Lacinonas salitolera gen. nov., sp. nov., isolated from surface water of a saline lake

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A Gram-stain-negative bacterium, strain TS-T30T, was isolated from a saline lake (Lake Tuosu) in Qaidam basin, Qinghai province, China, and its taxonomic position was determined by using a polyphasic approach. Cells were non-spor-forming rods, non-motile, 0.8–1.4 μm wide and 1.9–4.0 μm long. Strain TS-T30T was strictly heterotrophic and aerobic. Catalase- and oxidase-positive. Growth was observed in the presence of 0.5–11.0 % (w/v) NaCl (optimum 3.0 %), and at 10–35 °C (optimum 25 °C) and pH 6.5–10.0 (optimum pH 8.5). Strain TS-T30T contained C18:1ω7c as the only predominant fatty acid. The major respiratory quinone was Q-10. The DNA G+C content was 62 mol % (Tm). Phylogenetic trees based on 16S rRNA gene sequences showed that strain TS-T30T formed a distinct lineage that was independent of other most closely related genera: Lutimaribacter (95.2–95.9 % 16S rRNA gene sequence similarities), Poseidonocella (95.4 %), Ruegeria (92.8–94.9 %), Marivita (93.6–94.9 %), Seohaelicola (94.7 %), Sediminimonas (94.7 %), Shimia (93.9–94.7 %), Oceanicola (92.6–94.5 %) and Roseicyclus (94.5 %). The major polar lipids were phosphatidylglycerol, one unidentified phospholipid and an unknown aminolipid; phosphatidylcholine was not detected. These data demonstrated that strain TS-T30T represents a novel species of a new genus in the family Rhodobacteraceae, for which the name Lacinonas salitolera gen. nov., sp. nov. is proposed. The type strain of the type species is TS-T30T (=CGMCC 1.12477T=NBRC 110969T).

The family Rhodobacteraceae, belonging to the order Rhodobacterales in the class Alphaproteobacteria (Garrity et al., 2005, 2006), mainly comprises bacteria from aquatic environment, such as tidal flat sediment (Park et al., 2014a, b; Yoon et al., 2013b), seawater (Hahnke et al., 2013), aquaculture pond (Srinivas et al., 2007), saline pond (Chakravarthy et al., 2009), ancient salt mine sediment (Wang et al., 2009) and hypersaline lake water (Labrenz et al., 2005). Some genera classified within the family Rhodobacteraceae according to LPSN (2015) have been categorized into other genera, including the genera Catellibacterium, Gaetbulicola, Silicibacter, Staleyia and Thiosphaera, which have been reclassified as genera Gemmobacter (Chen et al., 2013), Marivita (Yoon et al., 2012), Ruegeria (Yi et al., 2007), Sulfitobacter (Yoon et al., 2007) and Paracoccus (Ludwig et al., 1993), respectively. At the time of writing, there were 111 genera in the family Rhodobacteraceae with validly published names. Species from some genera in this family, such as genera Rhodobacter (Imhoff et al., 1984), Roseicyclus (Rathgeber et al., 2005) and Roseibium (Suzuki et al., 2000) are capable of aerobic anoxygenic photosynthesis, which was considered as an important genus-specific property (Imhoff & Caumette, 2004; Uchino et al., 2002; Wang et al., 2014). During a survey of bacterial diversity of a saline lake (Lake Tuosu), a novel Rhodobacteraceae-like strain, designated TS-T30T, was obtained and investigated for its taxonomic position through a polyphasic approach.

A water sample was collected from Lake Tuosu (37° 11′ 15″ N 96° 53′ 30″ E; pH 8.8 and temperature 20.1 °C) in Qaidam basin, Qinghai province, China. Strain TS-T30T was isolated by the standard dilution plating technique on diluted Luria–Bertani (LB) agar (0.5 g tryptone, 0.25 g yeast extract, 50.0 g NaCl and 1 l distilled water).
water, pH 9.0) at 30 °C, and cultured on marine agar 2216 (MA; Difco). Strain TS-T30T could also grow well in marine broth 2216 (MB; Difco), but not in LB broth, trypticase soy broth (TSB; Bacto), or modified LB or TSB supplemented with 2.0–4.0 % (w/v) NaCl. The strain was preserved as glycerol stocks at −80 °C. Biomass for all analysis was obtained after cultivation on MA or in MB at 30 °C for 48 h unless otherwise stated.

Cell morphology was observed by optical microscopy (BH-2; Olympus) and transmission electron microscopy (H-600; Hitachi) after negative staining with 1 % (w/v) phosphotungstic acid. The Gram reaction was performed according to Dong & Cai (2001). Growth at 5, 10, 15, 20, 25, 30, 35 and 40 °C was measured in MB. Growth at pH 6.0–10.5 (at intervals of 0.5 pH units) was determined in MB using appropriate buffers (final concentration 50 mM): sodium phosphate buffer (for pH 6.0–8.0) and Tris/HCl buffer (for pH 8.0–10.5). Tolerance to NaCl was examined in modified MB by adjusting the final concentration of NaCl to 0–11.5 % (w/v, at intervals of 0.5 %) as described previously (Zhong et al., 2014).

The requirement for oxygen was tested in an anaerobic system (Oxoid AnaeroGen System). Production of hydrogen sulfide was assessed with lead acetate paper. Catalase and oxidase activities and hydrolysis of casein, l-tyrosine, starch and Tweens 20, 40, 60 and 80 were determined according to Dong & Cai (2001). Utilization of carbon substrates (0.5 %, w/v) was tested according to Dong & Cai (2001) with artificial seawater instead of distilled water, 0.01 % (w/v) yeast extract was added as a supplement. The artificial seawater contained (per litre distilled water): 24.0 g NaCl, 5.1 g MgCl₂, 4 g Na₂SO₄, 1.1 g CaCl₂, 0.7 g KCl, 0.2 g NaHCO₃, 0.1 g KBr, 0.027 g H₂BO₃, 0.024 g SrCl₂ and 0.003 g NaF. Additionally, API ZYM and API 20NE systems (bioMérieux) were used for tests of enzyme activities and other physiological and biochemical traits according to the manufacturer’s instructions. The pigments of strain TS-T30T were extracted by adding acetone/methanol (7 : 2, v/v) onto an MA slant after negative staining with 1 % (w/v) sodium phosphate buffer (for pH 6.0–8.0) and Tris/HCl buffer (for pH 8.0–10.5). Absorption spectra showed no absorption peaks, indicating that bacteriochlorophyll a (Bchl a) was absent. More characteristics of strain TS-T30T are specified in Table 1 and in the genus and species descriptions.

The 16S rRNA gene of strain TS-T30T was amplified with primers 27F and 1492R (Weisburg et al., 1991), and cloned into the pEASY-T1 vector and sequenced by Sino-GenoMax, China with primers M13f and M13r. An almost-complete 16S rRNA gene sequence (1425 nt) was obtained and compared with available sequences in the GenBank database using the BLAST program (Altschul et al., 1990) at NCBI (http://www.ncbi.nlm.nih.gov) and also on the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon/) by using identity analysis (Kim et al., 2012a). The 16S rRNA gene sequences of strain TS-T30T and related taxa were aligned with CLUSTAL x software 2.0 (Larkin et al., 2007). The neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) trees were reconstructed using MEGA program version 5 (Tamura et al., 2011). Evolutionary distances were calculated by Kimura’s two-parameter model (Kimura, 1980) and the gaps/missing data treatment option chosen was pairwise-deletion.

The neighbour-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 1. Strain TS-T30T formed a distinct lineage that was independent of other most closely related genera including Lutimaribacter, Poseidonocella, Ruegeria, Maritiva, Seohaeicola, Sediminimonas, Shinnia, Oceanicola and Roseicyclus, to which strain TS-T30T showed 95.1–95.8 %, 95.3 %, 92.7–94.9 %, 93.6–94.8 %, 94.7 %, 94.5 %, 93.8–94.6 %, 92.5–94.5 % and 93.5 % 16S rRNA gene sequence similarities, respectively. Similar topologies were recovered in maximum-parsimony (MP) and maximum-likelihood (ML) trees, albeit strain TS-T30T formed a clade with Sediminimonas qiaohouensis DSM 21189T in the ML tree with only 31 % bootstrap support. In the trees constructed with the type species of all genera in the family Rhodobacteraceae by the same method, strain TS-T30T joined with Roseicyclus mahoneyensis ML6T and Poseidonocealla pacifica KMM 9010T with low bootstrap values (<50 %) in both NJ and MP trees, and joined with Lutimaribacter saemankumensis SMK-117T with 36 % bootstrap value in the ML tree (data not shown). In addition, all of above related genera belonged to the Roseobacter group within the family Rhodobacteraceae. These phylogenetic data strongly suggested that strain TS-T30T cannot be assigned to any known genus of the family Rhodobacteraceae, and thus represents a novel genus belonging to the Roseobacter group.

Colonies of strain TS-T30T were circular, smooth, pale yellow, glistening and 0.5–1.2 mm in diameter after cultivation on MA (pH 7.5) at 30 °C for 4 days. Cells of strain TS-T30T were Gram-stain-negative, non-motile, non-spore-forming rods, 0.8–1.4 µm wide and 1.9–4.0 µm long (Fig. S1, available in the online Supplementary Material). Strain TS-T30T was strictly heterotrophic and aerobic, and catalase- and oxidase-positive. The novel strain was able to grow in the presence of 0.5–11.0 % (w/v) NaCl (optimum 3.0 %), and at 10–35 °C (optimum 25 °C) and pH 6.5–10.0 (optimum pH 8.5). No growth occurred at 5 °C or 40 °C, at pH 6.0 or pH 10.5, in the absence of NaCl or in the presence of 11.5 % (w/v) NaCl. H₂S was not produced. Strain TS-T30T did not hydrolyse l-tyrosine, starch, casein and Tweens 20, 40, 60 and 80. In addition, in vivo absorption spectra showed no absorption peaks, indicating that bacteriochlorophyll a (Bchl a) was absent. More characteristics of strain TS-T30T are specified in Table 1 and in the genus and species descriptions.

Biomass for chemotaxonomic analyses was cultivated on MA at 30 °C for 2 days to late exponential phase of growth. Genomic DNA was extracted by using a bacterial genomic kit (D3550-1; Omega Bio-Tek). The DNA base
Table 1. Differential characteristics of strain TS-T30ᵀ and phylogenetically related genera

Genera: 1, *Lacimonas* (strain TS-T30ᵀ, data from this study); 2, *Lutimaribacter* (Iwaki et al., 2013; Yoon et al., 2009a; Yuan et al., 2009); 3, *Poseidonocella* (Romanenko et al., 2012); 4, *Ruegeria* (Huo et al., 2011; Kämpfer et al., 2013; Kim et al., 2012b, 2014; Lee et al., 2012; Muramatsu et al., 2007; Oh et al., 2011; Park & Yoon, 2012; Uchino et al., 1998; Vandecandelaere et al., 2008; Yi et al., 2007); 5, *Marivita* (Hwang et al., 2009; Yoon et al., 2010, 2012, 2013a); 6, *Seohaeicola* (Yoon et al., 2009b); 7, *Sediminimonas* (Wang et al., 2009); 8, *Shimia* (Chen et al., 2011; Choi & Cho, 2006; Hameed et al., 2013; Hyun et al., 2013); 9, *Oceanicola* (Cho & Giovannoni, 2004; Gu et al., 2007; Huo et al., 2014; Lin et al., 2007; Park et al., 2013; Zheng et al., 2010). All genera are Gram-stain-negative, oxidase- and catalase-positive, and positive for esterase (C4) and esterase lipase (C8) activities. All genera are negative for cystine arylamidase, β-glucuronidase, α-mannosidase and α-fucosidase activities, and are absent of BChl a. +, Positive; −, negative; v, variable among type strains; NR, not reported; A, aerobic; F, facultative aerobic.

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<td>Predominant polar lipids§</td>
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<td>PC, PG, DPG, PL, AL</td>
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<td>PC, PG, DPG, PLs</td>
<td>PC, PG, DPG, AL, L</td>
<td>PC, PG, PE, AL</td>
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†All were positive except for *Marivita byunsanensis.*

‡All were negative except for *Marivita geojedonensis.*

§PG, phosphatidylocholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; PL, unknown phospholipid; AL, unknown aminolipid; L, unknown lipid.
sequences showing the phylogenetic position of strain TS-T30T and representatives of the most closely related genera. Bootstrap values (expressed as percentages of 1000 replications) >70 % are shown at the branch points. Filled circles indicate branches that were also recovered in both maximum-likelihood and maximum-parsimony trees. Rhodocyclus purpureus DSM 168 T was used as an outgroup. Bar, evolutionary distance (K_{nucl}) of 0.01.

The DNA G+C content of strain TS-T30T was 62 mol% (T_m). The major respiratory quinone was ubiquinone 10 (Q-10), which is typical of the vast majority of members within class Alphaproteobacteria. The detailed fatty acid profiles of strain TS-T30T and the type strains of the type species of the most closely related genera are shown in Table S1. The only major fatty acid of strain TS-T30T was C18 : 1ω7c (70.5 %), which was the major fatty acid for all type species of genera in the family Rhodobacteraceae (Table S1). However, strain TS-T30T could be distinguished from Poseidonocella pacifica KMM 9010 T by the presence of a lesser amount of C18 : 1ω7c 11-methyl and greater amounts of C17 : 0 3-OH and summed feature 7 (C19 : 1ω11c, C19 : 0 cyclo ω10c and/or ECL 18.846) (Table S1). In addition, strain TS-T30T could be differentiated from the type strains of the type species of other Ruegeria, Marinimimibacter, Marivita, Seohaeicola, Sediminimonas, Shimia and Oceanicola, by lack of other major fatty acids (Table S1). The polar lipid profile of strain TS-T30T contained phosphatidylglycerol, one unidentified phospholipid and an unknown aminolipid as the major polar lipids, and one unidentified aminolipid and five unknown lipids as the minor lipids (Fig. S2). The absence of phosphatidylcholine could distinguish strain TS-T30T from all of the most closely related genera in the family Rhodobacteraceae. Strain TS-T30T could be differentiated from members of the genera Lutimaribacter, Ruegeria, Marivita, Seohaeicola and Oceanicola by the absence of phosphatidylethanolamine, and from members of the genus Poseidonocella, Sediminimonas and Shimia by the absence of diphosphatidylglycerol (Table 1). In addition to data of cellular fatty acids, polar lipids and phylogenetic analysis, strain TS-T30T could also be differentiated from the most closely related genera by some physiological and genotypic characteristics as shown in Table 1, such as motility, oxygen requirement and hydrogen sulfide production, nitrate reduction, tolerance to NaCl, temperature and pH, hydrolysis of urea, l-arginine,
L-tyrosine, casein, starch, aesculin, gelatin and Tweens 20, 40, 60 and 80, some enzyme activities, utilization of some substrates and DNA G+C content.

Combining the above phenotypic, chemotaxonomic and genotypic results, it is concluded that strain TS-T30T represents a novel species of a new genus in the family Rhodobacteraceae, for which the name Lacimonas salitolerans gen. nov., sp. nov. is proposed.

Description of Lacimonas gen. nov.

Lacimonas (la.ci.mo’nas. L. masc. n. lacus lake; L. fem. n. monas a unit, monad; N.L. fem. n. Lacimonas a lake monad).

Strictly heterotrophic and aerobic. Oxidase- and catalase-positive. Cells are Gram-stain-negative, rod-shaped and non-motile. Negative for production of H2S and indole, fermentation of D-glucose, nitrate reduction, and hydrolysis of urea, starch, aesculin and gelatin. Contains Q-10 as the major respiratory quinone, C18:1ω7c as the major fatty acid and phosphatidylglycerol, an unknown phospholipid and an unidentified aminolipid as the major lipids. BCHl a is absent.

The type species is Lacimonas salitolerans. The DNA G+C content of the type strain of the type species is 62 mol% (Tm).

Description of Lacimonas salitolerans sp. nov.

Lacimonas salitolerans (sa.li.to’le.rans. L. n. sal, salis salt; L. part. adj. tolerans tolerating; N.L. part. adj. salitolerans salt-tolerating, originating from a saline habitat).

Displays the following characteristics in addition to those described for the genus. Cells are 0.8–1.4 μm wide and 1.9–4.0 μm long. Colonies are 0.5–1.2 mm in diameter, circular, smooth, pale yellow and glistening after cultivation on MA (pH 7.5) at 30 °C for 4 days. Negative for hydrolysis of L-arginine, and Tweens 20, 40, 60 and 80. Growth occurs at 10–35 °C (optimum 25 °C), at pH 6.5–10.0 (optimum pH 8.5), and in the presence of 0.5–11.0 % (w/v) NaCl (optimum 3.0 %). Utilizes D-glucose, D-fructose, sucrose, maltose, D-mannose, lactose, glutamate, succinate, D-xylose, D-galactose and citrate; but not trehalose, pyruvate and L-tyrosine, casein, starch, aesculin, gelatin and Tweens 20, 40, 60 and 80, some enzyme activities, utilization of some substrates and DNA G+C content.

The type strain is TS-T30T (=CGMCC 1.12477T=NBRC 110969T), isolated from surface water of Lake Tuosu in Qaidam basin, Qinghai province, China. The DNA G+C content of the type strain is 62 mol% (Tm).

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References


Lacimonas salitolerans gen. nov., sp. nov.


