Haloterrigena saccharevitans sp. nov., an extremely halophilic archaeon from Xin-Jiang, China

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A novel extremely halophilic strain, isolated from Aibi salt lake, Xin-Jiang, China, was subjected to polyphasic taxonomic characterization. This strain, designated AB14^T, is neutrophilic, motile and requires at least 10 % (w/v) NaCl for growth. Strain AB14^T grows at 24–58 °C, with optimal growth at 42–45 °C. Mg^{2+} is not required, but growth is observed in $MgCl_2$ concentrations as high as 1·0 M. Strain AB14^T possesses the diphytanyl ($C_{20}C_{20}$) and phytanyl-sesterterpanyl diether ($C_{20}C_{25}$) derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and mannose-2,6 disulfate 1→2 glucose-glycerol diether. The genomic DNA G+C content is 66·6 mol%. The 16S rRNA gene sequence similarity values of strain AB14^T with its nearest phylogenetic neighbours (*Haloterrigena thermotolerans* and *Haloterrigena turkmenica*) are 98·6 and 96·0 %, respectively. DNA–DNA hybridization revealed 54 % relatedness between strain AB14^T and *Haloterrigena thermotolerans* JCM 11050^T and 21 % between strain AB14^T and *Haloterrigena turkmenica* JCM 9101^T. It is therefore proposed that strain AB14^T represents a novel species, for which the name *Haloterrigena saccharevitans* sp. nov. is proposed. The type strain is AB14^T (=AS 1.3730^T=JCM 12889^T).

The genus *Haloterrigena* currently contains two species of extremely halophilic archaea, *Haloterrigena turkmenica* (Ventosa *et al.*, 1999) and *Haloterrigena thermotolerans* (Montalvo-Rodríguez *et al.*, 2000). In phylogenetic trees based on 16S rRNA gene sequences, species of the genera *Haloterrigena* and *Natrinema* sometimes cluster together (Montalvo-Rodríguez *et al.*, 2000; Xin *et al.*, 2000; Tindall, 2003). However, there are striking differences in the polar lipid composition among species of these two genera. The type species of *Haloterrigena* possesses mannose-2,6 disulfate 1→2 glucose-glycerol diether (S_2-DGD), but lacks phosphatidylglycerol sulfate (Ventosa *et al.*, 1999; Montalvo-Rodríguez *et al.*, 2000). The opposite is true for the type species of *Natrinema* (McGenity *et al.*, 1998; Xin *et al.*, 2000). Based on a combination of other morphological and chemotaxonomic characters, species of these two genera can thus be distinguished from each other.

Abbreviations: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester; S_2-DGD, mannose-2,6 disulfate 1→2 glucose-glycerol diether.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AB14^T is AY820137.

A thin-layer chromatogram of lipids from strain AB14^T and *Haloterrigena thermotolerans* JCM 11050^T is available as supplementary material in IJSEM Online.

Strain AB14^T was isolated from a soil sample collected from the near-edge floor of Aibi salt lake located in Xin-Jiang, China. The isolate was grown and maintained aerobically at 37 °C in S-G medium (Sehgal & Gibbons, 1960). A pure culture was obtained by repeated restreaking. Phenotypic tests were performed according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). The optimal conditions for growth were determined in S-G medium with 0·85–5·10 M NaCl and 0–1·0 M Mg^{2+}, respectively. The pH range for growth (assayed from pH 5·0 to 9·5 at intervals of 0·5) was determined by adding MES (pH 5·0–6·0), PIPES (pH 6·5–7·0), Tricine (pH 7·5–8·5) and CHES (pH 9·0–9·5) to S-G medium at a concentration of 50 mM. The temperature range for growth of strain AB14^T in S-G medium (pH 7·5) with optimal NaCl and Mg^{2+} concentrations was determined using a TN3F temperature gradient incubator (ADVANTEC). Cell morphology and motility were examined by optical and transmission electron

microscopy (H-600; Hitachi). Gram staining was performed using acetic acid-fixed samples, as described by Dussault (1955).

Anaerobic growth was tested in the presence of nitrate, L-arginine or DMSO (each at 5 g l^{-1}) in filled stoppered tubes. Gelatin hydrolysis was determined as described by Oren *et al.* (2002). The following characteristics were tested according to Xin *et al.* (2000) as described previously (Oren *et al.*, 1997): hydrolysis of starch, casein, Tween 40 and Tween 80; nitrate reduction; production of indole and H_2S; catalase and oxidase activities; and utilization of sugars, alcohols, amino acids and organic acids. *Halorubrum sodomense* JCM 8880^T and *Haloterrigena thermotolerans* JCM 11050^T were used as controls in tests.

Total lipids were extracted by the modified method of Kamekura & Kates (1988). Phospholipids and glycolipids were separated by TLC on silica gel plates (10 × 10 cm) and analysed according to Xin *et al.* (2000). Genomic DNA was prepared by the method of Marmur (1961) and the purity was checked spectrophotometrically. The DNA G+C content was determined by thermal denaturation (T_m) (Marmur & Doty, 1962) using *Escherichia coli* K-12 DNA as calibration standard. The 16S rRNA gene sequence was amplified under conditions described by Feng *et al.* (2005) with the following primers (position given according to *E. coli* 16S rRNA gene): primer 1, 5′-ATTCCGGTTGATCCTGC-3′ (positions 6–22); and primer 2, 5′-AGGAGGTGATCCAGCCGCAG-3′ (positions 1540–1521).

The sequence was compared with closely related sequences of reference organisms from the FASTA network service. Sequence data were aligned with CLUSTAL_W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining method with the MEGA3 program package (Kumar *et al.*, 2004). DNA–DNA hybridizations were performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970), as modified by Huß *et al.* (1983), using a Beckman DU 800 spectrophotometer.

The 16S rRNA gene sequence similarity values between strain AB14^T and the type strains of *Haloterrigena thermotolerans* JCM 11050^T and *Haloterrigena turkmenica* JCM 9101^T were 98·6 and 96·0 %, respectively. Phylogenetic analysis based on 16S rRNA gene sequence comparison showed that strain AB14^T formed a coherent cluster with *Haloterrigena thermotolerans* with a bootstrap resampling value of 99 % (Fig. 1). The polar lipid profile of strain AB14^T, which possesses the $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and S_2-DGD, was consistent with that of *Haloterrigena* species. The contents of PG and S_2-DGD of strain AB14^T, however, were different from those in *Haloterrigena thermotolerans* JCM 11050^T (see the supplementary figure in IJSEM Online). S_2-DGD was present in strain AB14^T in greatest abundance, whereas the amount of PG was lower than that in *Haloterrigena thermotolerans* JCM 11050^T. Strain AB14^T and *Haloterrigena thermotolerans* JCM 11050^T were incubated at 37 °C for 6 days and the polar lipids were extracted under identical conditions. The DNA G+C content of strain AB14^T (66·6 mol%) was notably higher than that of *Haloterrigena thermotolerans* (63·3 mol%) (Montalvo-Rodríguez *et al.*, 2000) and *Haloterrigena turkmenica* (59·2–60·2 mol%) (Ventosa *et al.*, 1999). The DNA–DNA relatedness levels of strain AB14^T to *Haloterrigena thermotolerans* JCM 11050^T and *Haloterrigena turkmenica* JCM 9101^T were 54 ± 2 % and 21 ± 2 %, respectively (mean values of two determinations). Comparison of phenotypic properties (Table 1) also indicated differences between strain AB14^T and *Haloterrigena thermotolerans*. The optimal growth temperature of strain AB14^T is 42–45 °C, which is lower than that of *Haloterrigena thermotolerans* (50 °C). Strain AB14^T could reduce nitrate under anaerobic conditions and some cells deposited under the tube, whereas *Haloterrigena thermotolerans* was strictly aerobic. In addition, strain AB14^T could be distinguished from *Haloterrigena thermotolerans* by its hydrolysis of gelatin and its sensitivity to tetracycline (Table 1); results were observed after 14 days, with weakly hydrolysable or sensitive

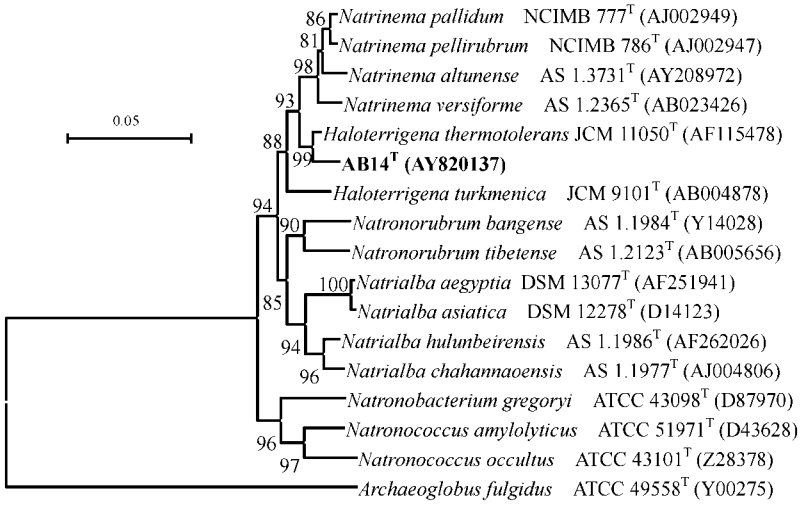

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data showing the phylogenetic positions of strain AB14^T, *Haloterrigena* species and some other related taxa. Bootstrap values (1000 replications) are shown as percentages at each node. Bar, 5 substitutions per 100 nt.

Table 1. Some characteristics that distinguish AB14^T from *Haloterrigena thermotolerans*

−, Negative; W, weak. Strains: 1, AB14^T; 2, *Haloterrigena thermotolerans* JCM 11050^T.

Characteristic	1	2
NaCl range for growth (M)	>1·7	>2·0
Optimum growth temperature (°C)	42–45	50
Anaerobic growth with nitrate	W	−
Hydrolysis of gelatin	−	W
Sensitivity to tetracycline	W	−
DNA G+C content (mol%)	66·6	63·3

being defined as the appearance of a zone of hydrolysis or inhibition of approximately 0·5–1·0 mm.

On the basis of the phylogenetic, genotypic, chemotaxonomic and phenotypic data, it is proposed that strain AB14^T should be classified as the type strain of a novel species within the genus *Haloterrigena*, *Haloterrigena saccharevitans* sp. nov.

Description of *Haloterrigena saccharevitans* sp. nov.

Haloterrigena saccharevitans (sac.char.e.vi′tans. L. neut. n. *saccharon*, *-i* a kind of sugar; L. part. adj. *evitans* shunning, avoiding; N.L. part. adj. *saccharevitans* sugar-avoiding, because it uses very few sugars).

Cells are Gram-negative, motile, rod-shaped (3–10 × 0·4–1·0 μm) and become coccoid in stationary cultures. Colonies on complex agar medium are 0·5–1·0 mm in diameter, smooth, circular, elevated and light red. At least 1·7 M NaCl is required for growth and growth is optimal at 3·0–3·4 M NaCl. Mg^{2+} range for growth is 0–1·0 M, with an optimum around 0–0·2 M. The pH and temperature ranges for growth are 6·5–8·5 (optimum at pH 7·5) and 24–58 °C (optimum at 42–45 °C), respectively. Chemo-organotrophic. Grows anaerobically in the presence of nitrate. Oxidase- and catalase-positive. Indole formation is negative. Nitrate is reduced without production of gas. H_2S is produced from thiosulfate. Tweens 40 and 80 are hydrolysed. Gelatin, starch and casein are not hydrolysed. The following substrates are utilized for growth: glycerol, arginine, ornithine, acetate, fumarate, malate, propionate, pyruvate, succinate and lactate. Fructose, glucose, mannose, starch, arabinose, lactose, mannitol, rhamnose, sorbitol, maltose, galactose, D-ribose, sucrose, D-xylose, glutamate, lysine, aspartate, glycine, alanine and citrate are not utilized for growth. Acid is only produced from glycerol. Sensitive to tetracycline, but not to ciprofloxacin, streptomycin, norfloxacin, kanamycin, ampicillin or vancomycin. The major polar lipids are the $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of PG, PGP-Me and S_2-DGD. The DNA G+C content of the type strain is 66·6 mol% (T_m).

The type strain, AB14^T (=AS 1.3730^T=JCM 12889^T), was isolated from Aibi salt lake, Xin-Jiang, China.

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