness analyses showed that strains PAMC 26568 and PAMC 26569$^T$ are clearly distinguished from other genera in the family *Acetobacteraceae* by average nucleotide identity values (<72.8%) and the genome-to-genome distance values (<22.5%). Genomic analysis revealed that strains PAMC 26568 and PAMC 26569$^T$ do not contain genes involved in atmospheric nitrogen fixation and utilization of sole carbon compounds such as methane and methanol. Instead, strains PAMC 26568 and PAMC 26569$^T$ possess genes to utilize nitrate and nitrite and certain monosaccharides and disaccharides. The major fatty acids (>10%) are summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$; 40.3–40.4%), $C_{18:1}$ 2OH (22.7–23.7%) and summed feature 2 ($C_{14:0}$ 3OH and/or $C_{16:1}$ iso I; 12.0% in PAMC 26568). The major respiratory quinone is Q-10. The genomic DNA G+C content of PAMC 26568 and PAMC 26569$^T$ is 64.6%. Their distinct phylogenetic position and some physiological characteristics distinguish strains PAMC 26568 and PAMC 26569$^T$ from other genera in the family *Acetobacteraceae* supporting the proposal of *Lichenicola* gen. nov., with the type species *Lichenicola cladoniae* sp. nov. (type strain, PAMC 26569$^T$=KCCM 43315$^T$=JCM 33604$^T$).

Lichens are symbiotic associations of lichenized fungi (mycobiont) and green algae and/or cyanobacteria (photobiont). In addition to mycobionts and photobionts, diverse microorganisms such as lichen-associated fungi, algae and non-photosynthetic lichen-associated bacteria have been revealed by culture-dependent and -independent approaches [1–11]. Among them, diverse non-photosynthetic lichen-associated bacteria are considered to participate in nutrient cycling through lytic activities, hormone production, phosphate mobilization and solubilization, and antagonistic activity [5, 7, 10, 12]. Among the bacterial groups, the orders *Rhodospirillales* and *Rhizobiales* of the class *Alphaproteobacteria* are known to be one of the most predominant bacterial groups in diverse lichens [6–9, 13–15]. In particular, the family *Acetobacteraceae* of *Rhodospirillales* dominates in lichens from Antarctic areas or in lichen of the genera *Clanonia*, *Umbilicaria* and *Rhizoplaca* [8, 13, 14, 16]. *Acetobacteraceae* is also known to be predominant in ants and plants [13, 17, 18]. Since strains of *Gluconacetobacter diazotrophicus*, *Swaminathania salitolerans* and *Acetobacter peroxydans* of the *Acetobacteraceae*, which were isolated from plants are known to promote plant growth by nitrogen fixation [18–21], it has been presumed that members of the *Acetobacteraceae* may play roles as nitrogen fixers in lichen [13, 14]. However, only a few bacterial isolates

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**Keywords:** Antarctica; *Lichenicola cladoniae*; new genus; polyphasic taxonomy; lichen.

**Abbreviations:** AAI, average amino acid identity; ANI, average nucleotide identity; DDH, DNA–DNA hybridization; ML, maximum-likelihood; NJ, neighbour-joining; POCP, percentage of conserved protein; R2A, Reasoner's 2A.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequence of strain PAMC 26569$^T$ are KJ606804 and CP053708-CP053715 under the BioProject number PRJNA632689, respectively. Those for the 16S rRNA gene sequence and the draft genome sequence of strain PAMC 26568 are KJ606803 and JABKAT000000000 under the BioProject number PRJNA632689, respectively.

Two supplementary figures and two supplementary tables are available with the online version of this article.

of *Acetobacteraceae* from lichens have been reported without appropriate nomenclature and determination of physiological characteristics [10]. In this study, we performed polyphasic analysis of two strains, PAMC 26568 and PAMC 26569$^T$, of the family *Acetobacteraceae* and isolated from an Antarctic lichen [10], to determine their taxonomic position.

Two strains, PAMC 26568 and PAMC 26569$^T$, which were isolated from a lichen specimen of *Cladonia borealis* (sampled at King George Island, Antarctica; 62° 13.563′ S, 58° 47.014′ W) were used [10]. In brief, for bacteria isolation, lichen thallus was immersed in 1 ml sterile distilled water in a petri dish for 1 min and this step was repeated four times. After the final wash, the samples were crushed in a TissueLyzer II containing steel beads (Qiagen) twice for 2 min. One hundred microlitres of the final suspension were spread on MY (20 g malt extract and 2 g yeast extract in 1 l distilled water) solid medium and incubated at 10 °C for 29 days. Strains PAMC 26568 and PAMC 26569$^T$ were isolated and subsequently streaked on Reasoner's 2A (R2A) agar plates three times to obtain pure cultures. These strains were maintained on R2A agar at 15 °C after the determination of optimal temperature and preserved as 20% (v/v) glycerol at −80 °C.

The 16S rRNA gene sequences (1352 nt) of PAMC 26568 and PAMC 26569$^T$ with 100% similarity were available under the accession numbers KJ606803 and KJ606804, respectively [10]. For confirmation, genomic DNA was extracted by using the genomic DNA isolation kit (Cosmo Genetech) according to the manufacturer's instructions. The 16S rRNA gene was amplified with two universal primers, 27F and 1492R [22]. PCR products were purified using the LaboPass PCR purification kit (Cosmo Genetech) and sequenced using primers, 785F and 926R [22]. The 16S rRNA sequences of PAMC 26568 and PAMC 26569$^T$ obtained by 785F and 926R were consistent with KJ606803 and KJ606804, respectively. Thus, 16S rRNA gene sequences available under the accession numbers KJ606803 and KJ606804 were used for comparison with those of all type strains in the EzBioCloud database [23] and aligned with closely related type strains in the family *Acetobacteraceae* using jPhydit [24]. Phylogenetic trees of the 16S rRNA gene sequences were reconstructed using the neighbour-joining (NJ) [25] and maximum-likelihood (ML) [26] methods using the MEGA X program [27]. The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications of the sequences. Comparison of 16S rRNA gene sequences showed that strains PAMC 26568 and PAMC 26569$^T$ were closely related to *Gluconacetobacter takamatsuzukensis* (96.1% sequence similarity), followed by *Gluconacetobacter tumulisoli* (95.9% sequence similarity) and *Gluconacetobacter sacchari* (95.7% sequence similarity). In phylogenetic trees inferred from the two algorithms, PAMC 26568 and PAMC 26569$^T$ were clearly separated from other genera of the family *Acetobacteraceae* (Fig. 1).

Genomic sequences of PAMC 26568 were obtained by sequencing with Illumina MiSeq apparatus and assembled with CLC Genomics Workbench version 9.0 (Qiagen). Genomic sequences of PAMC 26569$^T$ were obtained by hybrid *de novo* assembly of reads from HiSeq sequencing and MinION sequencing (Phyzen) using Unicycler assembler version 0.4.8 [28]. Genome annotation was performed using the Rapid Annotation using Subsystems Technology (RAST) server [29] and the NCBI Prokaryotic Genome Annotation Pipeline using a best-placed reference protein set and GeneMarkS-2+ [30, 31]. Analysis of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) orthology was performed using the KEGG Automatic Annotation Server (KAAS) [32]. Details of the PAMC 26568 and PAMC 26569$^T$ genomes are summarized in Table S1 (available in the online version of this article). In brief, the draft genome of PAMC 26568 comprised 154 contigs containing 5332 protein-coding genes, 44 tRNA genes and one rRNA operon (Table S1). The complete genome of PAMC 26569$^T$ comprised eight circular contigs containing 5411 protein-coding genes, 50 tRNA genes and three rRNA operons (Table S1). Genomic analysis revealed that strains PAMC 26568 and PAMC 26569$^T$ do not possess genes for nitrogen fixation such as nitrogenase and methane monooxygenases and methanol dehydrogenases (Tables 1 and 2). A complete set of enzymes involved in the glycolysis and tricarboxylic acid cycle (TCA) for ATP and NADH production were encoded by strains PAMC 26568 and PAMC 26569$^T$, and genes encoding lactate dehydrogenase and alcohol dehydrogenase for bacterial fermentation were identified. Strains PAMC 26568 and PAMC 26569$^T$ possessed ABC transporters for various carbon sources: sorbitol/mannitol, sugar, ribose, D-xylose, galactofuranose, rhamnose, erythritol and glycerol. In addition, genes involved in nitrate and nitrite uptake and utilization were identified. Genes involved in the Calvin–Benson pathway of carbon dioxide fixation except for phosphoribulokinase were found in the genomes.

The degree of pairwise genome-based relatedness was estimated by average nucleotide identity (ANI) calculation [33, 34], *in silico* DNA–DNA hybridization (DDH) inferred by using the Genome-to-Genome Distance Calculator (GGDC) [35], average amino acid identity (AAI) values calculated by the orthologous ANI algorithm [36], and the percentage of conserved proteins (POCP) values [37]. The ANI value between PAMC 26568 and PAMC 26569$^T$ was 99.9% (Table 2) and this level is above the ANI cut-off value (95–96%) used to delineate bacterial species [38]. In addition, the DDH value between PAMC 26568 and PAMC 26569$^T$ was 92.6% (Table 2), indicating that strains PAMC 26568 and PAMC 26569$^T$ are conspecific [39]. In contrast, ANI values between PAMC 26568 and PAMC 26569$^T$ and other related type strains of *Acetobacteraceae* were below 72.8%, and the DDH values were below 22.5% (Table 2). The AAI value between PAMC 26568 and PAMC 26569$^T$ was 100% (Table 2) and this level is above the AAI cut-off value (85–90%) used to delineate bacterial species. The POCP value between PAMC 26568 and PAMC 26569$^T$ was 97.6%. In contrast, AAI values between PAMC 26568 and PAMC 26569$^T$ and other related type strains of *Acetobacteraceae* were below 59.8% and POCP values were 45.9% (Table 2), supporting that these strains belong to a separate genus (45–65% AAI values and <50% POCP values for the same family) [36, 37].

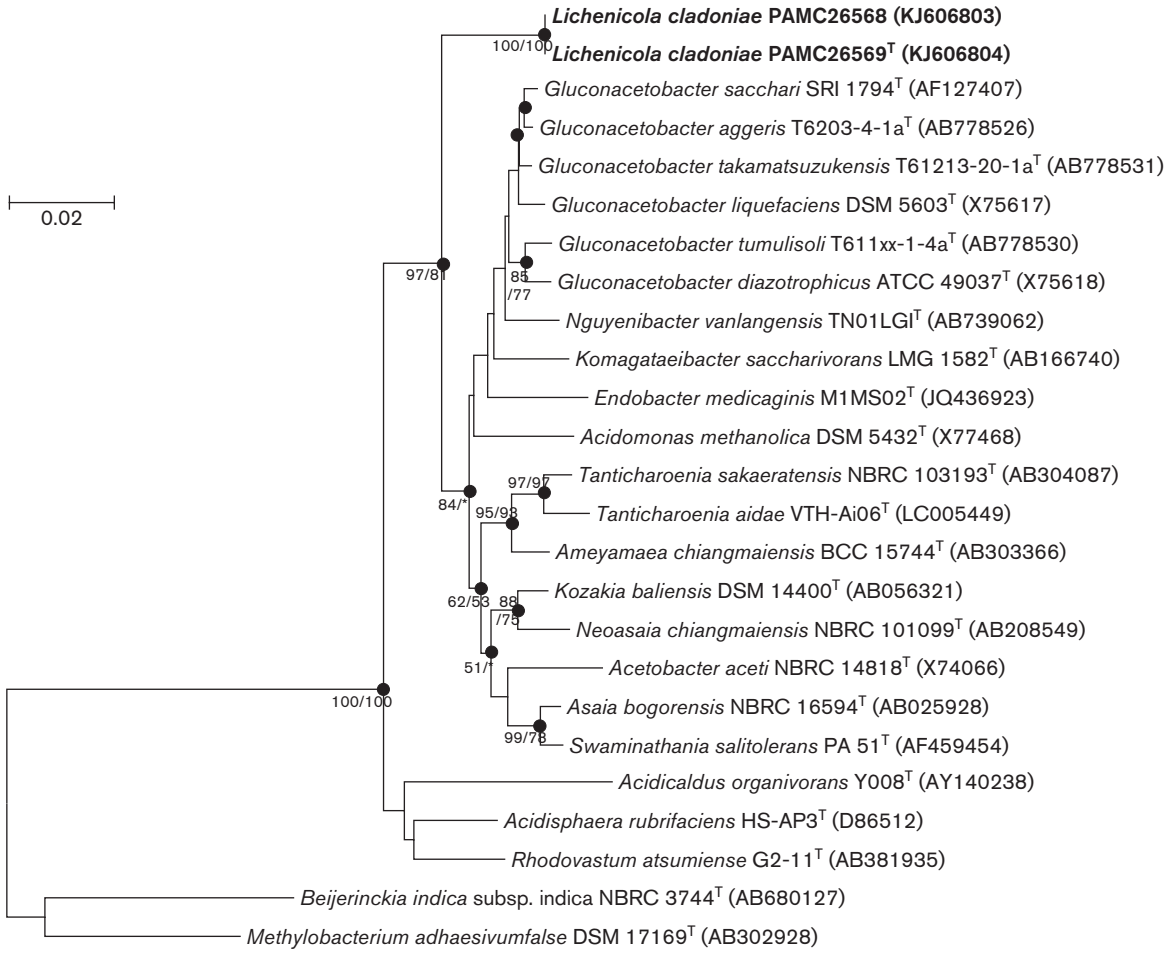


**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences of strains PAMC 26568 and PAMC 26569$^T$ and members of related genera of the family *Acetobacteraceae*. Bootstrap values (>50%) based on 1000 replications were shown on corresponding branches (neighbour-joining/maximum likelihood). Asterisks denote bootstrap values less than 50%. Filled circles indicate conserved nodes in both neighbour-joining and maximum-likelihood trees. *Beijerinckia indica* subsp. *Indica* NBRC 3744$^T$ and *Methylobacterium adhaesivum* DSM 17169$^T$ of the order *Rhizobiales* were used as outgroups. Bar, 0.02 substitutions per nucleotide position.

Multiple sequence alignment of the concatenated 120 ubiquitous single-copy proteins [40] for the available genomes in the family *Acetobacteraceae* was performed by GTDB-Tk [41]. Phylogenomic trees using the NJ and ML algorithms were reconstructed based on 1000 sets of sequence replications using MEGA X [26]. In agreement with the phylogenetic tree based on the 16S rRNA gene sequences, the phylogenomic tree also showed that strains PAMC 26568 and PAMC 26569$^T$ formed a distinct clade from other genera in the family *Acetobacteraceae* (Fig. S1). The 16S rRNA gene sequences of strains PAMC 26569 and PAMC 26569$^T$ determined by direct sequencing were identical to those retrieved from their genome sequences. The genomic DNA G+C content of strains PAMC 26568 and PAMC 26569$^T$, which was calculated from genome sequences, was 64.6% (Table 1).

The temperature range and optimal temperature for growth were determined by culturing strains on R2A solid medium at different temperatures (0, 4, 10, 15, 20, 25 and 30 °C) for 14 days. The range and optimal pH for growth were determined in R2A liquid medium. The pH was adjusted using the following buffering systems; $Na_2HPO_4$-buffered citric acid, pH 4.0–5.0; MES, pH 5.5–6.0; MOPS, pH 6.5–7.0; AMPD, pH 8.0–9.5; CAPS, pH 10.0. The growth of each culture was assessed by measuring optical density at 600 nm (EnVision plate reader, PerkinElmer) every day for up to 14 days. NaCl tolerance tests were carried on R2A solid medium supplemented with 0, 0.5, 1, 2, 3, 4 and 5% NaCl (w/v). Catalase activity was tested with 3% $H_2O_2$ and oxidase activity was determined using tetramethyl-*p*-phenylenediamine following the methods described by Kovacs *et al.* [42]. Anaerobic growth was tested on R2A agar, minimal salt solid (MSS) medium [43] and MSS supplemented with glucose (1%) in a jar containing an AnaeroPak (Mitsubishi Gas Chemical) for up to 14 days at 15 °C.

Gram staining was carried out using Gram-stain kit (Sigma) according to the manufacturer's instructions. Motility was

**Table 1.** Major characteristics that distinguish PAMC 26569$^T$ and PAMC 26568 and other genera of *Acetobacteraceae*

Strains: 1, PAMC 26569$^T$; 2, *Gluconacetobacter takamatsuzukensis* T61213-20-1a$^T$; 3, *Gluconacetobacter tumulisoli* T611xx-a-4a$^T$; 4, *Gluconacetobacter sacchari* SRI1794$^T$; 5, *Gluconacetobacter diazotrophicus* ATCC 49037$^T$; 6, *Gluconacetobacter liquefaciens* DSM 5603$^T$; 7, *Gluconacetobacter aggeris* T6203-4-1a$^T$; 8, *Gluconacetobacter tumulicola* K5929-2-1b$^T$; 9, *Gluconacetobacter asukensis* K8617-1-1b$^T$; 10, *Gluconacetobacter johannae* CFN-Cf55$^T$; 11, *Gluconacetobacter azotocaptans* CFN-Ca54$^T$; 12, *Kozakia baliensis* DSM 14400$^T$; 13, *Ameyamaea chiangmaiensis* BCC 15744$^T$; 14, *Tanticharoenia aidae* VTH-Ai06$^T$; 15, *Nguyenibacter vanlangensis* TN01LGI$^T$; 16, *Acidomonas methanolica* DSM 5432$^T$. Data for strains PAMC 26569$^T$ and PAMC 26568 were obtained from the present study and all characteristics of strain PAMC 26568 were identical to those of strain PAMC 26569$^T$. Other data were from previously published sources as indicated. +, Positive; –, negative; Pt, peritrichous flagellation; Pl, polar flagellation; SA, strictly aerobic; A, aerobic; Aa, anaerobic; F, facultative anaerobic; ND, not determined.

| **Characteristics*** | **1** | **2**[a] | **3**[a] | **4**[b] | **5**[c] | **6**[d] | **7**[a] | **8**[e] | **9**[e] | **10**[f] | **11**[f] | **12**[g] | **13**[h] | **14**[i] | **15**[j] | **16**[k] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Optimum temperature (range) for growth (°C) | 15 (4–20) | 20–30 (15–30) | 20–30 (15–30) | 28–30 | 30 | 28 (25–30) | 20–30 (15–30) | 20–30 (15–30) | 20–30 (15–30) | 29 | 29 | 30 | ND | 20–30 (10–32) | ND | 30–32 (30–37) |
| Flagellation | – | Pt | – | Pt | Pt | Pt | Pt | Pt | Pt | Pt | Pt | – | Pl | – | Pt | Pl |
| Oxygen requirement | F | SA | SA | A | A | A, Aa | SA | SA | SA | A | A | SA | ND | ND | ND | A |
| pH range for growth | 5.5–6.5 | 3.0–6.8 | 3.0–6.8 | ND | 5.5 | 5.4–6.3 | 3.0–6.8 | 3.0–6.8 | 3.0–6.8 | 4.0–7.0 | 4.0–7.0 | 3.0 | ND | 2.5–9.0 | ND | 2.0–5.5 |
| G+C content (mol%) | 64.6† | 66.6 | 66.5 | 66.0 | 61.0 | 64.6 | 65.4 | 64.7 | 65.4 | 57.9 | 64.0 | 57.2 | 66.0 | 65.4 | 69.4 | 64.4 |
| Reduction of nitrate to nitrite | +† | ND | ND | – | – | –† | ND | ND | ND | – | – | – | ND | –† | ND | – |
| Nitrogen fixation | –† | – | – | – | + | –† | – | ND | ND | + | + | –† | ND | –† | ND | –† |

*Data from: *a*, Nishijima *et al.* [46]; *b*, Franke *et al.* [47]; *c*, Gillis *et al.* [20]; *d*, Gossele *et al.* [48]; *e*, Tazato *et al.* [49]; *f*, Fuentes-Ramírez *et al.* [50]; *g*, Lisdiyanti *et al.* [51]; *h*, Yukphan *et al.* [53]; *i*, Vu *et al.* [54]; *j*, Vu *et al.* [55]; *k*, Urakami *et al.* [52].
†The data were inferred from genome sequences.

**Table 2.** Results of genomic relatedness analyses based on average nucleotide identity (ANI; [34]), *in silico* DNA–DNA hybridization (DDH) inferred by the genome-to-genome distance [35], average amino acid identity (AAI; [36]) and percentage of conserved proteins (POCP; [37])

Strain (accession number): 1, PAMC 26568 (PRJNA632689); 2, PAMC 26569$^T$ (PRJNA632689); 3, *Gluconacetobacter liquefaciens* DSM 5603$^T$ (PRJNA439994); 4, *Gluconacetobacter diazotrophicus* ATCC 49037$^T$ (PRJNA21071); 5, *Acidomonas methanolica* DSM 5432$^T$ (PRJNA500315); 6, *Tanticharoenia sakaeratensis* NBRC 103193$^T$ (PRJDB503); 7, *Asaia bogorensis* NBRC 16594$^T$ (PRJDB519); 8, *Neoasaia chiangmaiensis* NBRC 101099$^T$ (PRJNA311264); 9, *Kozakia baliensis* DSM 14400$^T$ (PRJNA311264).

| | ANI (%) | | | | | | | | | AAI (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strain** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| 1 | – | 99.9 | 72.2 | 72.8 | 70.4 | 70.4 | 69.4 | 70.1 | 69.2 | – | 100.0 | 58.8 | 59.8 | 56.0 | 56.7 | 55.8 | 56.8 | 56.2 |
| 2 | 92.6 | – | 72.1 | 72.8 | 70.4 | 70.5 | 69.5 | 70.1 | 69.1 | 97.6 | – | 58.8 | 59.7 | 56.1 | 56.7 | 55.8 | 56.8 | 56.1 |
| 3 | 19.3 | 19.5 | – | 80.7 | 73.9 | 71.9 | 70.5 | 72.5 | 71.1 | 42.8 | 42.7 | – | 77.7 | 62.7 | 61.6 | 60.5 | 62.4 | 61.6 |
| 4 | 19.1 | 19.2 | 24.3 | – | 73.8 | 72.4 | 70.9 | 72.9 | 71.7 | 45.2 | 45.3 | 70.7 | – | 62.6 | 62.2 | 60.5 | 63.1 | 62.0 |
| 5 | 18.7 | 19.1 | 21.5 | 22.6 | – | 72.4 | 72.0 | 74.5 | 72.5 | 40.0 | 40.2 | 58.9 | 59.2 | – | 64.1 | 64.5 | 69.8 | 66.7 |
| 6 | 18.8 | 19.1 | 19.3 | 20.3 | 19.8 | – | 71.0 | 73.2 | 71.2 | 43.1 | 43.1 | 58.6 | 58.6 | 59.1 | – | 62.6 | 66.8 | 65.0 |
| 7 | 21.4 | 22.1 | 20.5 | 20.9 | 19.7 | 20.1 | – | 73.3 | 72.3 | 41.1 | 41.2 | 57.4 | 58.3 | 58.4 | 65.0 | – | 66.8 | 69.0 |
| 8 | 19.9 | 20.5 | 21.2 | 22.6 | 20.5 | 22 | 19.8 | – | 73.8 | 44.0 | 43.9 | 60.0 | 62.6 | 66.8 | 70.7 | 68.7 | – | 71.5 |
| 9 | 21.7 | 22.5 | 22.7 | 24 | 20.2 | 21.2 | 19.8 | 19.7 | – | 45.3 | 45.9 | 56.8 | 60.3 | 61.7 | 66.8 | 69.8 | 71.5 | – |
| | DDH (%) | | | | | | | | | POCP (%) | | | | | | | | |

determined by the observation of growth after inoculation in the R2A liquid medium with 0.4% agar. Morphology of cells was examined by transmission electron microscopy (TEM; CM200, Philips). For TEM, cells were negatively stained with 2.0% uranyl acetate on a carbon-coated copper grid. Biochemical activities were determined by using the API 20NE, API ZYM and API 50CH kits (bioMérieux) according to the manufacturer's instructions. The utilization of methanol, formaldehyde, formate, methylamine and glucose as a sole carbon sourcewas determined by cultivation on minimal salt liquid medium at 0.1% concentration (v/v) of each substrate with the exception of glucose (0.4 and 1.0%) by measuring optical density at 600 nm (EnVision plate reader, PerkinElmer) every 3 days for up to 14 days. Nitrogen fixation was tested for strains PAMC 26568 and PAMC 26569$^T$, with a strain of the genus *Sinorhizobium* as a positive control by cultivation on the nitrogen-free solid medium [43].

The morphological, physiological and biochemical characteristics of strains PAMC 26568 and PAMC 26569$^T$ are described in Fig. S2, Table 1 and the species description. Strains PAMC 26568 and PAMC 26569$^T$ grew at 4–20 °C (optimally at 15 °C). The pH range for growth of strains PAMC 26568 and PAMC 26569$^T$ was pH 5.5–6.5. The strains did not require NaCl for growth. Strains PAMC 26568 and PAMC 26569$^T$ grew under aerobic and anaerobic conditions. Catalase was positive and oxidase was negative. A transmission electron microscope image showed that cells are rod-shaped (Fig. S2). In addition, strains PAMC 26568 and PAMC 26569$^T$ did not have peritrichous flagella. Strains PAMC 26568 and PAMC 26569$^T$ were capable of reducing nitrate to nitrite with nitrate reductase and no genes involved in nitrogen fixation were found with very weak growth on nitrogen-free medium.

For cellular fatty acid analysis, strains PAMC 26568 and PAMC 26569$^T$ were grown on R2A agar at 15 °C for 7 days. Analysis was performed according to the method described by the Sherlock Microbial Identification System version 6.1 (MIDI) using the TSBA6 database [44]. The major fatty acids (>10%) of strains PAMC 26568 and PAMC 26569$^T$ were summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*; 40.4 and 40.3%), $C_{18:1}$ 2OH (23.7 and 22.7%) and summed feature 2 ($C_{14:0}$ 3OH and/or $C_{16:1}$ iso I; 12.0 and 5.1%) (Table 3). Quinones were extracted as described by Collins and Jones [45] and analysed by high-performance liquid chromatography (HPLC) by the Korean Culture Centre of Microorganisms (Republic of Korea). Q-10 was the only menaquinone present in strains PAMC 26568 and PAMC 26569$^T$.

Strains PAMC 26568 and PAMC 26569$^T$ showed several differential physiological and genomic characteristics from other genera of the family *Acetobacteraceae*. Strains PAMC 26568 and PAMC 26569$^T$ grew at lower temperatures (4–20 °C, optimally at 15 °C; Table 1) compared to related type strains of the genus *Gluconacetobacter* (15–30 °C, optimally at 20–30 °C) [20, 46–50]. The pH range for growth of the type strains of *Gluconacetobacter* was more acidic (pH 3–7) than that of strains PAMC 26568 and PAMC 26569$^T$ (pH 5.5–6.5; Table 1) [46]. Strains PAMC 26568 and PAMC 26569$^T$ did not grow as well under anaerobic conditions compared to growth under aerobic conditions. However, the observed anaerobic growth of PAMC 26568 and PAMC 26569$^T$ is in contrast to the type strains of the genera in *Acetobacteraceae* except for *G. liquefaciens*, which grows aerobically [20, 46–52]. In addition, strains PAMC 26568 and PAMC 26569$^T$ do not have flagella, unlike the type strains of the genus *Gluconacetobacter*, which have peritrichous flagella [20, 46–50]. Unlike *G. diazotrophicus*, *G. johannae* and *G. azotocaptan*, strains PAMC 26568 and PAMC 26569$^T$ do not fix atmospheric nitrogen [20, 50]. Unlike the strains of the genus *Gluconacetobacter* with available genomes, strains PAMC 26568 and PAMC 26569$^T$ possess genes for the utilization of nitrite and nitrate-encoding nitrate reductase.

**Table 3.** Fatty acid profiles of strains PACM 26569$^T$ and PAMC 26568

All strains were grown on R2A plates at 15 °C for 7 days. Results are shown as percentages of the total fatty acids. Fatty acids accounting for up to 10% or more are presented in bold. ND, Not detected.

| Fatty acid | PAMC 26569$^T$ | PAMC 26568 |
|---|---|---|
| $C_{12:0}$ | 0.6 | 0.9 |
| $C_{16:1}$ *ω11c* | 1.9 | 1.8 |
| $C_{16:0}$ | 2.6 | 2.6 |
| $C_{16:0}$ 2OH | 4.7 | 3.8 |
| $C_{16:0}$ 3OH | 4.7 | 2.0 |
| $C_{18:3}$ *ω6c* (6, 9, 12) | 0.7 | 1.7 |
| $C_{18:0}$ iso | 8.3 | 4.5 |
| $C_{18:1}$ *ω9c* | 0.4 | ND |
| $C_{18:1}$ *ω5c* | 0.5 | 0.5 |
| $C_{18:0}$ | 0.5 | 0.7 |
| $C_{19:0}$ cyclo *ω8c* | 2.4 | 3.3 |
| $C_{18:1}$ 2OH | 22.7 | 23.7 |
| $C_{18:0}$ 3OH | 2.8 | 0.7 |
| $C_{20:2}$ *ω6,9c* | 0.6 | ND |
| Summed feature 2* | 5.1 | 12.0 |
| Summed feature 3* | 0.5 | ND |
| Summed feature 7* | 0.8 | 1.4 |
| Summed feature 8* | 40.3 | 40.4 |

*Summed features represent fatty acids that could not be separated by GLC with the MIDI system; summed feature 2 comprises $C_{14:0}$ 3OH and/or $C_{16:1}$ iso I, summed feature 3 comprises $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*, summed feature 7 comprises $C_{19:1}$ *ω7c* and/or $C_{19:1}$ *ω6c* and summed feature 8 comprises $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*.

Based on the clearly separated phylogenetic cluster of PAMC 26568 and PAMC 26569$^T$ within *Acetabacteraceae* (Fig. 1), genome-based relatedness data that differentiates PAMC 26568 and PAMC 26569$^T$ from type strains of the genera of the family *Acetobacteraceae*, and differential physiological characteristics, we propose the genus *Lichenicola* gen. nov., a new member of the family *Acetobacteraceae*.

## DESCRIPTION OF *LICHENICOLA* GEN. NOV.

*Lichenicola* (Li.che.ni′co.la L. masc. n. *lichen*, lichen; L. masc/fem. suff. *–cola*, an inhabitant; from L. masc./fem. n. *incola*; N.L. masc. n. *Lichenicola*, inhabitant of lichens).

Cells are Gram-stain-negative, rod-shaped, non-motile and facultatively anaerobic. Cells are oxidase-negative and catalase-positive. The dominant fatty acids include summed feature 8 ($C_{18:1}$ *ω*7c and/or $C_{18:1}$ *ω*6*c*), $C_{18:1}$ 2OH and summed feature 2 ($C_{14:0}$ 3OH and/or $C_{16:1}$ iso I). The major isoprenoid quinone is Q-10. DNA G+C content is 64.6%. No genes involved in nitrogen fixation and methane or methanol utilization are detected. Contains a complete set of genes involved in glycolysis, the TCA cycle and oxidative phosphorylation. Member of the family *Acetobacteraceae*, order *Rhodospirillales*. The type species is *Lichenicola cladoniae*.

## DESCRIPTION OF *LICHENICOLA CLADONIAE* SP. NOV.

*Lichenicola cladoniae* (cla.do.ni′a.e N.L. gen. n. *cladoniae*, of a *Cladonia*, the lichen that was the source of the type strain).

Cells are Gram-stain-negative, rod-shaped, non-motile, facultative anaerobic, 0.7–1.2 µm wide and 1.0–2.1 µm long. Colonies are irregular, convex and pink-coloured on R2A plates after 2 weeks incubation at 15 °C. Growth occurs at 4–20 °C (optimum, 15 °C), pH 5.5–7.0 (optimum, pH 6.5) and in the absence of NaCl after 2 weeks of incubation on R2A agar at 15 °C. No capacity to utilize methanol, formaldehyde, formate and methylamine. No genes involved in nitrogen fixation and methane or methanol utilization. In the API 20NE system, nitrate reduction, urease, hydrolysis of β-glucosidase and β-galactosidase are positive but indole production, arginine dihydrolase and hydrolysis of gelatin are negative. In the API ZYM system, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-galactosidase, α-glucosidase and β-glucuronidase are positive. Acid is produced from D-fucose, D-glucose, melibiose, D-ribose, D-xylose and L-arabinose, but not from starch, amygdalin, arbutin, D-adonitol, D-arabinose, D-arabitol, cellobiose, D-fructose, D-lyxose, maltose, D-mannitol, melezitose, raffinose, D-sorbitol, D-tagatose, trehalose, turanose, dulcitol, erythritol, aesculin ferric citrate, gentiobiose, glycerol, glycogen, inositol, inulin, L-arabitol, L-fucose, L-rhamnose, L-sorbose, L-xylose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-zylopyranoside, *N*-acetylglucosamine, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, salicin and xylitol. The type genus is *Lichenicola*. The type strain, PAMC 26569$^T$ (=KCCM 43315$^T$=JCM 33604$^T$), was isolated from lichen, *Cladonia borealis*, collected from King George Island, Antarctica.

The GenBank/EMBL/DBBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequences of strain PAMC 26569$^T$ are KJ606804 and CP053708–CP053715, respectively. The genomic DNA G+C content of the type strain calculated from the genome sequence is 64.6%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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