Haloterrigena longa sp. nov. and *Haloterrigena limicola* sp. nov., extremely halophilic archaea isolated from a salt lake

Heng-Lin Cui,^{1,2} Dilbr Tohty,³ Pei-Jin Zhou¹ and Shuang-Jiang Liu¹

Correspondence

Shuang-Jiang Liu
shuangjiang@hotmail.com

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

²Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

³College of Life Sciences, Xin-Jiang Normal University, Urumqi 830053, People's Republic of China

Halophilic archaeal strains ABH32^T and AX-7^T were isolated from Aibi salt lake in Xin-Jiang, China, and were subjected to taxonomic studies. The cells of both strains were rod-shaped, and growth required at least 10 % (w/v) NaCl. The cellular polar lipids were identified as phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and mannose-2,6-disulfate (1→2)-glucose glycerol diether (S₂-DGD). The isolates were different in that strain ABH32^T did not require magnesium and had strong ability to assimilate sugars for growth, whereas strain AX-7^T required magnesium and did not assimilate glucose, sucrose or maltose for growth. Both strain ABH32^T and strain AX-7^T were most closely related to members of the genera *Haloterrigena* and *Natrinema*. On the basis of phenotypic and genotypic characteristics, especially their cellular polar lipids, the two isolates were assigned to the genus *Haloterrigena*. Moreover, the DNA relatedness values for strains ABH32^T and AX-7^T with respect to members of the genus *Haloterrigena* were found to be well below 70 %. Thus, strains ABH32^T and AX-7^T represent two novel species within the genus *Haloterrigena*, for which the names *Haloterrigena longa* sp. nov. (type strain ABH32^T = CGMCC 1.5334^T = JCM 13562^T) and *Haloterrigena limicola* sp. nov. (type strain AX-7^T = CGMCC 1.5333^T = JCM 13563^T) are proposed.

On the basis of phylogenetic analysis and DNA–DNA hybridization data, the genus *Haloterrigena* was established by Ventosa *et al.* (1999) as accommodating a single species, *Haloterrigena turkmenica*. *Haloterrigena thermotolerans* (Montalvo-Rodríguez *et al.*, 2000) and *Haloterrigena saccharevitans* (Xu *et al.*, 2005) were described subsequently. 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) depending on the selection of sequences used. However, there are differences in the polar lipid composition among species of these two genera. All type strains of the species of *Haloterrigena* possess mannose-2,6-disulfate (1→2)-glucose glycerol diether (S₂-DGD), but

lack phosphatidylglycerol sulfate (or contain levels below the limit of detection) (Ventosa *et al.*, 1999; Montalvo-Rodríguez *et al.*, 2000). The opposite is true for the species of *Natrinema* (McGenity *et al.*, 1998; Xin *et al.*, 2000), and two strains of *Htg. turkmenica*, JCM 9743 and strain GSL-11, have been shown to possess glycolipids other than S₂-DGD (Ventosa *et al.*, 1999). Thus, on the basis of a combination of phylogenetic, morphological and chemotaxonomic characteristics, the currently described species (represented by their type strains) of these genera can be distinguished from each other. During our surveys on the biodiversity of the halophilic archaea of Aibi salt lake in the Xin-Jiang region of China (Cui *et al.*, 2006), we obtained two strains that showed the same polar lipid pattern as that of members of the genus *Haloterrigena*. In this paper, we describe the characterization and the taxonomy of the two strains, ABH32^T and strain AX-7^T. The results indicated that these two strains represent two novel species of the genus *Haloterrigena*.

Strain ABH32^T and strain AX-7^T were isolated from sediment of Aibi salt lake. The isolation procedure was

Abbreviation: S₂-DGD, mannose-2,6-disulfate (1→2)-glucose glycerol diether.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ABH32^T and AX-7^T are DQ367242 and DQ367241, respectively.

Thin-layer chromatograms showing the polar lipids of strains ABH32^T and AX-7^T are available as supplementary figures in IJSEM Online.

the same as that described previously (Xin *et al.*, 2000; Feng *et al.*, 2004, 2005; Xu *et al.*, 2005). The characterization of strains ABH32^T and AX-7^T was performed according to the minimal standards proposed for the description of novel taxa of the order *Halobacteriales* (Oren *et al.*, 1997). Morphological observation, growth characteristic tests, miscellaneous biochemical tests, nutrition tests, sensitivity to antimicrobial agents, lipid analysis and nucleic acid characterization were performed as described or cited by Xu *et al.* (2005).

The cells of strains ABH32^T and AX-7^T are rods, Gram-negative and able to grow in a wide range of salinities (1.7–5.1 M NaCl). Colonies on agar medium are red-pigmented. Strain ABH32^T is different from strain AX-7^T in that the former does not require magnesium, is unable to reduce nitrate and is able to assimilate sugars (glucose, sucrose and maltose). Detailed results of morphological analyses, assessments of growth characteristics, nutritional experiments, antibiotic-sensitivity determinations and miscellaneous biochemical tests conducted with strains ABH32^T and AX-7^T are given in the species descriptions. The physiological and biochemical differences among strain

ABH32^T, strain AX-7^T and other members of the genus *Haloterrigena* are listed in Table 1.

Polar lipid analysis indicated that both strain ABH32^T and strain AX-7^T contained C₂₀C₂₀ and C₂₀C₂₅ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and bis-sulfated diglycosyl diether (see the thin-layer chromatograms available as Supplementary Figs S1 and S2 in IJSEM Online). The DNA G+C contents (Marmur & Doty, 1962) of strain ABH32^T and strain AX-7^T were 63.2 and 61.9 mol%, respectively.

The 16S rRNA gene sequence similarity between strain ABH32^T and strain AX-7^T was 94.0%. Phylogenetic analysis based on 16S rRNA gene similarities showed that strains AX-7^T and ABH32^T are closely related to *Htg. thermotolerans* PRS^T (97.0 and 95.4% similarity, respectively), *Htg. saccharevitans* AB14^T (96.2 and 94.7%), *Htg. turkmenica* VKM B-1734^T (96.0 and 93.9%), *Natrinema pallidum* NCIMB 777^T (96.4 and 95.1%), *Natrinema versiforme* XF10^T (96.2 and 95.3%), *Natrinema pellirubrum* NCIMB 786^T (96.0 and 94.7%) and *Natrinema altunense* AJ2^T (96.0 and 94.6%). A phylogenetic tree constructed on the basis of the 16S rRNA gene showed that neither strain ABH32^T nor strain AX-7^T clustered with *Natrinema*; they formed a loose group with other members of the genus *Haloterrigena* (Fig. 1). As strains ABH32^T and AX-7^T contain bis-sulfated diglycosyl diether, a polar lipid characteristic of the genus *Haloterrigena*, we concluded that these strains should be classified within the genus *Haloterrigena*.

Levels of DNA–DNA relatedness was determined as described by De Ley *et al.* (1970) as modified by Huß *et al.* (1983). The DNA–DNA relatedness values for strain ABH32^T and strain AX-7^T with respect to *Htg. turkmenica* JCM 8891^T, *Htg. thermotolerans* CGMCC 1.3709^T and *Htg. saccharevitans* CGMCC 1.3730^T were 43.1, 38.2, 40.3% and 42.5, 45.9 and 43.0%, respectively. The DNA–DNA relatedness between strain ABH32^T and strain AX-7^T was 32.3%.

On the basis of the phenotypic and genotypic data, it is concluded that strain ABH32^T and strain AX-7^T represent two novel species of the genus *Haloterrigena*, for which the names *Haloterrigena longa* sp. nov. and *Haloterrigena limicola* sp. nov., respectively, are proposed.

Description of *Haloterrigena longa* sp. nov.

Haloterrigena longa (lon'ga. L. fem. adj. *longa* long, referring to the production of long rods in liquid medium).

Cells are non-motile, rod-shaped (0.5–0.6 × 2.8–11 µm) and Gram-negative. Colonies on agar plates containing 3.1 M NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 1.7–5.1 M, at Mg²⁺ concentrations of 0–1.0 M, at pH values in the range 6.5–9.0 and at temperatures of 30–56 °C. The optimal NaCl and Mg²⁺ concentrations for growth are 3.1 M and 0.1 M, respectively. Optimal pH and

Table 1. Characteristics that distinguish strains ABH32^T and AX-7^T from other members of the genus *Haloterrigena*

Strains: 1, strain ABH32^T; 2, strain AX-7^T; 3, *Htg. turkmenica* JCM 8891^T (data from Zvyagintseva & Tarasov, 1987; Ventosa *et al.*, 1999); 4, *Htg. thermotolerans* CGMCC 1.3709^T (Montalvo-Rodríguez *et al.*, 2000); 5, *Htg. saccharevitans* CGMCC 1.3730^T (Xu *et al.*, 2005). +, Positive; –, negative; W, weak; ND, no data available.

Characteristic	1	2	3	4	5
Cell shape	Rod	Rod	Coccoid	Rod	Rod
Motility	–	+	–	–	+
Mg ²⁺ requirement	–	+	ND	–	–
H ₂ S formation	+	+	–	ND	+
Nitrate reduction	–	+	+	+	+
Indole formation	+	–	–	–	–
Hydrolysis of:					
Tween 20	+	+	ND	ND	ND
Tween 40	+	–	ND	ND	+
Tween 60	+	–	ND	ND	ND
Tween 80	–	–	ND	+	+
Gelatin liquefaction	–	–	–	W	–
Utilization of:					
Glucose	+	–	+	–	–
Fructose	–	–	+	–	–
Mannose	–	–	+	–	–
D-Ribose	–	–	+	–	–
Sucrose	+	–	+	–	–
Maltose	+	–	ND	–	–
DNA G+C content (mol%)	63.2	61.9	59.8	63.3	66.6

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the members of the genus *Haloterrigena* and surrounding genera within the family *Halobacteriaceae*. The tree was constructed using MEGA, version 3.1 (Kumar *et al.*, 2004). Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 70% bootstrap support. Bar, 0.02 expected changes per site.

temperature values are 7.0–7.5 and 41–45 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is not observed. H₂S is produced from Na₂S₂O₃. Positive for indole formation. Tweens 20, 40 and 60 are hydrolysed but Tween 80 is not. Negative for caseinase, amylase and gelatinase. The following substrates are utilized as carbon sources: glucose, sucrose, maltose, pyruvate and acetate. Fructose, sorbose, xylose, galactose, mannose, D-ribose, lactose, mannitol and sorbitol are not utilized as carbon sources. Acid is produced in unbuffered medium from glucose and sucrose. Sensitive to rifampicin (5 µg per disc) and novobiocin (30 µg per disc). Resistant to the following antibiotics (µg per disc, unless otherwise indicated): erythromycin (15), neomycin (30), chloramphenicol (30), ampicillin (10), norfloxacin (10), ciprofloxacin (5), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), bacitracin (0.04 IU per disc) and penicillin G (10 IU per disc). The major polar lipids are the C₂₀C₂₀ and C₂₀C₂₅ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and S₂-DGD. The DNA G+C content of the type strain is 63.2 mol% (T_m).

The type strain, strain ABH32^T (= CGMCC 1.5334^T = JCM 13562^T), was isolated from Aibi salt lake in Xin-Jiang, China.

Description of *Haloterrigena limicola* sp. nov.

Haloterrigena limicola (li.mi'co.la. L. n. *limus* mud; L. suff. -cola from L. n. *icola* dweller; N.L. masc. or fem. n. *limicola* mud-dweller).

Cells are motile, rod-shaped (0.6–0.8 × 1.8–3.6 µm) and Gram-negative. Colonies on agar plates containing 2.6 M NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 1.7–5.1 M, at Mg²⁺ concentrations of 0.005–0.7 M, at pH values in the range 6.5–9.0 and at temperatures of 30–61 °C.

Optimal NaCl concentration, Mg²⁺ concentration, pH and temperature for growth are 3.1 M, 0.05 M, pH 7.0 and 45–50 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is observed. H₂S is produced from Na₂S₂O₃. Negative for indole formation. Tween 20 is hydrolysed, but Tweens 40, 60 and 80 are not. Negative for caseinase, amylase and gelatinase. Glucose, sucrose, maltose, fructose, sorbose, xylose, galactose, mannose, D-ribose, lactose, mannitol and sorbitol are not utilized as carbon sources. Pyruvate and acetate are utilized for growth. Sensitive to rifampicin (5 µg per disc) and novobiocin (30 µg per disc). Resistant to the following antibiotics (µg per disc, unless otherwise indicated): erythromycin (15), neomycin (30), chloramphenicol (30), ampicillin (10), norfloxacin (10), ciprofloxacin (5), streptomycin (10), kanamycin (30), tetracycline (30), vancomycin (30), bacitracin (0.04 IU per disc) and penicillin G (10 IU per disc). The major polar lipids are the C₂₀C₂₀ and C₂₀C₂₅ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and S₂-DGD. The DNA G+C content of the type strain is 61.9 mol% (T_m).

The type strain, strain AX-7^T (= CGMCC 1.5333^T = JCM 13563^T), was isolated from Aibi salt lake in Xin-Jiang, China.

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