

# *Siculibacillus lacustris* gen. nov., sp. nov., a new rosette-forming bacterium isolated from a freshwater crater lake (Lake St. Ana, Romania)

Tamás Felföldi,<sup>1,2,\*</sup> Zsuzsanna Márton,<sup>1</sup> Attila Szabó,<sup>1</sup> Anikó Mentés,<sup>1</sup> Károly Bóka,<sup>3</sup> Károly Márialigeti,<sup>1</sup> István Máthé,<sup>2</sup> Mihály Koncz,<sup>2†</sup> Peter Schumann<sup>4</sup> and Erika Tóth<sup>1</sup>

## Abstract

A new aerobic alphaproteobacterium, strain SA-279<sup>T</sup>, was isolated from a water sample of a crater lake. The 16S rRNA gene sequence analysis revealed that strain SA-279<sup>T</sup> formed a distinct lineage within the family *Ancalomicrobiaceae* and shared the highest pairwise similarity values with *Pinisolibacterravus* E9<sup>T</sup> (96.4%) and *Ancalomicrobium adetum* NBRC 102456<sup>T</sup> (94.2%). Cells of strain SA-279<sup>T</sup> were rod-shaped, motile, oxidase and catalase positive, and capable of forming rosettes. Its predominant fatty acids were C<sub>18:1</sub>ω7c (69.0%) and C<sub>16:1</sub>ω7c (22.7%), the major respiratory quinone was Q-10, and the main polar lipids were phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine, phosphatidylglycerol, an unidentified aminophospholipid and an unidentified lipid. The G+C content of the genomic DNA of strain SA-279<sup>T</sup> was 69.2 mol%. On the basis of the phenotypic, chemotaxonomic and molecular data, strain SA-279<sup>T</sup> is considered to represent a new genus and species within the family *Ancalomicrobiaceae*, for which the name *Siculibacillus lacustris* gen. nov., sp. nov. is proposed. The type strain is SA-279<sup>T</sup> (=DSM 29840<sup>T</sup>=JCM 31761<sup>T</sup>).

The order *Rhizobiales* (class *Alphaproteobacteria*) currently contains more than 15 families, such as ‘*Aurantimonadaceae*’, *Bartonellaceae*, *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Brucellaceae*, *Chelatococcaceae*, *Cohaesibacteraceae*, *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Methylocystaceae*, *Notoacmeibacteraceae*, *Phyllobacteriaceae*, *Rhizobiaceae*, *Rhodobiaceae* and *Xanthobacteraceae* [1–3]. Although many well-known genera from this order are pathogenic to humans and animals (e.g. *Bartonella*, *Brucella*), associated with plants (e.g. *Phyllobacterium*, *Rhizobium*) or inhabitants of soil (e.g. *Nitrobacter*) and wastewater-treating bioreactors (e.g. *Chelatococcus*) [2], yet-not-cultivated members of *Rhizobiales* could be important members of bacterioplankton in some aquatic environments (e.g. some lakes and special oceanic habitats [4–6]). In our recent study [7], we gave the description of a new *Rhizobium* species isolated from a water sample collected from a crater lake. In this paper,

another new strain, SA-279<sup>T</sup>, was characterized in detail, which was isolated from the same locality. Based on the obtained results, this strain is supposed to represent a novel genus for which the name *Siculibacillus lacustris* gen. nov., sp. nov. is proposed. The new genus is the member of the recently described new family, *Ancalomicrobiaceae*, which currently contains only two other genera, *Pinisolibacter* and *Ancalomicrobium* [8].

Strain SA-279<sup>T</sup> was isolated from a freshwater crater lake, Lake St. Ana (46° 07′ 34.7″ N 25° 53′ 15.8″ E; located in Ciomad Mountains, Harghita County, Romania; in Romanian: Lacul Sfânta Ana) in August 2012. A detailed site description including the physical and chemical characteristics of the lake water is given by Felföldi et al. [9]. For isolation, plates containing only lake water solidified with 20 g l<sup>-1</sup> agar were used. The standard dilution plating technique (spread plate method) was applied to obtain isolates by

**Author affiliations:** <sup>1</sup>Department of Microbiology, ELTE Eötvös Loránd University, Pázmány Péter stny. 1/c, 1117 Budapest, Hungary; <sup>2</sup>Department of Bioengineering, Sapientia Hungarian University of Transylvania, Piața Libertății 1, 530104 Miercurea Ciuc, Romania; <sup>3</sup>Department of Plant Anatomy, ELTE Eötvös Loránd University, Pázmány Péter stny. 1/c, 1117 Budapest, Hungary; <sup>4</sup>Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany.

\*Correspondence: Tamás Felföldi, [tamas.felfoldi@gmail.com](mailto:tamas.felfoldi@gmail.com)

**Keywords:** rosette; *Alphaproteobacteria*; new genus; *Ancalomicrobiaceae*.

**Abbreviations:** AL, unidentified aminolipid(s); APL, unidentified aminophospholipid(s); DPG, diphosphatidylglycerol; GL, unidentified glycolipid(s); L, unidentified lipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid(s); PME, phosphatidylmonomethylethanolamine; PS, phosphatidylserine.

†Present address: Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, 6726 Szeged, Hungary.

The GenBank accession number for the 16S rRNA gene and the genome sequence of strain SA-279<sup>T</sup> is KM083137 and SJFN00000000, respectively. Four supplementary figures and two supplementary tables are available with the online version of this article.

incubation at room temperature (20–22 °C). Subsequently, strain SA-279<sup>T</sup> was maintained on a modified Reasoner's 2A agar medium (mR2A, pH 5.5), which contained only a half amount of the carbon sources as given in the original description (DSMZ medium 830, www.dsmz.de; 0.25 g l<sup>-1</sup> yeast extract, 0.25 g l<sup>-1</sup> proteose peptone, 0.25 g l<sup>-1</sup> caseamino acids, 0.25 g l<sup>-1</sup> glucose, 0.25 g l<sup>-1</sup> soluble starch, 0.15 g l<sup>-1</sup> sodium pyruvate, 0.3 g l<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.05 g l<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O). Later, strain SA-279<sup>T</sup> was grown on mR2A or R2A agar medium at room temperature (~22 °C). For side-by-side analyses, strains *Pleomorphomonas oryzae* DSM 16300<sup>T</sup> and *Phreatobacter oligotrophus* DSM 25521<sup>T</sup> were also maintained on R2A agar.

Temperature and pH optima as well as salt tolerance were determined based on the observed growth intensity at 4, 10, 15, 20, 25, 28, 37, 45, 55 and 65 °C, at pH from 4 to 11 (with intervals of 0.5), and from 0 to 5 % (w/v) NaCl concentration (with intervals of 0.5 %), as described previously [10]. Colony morphology of strain SA-279<sup>T</sup> was tested by direct observation of single colonies. Cell morphology was studied with Gram staining according to Claus [11], with phase contrast microscopy and with electron microscopy as described by Tóth *et al.* [12]. The presence of flagella was checked also as described by Heimbrook *et al.* [13], while motility was also inferred based on the spreading growth in semisolid agar [14] using mR2A medium containing 4 g l<sup>-1</sup> agar. Oxidase activity was determined as described by Tarrand and Gröschel [15], while catalase reaction was examined according to Cowan and Steel [14]. Metabolic tests were performed with API 50 CH, API 20 NE and API ZYM (bioMérieux) systems according to instructions of the manufacturer, while chemotaxonomic analyses (determination of isoprenoid quinones using HPLC, cellular fatty acids using GC and polar lipids using two-dimensional TLC) were performed as described in detail previously [16].

The 16S rRNA gene sequence of strain SA-279<sup>T</sup> was amplified using the protocol described by Felföldi *et al.* [17], and sequenced by the Biomi Ltd. (Gödöllő, Hungary). Closest related species represented by the type strains were identified by EzBioCloud's online Identify service [3], 16S rRNA gene sequences were retrieved from GenBank, and sequence alignment was performed with the ARB-SINA Alignment Service [18]. Phylogenetic analysis (including the search for the best-fit model parameters) was conducted with the MEGA7 software [19].

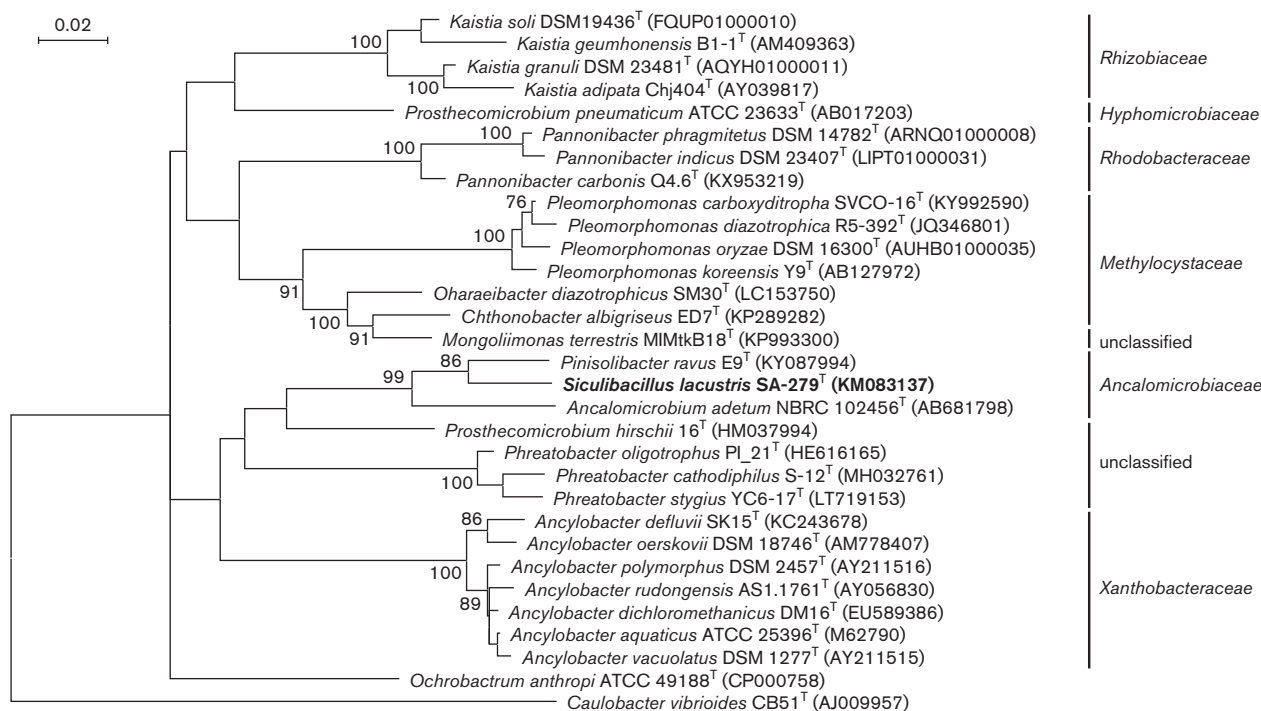
For the whole genome project of strain SA-279<sup>T</sup>, genomic DNA was extracted with the DNeasy PowerLyzer Microbial Kit (Qiagen) including an RNase A treatment at 37 °C for 20 min. Illumina sequencing was performed by the Genomics Facility RTSF, Michigan State University (USA) with the following main steps: library preparation using the SMARTer ThruPLEX DNA-Seq kit (Takara); quality control using a combination of Qubit dsDNA HS assay (Thermo Fisher Scientific), 4200 TapeStation High Sensitivity DNA 1000 assay (Agilent) and the Illumina Library Quantification qPCR kit (Kapa Biosystems); sequencing

which was performed in a 2×250 bp paired-end format using a MiSeq Standard v2 flow cell and a MiSeq 500 cycle v2 reagent cartridge (Illumina). Base calling was performed by Illumina Real Time Analysis (RTA) version 1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq version 2.19.1. Sequence read quality was checked with FastQC [20]. *De novo* assembly of sequence reads was performed using A5-miseq [21], which resulted 99 contigs (all contigs were longer than 500 nt) with N50 value of 120 665 nt and 85.9× genome coverage. The ContEst16S software [22] was used to check possible contamination.

Sequencing the 16S rRNA gene of strain SA-279<sup>T</sup> resulted in a stretch of 1403 nucleotides. The closest type strains of bacterial species were identified as *Pinisolibacter ravus* E9<sup>T</sup> with 96.4 %, *Ancalomicrobium adetum* NBRC 102456<sup>T</sup> with 94.2 % (both strains are members of family *Ancalomicrobiaceae*), *Prosthecomicrobium hirschii* 16<sup>T</sup> with 93.5 % (unclassified), *Kaistia algarum* LYH11<sup>T</sup> with 92.8 % (family *Rhizobiaceae*), *Chthonobacter albigriseus* ED7<sup>T</sup> with 92.8 %, *Pleomorphomonas oryzae* DSM 16300<sup>T</sup> and *Oharaeibacter diazotrophicus* SM30<sup>T</sup> both with 92.7 % (the former three strains, family *Methylocystaceae*), and *Phreatobacter oligotrophus* PI\_21<sup>T</sup> (=DSM 25521<sup>T</sup>) (unclassified) with 92.6 % sequence similarity values. Other species shared <92.3 % pairwise similarity values [other related type strains belonged to genera *Ancylobacter* (family *Xanthobacteraceae*), *Ochrobactrum* (family *Brucellaceae*) and *Pannonibacter* (family *Rhodobacteraceae*)]. Although the closest related type strain showed slightly higher value than threshold value (95 %) suggested for the genus-level by Tindall *et al.* [23], in the case of a related genus, similar values could be found, since *Chthonobacter albigriseus* ED7<sup>T</sup> shows 96.7 % 16S rRNA gene sequence similarity value to *Mongoliiimonas terrestris* MIMtkB18<sup>T</sup> and 96.4 % to *Oharaeibacter diazotrophicus* SM30<sup>T</sup>. The phylogenetic analysis based on the 16S rRNA gene (Figs 1 and S1, available in the online version of this article) supported that the new strain is the member of family *Ancalomicrobiaceae*, since it formed a cluster with *Pinisolibacter* and *Ancalomicrobium* with high bootstrap support (99–100); on the other hand, moderate bootstrap values (78–88) supported that strain SA-279<sup>T</sup> represents a separate genus from *Pinisolibacter*.

The assembled genome of strain SA-279<sup>T</sup> had a total length of 5.0 Mb. The G+C content of the genomic DNA of strain SA-279<sup>T</sup> was 69.2 mol%. The full-length 16S rRNA gene sequence of strain SA-279<sup>T</sup> obtained by Sanger method was compared with the extracted 16S rRNA gene sequence from the genome assembly and showed 100 % similarity. Base composition of genomic DNA was determined also by reversed-phase HPLC as described in detail previously [16], which resulted in the same value.

Cells of strain SA-279<sup>T</sup> were rod-shaped, Gram-stain-negative, motile by a subpolar flagellum, capable to form rosettes (Figs S2 and S3), aerobic and mesophilic with a characteristic heterotrophic metabolism (Table S1). Some



**Fig. 1.** Phylogenetic position of SA-279<sup>T</sup> and related type strains based on the 16S rRNA gene. The phylogenetic tree has been reconstructed based on 1372 positions using the maximum likelihood method and the Tamura three-parameter nucleotide substitution model. Bootstrap values >70 % are shown at the nodes. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide.

distinguishing characters (e.g. motility, negative aesculin hydrolysis and trypsin enzyme activity and capability to use malate as a sole carbon source) which could be used for the discrimination of the new genus from related genera are given in Table 1.

The isoprenoid quinones of strain SA-279<sup>T</sup> were Q-10 and Q-9 in the ratio 94:4. The fatty acid pattern of strain SA-279<sup>T</sup> was predominated by C<sub>18:1</sub>ω7c (69.0 %) and C<sub>16:1</sub>ω7c (22.7 %), while C<sub>16:0</sub> (6.4 %) was also present in a notable amount (Table S2). The dominance of fatty acid C<sub>18:1</sub>ω7c and ubiquinone Q-10 is a characteristic chemotaxonomic trait in the case of other related members of *Rhizobiales* (Table 1, Table S2). The polar lipid profile of strain SA-279<sup>T</sup> was dominated by phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), phosphatidylcholine (PC), phosphatidylglycerol (PG) and an unidentified aminophospholipid (AL), while an unidentified lipid (L) was also detected (Fig. S4). The lack of diphosphatidylglycerol (DPG) distinguishes the new strain from the members of closest related genera, *Pinisolibacter* and *Ancalomicrobium* (Table 1).

In conclusion, based on the data discussed above, strain SA-279<sup>T</sup> is considered to represent a novel genus and a novel species within family *Ancalomicrobiaceae*, for which the name *Siculibacillus lacustris* gen. nov., sp. nov. is proposed.

## DESCRIPTION OF *SICULIBACILLUS* GEN. NOV.

*Siculibacillus* [Si.cu.li.ba.cil'lus, M.L. masc. pl. n. *Siculi* Székely, referring to people living in Terra Siculorum (i.e. Transylvania, Romania) from where the type strain was isolated, L. masc. n. *bacillus* a rod and also a bacterial generic name); N.L. masc. n. *Siculibacillus*, Székely bacillus)].

Cells are Gram-negative, motile rods and capable to form rosettes. Aerobic and mesophilic. Oxidase- and catalase-positive. The major respiratory quinone is Q-10. Major cellular fatty acids are C<sub>18:1</sub>ω7c and C<sub>16:1</sub>ω7c. Polar lipids are dominated by PE, PME, PC, PG, APL and L.

The type species is *Siculibacillus lacustris*.

## DESCRIPTION OF *SICULIBACILLUS LACUSTRIS* SP. NOV.

*Siculibacillus lacustris* (la.cus'tris. N.L. masc. adj. *lacustris* of a lake)

Cells are rod-shaped (0.6–0.8 × 1.3–2.5 μm) and motile. Colonies on mR2A medium are greyish-white in colour, circular and raised with a diameter of 1–2 mm. Growth occurs at 15–37 °C (with an optimum between 20–28 °C) and pH 5.0–7.5 (optimum, pH 5.0–6.0). Positive for acid phosphatase (weak), alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and urease

**Table 1.** Differential characteristics of *Sicilibacillus* and related genera

Genera: 1, *Sicilibacillus* (this study); 2, *Pinisolibacter* [8]; 3, *Ancalomicrobium* [8, 24]; 4, Putative new genus represented by *Prosthecomicrobium hirschii* 16<sup>T</sup> (as suggested in Yee et al. [25]) [8, 25–27]; 5, *Chthonobacter* [28]; 6, *Pleomorphomonas* [29–32]; 7, *Oharaebacter* [33]; 8, *Phreatobacter* (this study, [34–36]); 9, *Pannonibacter* [37–39]. Polar lipids: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PME, phosphatidylmonomethyl ethanolamine; PS, phosphatidylserine; APL, unidentified aminophospholipid(s); AL, unidentified aminolipid(s); GL, unidentified glycolipid(s); PL, unidentified phospholipid(s); L, unidentified lipid. Fatty acids in parentheses are present but not reaching 10% in all type strains (or contradictory data are available in the literature). Test results and polar lipids shown in parentheses were detected only in one type strain. In all cases, the major isoprenoid quinone is Q-10. +, Present; –, absent; w, weak reaction; ND, no data. Most data are not available for *Pleomorphomonas carboxyditropha* SVCO-16<sup>T</sup>.

| Characteristic                    | 1  | 2                           | 3  | 4   | 5                   | 6  | 7                               | 8   | 9   |
|-----------------------------------|--|-----------------------------|--|---|---------------------|--|---------------------------------|---|---|
| Family                            | Ancalomicrobiaceae                           | Ancalomicrobiaceae          | Ancalomicrobiaceae                             | Unclassified  | Methylocystaceae    | Methylocystaceae   | Methylocystaceae                | Unclassified  | Rhodobacteraceae                          |
| Number of species*                | 1  | 1                           | 1  | 1   | 1                   | 4  | 1                               | 3   | 3   |
| Colony colour                     | Greyish-white                                | Straw-coloured              | ND   | Light pink  | Greyish-white       | Pale-white, white  | White                           | Whitish, pale yellow  | Whitish cream                             |
| Motility                          | +  | –                           | –  | +   | –                   | –  | +                               | +   | +   |
| Aesculin hydrolysis               | –  | +                           | +  | –   | +                   | +  | –                               | +/-   | -/+                                       |
| Alkaline phosphatase activity     | +  | +                           | w  | w   | –                   | +  | ND                              | +   | (+)                                       |
| Citrate utilization               | +  | +                           | +  | –   | ND                  | –  | –                               | –   | +   |
| Malate utilization                | +  | –                           | –  | +   | +                   | +/-  | +                               | –   | ND  |
| Trypsine activity                 | –  | +                           | +  | –   | +                   | +  | ND                              | +/-   | (+)                                       |
| Urease activity                   | +  | +                           | +  | –   | +                   | +/-  | –                               | +/-   | +   |
| Major fatty acids (at least 10%)† | C <sub>18:1</sub> ω7c, C <sub>16:1</sub> ω7c | SF8, SF3, C <sub>16:0</sub> | SF8, C <sub>14:0</sub> 2-OH, C <sub>16:0</sub> | C <sub>18:1</sub> ω7c, C <sub>16:1</sub> ω7c, C <sub>16:0</sub> | SF8, SF2            | C <sub>18:1</sub> ω7c/SF8, (cyclo)C <sub>19:0</sub> ω8c, C <sub>16:0</sub> , C <sub>18:0</sub> | SF8, cycloC <sub>19:0</sub> ω8c | C <sub>18:1</sub> ω7c, 11-methyl-C <sub>18:1</sub> ω7c, (SF3) | C <sub>18:1</sub> ω7c                     |
| Detected polar lipids‡            | PE, PME, PC, APL, PG, L                      | PE, PME, PC, DPG, PG, L     | PE, DPG, PG, PC, L                             | PG, PME, PC, C <sub>16:0</sub>                                  | PC, PG, PE, APL, PL | PC, PE, PME, PG, DPG   | ND                              | PC, PE, DPG, L (PL, GL, PG)                                   | PG, PC, DPG, PE, PL, AL (PME, PS, L)      |
| DNA G+C content (mol%)            | 69.2   | 68.4                        | 70.4   | 68.9  | 71.8                | 63.1–66.4  | 74.6                            | 64.4–69.3   | 63.3–64.6                                 |
| Isolation source of type strains  | Lake water                                   | Soil                        | Freshwater                                     | Pond  | Grass-field soil    | Paddy soil, root tissue, contaminated culture, anaerobic sludge                                | Rhizosphere                     | Ultrapure water, pieces of wood, microbial fuel cell          | Reed rhizome, hot spring, coal mine water |

\*Based on the search performed with Prokaryotic Nomenclature Up-To-Date [40] on 17 February 2019.

†SF2, summed feature 2 (consisted of C<sub>14:0</sub> 3-OH and/or C<sub>16:1</sub> iso I); SF3, summed feature 3 (consisted of C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c); SF8, summed feature 8 (consisted of C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c).

‡Not available for *Oharaebacter diazotrophicus* SM30<sup>T</sup> and *Pleomorphomonas koreensis* Y9<sup>T</sup>.

enzyme activities; assimilation of D-arabinose, L-arabinose, citrate, D-fructose, L-fucose, gluconate (weak), D-glucose, D-lyxose, D-mannitol (weak), D-mannose, malate, maltose (weak), L-rhamnose, D-ribose (weak) and D-xylose. Negative for  $\alpha$ -chymotrypsine, cystine arylamidase,  $\alpha$ -fucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase, gelatinase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, leucine arylamidase, lipase (C14),  $\alpha$ -mannosidase, N-acetyl- $\beta$ -glucosaminidase and trypsin enzyme activities; assimilation of adipate, D-adonitol, aesculin, amygdalin, D-arabitol, L-arabitol, L-arginine, arbutin, capric acid, cellobiose, dulcitol, erythritol, D-fucose, D-galactose, gentiobiose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, melezitose, melibiose, methyl  $\alpha$ -D-glucopyranoside, methyl  $\alpha$ -D-mannopyranoside, methyl  $\beta$ -D-xylopyranoside, N-acetylglucosamine, phenylacetic acid, raffinose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-tagatose, trehalose, turanose, xylitol and L-xylose.

The G+C content of the genomic DNA is 69.2 mol%.

The type strain is SA-279<sup>T</sup> (=DSM 29840<sup>T</sup>=JCM 31761<sup>T</sup>), which was isolated from lake water.

The GenBank accession numbers for the 16S rRNA gene and the genome sequence of strain SA-279<sup>T</sup> are KM083137 and SJFN00000000, respectively.

## EMENDED DESCRIPTION OF THE FAMILY ANCALOMICROBIACEAE DAHAL ET AL. 2018

The description of family the *Ancalomicrobiaceae* is as given by Dahal et al. [8], with the following amendments. Cells are motile or non-motile. The major polar lipids are PE, PC, PME, PG and DPG.

### Funding information

This work was completed in the ELTE Institutional Excellence Program (1783-3/2018/FEKUTSRAT) supported by the Hungarian Ministry of Human Capacities. Attila Szabó was supported by the ÚNKP-18-3-III-ELTE-709 New Excellence Program of the Ministry of Human Capacities.

### Acknowledgements

The authors are thankful to Anikó Lajosné Balogh, Zsuzsa Kéki, Hajnalka Nagy and Zsuzsanna Halász for their technical assistance.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

### References

- Sayers EW, Barrett T, Benson DA, Bryant SH, Canese K et al. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2009;37:D5–D15.
- Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F et al. *The Prokaryotes, Alphaproteobacteria and Betaproteobacteria*, 4th ed. Berlin: Springer-Verlag; 2014.
- Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017; 67:1613–1617.
- Bowman JS, Berthiaume CT, Armbrust EV, Deming JW. The genetic potential for key biogeochemical processes in Arctic frost flowers and young sea ice revealed by metagenomic analysis. *FEMS Microbiol Ecol* 2014;89:376–387.
- Szabó A, Korponai K, Kerepesi C, Somogyi B, Vörös L et al. Soda pans of the Pannonian steppe harbor unique bacterial communities adapted to multiple extreme conditions. *Extremophiles* 2017; 21:639–649.
- Mentes A, Szabó A, Somogyi B, Vajna B, Tugyi N et al. Differences in planktonic microbial communities associated with three types of macrophyte stands in a shallow lake. *FEMS Microbiol Ecol* 2018; 94:fix164.
- Máthé I, Tóth E, Mentes A, Szabó A, Márialigeti K et al. A new *Rhizobium* species isolated from the water of a crater lake, description of *Rhizobium aquaticum* sp. nov. *Antonie van Leeuwenhoek* 2018;111:2175–2183.
- Dahal RH, Chaudhary DK, Kim J. *Pinisolibacter ravus* gen. nov., sp. nov., isolated from pine forest soil and allocation of the genera *Ancalomicrobium* and *Pinisolibacter* to the family *Ancalomicrobiaceae* fam. nov., and emendation of the genus *Ancalomicrobium* Staley 1968. *Int J Syst Evol Microbiol* 2018;68:1955–1962.
- Felföldi T, Ramganesh S, Somogyi B, Krett G, Jurecska L et al. Winter planktonic microbial communities in highland aquatic habitats. *Geomicrobiol J* 2016;33:494–504.
- Felföldi T, Vengring A, Kéki Z, Márialigeti K, Schumann P et al. *Eoetvoesia caeni* gen. nov., sp. nov., isolated from an activated sludge system treating coke plant effluent. *Int J Syst Evol Microbiol* 2014;64:1920–1925.
- Claus D. A standardized Gram staining procedure. *World J Microbiol Biotechnol* 1992;8:451–452.
- Tóth E, Szuróczi S, Kéki Z, Bóka K, Szili-Kovács T et al. *Gelleriella hungarica* gen. nov., sp. nov., a novel bacterium of the family *Rhizobiaceae* isolated from a spa in Budapest. *Int J Syst Evol Microbiol* 2017;67:4565–4571.
- Heimbrook ME, Wang WL, Campbell G. Staining bacterial flagella easily. *J Clin Microbiol* 1989;27:2612–2615.
- Barrow GI, Cowan RKA. *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd ed. Cambridge: Cambridge University Press; 2003.
- Tarrand JJ, Gröschel DH. Rapid, modified oxidase test for oxidase-variable bacterial isolates. *J Clin Microbiol* 1982;16:772–774.
- Felföldi T, Kéki Z, Sipos R, Márialigeti K, Tindall BJ et al. *Ottowia pentelensis* sp. nov., a floc-forming betaproteobacterium isolated from an activated sludge system treating coke plant effluent. *Int J Syst Evol Microbiol* 2011;61:2146–2150.
- Felföldi T, Fikó RD, Mentes A, Kovács E, Máthé I et al. *Quisquilliibacterium transsilvanicum* gen. nov., sp. nov., a novel betaproteobacterium isolated from a waste-treating bioreactor. *Int J Syst Evol Microbiol* 2017;67:4742–4746.
- Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
- Andrews S. FastQC: a quality control tool for high throughput sequence data. 2010. Available online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- Coil D, Jospin G, Darling AE. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* 2015;31:587–589.
- Lee I, Chalita M, Ha SM, Na SI, Yoon SH et al. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
- Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;60:249–266.
- Staley JT. *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* 1968;95:1921–1942.

25. Yee B, Oertli GE, Fuerst JA, Staley JT. Reclassification of the polyphyletic genus *Prosthecomicrobium* to form two novel genera, *Vasilyevaea* gen. nov. and *Bauldia* gen. nov. with four new combinations: *Vasilyevaea enhydra* comb. nov., *Vasilyevaea mishustinii* comb. nov., *Bauldia consociata* comb. nov. and *Bauldia litoralis* comb. nov. *Int J Syst Evol Microbiol* 2010;60:2960–2966.
26. Staley JT. *Prosthecomicrobium hirschii*, a new species in a redefined genus. *Int J Syst Bacteriol* 1984;34:304–308.
27. Sittig M, Schlesner H. Chemotaxonomic investigation of various prosthecate and/or budding bacteria. *Syst Appl Microbiol* 1993;16:92–103.
28. Kim D, Kang K, Ahn TY. *Chthonobacter albigriseus* gen. nov., sp. nov., isolated from grass-field soil. *Int J Syst Evol Microbiol* 2017;67:883–888.
29. Xie CH, Yokota A. *Pleomorphomonas oryzae* gen. nov., sp. nov., a nitrogen-fixing bacterium isolated from paddy soil of *Oryza sativa*. *Int J Syst Evol Microbiol* 2005;55:1233–1237.
30. Im WT, Kim SH, Kim MK, Ten LN, Lee ST. *Pleomorphomonas koreensis* sp. nov., a nitrogen-fixing species in the order *Rhizobiales*. *Int J Syst Evol Microbiol* 2006;56:1663–1666.
31. Madhaiyan M, Jin TY, Roy JJ, Kim SJ, Weon HY et al. *Pleomorphomonas diazotrophica* sp. nov., an endophytic N-fixing bacterium isolated from root tissue of *Jatropha curcas* L. *Int J Syst Evol Microbiol* 2013;63:2477–2483.
32. Esquivel-Elizondo S, Maldonado J, Krajmalnik-Brown R. Anaerobic carbon monoxide metabolism by *Pleomorphomonas carboxyditropha* sp. nov., a new mesophilic hydrogenogenic carboxydrotroph. *FEMS Microbiol Ecol* 2018;94.
33. Lv H, Masuda S, Fujitani Y, Sahin N, Tani A. *Oharaebacter diazotrophicus* gen. nov., sp. nov., a diazotrophic and facultatively methylotrophic bacterium, isolated from rice rhizosphere. *Int J Syst Evol Microbiol* 2017;67:576–582.
34. Tóth EM, Vengring A, Homonnay ZG, Kéki Z, Spröer C et al. *Phreatobacter oligotrophus* gen. nov., sp. nov., an alphaproteobacterium isolated from ultrapure water of the water purification system of a power plant. *Int J Syst Evol Microbiol* 2014;64:839–845.
35. Lee SD, Joung Y, Cho JC. *Phreatobacter stygius* sp. nov., isolated from pieces of wood in a lava cave and emended description of the genus *Phreatobacter*. *Int J Syst Evol Microbiol* 2017;67:3296–3300.
36. Kim SJ, Ahn JH, Heo J, Cho H, Weon HY et al. *Phreatobacter cathodiphilus* sp. nov., isolated from a cathode of a microbial fuel cell. *Int J Syst Evol Microbiol* 2018;68:2855–2859.
37. Borsodi AK, Micsinai A, Kovács G, Tóth E, Schumann P et al. *Pannonibacter phragmitetus* gen. nov., sp. nov., a novel alkalitolerant bacterium isolated from decomposing reed rhizomes in a Hungarian soda lake. *Int J Syst Evol Microbiol* 2003;53:555–561.
38. Bandyopadhyay S, Schumann P, Das SK. *Pannonibacter indica* sp. nov., a highly arsenate-tolerant bacterium isolated from a hot spring in India. *Arch Microbiol* 2013;195:1–8.
39. Xi L, Qiao N, Liu D, Li J, Zhang J et al. *Pannonibacter carbonis* sp. nov., isolated from coal mine water. *Int J Syst Evol Microbiol* 2018;68:2042–2047.
40. Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures. Prokaryotic Nomenclature up-to-date, update. 2019 <http://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date>.

#### Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at [microbiologyresearch.org](http://microbiologyresearch.org).