

Alkalilacustris brevis gen. nov., sp. nov., isolated from a soda lake

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Abstract

A Gram-stain-negative, aerobic, non-pigmented and short-rod-shaped bacterium, designated 34079^T, was isolated from a water sample of a soda lake in Jilin, a province of China. Strain 34079^T grew at 10–50 °C (optimum, 35 °C), pH 7–10 (optimum, pH 8.0–8.5). NaCl was required for growth at the concentration range 1–10.0 % (w/v), with an optimum at 2.5–4 % (w/v). Chemotaxonomic analysis indicated that the sole respiratory quinone was Q-10. The predominant cellular fatty acids (>5 %) were summed feature 8 (C_{18:1}ω7c/C_{18:1}ω6c) and C_{16:0}. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, three unidentified amino lipids, one unidentified amino phosphoglycolipid, one phosphoglycolipid, one unidentified glycolipid, three unidentified phospholipids and two unidentified lipids. The DNA G+C content was 65.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 34079^T formed a distinct lineage in the clade of the family 'Rhodobacteraceae' with the highest sequence similarity of 96.1 % to *Pararhodobacter aggregans*, followed by *Rhodobaca bogoriensis* DSM 18756^T (95.7 %) and *Roseibaca ekhonensis* DSM 11469^T (94.7 %). The distinct biochemical, chemotaxonomic and phylogenetic differences from the previously described taxa supported that strain 34079^T represents a novel species of a new genus, for which the name *Alkalilacustris brevis* gen. nov., sp. nov. is proposed. The type strain is 34079^T (=KCTC 62428^T=MCCC 1K03493^T).

The family 'Rhodobacteraceae' (in order Rhodobacterales [1], class Alphaproteobacteria [2]) was proposed by Garrity *et al.* [3] based on the phylogenetic analysis of 16S rRNA gene sequences. At present, it comprises over 180 genera (www.ncbi.nlm.nih.gov/taxonomy). The family 'Rhodobacteraceae' shows phenotypical, metabolic and ecological diversities. Many members of this family are aquatic, require sodium ions or combined salts for growth and were isolated from lake [4, 5], sludge [6, 7], sea water [8], marine sediment [9] and soil [10]. Most species of this family are Gram-stain-negative and oxidase-positive. In addition, the major or only respiratory quinone is ubiquinone 10, which occurs almost universally in the class Alphaproteobacteria. The cellular fatty acids are usually dominated by C_{18:1}ω7c, which frequently constitutes more than 50 % of the total [11]. In this study, a novel strain, designated 34079^T, showing all the common characteristics of family 'Rhodobacteraceae' described above, was isolated

from a soda lake. Based on the polyphasic taxonomic characterization, strain 34079^T is considered to represent a novel species of a novel genus in this family.

A water sample was collected from a soda lake located in Jilin, China (44° 03' N, 126° 57' E), in Autumn 2016 and was spread onto marine agar 2216 (MA) after treatment with the tenfold dilution series method. After cultivation at 30 °C for 5 days, distinct colonies were observed, including strain 34079^T. Purified strains were obtained by streaking several times. The isolate was routinely cultured in marine broth 2216 (MB) medium and preserved at –80 °C with 30 % (v/v) glycerol for further study.

Genomic DNA of strain 34079^T was extracted using the quick genomic extraction kit (Dongsheng Biotech) to obtain a high-quality template. The 16S rRNA gene was amplified, cloned and sequenced according to Zhang *et al.* [12]. The nearly complete 16S rRNA gene sequence (1431 bp) was

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Abbreviations: bchl *a*, bacteriochlorophyll *a*; MA, marine agar; MB, marine broth; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 34079^T is MF664222. The GenBank accession number for the whole genome sequence of strain 34079^T is QNVJ000000000.

One supplementary table and two supplementary figures are available with the online version of this article.

subjected to pairwise sequence alignment by the EzTaxon-e server [13]. Multiple sequences were aligned with CLUSTAL_W program [14]. Phylogenetic trees were reconstructed using the following three statistical methods: neighbour-joining [15], maximum-evolution [15] and maximum-likelihood [16] with MEGA 7 [17]. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model [18] for the neighbour-joining tree. Bootstrap analysis was based on 1000 replications [19]. Phylogenetic analysis on the basis of 16S rRNA gene sequences exhibited that strain 34079^T had highest sequence similarities to the genera *Pararhodobacter* (96.1 %) and *Rhodobaca* (95.3–95.7 %), while having a close relationship with the genus *Roseibaca* (Fig. 1). According to this result, *Pararhodobacter aggregans* DSM 18938^T (96.1 %), *Rhodobaca bogoriensis* DSM 18756^T (95.7 %) and *Roseibaca ekhonensis* DSM 11469^T (94.7 %) were used as the reference type strains.

The genome of strain 34079^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Novogene Technology Company). The sequencing generated approximate 1.3 Gb of clean data. The *de novo* assembly was performed using ABySS 1.5.2 [20]. The assembly k-value was tested from 40 to 64 to find the optimal k-value using the abyss-pe script. The quality of the genome was assessed using CheckM 1.0.8 [21]. As a result, the genomic sequence was estimated to be ≥ 95 % complete (97.3 %), with ≤ 5 % contamination (0.0 %), and was considered as an excellent reference genome for further analyses [21].

The result of G+C content of the genomic DNA calculated by genome sequencing was 65.6 mol%, which is higher than those of members of the genera *Roseibaca* (61 %) and *Rosenatronobacter* (59.0–61.5 %), and lower than the genus *Pararhodobacter* (68 %). The G+C content of the genomic DNA was determined to be 62.7 % by HPLC according to the method of Tamaoka and Komagata [22].

The temperature range for growth was tested at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C in MB. The pH range for growth was examined at 35 °C and pH 5.0–10.0 (in pH unit increments of 0.5) by supplementing 40 mM buffering agents in MB, including MES for pH 5.0–5.5, PIPES for pH 6.0–7.5, Tricine for pH 8.0–8.5 and CAPSO for pH 9.0–10.0. Salt tolerance at various Na⁺ ion concentrations (0, 0.5 and 1.0–15.0 %, at increments of 1 %, w/v) was investigated in modified MB medium without Na⁺ ions. After incubation for 3 days, OD₅₉₀ values were measured with a UV/visible spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences) to determine the optical growth, and the growth limits were tested after 14 days of incubation. Anaerobic growth was tested by adding various electronic acceptors into MB, including sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM) and L-arginine (5 g l⁻¹). Na₂S·9H₂O (0.05 g l⁻¹) and L-cysteine (0.04 g l⁻¹) were used as reductants, and resazurin (1 mg l⁻¹) as an oxygen indicator. Anaerobic tubes were filled with 80 % N₂, 10 % CO₂ and

10% H₂ to create an anaerobic condition. All anaerobic tubes were cultivated under optimum temperature (35 °C for strain 34079^T, 30 °C for *P. aggregans* DSM 18938^T, 39 °C for *R. bogoriensis* DSM 18756^T, 16 °C for *R. ekhonensis* DSM 11469^T, unless otherwise stated) for 1 month with the colour of the medium unchanged.

The Gram-stain reaction was performed as described by Dong and Cai [23]. Cell morphology and motility were observed by using optical microscope (BX40, Olympus), and transmission electron microscopy (JEM-1230, JEOL). Carotenoid and poly- β -hydroxybutyrate (PHB) production were investigated according to the methods of Zhu *et al.* [24]. Bacteriochlorophyll *a* was extracted and tested using method of Kumar *et al.* [25]. Catalase activity was tested by using 3 % (v/v) H₂O₂ to observe bubbles. Oxidase activity was determined by observing colour change on reaction with *p*-aminodimethylaniline oxalate solution. H₂S production, methyl red and Voges-Proskauer reactions were tested according to Wu *et al.* [26]. 0.5 % tyrosine, 0.05 % xanthine and 0.05 % hypoxanthine (w/v) were added into MA to check the hydrolysis activities. Degradation of starch and hydrolysis of Tweens 20, 40, 60 and 80 were examined as described by Sun *et al.* [27].

Single carbon source assimilation tests were performed using basal medium (MB without peptone and with 0.005 % yeast extract as growth factors). All carbon sources, including sugar, alcohol, organic acid and amino acids, were tested at concentration of 0.4 % (w/v) and were filter-sterilized. Basal medium with substrates but without inoculation was used as a blank control and that with inoculation but without substrates was served as a negative control. Growth was evaluated by measuring turbidity (OD₅₉₀). Positive results were considered only when OD₅₉₀ values were twice higher than the negative control. Other biochemical properties, enzyme activities and acid production were tested using API ZYM, API 20NE and API 50CH systems (bioMérieux) following the manufacturer's instructions.

Antibiotics susceptibility was measured according to the disc agar diffusion method [28]. The antibiotic discs used included (per piece) ampicillin (10 μ g), bacitracin (0.04 U), cefoxitin (30 μ g), ceftriaxone (30 μ g), chloramphenicol (30 μ g), ciprofloxacin (5 μ g), compound sulfamethoxazole (19 μ g), erythromycin (15 μ g), lincomycin (2 μ g), minocycline (30 μ g), nalidixan (30 μ g), novobiocin (30 μ g), penicillin G (10 U), polymyxin B (300 U), piperacillin (100 μ g), rifampicin (5 μ g), streptomycin (10 μ g), tetracycline (30 μ g) and vancomycin (30 μ g). The plates with antibiotics discs were incubated for a week at optimum temperature and the diameter of the inhibition zone was measured according to Nokhal *et al.* [29].

All results of the phenotypic identification mentioned above were shown in detail in the genus and species descriptions and in Table 1.

For fatty acid analyses, cells of strain 34079^T, *P. aggregans* DSM 18938^T, *R. bogoriensis* DSM 18756^T and *R. ekhonensis*

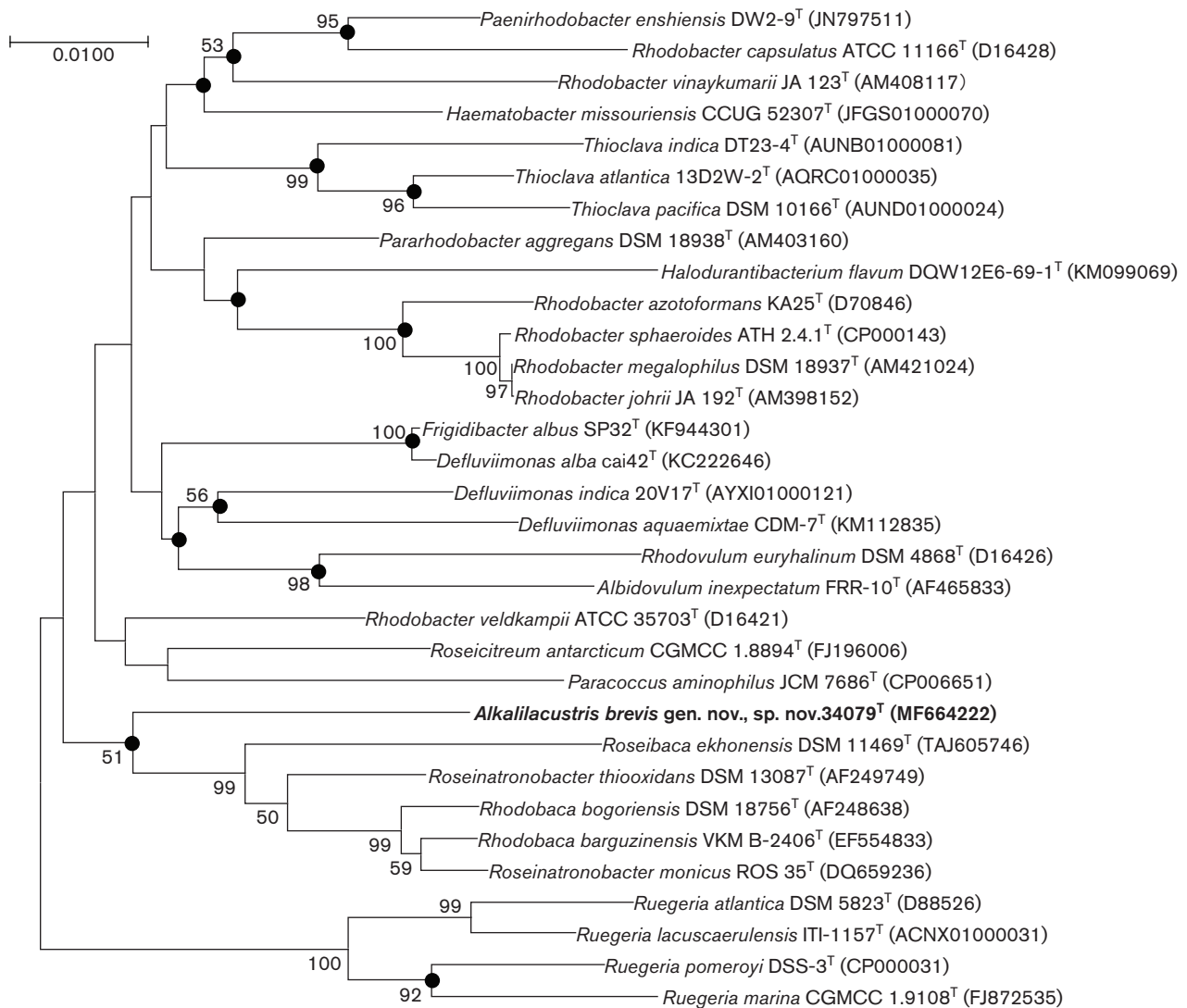


Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1431 bp) showing the position of strain 34079^T and some closely related taxa. Bootstrap values were expressed as a percentage of 1000 replicates and only those higher than 50% are shown. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-evolution trees. Bar, 0.01 substitutions per nucleotide position.

DSM 11469^T in late exponential phase were collected after incubation on MA for 3, 4, 4 and 8 days, respectively at optimum temperature (35, 30, 39 and 16 °C, respectively) and freeze-dried. Whole cell fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI) using the RTSBA6 database. For polar lipid analysis, cells of strain 34079^T and related type strains were collected after incubation in MB at optimum temperature. Polar lipids were extracted by 80 ml of chloroform/methanol/water (1:2:1, by vol) and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) which were dried for 30 min at 55 °C before use, and then analysed according to Tindall [30] and Cui [31]. The first dimension was chloroform/methanol/water (65:25:4, by vol.) and the

second dimension was chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) [32]. Molybdophosphoric acid, ninhydrin reagent, molybdenum blue and α -naphthol/H₂SO₄ were used as spraying reagents for the detection of total lipids, aminolipids, phospholipids and glycolipids, respectively [33–35]. Isoprenoid quinones were analysed as described by Komagata [34] using HPLC-MS. The predominant cellular fatty acids ($\geq 5\%$) detected in strain 34079^T were summed feature 8 (C_{18:1} $\omega 7c$ and/or C_{18:1} $\omega 6c$, 81.5%) and C_{16:0} (6.4%), with summed feature 3 (C_{16:1} $\omega 7c$ and/or C_{16:1} $\omega 6c$, 2.5%), C_{18:0} (2.8%), 11-methyl C_{18:1} $\omega 7c$ (2.1%), C_{10:0} 3-OH (1.7%) present in lower amounts (Table S1, available in the online version of this article). The sole respiratory quinone was Q-10. The polar lipid profile of

Table 1. Differential phenotypic and genotypic characteristics between strain 34079^T and related type strains

Strains: 1, 34079^T; 2, *P. aggregans* DSM 18938^T, 3, *R. bogoriensis* DSM 18756^T 4, *R. ekhonensis* DSM 11469^T. All data was obtained from this study under identical growth conditions at proper temperature, except where indicated otherwise. All strains are positive for catalase and oxidase. All strains are negative for hydrolysis of starch, xanthine and hypoxanthine, Tweens (20, 40, 60 and 80), methyl red test and Voges–Proskauer reaction. All strains are no growth anaerobic. +, Positive; –, negative; w, weakly positive; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 |
|-------------------------------------|-----------|----------|----------------|----------------|
| Cell morphology | Short rod | Rod* | Ovoid to rods† | Rod‡ |
| bchl <i>a</i> | – | –* | +† | +‡ |
| Carotenoid | – | –* | +† | –‡ |
| Temperature range for growth (°C) | 10–50 | 15–40 | 30–43 | 10–30 |
| Optimum temperature for growth (°C) | 35 | 30–40* | 39† | 16‡ |
| pH range for growth | 7–10 | 6–10 | 7.5–10 | ND |
| Optimum pH for growth | 8.0–8.5 | 7.0–8.5* | 9† | 7.0–9.5‡ |
| NaCl tolerance (%; w/v) | 1–10 | 0–5 | 0–10 | 0–1.0, 3.5–4.0 |
| Optimum NaCl (%; w/v) | 2.5–4 | 1–3* | 1† | 2.5‡ |
| H ₂ S production | – | + | + | – |
| Hydrolysis of tyrosine | – | – | + | – |
| Utilization of: | | | | |
| Acetate | + | – | + | + |
| Fructose, fumaric acid, mannitol | – | + | + | + |
| Inositol, lactate | + | – | + | + |
| Xylose | + | + | – | + |
| API 20NE test results: | | | | |
| Aesculin, β-galactosidase | – | – | + | + |
| Urease | – | + | + | – |
| API ZYM test results: | | | | |
| Alkaline phosphatase | w | w | + | + |
| Esterase | + | + | + | w |
| Esterase lipase | w | + | + | w |
| Lipase | w | – | w | – |
| Leucine arylamidase | + | w | + | + |
| Valine arylamidase | + | – | + | w |
| Cystine arylamidase, trypsin | w | w | w | – |
| α-Chymotrypsin | – | w | w | w |
| Acid phosphatase | – | + | w | w |
| Naphthol-AS-BI-phosphohydrolase | w | w | + | w |
| β-Galactosidase | – | – | – | w |
| α-Glucosidase | – | – | w | – |
| β-Glucosidase | – | – | w | w |
| API 50CH test results: | | | | |
| Glycerol, inositol | – | – | + | + |
| D-Arabinose, L-arabinose | – | – | – | + |
| Ribose | – | – | + | – |
| D-Xylose, L-rhamnose | – | + | + | – |
| D-Galactose, D-sorbitol, maltose | – | – | – | + |
| D-Glucose, D-fructose, D-mannose | – | – | – | + |
| D-Mannitol, sucrose | – | + | – | + |
| Methyl α-D-mannoside, arbutin | – | – | + | – |
| N-Acetylglucosamine, salicin | – | – | – | + |
| Cellobiose | + | + | – | + |
| Melezitose, raffinose | – | – | + | – |
| Amidone, glycogen | – | – | + | – |
| D-Fucose | – | + | – | + |
| Potassium 2-ketogluconic | + | + | – | + |
| G+C content (mol%) | 65.6 | 68.0* | 59.0† | 58.8‡ |

*Data are from Foesel et al. (2011).

†Data are from Milford et al. (2000).

‡Data are from Labrenz et al. (2009).

strain 34079^T consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, three unidentified amino lipids, one unidentified amino phosphoglycolipid, one phosphoglycolipid, one unidentified glycolipid, three unidentified phospholipids and two unidentified lipids (Fig. S2).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 34079^T is MF664222. The draft genome of strain 34079^T was 3 480 246 bp long assembled into 103 contigs. The N50 value of the genome was 58 978 bp and the largest contig was 368 150 bp. The GenBank accession number for the whole genome sequence of strain 34079^T is QNVJ00000000. Compared with the 16S rRNA gene obtained from TA cloning and genome sequencing, there was no difference (100 % similarity) between the two sequences.

According to the data obtained in this study, strain 34079^T exhibits typical characteristics of the family 'Rhodobacteraceae', such as having ubiquinone 10 as the only respiratory quinone and summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c) accounting for more than 50 % of the total cellular fatty acids. However, there are also a number of differences between strain 34079^T and the most closely related 'Rhodobacteraceae' genera, for example, strain 34079^T cannot use fructose and the optimum growth range of Na⁺ ions is higher than members of the related taxa. A summary of the principal characteristics that differentiate strain 34079^T from other closely related genera within the family 'Rhodobacteraceae' is provided in Table 2.

Strain 34079^T had a wider temperature tolerance range (10–50 °C) than *P. aggregans* DSM 18938^T (15–40 °C), *R. bogoriensis* DSM 18756^T (30–43 °C) and *R. ekhonensis* DSM 11469^T (10–30 °C). NaCl tolerance of strain 34079^T (10 %) was higher than *P. aggregans* DSM 18938^T (5 %) and *R. ekhonensis* DSM 11469^T (4 %). *R. bogoriensis* DSM 18756^T and *R. ekhonensis* DSM 11469^T contained bchl *a*, which was not detected in strain 34079^T. In contrast to *R. bogoriensis* DSM 18756^T and *P. aggregans* DSM 18938^T, strain 34079^T did not produce H₂S from Na₂S₂O₃. The other differences of acid production and enzyme activity are shown in Table 1.

As for the chemotaxonomic characteristics, strain 34079^T lacked C_{12:1} 3-OH, which was detected in *P. aggregans* DSM 18938^T (1.6 %). *R. ekhonensis* DSM 11469^T had C_{17:1} ω8c and summed feature 7 (1.8 % and 2.8 %, respectively). In addition, compared with *R. bogoriensis* DSM 18756^T and *R. ekhonensis* DSM 11469^T, strain 34079^T had C_{10:0} 3-OH (1.7 %). On the other hand, the polar lipid profiles shows great differences. Firstly, the absence of PE in strain 34079^T clearly distinguished it from the related type strains.

Secondly, an unidentified amino lipid and an unidentified amino phosphoglycolipid were detected in strain 34079^T while not in the related type strains. Strain 34079^T was susceptible to ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), compound sulfamethoxazole (19 μg), eftriaxone (30 μg), novobiocin (30 μg), penicillin G (10 U), piperacillin (100 μg), polymyxin B (300 U), rifampicin (5 μg) and vancomycin (30 μg), and resistant to bacitracin (0.04 U), cefoxitin (30 μg), erythromycin (15 μg), lincomycin (2 μg), minocycline (30 μg), nalidixan (30 μg), streptomycin (10 μg) and tetracycline (30 μg).

On the basis of the phylogenetic, genotypic, phenotypic and chemotaxonomic characterization described above, it can be concluded that strain 34079^T should represent a novel species of a new genus in the family 'Rhodobacteraceae', for which the name *Alkalilacustris brevis* gen. nov., sp. nov. is proposed.

DESCRIPTION OF ALKALILACUSTRIS GEN. NOV.

Alkalilacustris [Al.ka.li.la.cus'tris. N.L. n. *alkali* (from Arabic article *al*, the; Arabic n. *qaliy*, ashes of saltwort) alkali; L. masc. n. *lacustris*, belonging to a lake; N.L. masc. n. *Alkalilacustris*, of an alkaline lake].

Cells are Gram-stain-negative and strictly aerobic short-rods, non-motile and non-pigmented. Catalase and oxidase are positive. The sole respiratory quinone is Q-10. The major cellular fatty acid (≥5 %) is summed feature 8 (C_{18:1} ω7c/C_{18:1} ω6c) and C_{16:0}. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. The genus is affiliated to the family 'Rhodobacteraceae' of the order Rhodobacterales. The type species is *Alkalilacustris brevis*.

DESCRIPTION OF ALKALILACUSTRIS BREVIS GEN. NOV., SP. NOV.

Alkalilacustris brevis (bré'vis. L. masc. adj. *brevis* short, referring to the short cells).

Following characteristics are displayed in addition to those given in the genus description. Cells are 0.9–1.6 μm long, 0.4–0.6 μm wide. After incubation on MA at 35 °C for 3 days, colonies are 1.0–1.5 mm in diameter with a smooth, opaque, convex surface and glossy margins. Growth occurs at 10–50 °C (optimum, 35 °C) and pH 7–10 (pH 8.0–8.5). Growth occurs in 1–10 % NaCl (w/v), with an optimum at 2.5–4 % (w/v). Carotenoid, bchl *α* and PHB are not detected. Negative for hydrolysis of starch, tyrosine, Tweens (20, 40, 60, 80), xanthine and hypoxanthine, as well as for H₂S production, the methyl red test and the Voges–

Table 2. Differential phenotypic characteristics of strain 34079^T and closely related genera of the family 'Rhodobacteraceae'

Strains: 1, 34079^T; 2, *Pararhodobacter* (data from Foessel et al., 2011); 3, *Rhodobaca* (data from two species, Milford et al., 2000; Boldareva et al., 2008); 4, *Roseibaca* (data from Labrenz et al., 2009). All the taxa are Gram-stain-negative, and Q-10 is the sole respiratory quinone. +, Positive; -, negative; ND, not detected; TR, trace (<1 %); NA, not available in all species.

| | 1 | 2 | 3 | 4 |
|---|---------|---------|-----------|---------|
| Motility | - | - | + | - |
| Anaerobic growth | - | - | + | - |
| bchl <i>a</i> | - | - | + | + |
| Carotenoids | - | - | + | - |
| Poly- β -hydroxybutyrate | - | NA | + | - |
| Temperature optimum for growth (°C) | 35 | 30-40 | 20-39 | 16 |
| pH optimum for growth | 8.0-8.5 | 7.0-8.5 | 8.2-9.0 | 7.0-9.5 |
| NaCl tolerance optimum (%) | 2.5-4.0 | 1.0-3.0 | 1.0-3.0 | 2.5 |
| Utilization of: | | | | |
| Fructose | - | + | + | + |
| Xylose | + | + | + | - |
| Mannitol | - | - | + | + |
| Fatty acids (%): | | | | |
| C _{10:0} 3-OH | 1.71 | 2.0 | NA | NA |
| C _{14:0} 3-OH | ND | NA | NA | 7.2 |
| C _{14:1} | ND | NA | 2.1-2.2 | ND |
| Summed feature 3* | 2.5 | 0.8 | 2.4-2.6 | ND |
| C _{16:1} ω 9c | ND | NA | NA | 5.9 |
| C _{16:0} | 6.4 | 7.9 | 9.7-18.7 | 1.3 |
| C _{18:0} | 2.6 | 5.5 | 1.6-2.4 | NA |
| Summed feature 8* | 81.5 | 78.2 | 67.3-79.1 | 80.5 |
| C _{18:2} ω 6,9c | ND | NA | NA | ND |
| 11-Methyl C _{18:1} ω 7c | 2.1 | 2.8 | 3.9-4.6 | ND |
| C _{18:1} ω 9c | ND | NA | TR/ND | 1.5 |
| G+C content (mol%) | 65.6 | 68.0 | 59-59.8 | 61 |

*Summed feature 3 comprised C_{16:1} ω 7c and/or C_{16:1} ω 6c, summed Feature 8 comprised C_{18:1} ω 7c and/or C_{18:1} ω 6c.

Proskauer reaction. Growth is detected on acetate, D-arabinose, L-arabinose, citrate, erythrose, inositol, glucose, glutamate, lactate, malate, pyruvate, rhamnose, ribose, sorbate, sodium malonate, sucrose, succinic acid, valine, xylose and xylitol, while not on ethanol, fructose, fucose, fumaric acid, galactose, α -ketopamyl diacid, mannitol, oxalate, oxalate acid and sorbitol. Positive for esterase (C4), leucine arylamidase and valine arylamidase, weakly positive for alkaline phosphatase, cystine arylamidase, esterase lipase, lipase (C14), naphthol-AS-BI-phosphohydrolase and trypsin; negative for acid phosphatase, α -chymotrypsin, α -fucosidase, α -mannosidase, *N*-acetyl- β -glucosaminidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase and β -glucuronidase. Acids were produced from potassium 2-ketogluconic and cellobiose. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, three unidentified amino lipids, one unidentified amino phosphoglycolipid, one phosphoglycolipid, one unidentified glycolipid, three unidentified phospholipids and two unidentified lipids. The DNA G+C content was 65.6 mol% (by genome).

The type strain, 34079^T (=KCTC 62428^T=MCCC 1K03493^T), was isolated from a soda lake (44° 03' N 126° 57' E) located in Jilin, PR China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 34079^T is MF664222. The GenBank accession number for the whole genome sequence of strain 34079^T is QNVJ00000000.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- Garrity GM, Bell JA, Lilburn T, Order III. *Rhodobacteriales* ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (editors). *Bergey's Manual of Systematic Bacteriology*, 2nd ed, vol. 2 (*The Proteobacteria*), part C (*The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*). New York: Springer; 2005. pp. 161.
- Garrity GM, Bell JA, Lilburn T, Class I. Alphaproteobacteria class. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (editors).

- Bergey's Manual of Systematic Bacteriology*, 2nd ed, vol. 2 (*The Proteobacteria*), part C (The *Alpha*-, *Beta*-, *Delta*-, and *Epsilon*proteobacteria). New York: Springer; 2005. pp. 1.
3. Garrity GM, Bell JA, Lilburn T. Family I. *Rhodobacteraceae* fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (editors). *Bergey's Manual of Systematic Bacteriology*, 2nd ed, vol. 2 (*The Proteobacteria*), part C (The *Alpha*-, *Beta*-, *Delta*-, and *Epsilon*proteobacteria). New York: Springer; 2005. pp. 161.
 4. Suzuki T, Muroga Y, Takahama M, Shiba T, Nishimura Y. *Rubrimonas cliftonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from a saline lake. *Int J Syst Bacteriol* 1999;49 Pt 1:201–205.
 5. Kompantseva EI, Komova AV, Kostrikin NA. *Rhodovulum step-pense* sp. nov., an obligately haloalkaliphilic purple nonsulfur bacterium widespread in saline soda lakes of Central Asia. *Int J Syst Evol Microbiol* 2010;60:1210–1214.
 6. Maszenan AM, Seviour RJ, Patel BK, Rees GN, McDougall BM et al. *Amaricoccus* gen. nov., a gram-negative coccus occurring in regular packages or tetrads, isolated from activated sludge biomass, and descriptions of *Amaricoccus veronensis* sp. nov., *Amaricoccus tam-worthensis* sp. nov., *Amaricoccus macauensis* sp. nov., and *Amaricoccus kaplicensis* sp. nov. *Int J Syst Bacteriol* 1997;47:727–734.
 7. Hiraishi A, Muramatsu K, Ueda Y. Molecular genetic analyses of *Rhodobacter azotoformans* sp. nov. and related species of phototrophic bacteria. *Syst Appl Microbiol* 1996;19:168–177.
 8. Abraham WR, Strömpl C, Meyer H, Lindholm S, Moore ER et al. Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int J Syst Bacteriol* 1999;49:1053–1073.
 9. Hiraishi A, Ueda Y. Intrageneric structure of the genus *Rhodobacter*: transfer of *Rhodobacter sulfidophilus* and related marine species to the genus *Rhodovulum* gen. nov. *Int J Syst Bacteriol* 1994;44:15–23.
 10. Sun X, Luo P, Li M. *Paracoccus angustae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2015;65:3469–3475.
 11. Pujalte MJ, Lucena T. The family Rhodobacteraceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F et al. (editors). *The Prokaryotes*. Berlin Heidelberg: Springer; 2014.
 12. Zhang XQ, Sun C, Wang CS, Zhang X, Zhou X et al. *Sinimariniibacterium flocculans* gen. nov., sp. nov., a gammaproteobacterium from offshore surface seawater. *Int J Syst Evol Microbiol* 2015;65:3541–3546.
 13. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al. Introducing EzBio-Cloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
 14. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.
 15. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
 16. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
 17. Kumar S, Tamura K, Nei M. MEGA: molecular evolutionary genetics analysis software for microcomputers. *Comput Appl Biosci* 1994;10:189–191.
 18. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
 19. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
 20. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ et al. ABySS: a parallel assembler for short read sequence data. *Genome Res* 2009;19:1117–1123.
 21. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
 22. Tamaoka J, Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 1984;25:125–128.
 23. Dong XZ, Cai MY. *Determinative Manual for Routine Bacteriology*, 1st ed. Beijing: Scientific Press; 2001. pp. 353–364.
 24. Zhu XF, Jia XM, Zhang XQ, Yh W. *Modern Experimental Technique of Microbiology*. Hangzhou: Zhejiang University Press (English translation); 2011.
 25. Kumar PA, Srinivas TN, Manasa P, Madhu S, Shivaji S et al. *Lutibaculum baratangense* gen. nov., sp. nov., a proteobacterium isolated from a mud volcano. *Int J Syst Evol Microbiol* 2012;62:2025–2031.
 26. Wu XY, Zheng G, Zhang WW, Xu XW, Wu M et al. *Amphibacillus jiliniensis* sp. nov., a facultatively anaerobic, alkaliphilic bacillus from a soda lake. *Int J Syst Evol Microbiol* 2010;60:2540–2543.
 27. Sun C, Huo YY, Liu JJ, Pan J, Qi YZ et al. *Thalassomonas eurytherma* sp. nov., a marine proteobacterium. *Int J Syst Evol Microbiol* 2014;64:2079–2083.
 28. Dornbusch K, Nord CE, Olsson B. Antibiotic susceptibility testing of anaerobic bacteria by the standardized disc diffusion method with special reference to *bacteroides fragilis*. *Scand J Infect Dis* 1975;7:59–66.
 29. Nokhal T-H, Schlegel HG. Taxonomic study of *Paracoccus denitrificans*. *Int J Syst Bacteriol* 1983;33:26–37.
 30. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
 31. Cui HL, Gao X, Yang X, Xu XW, Xw X. Halolamina pelagica gen. nov., sp. nov., a new member of the family Halobacteriaceae. *Int J Syst Evol Microbiol* 2011;61:1617–1621.
 32. Sheu SY, Lin KR, Hsu MY, Sheu DS, Tang SL et al. *Endozoicomonas acroporae* sp. nov., isolated from Acropora coral. *Int J Syst Evol Microbiol* 2017;67:3791–3797.
 33. Chen C, Su Y, Tao T, Fu G, Zhang C et al. *Maripseudobacter aurantiacus* gen. nov., sp. nov., a novel member of the family Flavobacteriaceae isolated from a sedimentation basin. *Int J Syst Evol Microbiol* 2017;67:778–783.
 34. Komagata K, Suzuki K. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1988;19:161–207.
 35. Worliczek HL, Kämpfer P, Rosengarten R, Tindall BJ, Busse HJ. Polar lipid and fatty acid profiles-re-vitalizing old approaches as a modern tool for the classification of *mycoplasmas*? *Syst Appl Microbiol* 2007;30:355–370.