***Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China**

Huawei Xin,^{1,2} Takashi Itoh,² Peijin Zhou,¹ Ken-ichiro Suzuki,² Masahiro Kamekura³ and Takashi Nakase²

Author for correspondence: Takashi Itoh. Tel: +81 48 467 9560. Fax: +81 48 462 4617.
e-mail: ito@jcm.riken.go.jp

¹ The Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100080, PR China

² Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-0198, Japan

³ Noda Institute for Scientific Research, 399 Noda, Noda-shi, Chiba 278-0037, Japan

A novel extremely halophilic archaeon, strain XF10^T, was isolated from a salt lake in China. This organism was neutrophilic, non-motile and pleomorphic, and was rod, coccus or irregularly shaped. It required at least 1.5 M NaCl for growth and grew in a wide range of MgCl₂ concentrations (0.005–0.5 M). Lipid extract of whole cells contained two glycolipids with the same chromatographic properties as two unidentified glycolipids found in the two described *Natrinema* species, *Natrinema pellirubrum* and *Natrinema pallidum*. Phylogenetic analysis based on 16S rDNA sequence comparison revealed that strain XF10^T clustered with the two described *Natrinema* species and several other strains (strains T5.7, GSL-11 and *Haloterrigena turkmenica* JCM 9743) with more than 98.1% sequence similarities, suggesting that strain XF10^T belongs to the genus *Natrinema*. Comparative analysis of phenotypic properties and DNA–DNA hybridization between strain XF10^T and the *Natrinema* species supported the conclusion that strain XF10^T is a novel species within the genus *Natrinema*. The name *Natrinema versiforme* sp. nov. is proposed for this strain. The type strain is XF10^T (= JCM 10478^T = AS 1.2365^T = ANMR 0149^T).

Keywords: *Natrinema versiforme* sp. nov., halobacteria, archaea

INTRODUCTION

Halobacteria (the family *Halobacteriaceae*; Grant & Larsen, 1989) are a diverse group of extremely halophilic archaea that require at least 1.5 M NaCl for growth. In the last few years, the numbers of halobacterial genera and species have increased rapidly. Several new genera, e.g. *Halogeometricum* (Montalvo-Rodríguez *et al.*, 1998) and *Natronorubrum* (Xu *et al.*, 1999), have been created to accommodate newly isolated strains, and the others have been proposed by re-evaluation of misidentified or insufficiently described strains, e.g. *Natrinema* (McGenity *et al.*, 1998) and *Haloterrigena* (Ventosa *et al.*, 1999).

Abbreviations: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate-methyl ester; PGS, phosphatidylglycerosulfate; S₂-DGD-1, 2,6-HSO₃-Manp- α (1 $\rightarrow$ 2)-Glc- α (1 $\rightarrow$ 1)-sn-glyceroldiether; TMAO, trimethylamine N-oxide.

The DDBJ accession number for the 16S rDNA sequence of *Natrinema versiforme* XF10^T is AB023426.

Hypersaline environments are commonly found in China. In addition to many coastal salterns, a number of salt lakes, soda lakes and salt-rich deserts are located in the west to northwest part of China, i.e. from Inner Mongolia, Qinghai Province, to Xinjiang and Tibet Autonomous Regions. From these saline environments, a number of halobacteria have been isolated, including strains A5 and B2, which have been shown to be related to the genus *Haloarcula* based on polar lipid composition and 16S rDNA sequence (Zhou *et al.*, 1994; Xu *et al.*, 1995), strain HAM-2, which has been shown to be a close relative of *Natrialba magadii* on the basis of 16S rDNA sequence (Tian *et al.*, 1997; Xu *et al.*, 1999), two species of the genus *Natronorubrum* (*Natronorubrum bangense* and *Natronorubrum tibetense*) (Xu *et al.*, 1999), and some incompletely characterized strains (Wang *et al.*, 1984; Zhou *et al.*, 1990). Isolation of novel strains of the genera *Haloarcula*, *Natrialba* and *Natrinema* (H. Xin and others, unpublished work) has also exemplified the wide diversity of halobacteria in the hypersaline habitats in China.

Fig. 1. Phase-contrast micrograph of strain XF10^T. Cells were pleomorphic and rod, coccus or irregularly shaped. Bar, 10 µm.

In this paper, a novel halobacterial isolate, XF10^T, which was isolated from a salt lake in the northwest part of China, is described.

METHODS

Isolation procedure, strains and growth conditions. Strain XF10^T was isolated from clay collected from the shallow near-edge floor of Aibi salt lake in Xinjiang Autonomous Region, China. After enrichment of the sample in Sehgal and Gibbons medium (Sehgal & Gibbons, 1960) at 37 °C with shaking for 1–2 weeks, a pure culture was obtained by plating serial dilutions of enrichment cultures and repeated transfers of separate colonies on agar plates of the same medium. The purity of the strain was checked by colony morphology and randomly amplified polymorphic DNA patterns of total DNAs derived from different colonies on the agar plate. The growth medium used for the following studies contained (l⁻¹): 5 g Casamino acids (Difco); 5 g yeast extract (Difco); 1 g sodium glutamate; 3 g trisodium citrate dihydrate; 30 g MgCl₂·6H₂O; 5 g K₂SO₄; 36 mg FeCl₂·6H₂O; 0.36 mg MnCl₂·4H₂O; and 220–250 g NaCl (pH 7.0). Agar slants and plates were prepared by adding 20 g agar l⁻¹. The strain was maintained on agar slants for short periods (several months) or stored in growth medium with glycerol (final 20%, v/v) at –80 °C for long-term preservation. *Haloterrigena turkmenica* JCM 9743 (for genus and species designation, see Discussion; deposited in JCM by M. Kamekura in 1995), *Haloterrigena turkmenica* JCM 9101^T (= VKM B-1734^T; transferred from VKM to JCM in 1993), *Natrinema pallidum* JCM 8980^T (= NCIMB 777^T; transferred from NCIMB to JCM in 1993) and *Natrinema pellirubrum* JCM 10476^T (= NCIMB 786^T; transferred from NCIMB to JCM in 1999) were used as reference strains and cultivated in the above growth medium with 20 g MgSO₄·7H₂O l⁻¹ and 2 g KCl l⁻¹ (instead of MgCl₂·6H₂O and K₂SO₄), and 200 g NaCl l⁻¹. If not specified, strains were cultivated at 37 °C with shaking at 180 r.p.m. in 500 ml Erlenmeyer flasks containing 100 ml medium. Inoculated agar plates were wrapped in plastic bags and incubated at 37 °C.

Morphology and growth characteristics of strains. Cells were observed under a phase-contrast light microscope (Optiphot-2; Nikon). Gram staining was performed with acetic-acid-fixed cells as described by Dussault (1955). Cell lysis was observed by diluting dense cell suspensions with the

diluted medium or distilled water. Growth ranges and optima of NaCl and MgCl₂ levels were determined using growth medium containing various concentrations of NaCl (1.2–5.2 M) and MgCl₂ (0.005–0.5 M), respectively. Various buffer systems were employed in the determination of growth pH (50 mM of each): MES (pH 5.5–6.5), PIPES (pH 6.5–7.5), HEPES (pH 7.0–8.0), Tricine (pH 7.5–9.0) and CHES (pH 9.0–10.0). Growth temperature was determined in the range 4–60 °C by using a temperature gradient incubator (model TN-3; ADVANTEC). Growth rate was determined by measuring culture turbidity at 660 nm.

Nutrition. Anaerobic growth in the presence of arginine, DMSO, trimethylamine *N*-oxide (TMAO) and nitrate (5 g l⁻¹ of each) was determined in rubber-stoppered tubes completely filled with the growth medium in the dark for 1–3 weeks and compared to growth on medium without the test compounds. Reduction of nitrate was detected by using the sulfanilic acid and α-naphthylamine reagent (Smibert & Krieg, 1981). Formation of gas from nitrate was detected by using Durham tubes under anaerobic conditions.

To estimate the utilization of various carbohydrates as carbon and energy sources, the basal medium [0.1 g yeast extract l⁻¹ (Difco), 0.5 g NH₄Cl l⁻¹, 0.05 g KH₂PO₄ l⁻¹, at pH 7.0 with 50 mM HEPES] was supplemented with 10 g test carbohydrate l⁻¹. Production of acids from carbohydrates was tested in the basal medium supplemented with 0.5 g test substrate l⁻¹ without buffer. The cultures were incubated at 37 °C without shaking for 2 weeks. Growth was determined visually and pH was measured with a pH electrode.

Biochemical tests. Tests for catalase and oxidase activities, formation of indole, and hydrolysis of starch, gelatin, casein and Tween 80 were performed according to the standard or modified procedures of Oren *et al.* (1997). Formation of sulfide was determined by incubating cells in the growth medium supplemented with elemental sulfur (6.4 g l⁻¹) or sodium thiosulfate (Na₂S₂O₃·5H₂O, 5.0 g l⁻¹) without shaking, and detecting sulfide with a strip of paper impregnated with 10% (w/v) lead acetate solution.

Sensitivity to antimicrobial agents. Sensitivity to antimicrobial agents was determined in the growth medium containing each antimicrobial agents at 50 mg l⁻¹ for at least 2 weeks. Antimicrobial agents used were ampicillin, anisomycin, bacitracin, chloramphenicol, erythromycin, neomycin, novobiocin, penicillin G and rifampicin.

Lipid analyses. Total lipids were extracted by the modified method of Kamekura (1993) and separated by TLC on Merck Kieselgel 60-HPTLC. For one-dimensional development, the solvent was chloroform/methanol/acetic acid/water (85:22.5:10:4, v/v). For two-dimensional development, the solvents were chloroform/methanol/water (65:25:4, v/v) followed by chloroform/methanol/acetic acid/water (80:12:15:4, v/v). Phospholipids were detected with the Zinzadze reagent of Dittmer & Lester (1964). Glycolipids were detected by spraying the plate with 0.5% 1-naphthol in methanol/water (1:1) and then with sulfuric acid/ethanol (1:1) followed by heating at 120 °C for 5–10 min; all the other polar lipids were detected by further heating of the plate at about 250 °C for several minutes (Ihara *et al.*, 1997).

Sequencing of 16S rDNA. Total DNAs were extracted by the method of Cline *et al.* (1989). The 16S rRNA genes were amplified by PCR with the following forward and reverse primers: 5'-TCCGGTTGATCCTGCCG (positions 8–24 according to *Escherichia coli* numbering) and 5'-GGAGG-TGATCCAGCCG (positions 1540–1525). The amplified

16S rDNAs were cloned into pT7Blue T-vector (Novagen) and sequenced using the SequiTherm Long-Read Cycle Sequencing kit (Epicentre Technologies) on the ALFred DNA sequencer (Pharmacia Biotech). The sequence obtained was aligned with the other reported halobacterial 16S rDNA sequences by using the CLUSTAL W 1.7 program (Thompson *et al.*, 1994). The phylogenetic tree was reconstructed by the neighbour-joining method (Saitou & Nei, 1987) and was evaluated by bootstrap sampling (Felsenstein, 1985).

G+C content and DNA–DNA hybridization. G+C content was determined by the HPLC method of Tamaoka (1994). DNA–DNA hybridization was assessed by the fluorometric method of Ezaki *et al.* (1989).

RESULTS

Morphology and growth characteristics

Cells of strain XF10^T growing exponentially under optimal condition were non-motile and pleomorphic,

with rods, cocci or irregular shapes (Fig. 1). The pleomorphism was confirmed in media containing various concentrations of NaCl (2.6–5.2 M) or MgCl₂ (0.005–0.5 M). Cell lysis occurred in the diluted medium containing less than 1.0 M NaCl or in distilled water. There were no gas vesicles formed inside cells. Cells stained Gram-negative. Colonies formed on agar plates were light-red, opaque, small and circular, 0.5–1.0 mm in diameter, and elevated. The strain grew at 1.5 M to saturation of NaCl and grew optimally at 3.4–4.3 M (in the presence of 0.15 M MgCl₂). The strain grew in a wide range of MgCl₂ concentrations from 0.005 to 0.5 M and grew optimally around 0.15 M. It was neutrophilic (pH 6.0–8.0) with an optimum pH at 6.5–7.0. Growth occurred in the temperature range 20–53 °C with an optimum at 37–46 °C. Under the optimal growth conditions (3.4–4.3 M NaCl, 0.15 M MgCl₂, at 37 °C and pH 7.0), the doubling time was 11.0 h.

Fig. 2. TLC of polar lipids extracted from strain XF10^T and some halobacteria by using the 1-naphthol/sulfuric acid-ethanol stain. (a) Two-dimensional TLC of polar lipids from strain XF10^T. The plate was heated to 120 °C to show the glycolipids and then to about 250 °C to show all the polar lipid components. First dimension, from left to right; second dimension, from bottom to top. (b) One-dimensional TLC (from bottom to top). The plate was heated to 120 °C to show the glycolipids. Lanes: 1, strain XF10^T; 2, *Natrinema pellirubrum* JCM 10476^T; 3, *Haloterrigena turkmenica* JCM 9101^T; 4, *Haloterrigena turkmenica* JCM 9743; 5, *Natrinema pallidum* JCM 8980^T. GL, unidentified glycolipid; other abbreviations are defined in the text. The designation pairs (1-1 and 1-2; 2-1 and 2-2) refer to the two adjacent glycolipids in each set which may contain the same sugar residues but different diether core lipids (C₂₀C₂₀ or C₂₀C₂₅).

Fig. 3. Phylogenetic tree showing the position of *Natrinema versiforme* strain XF10^T. The tree was constructed by the neighbour-joining method derived from the 16S rRNA/rDNA sequences. The numbers indicate the bootstrap scores of 1000 trials; values greater than 70% are shown. Bar, 1% nucleotide substitution. Accession numbers of nucleotide sequences cited are as follows: *Halobacterium salinarum* R1, M38280; *Haloterrigena turkmenica* JCM 9743, D14125; *Haloterrigena turkmenica* VKM B-1734^T, AB004878; *Natrialba asiatica* JCM 9576^T, D14123; *Natrialba asiatica* JCM 9577, D14124; *Natrialba magadii* NCIMB 2190^T, X72495; *Natrinema pallidum* NCIMB 777^T, AJ002949; *Natrinema pallidum* NCIMB 784, AJ002948; *Natrinema pellirubrum* NCIMB 786^T, AJ002947; *Natronobacterium gregoryi* NCIMB 2189^T, D87970; *Natronobacterium bangense* A33^T, Y14028; *Natronobacterium tibetense* GA33^T, AB005656; *Natronococcus amylolyticus* JCM 9655^T, D43628; *Natronococcus occultus* NCIMB 2192^T, Z28378; strain C112, AJ004806; strain GSL-11, D14126; strain HAM-2, AF009601; strain SR1.5, AJ002945; strain T5.7, AJ002946; and strain Y21, AJ001376.

Nutrition, biochemical tests and sensitivity to antimicrobial agents

Strain XF10^T showed anaerobic growth in the presence of nitrate but not arginine, DMSO or TMAO. Nitrate was reduced and gas formation was observed. Sulfide was formed from sulfur or thiosulfate. It exhibited positive catalase and oxidase reactions. Indole was formed. There was no hydrolysis of gelatin or casein, although weak hydrolysis of Tween 80 occurred. Hydrolysis of starch was doubtful. The strain utilized several carbohydrates. Among them, strong acid formation was observed from fructose, glucose and glycerol, and weak acid formation was observed from galactose, maltose and D-xylose; no acid was formed from mannose, D-ribose, starch or sucrose. The strain did not utilize arabinose, lactose, mannitol, raffinose, rhamnose or sorbitol. The strain was sensitive to anisomycin, bacitracin, novobiocin and rifampicin, while being insensitive to ampicillin, chloramphenicol, erythromycin, neomycin and penicillin G.

Lipid composition

Two-dimensional TLC revealed that strain XF10^T possessed the glycerol diether analogues of phosphatidylglycerol (PG), phosphatidylglycerophosphate-methyl ester (PGP-Me), phosphatidylglycerosulfate (PGS) and some glycolipids (Fig. 2a). The core lipids were C₂₀C₂₀ and C₂₀C₂₅ diethers, as shown from the two PG or PGP spots. Strain XF10^T showed two glycolipid spots, tentatively designated GL 1-1 and 1-2, which might correspond to molecules containing C₂₀C₂₀ and C₂₀C₂₅ diether moieties. These two glyco-

lipid spots were also found in varying amounts in the two described *Natrinema* species, *Natrinema pellirubrum* JCM 10476^T and *Natrinema pallidum* JCM 8980^T, as well as *Haloterrigena turkmenica* JCM 9743 (Fig. 2b, lanes 2, 5 and 4, respectively). Like *Natrinema pellirubrum* JCM 10476^T, strain XF10^T did not contain two glycolipids (tentatively designated GL 2-1 and 2-2) found in *Natrinema pallidum* JCM 8980^T and *Haloterrigena turkmenica* JCM 9743 (Fig. 2b, lanes 5 and 4), whereas it lacked two glycolipids found in *Natrinema pellirubrum* JCM 10476^T which appeared below GL 1-1 and 1-2 (Fig. 2b, lane 2). On the other hand, the type strain of *Haloterrigena turkmenica*, JCM 9101^T, contained S₂-DGD-1 [2,6-HSO₃-Manp-α(1 → 2)-Glc-α(1 → 1)-sn-glyceroldiether] as a major glycolipid (Fig. 2b, lane 3), which was not detected in strain XF10^T.

Sequencing of 16S rRNA gene, alignment and reconstruction of phylogenetic tree

Sequences of several clones containing PCR-amplified 16S rDNA were determined. There were few heterogeneities among the sequences. The sequence determined was 1438 bp. Phylogenetic analysis of this 16S rDNA sequence with those of relevant halobacteria available from the database was conducted by comparing 1367 bases of each sequence, excluding gaps and uncertain bases. On the phylogenetic tree, strain XF10^T, the two described *Natrinema* species, strains T5.7, GSL-11 (formerly L-11: Kamekura & Dyall-Smith, 1995) and *Haloterrigena turkmenica* JCM 9743 formed a tight cluster (Fig. 3). This cluster was supported by a high bootstrap value (100%). Strain

Table 1. Levels of DNA–DNA relatedness between strain XF10^T and related strains

Strain	Relatedness (%) with biotinylated DNA from:				
	Strain XF10 ^T	JCM 10476 ^T	JCM 8980 ^T	JCM 9743	JCM 9101 ^T
Strain XF10 ^T	100	17	19	11	6
<i>Natrinema pellirubrum</i> JCM 10476 ^T	26	100	50	26	18
<i>Natrinema pallidum</i> JCM 8980 ^T	29	48	100	114	17
<i>Haloterrigena turkmenica</i> JCM 9743	22	40	93	100	11
<i>Haloterrigena turkmenica</i> JCM 9101 ^T	5	6	7	5	100

XF10^T had 98.5% and 98.8% sequence similarities to the two described *Natrinema* species, *Natrinema pellirubrum* NCIMB 786^T and *Natrinema pallidum* NCIMB 777^T (also *Natrinema pallidum* NCIMB 784), respectively, 99.0% to strain T5.7, and 98.1–98.4% to the *Haloterrigena turkmenica* JCM 9743/strain GSL-11 group. The closest relatives to this cluster were strain SR1.5 and *Haloterrigena turkmenica* VKM B-1734^T (=JCM 9101^T), which had 97.1–98.0% and 96.2–97.2% sequence similarities, respectively, to the cluster members (97.3% and 96.6%, respectively, to strain XF10^T). Sequence similarities between strain XF10^T and the rest of halophilic archaea compared were 90.5–95.7%.

G + C content and DNA–DNA hybridization

The G + C content of total DNA of strain XF10^T was 64.2 mol%, which is similar to that of *Natrinema pellirubrum* JCM 10476^T, *Natrinema pallidum* JCM 8980^T and *Haloterrigena turkmenica* JCM 9743 (62.9, 63.9 and 64.4 mol%, respectively). The DNA–DNA hybridization results are shown in Table 1. Low levels of DNA–DNA hybridization were observed between strain XF10^T and the two described *Natrinema* species (17–29%), and *Haloterrigena turkmenica* JCM 9743 (11–22%), as well as *Haloterrigena turkmenica* JCM 9101^T (5–6%). The DNA–DNA hybridization values between *Natrinema pellirubrum* JCM 10476^T and *Natrinema pallidum* JCM 8980^T were almost the same as that reported by Ross & Grant (1985) (50%).

DISCUSSION

Strain XF10^T was isolated from a neutral salt lake in the northwest part of China. It was isolated along with many other halobacterial strains (and some eubacteria) after enrichment in complex medium containing 20% NaCl.

On the phylogenetic tree based on 16S rDNA sequences, strain XF10^T clustered with the two described *Natrinema* species as well as strains T5.7, GSL-11 and *Haloterrigena turkmenica* JCM 9743 ($\geq 98.1\%$ sequence similarities). This cluster is still phylogenetically coherent when compared with several halobacterial genera such as *Halorubrum* ($\geq 92.8\%$

sequence similarity), *Natrialba* ($\geq 93.3\%$) and *Natronorubrum* (95.2%) (McGenity & Grant, 1995; Kamekura *et al.*, 1997; Xu *et al.*, 1999). Therefore, all members of this cluster, including strain XF10^T, should belong to the genus *Natrinema*, which was proposed validly in 1998 (McGenity *et al.*, 1998). Although *Haloterrigena turkmenica* JCM 9743 should be renamed, this strain is still in taxonomic confusion as discussed below. The presence of the two unidentified glycolipid spots shared by the two described *Natrinema* species would support assignment of strain XF10^T to the genus *Natrinema*. Strain XF10^T was also similar to the two described *Natrinema* species in possessing C₂₀C₂₅ core lipids (McGenity *et al.*, 1998; Kamekura & Dyall-Smith, 1995). Although strain XF10^T (and the *Natrinema* species) showed slightly high 16S rDNA sequence similarity to *Haloterrigena turkmenica* VKM B-1734^T (96.6%), the latter had a significant amount of S₂-DGD-1 as a characteristic glycolipid (Ventosa *et al.*, 1999), which suggested that strain XF10^T should not belong to the genus *Haloterrigena*.

In terms of phenotypic properties, the difference between strain XF10^T and the two described *Natrinema* species (McGenity *et al.*, 1998) is clear despite the close phylogenetic relationships. Strain XF10^T does not exhibit the typical rod shape of the two described *Natrinema* species and it differs from them in several biochemical and physiological properties as shown in Table 2. Moreover, strain XF10^T shows some differences in polar lipid composition from both the two described *Natrinema* species. Low DNA–DNA relatedness between strain XF10^T and the two described *Natrinema* species clearly indicated that strain XF10^T is independent from them. Likewise, differences in polar lipid composition (Fig. 2b, lane 4) and low DNA–DNA relatedness between strain XF10^T and *Haloterrigena turkmenica* JCM 9743 support the proposal that strain XF10^T is also separate from *Haloterrigena turkmenica* JCM 9743. According to the properties described above, strain XF10^T should be a new species of the genus *Natrinema*, and the name *Natrinema versiforme* sp. nov. is proposed. The type strain is strain XF10^T, which has been deposited in the Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Saitama,

Table 2. Major differential properties between strain XF10^T and *Natrinema* species

Property	Strain XF10 ^T	<i>Natrinema pellirubrum</i> *	<i>Natrinema pallidum</i> *
Cell morphology	Pleomorphic	Rod	Rod
Anaerobic growth with nitrate	+	—	—
Gas formation from nitrate	+	—	—
Indole formation	+	—	—
Hydrolysis of gelatin	—	+	+
Utilization of:			
Lactose	—	+	+
Ribose	+	+	—
Sensitivity to rifampicin	+	—	Slight

* From McGenity *et al.* (1998).

Japan as JCM 10478^T, and in the Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, PR China as AS 1.2365^T. It is also registered as ANMR 0149^T as the strain belongs to the 'Asian Network on Microbial Research' project.

Recently, it was shown that *Haloterrigena turkmenica* JCM 9743 (formerly *Halobacterium trapanicum* JCM 9743), which had been thought to be the descendant of the original type strain of *Halobacterium trapanicum* (NRC 34021^T), was not identical to the original strain (Kamekura, 1998). Ventosa *et al.* (1999) regarded it as a strain of *Haloterrigena turkmenica*, although this needs to be reconsidered. Our DNA–DNA hybridization data revealed that *Haloterrigena turkmenica* JCM 9743 was unrelated to *Haloterrigena turkmenica* JCM 9101^T (an equivalent strain of VKM B-1734^T) (Table 1). Moreover, the two strains differed in their polar lipid composition (Ventosa *et al.*, 1999; also shown in Fig. 2b, lanes 3 and 4), and the 16S rDNA sequence similarity between both the strains was too low (96.6%) for inclusion in the same species. On the basis of the very high DNA–DNA hybridization value (Table 1), it appears that *Haloterrigena turkmenica* JCM 9743 and *Natrinema pallidum* JCM 8980^T are the same species. However, a final conclusion should not be drawn until a thorough phenotypic comparison with *Natrinema pallidum* is accomplished.

Inclusion of strain XF10^T as a new species in the genus *Natrinema* seems to require the amendment of the description of this genus, which is given below.

Amended description of the genus *Natrinema* McGenity, Gemmell and Grant 1998

Cells rod-shaped, 1–5 µm by 0.6–1.0 µm, or pleomorphic. Cells lyse at low NaCl concentration (<1.0 M). Colonies light orange-red or pale orange, 0.5–2.0 mm in diameter, smooth, circular, convex. Gram-negative. Chemo-organotroph. Some species are strict aerobes, whereas others show anaerobic growth with nitrate. Nitrogen source: Casamino acids. Carbon sources: Casamino acids and certain sugars. Requires

at least 1.5–1.7 M NaCl for growth; optimum 3.4–4.3 M NaCl. Optimum pH around 7.0. Possesses C₂₀C₂₀ and C₂₀C₂₅ diether core lipids (Ross *et al.*, 1985; Ross & Grant, 1985). Possesses several unidentified glycolipids (Ross *et al.*, 1985). Sensitive to anisomycin, bacitracin and novobiocin. Type species: *Natrinema pellirubrum*.

Description of *Natrinema versiforme* sp. nov.

Natrinema versiforme (ver.si.for'me. L. neut. adj. *versiforme* of various shapes; the various-shaped *Natrinema*).

Cells non-motile and pleomorphic. Lyse in dilute medium containing less than 1.0 M NaCl or in distilled water. Colonies light red, 0.5–1.0 mm in diameter, smooth, circular and elevated. Requires at least 1.5 M NaCl for growth; optimum 3.4–4.3 M (in the presence of 0.15 M MgCl₂). Growth occurs at 0.005–0.5 M MgCl₂; optimum around 0.15 M. Temperature range for growth 20–53 °C, optimum 37–46 °C. pH range for growth 6.0–8.0; optimum 6.5–7.0. Chemo-organotrophic. Anaerobic growth in the presence of nitrate. Carbon sources: Casamino acids and several sugars, such as fructose, glucose, glycerol (with strong acid formation), galactose, maltose, mannose, D-ribose, sucrose and D-xylose, but not lactose. Reduces nitrate and forms gas. Forms sulfide from sulfur or thio-sulfate. Forms indole. Does not hydrolyse gelatin or casein. Starch hydrolysis doubtful and weak hydrolysis of Tween 80. Possesses C₂₀C₂₀ and C₂₀C₂₅ diether core lipids and some unidentified glycolipids. Possesses phospholipids: PG, PGP-Me and PGS. Sensitive to anisomycin, bacitracin, novobiocin and rifampicin. Insensitive to ampicillin, chloramphenicol, erythromycin, neomycin and penicillin G. Type strain: XF10^T (=JCM 10478^T = AS 1.2365^T = ANMR 0149^T).

ACKNOWLEDGEMENTS

We thank Liu Dali for skilled assistance. This study was partially supported by special coordination funds for promoting science and technology of the Science and Technology Agency of the Japanese Government.

REFERENCES

- Cline, S. W., Schalkwyk, L. C. & Doolittle, W. D. (1989). Transformation of the archaeobacterium *Halobacterium volcanii* with genomic DNA. *J Bacteriol* **171**, 4987–4991.
- Dittmer, J. C. & Lester, R. L. (1964). A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J Lipid Res* **5**, 126–127.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Ezaki, T., Hashimoto, H. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Grant, W. D. & Larsen, H. (1989). Extremely halophilic archaeobacteria, order *Halobacteriales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2216–2233. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Ihara, K., Watanabe, S. & Tamura, T. (1997). *Haloarcula argentinensis* sp. nov. and *Haloarcula mukohataei* sp. nov., two new extremely halophilic archaea collected in Argentina. *Int J Syst Bacteriol* **47**, 73–77.
- Kamekura, M. (1993). Lipids of extreme halophiles. In *The Biology of Halophilic Bacteria*, pp. 135–161. Edited by R. H. Vreeland & L. I. Hochstein. Boca Raton: CRC Press.
- Kamekura, M. (1998). Diversity of extremely halophilic bacteria *Extremophiles* **2**, 289–295.
- Kamekura, M. & Dyal-Smith, M. L. (1995). Taxonomy of the family *Halobacteriaceae* and the description of two genera *Halorubrobacterium* and *Natrialba*. *J Gen Appl Microbiol* **41**, 333–350.
- Kamekura, M., Dyal-Smith, M. L., Upasani, V., Ventosa, A. & Kates, M. (1997). Diversity of alkaliphilic halobacteria: proposals for transfer of *Natronobacterium vacuolatum*, *Natronobacterium magadii*, and *Natronobacterium pharaonis* to *Halorubrum*, *Natrialba*, and *Natronomonas* gen. nov., respectively, as *Halorubrum vacuolatum* comb. nov., *Natrialba magadii* comb. nov., and *Natronomonas pharaonis* comb. nov., respectively. *Int J Syst Bacteriol* **47**, 853–857.
- McGenity, T. J. & Grant, W. D. (1995). Transfer of *Halobacterium saccharovorum*, *Halobacterium sodomense*, *Halobacterium trapanicum* NRC 34021 and *Halobacterium lacusprofundi* to the genus *Halorubrum* gen. nov. as *Halorubrum saccharovorum* comb. nov., *Halorubrum sodomense* comb. nov., *Halorubrum trapanicum* comb. nov., and *Halorubrum lacusprofundi* comb. nov. *Syst Appl Microbiol* **18**, 237–243.
- McGenity, T. J., Gemmell, R. T. & Grant, W. D. (1998). Proposal of a new halobacterial genus *Natrinema* gen. nov., with two species *Natrinema pellirubrum* nom. nov. and *Natrinema pallidum* nom. nov. *Int J Syst Bacteriol* **48**, 1187–1196.
- Montalvo-Rodríguez, R., Vreeland, R. H., Oren, A., Kessel, M., Betancourt, C. & López-Garriga, J. (1998). *Halogeometricum borinquense* gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico. *Int J Syst Bacteriol* **48**, 1305–1312.
- Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.
- Ross, H. N. M. & Grant, W. D. (1985). Nucleic acid studies on halophilic archaeobacteria. *J Gen Microbiol* **131**, 165–173.
- Ross, H. N. M., Grant, W. D. & Harris, J. E. (1985). Lipids in archaeobacterial taxonomy. In *Chemical Methods in Bacterial Systematics*, pp. 289–300. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sehgal, S. N. & Gibbons, N. E. (1960). Effect of metal ions on the growth of *Halobacterium cutirubrum*. *Can J Microbiol* **6**, 165–169.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Tamaoka, J. (1994). Determination of DNA base composition. In *Chemical Methods in Prokaryotic Systematics*, pp. 463–470. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tian, X., Xu, Y., Liu, H. & Zhou, P. (1997). New species of *Natronobacterium*. *Acta Microbiol Sin* **37**, 1–6.
- Ventosa, A., Gutiérrez, M. C., Kamekura, M. & Dyal-Smith, M. L. (1999). Proposal to transfer *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743 and strain GSL-11 to *Halo-terrigena turkmenica* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 131–136.
- Wang, D., Zhou, P., Tian, X. & Ma, G. (1984). Identification of new species of extreme halophilic bacteria. *Acta Microbiol Sin* **24**, 304–309.
- Xu, Y., Liu, H. & Zhou, P. (1995). Nucleotide sequence of the 16S rRNA from an archaea, *Haloarcula aidinensis* strain B2. *Acta Microbiol Sin* **35**, 77–85.
- Xu, Y., Zhou, P. & Tian, X. (1999). Characterization of two novel haloalkaliphilic archaea, *Natronorubrum bangense* gen. nov., sp. nov. and *Natronorubrum tibetense* gen. nov., sp. nov. *Int J Syst Bacteriol* **49**, 261–266.
- Zhou, P., Tian, X., Ma, Y., Xiao, C. & Wang, D. (1990). An extreme haloalkaligenous bacterium. *Acta Microbiol Sin* **30**, 1–6.
- Zhou, P., Xu, Y., Xiao, C., Ma, Y. & Liu, H. (1994). New species of *Haloarcula*. *Acta Microbiol Sin* **34**, 89–95.