Two halophilic archaeal strains, TRM20010T and TRM20345T, were isolated from saline soil of the Lop Nur region in Xinjiang, north-west China. Cells from the two strains were pleomorphic rods, stained Gram-negative and produced red-pigmented colonies. Strains TRM20010T and TRM20345T were able to grow at 30–62 °C (optimum 37 °C), 0.9–5.1 M NaCl (optimum 2.6 and 3.4 M, respectively) and pH 6.0–10.0 (optimum pH 7.0–7.5) and neither strain required Mg2+ or Ca2+ for growth. The major polar lipids of the two strains were phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), two glycolipids chromatographically identical to galactosyl mannosyl glucosyl diether (TGD-1) and disulfated mannosyl glucosyl diether (S2-DGD). Phylogenetic analysis based on 16S rRNA and rpOB genes revealed that strains TRM20010T and TRM20345T clustered together and formed a distinct clade separated from the related genera Halovivax, Haloterrigena, Halostagnicola, Natronolimnobius and Natrinema. The DNA G+C contents of strains TRM20010T and TRM20345T were 63.9 and 63.8 mol%, respectively. The DNA–DNA hybridization value between strain TRM20010T and strain TRM20345T was 42.8%. The phenotypic, chemotaxonomic and phylogenetic properties suggested that strains TRM20010T and TRM20345T represent two novel species in a new genus within the family Halobacteriaceae, for which the names Natribaculum breve gen. nov., sp. nov. (type strain TRM20010T = CCTCC AB2013112T = NRRL B-59996T) and Natribaculum longum sp. nov. (type strain TRM20345T = CCTCC AB2013113T = NRRL B-59997T) are proposed.
Two novel species of Natribaculum gen. nov.

morphology and motility in exponentially growing liquid cultures were examined using a Leica microscope equipped with phase-contrast optics. The Gram stain was performed following the method outlined by Dussault (1955). The minimum salt concentration preventing cell lysis was determined by suspending washed cells in serial sterile saline solutions containing NaCl ranging from 0 to 150 g l\(^{-1}\) and the stability of the cells was detected by light microscopic examination. The pH range for growth was determined at pH 5.0–10.0 (at intervals of 0.5 units) using the following buffers, each at a concentration of 25 mM: MES (pH 5.5–6.7), PIPEs (pH 6.1–7.5), MOPS (pH 6.5–7.9), HEPES (pH 6.8–8.2), Tricine (pH 7.4–8.8) and CHES (pH 8.6–10.0). The temperature range for growth was determined by incubating the strains at 28–65 °C at 5 °C intervals. Halovivax asiaticus CGMCC 1.4248\(^T\), Natribia limnobius CGMCC 1.1966\(^T\) and Natronolimnobium baerhuensis CGMCC 1.3597\(^T\) were selected as reference strains in phenotypic tests.

Polar lipids were extracted using a chloroform/methanol system and analysed using one- and two-dimensional TLC, as described previously (Cui et al., 2010). Merck silica gel 60 F\(_{254}\) aluminium-backed thin-layer plates were used for TLC analyses. In two-dimensional TLC, the first solvent was chloroform/methanol/water (65 : 25 : 4, by vol.), and the second solvent was chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). The latter solvent mixture was also used in one-dimensional TLC. Two specific detection spray reagents, phosphate stain reagent for phospholipids and a-naphthol stain for glycolipids, were used. The general detection reagent, sulfuric acid/ethanol (1 : 2, by vol.), was also used to detect total polar lipids.

Genomic DNA from halophilic archaeal strains was prepared as described previously (Cui et al., 2011). The 16S rRNA genes were amplified, cloned and sequenced according to the protocol of Shimane et al. (2011). PCR-mediated amplification and sequencing of the rpoB\(^9\) genes were performed as described by Minegishi et al. (2010). Multiple sequence alignments were performed using the CLUSTAL W program integrated in the MEGA 5 software (http://www.megasoftware.net/). Phylogenetic trees were reconstructed using the maximum-likelihood algorithm in the MEGA 5 software (Tamura et al., 2011). Gene sequence similarity among halophilic archaea was calculated using the pairwise-distance computing function of MEGA 5. The DNA G + C content was determined by the HPLC method (Mesbah et al., 1989). DNA–DNA hybridization analyses were performed according to the thermal denaturation and renaturation method of De Ley et al. (1970) as modified by Huss et al. (1983).

Cells of strains TRM20010\(^T\) and TRM20345\(^T\) were motile and pleomorphic rod-shaped when grown in liquid medium (Fig. S1, available in the online Supplementary Material). They stained Gram-negative and colonies were red-pigmented. Strains TRM20010\(^T\) and TRM20345\(^T\) were able to grow at 30–62 °C (optimum 37 °C), at 0.9–5.1 M NaCl (optimum 2.6 and 3.4 M, respectively) and at pH 6.0–10.0 (optimum pH 7.0–7.5) and neither strain required Mg\(^{2+}\) for growth. Cells of strain TRM20010\(^T\) were not lysed in distilled water while those of strain TRM20345\(^T\) were lysed and the minimum NaCl concentration that prevented cell lysis was 5 % (w/v). Strains TRM20010\(^T\) and TRM20345\(^T\) produced H\(_2\)S from sodium thiosulfate, and hydrolysed starch but not casein or Tween 80. The main phenotypic characteristics differentiating strains TRM20010\(^T\) and TRM20345\(^T\) from members of the genera Halovivax, Haloterrigena, Halostagnicola and Natronolimnobium are given in Table 1. More detailed results of phenotypic tests and nutritional features of strains TRM20010\(^T\) and TRM20345\(^T\) are given in the species descriptions.

The major polar lipids of strains TRM20010\(^T\) and TRM20345\(^T\) were phosphatidylglycerol (PG) and phosphatidylglycerol phosphate methyl ester (PGP-Me), two glycolipids chromatographically identical to galactosyl mannosyl glucosyl diether (TGD-1) and disulfated mannosyl glucosyl diether (S\(_2\)-DGD) (Fig. S2). The two glycolipids (GL1, GL2) were chromatographically identical to the two glycolipids detected in Natrinema pellirubrum JCM 10476\(^T\), Halostagnicola larseni CGMCC 1.5338\(^T\), Haloterrigena longa CGMCC 1.5334\(^T\) and Halovivax asiaticus CGMCC 1.4248\(^T\). The polar lipid profile differentiated strains TRM20010\(^T\) and TRM20345\(^T\) from members of the genera Natrinema, Halostagnicola, Haloterrigena and Halovivax.

Strains TRM20010\(^T\) and TRM20345\(^T\) had one type of 16S rRNA gene sequence, and the sequences were 99.1 % similar to each other, and both strains were closely related to members of the genera Halovivax (93.5–93.7 % similarity), Haloterrigena (93.4–93.5 %) and Natrinema (93.3 %). Phylogenetic tree reconstructions using the neighbour-joining algorithm revealed that strains TRM20010\(^T\) and TRM20345\(^T\) formed a distinct clade, separate from related recognized genera of the Halobacteriaceae, namely Halovivax, Haloterrigena, Halostagnicola and Natronolimnobium (Fig. 1a). The phylogenetic position was also confirmed in other trees generated using the maximum-parsimony and maximum-likelihood algorithms (data not shown).

The rpoB\(^9\) genes of both novel strains were sequenced and found to be identical in length (1830 bp). The nucleotide sequences were 99.2 % similar to each other, and were closely similar to the corresponding gene of members of the genera Halovivax (87.8–88.1 % similarity), Haloterrigena (89.0–89.7 %) and Natrinema (88.6–88.9 %). In the phylogenetic tree reconstructed based on rpoB\(^9\) gene sequences, strains TRM20010\(^T\) and TRM20345\(^T\) formed a monophyletic group separate from the related recognized genera of the Halobacteriaceae, namely Halovivax, Haloterrigena, Halostagnicola, Natronolimnobium and Natrinema (Fig. 1b).

The DNA G + C contents of strains TRM20010\(^T\) and TRM20345\(^T\) were 63.9 and 63.8 mol %, respectively. These
Table 1. Differential characteristics between strains TRM20010$^T$ and TRM20345$^T$ and closely related members of the family Halobacteriaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Colony colour</td>
<td>Red</td>
<td>Pale red</td>
<td>Orange/red</td>
<td>Pink/red</td>
<td>Red</td>
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<td>Rod/coccoid</td>
<td>Pleomorphic</td>
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<td>+</td>
<td>+/-</td>
<td>-</td>
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<tr>
<td>Cell lysis in distilled water</td>
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<td>+/-</td>
<td>+</td>
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<tr>
<td>Optimum NaCl (M)</td>
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<td>3.4</td>
<td>2.0–3.5</td>
<td>3.4</td>
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<tr>
<td>D-Glucose</td>
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<td>+</td>
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<tr>
<td>D-Galactose</td>
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<td>+/-</td>
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<td>+</td>
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<tr>
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<td>-</td>
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<td>+</td>
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<tr>
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<tr>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>63.9</td>
<td>63.8</td>
<td>59.3–67.0</td>
<td>60.3–65.0</td>
<td>59.2–63.1</td>
<td>59.8–61.0</td>
</tr>
</tbody>
</table>

Values are within the range of those of the genera *Haloterrigena* (59.3–67.0 mol%) and *Halovivax* (60.3–65.0 mol%), but higher than those of *Halostagnicola* (59.8–61.0 mol%) and *Natranolimnobius* (59.2–63.1 mol%) (Table 1). The DNA–DNA hybridization value between strains TRM20010$^T$ and TRM20345$^T$ was 42.8%, much lower than the accepted threshold value (70%) used to separate two species (Stackebrandt & Goebel, 1994).

The phenotypic, chemotaxonomic and phylogenetic properties thus suggest that strains TRM20010$^T$ and TRM20345$^T$ represent two novel species in a new genus within the family Halobacteriaceae, for which the names *Natribaculum breve* gen. nov., sp. nov. and *Natribaculum longum* sp. nov. are proposed. Characteristics distinguishing strains TRM20010$^T$ and TRM20345$^T$ from other genera within the family Halobacteriaceae are shown in Table 1.

Description of *Natribaculum gen. nov.*

*Natribaculum* (Na.tri.ba’cu.lum. N.L. n. *natron* arbitrarily derived from the Arabic n. *natrun* or *natron* soda, sodium carbonate; L. neut. n. *baculum* stick; N.L. neut. n. *Natribaculum* soda stick).

Cells are motile pleomorphic rods under optimal growth conditions, stain Gram-negative and are aerobic heterotrophs. Catalase- and oxidase-positive. Extremely halophilic, with growth occurring in media containing 0.9–5.1 M NaCl. Temperatures between 30 and 62 °C and pH between 6.0 and 10.0 may support growth. Sugars are metabolized, in some cases with the formation of acids. The polar lipids are phosphatidylglycerol (PG), phosphatidylylglycerol phosphate methyl ester (PGP-Me), two glycolipids chromatographically identical to galactosyl mannosyl glucosyl diether (TGD-1) and disulfated mannosyl glucosyl diether (S$_2$-DGD). The type species is *Natribaculum breve*. Recommended three-letter abbreviation: Nbl.

Description of *Natribaculum breve* sp. nov.

*Natribaculum breve* (bre’ve. L. neut. adj. breve short, the shape of the cells).

Has the following characteristics in addition to those given for the genus. Colonies on agar plates containing 2.6 M NaCl are red, elevated and round. Chemo-organotrophic. Growth occurs at 30–62 °C (optimum 37 °C), at 0.9–5.1 M NaCl (optimum 2.6 M), at 0–1.0 M MgCl$_2$ (optimum 0.05 M) and at pH 6.0–10.0 (optimum pH 7.0–7.5). Cells do not lyse in distilled water. Grows under anaerobic conditions using nitrate but not L-arginine or DSMO. Nitrate reduction to nitrite and gas formation from nitrate are not observed. H$_2$S is produced from sodium thiosulfate. Indole formation is positive. Hydrolyses starch and gelatin but not casein or Tween 80. The following substrates are utilized as single carbon and energy sources for growth: D-glucose and pyruvate. No growth occurs on D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, sucrose, lactose, glycerol, D-mannitol, D-sorbitol, acetate, DL-lactate, fumarate, citrate, starch, L-arginine, L-aspartate, L-alanine, L-glutamate, L-lysine or L-ornithine. Sensitive to the following antimicrobial compounds: novobiocin, mycostatin and nitrofurantoin. Resistant to the following antimicrobial compounds: bacitracin, rifampicin, trimethoprim, erythromycin, penicillin G, ampicillin, chloramphenicol, neomycin, norfloxacin, ciprofloxacin, streptomycin, kanamycin, tetracycline, vancomycin, gentamicin and nalidixic acid.
The type strain, TRM20010T (≡CCTCC AB2013112T = NRRL B-59996T), was isolated from saline soil of the Lop Nur region in Xinjiang, north-west China. The DNA G+C content of the type strain is 63.9 mol% (Tm).

**Description of Natribaculum longum sp. nov.**

*Natribaculum longum* (lon’gum. L. neut. adj. *longum* long, the shape of the cells).

Has the following characteristics in addition to those given for the genus. Colonies on agar plates containing 3.4 M NaCl are red, elevated and round. Chemo-organotrophic. Growth occurs at 30–62 °C (optimum 37 °C), at 0.9–5.1 M NaCl (optimum 3.4 M), at 0–1.0 M MgCl₂ (optimum 0.05 M) and at pH 6.0–10.0 (optimum pH 7.0–7.5). Cells lyse in distilled water and the minimal NaCl concentration to prevent cell lysis is 8 % (w/v). Grows under anaerobic conditions using nitrate but not L-arginine or DMSO. Nitrate reduction to nitrite and gas formation from nitrate are not observed. H₂S is produced from sodium thiosulphate. Indole formation is negative. Hydrolyses starch but not casein, gelatin or Tween 80. The following substrates are utilized as single carbon and energy sources for growth: D-glucose, D-mannose, D-galactose, D-ribose and pyruvate.

![Fig. 1. Neighbour-joining phylogenetic tree reconstructions based on 16S rRNA gene (a) and rpoB gene (b) sequences, showing the relationships between strains TRM20010T and TRM20345T and related members within the family Halobacteriaceae. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more 50 % bootstrap support. Bar, 0.02 (a) and 0.05 (b) expected changes per site.](http://ijs.sgmjournals.org)
L-aspartate is utilized as a single carbon, nitrogen or energy source for growth. No growth occurs on D-fructose, L-sorbose, D-xylose, maltose, sucrose, lactose, glycerol, D-mannitol, D-sorbitol, acetate, DL-lactate, fumarate, citrate, starch, L-arginine, L-alanine, L-glutamate, L-lysine or L-ornithine. Sensitive to the following antimicrobial compounds: novobiocin, rifampicin and nitrofurantoin. Resistant to the following antimicrobial compounds: mycostatin, bacitracin, trimethoprim, erythromycin, penicillin G, ampicillin, chloramphenicol, neomycin, norfl Roxacin, ciprofloxacin, streptomycin, kanamycin, tetracycline, vancomycin, gentamicin and nalidixic acid.

The type strain, TRM20010T (= CCTCC AB2013113T = NRRL B-59997T), was isolated from saline soil of the Lop Nur region in Xinjiang, north-west China. The DNA G+C content of the type strain is 63.8 mol% (Tm).

Acknowledgements

This research was supported by the National High-tech R&D Program of China (863 Program) (grant no. 2012AA021705) and the opening project of Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin (BRZD1206). We are grateful to Professor Aharon Oren for the etymology of the new names and Professor Heng-Lin Cui for helpful comments.

References


