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Halorhabdus utahensis gen. nov., sp. nov., an aerobic, extremely halophilic member of the *Archaea* from Great Salt Lake, Utah

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Strain AX-2^T (T = type strain) was isolated from sediment of Great Salt Lake, Utah, USA. Optimal salinity for growth was 27% (w/v) NaCl and only a few carbohydrates supported growth of the strain. Strain AX-2^T did not grow on complex substrates such as yeast extract or peptone. 16S rRNA analysis revealed that strain AX-2^T was a member of the phyletic group defined by the family *Halobacteriaceae*, but there was a low degree of similarity to other members of this family. The polar lipid composition comprising phosphatidyl glycerol, the methylated derivative of diphosphatidyl glycerol, triglycosyl diethers and sulfated triglycosyl diethers, but not phosphatidyl glycerosulfate, was not identical to that of any other aerobic, halophilic species. On the basis of the data presented, it is proposed that strain AX-2^T should be placed in a new taxon, for which the name *Halorhabdus utahensis* is appropriate. The type strain is strain AX-2^T (= DSM 12940^T).

Keywords: *Halorhabdus utahensis*, *Archaea*, extremely halophilic, taxonomy

INTRODUCTION

The increasing interest, in recent years, in microorganisms from hypersaline environments has led to the discovery of several new species and genera belonging to the *Bacteria* and the *Archaea*. Within the family *Halobacteriaceae*, comprising the aerobic, extremely halophilic members of the *Archaea*, there are, at the time of writing, 10 described genera whose names have been validly published: *Haloarcula*, *Halobacterium*, *Halobaculum*, *Halococcus*, *Haloferax*, *Halorubrum*, *Natrialba*, *Natronobacterium*, *Natrococcus* and *Natronomonas*.

Strains of aerobic, extremely halophilic members of the *Archaea* have been isolated from various hypersaline environments such as hypersaline lakes (e.g. Franzmann *et al*., 1988; Oren *et al*., 1995), soda lakes (e.g. Soliman & Trüper, 1982; Tindall *et al*., 1984), solar salterns (e.g. Nuttall & Dyall-Smith, 1993; Juez *et al*., 1986; Ihara *et al*., 1997), saline soils (e.g. Kobayashi *et al*., 1992; Zvyagintseva & Tarasov, 1987) or salt mines (e.g. Denner *et al*., 1994; Norton *et al*., 1993).

Abbreviations: PG, phosphatidyl glycerol; PHB, poly-β-hydroxybutyrate; MePGP, methylated-phosphatidyl glycerophosphate; S-TGD, sulfated triglycosyl glycolipid; TGD, triglycosyl glycolipid.

During a preliminary study of the distribution of halophilic members of the *Bacteria* and the *Archaea* in Great Salt Lake, UT, USA, three extremely halophilic strains were isolated. One strain, designated strain AX-2^T, was the subject of the current taxonomic study, while the other two strains were shown not to be taxonomically related to strain AX-2. Physiologically, strain AX-2^T only used a limited range of substrates for growth and was unique in its inability to utilize yeast extract or peptone for growth. Strain AX-2^T contained MK-8, MK-8(VIII-H_2) and diether-linked lipids, features common to all members of the family *Halobacteriaceae*. The major diether lipids present were diphytanyl derivatives (C_{20},C_{20}). The polar lipids present comprised the corresponding diether derivatives of phosphatidyl glycerol (PG), methylated-phosphatidyl glycerophosphate (MePGP), a triglycosyl glycolipid (TGD) and a sulfated triglycosyl glycolipid (S-TGD). This composition indicates that strain AX-2^T is a member of the family *Halobacteriaceae*, but is chemically distinctive and constitutes a member of a novel taxon within this family. Consequently, we consider that strain AX-2^T, on the basis of the biochemical and physiological properties, the chemical data and the 16S rDNA sequence, constitutes a new species within a new genus, for which we propose the name *Halorhabdus utahensis* gen. nov., sp. nov.

METHODS

Isolation procedure. Strain AX-2^T was isolated from a sediment sample collected from the southern arm of Great Salt Lake, UT, USA. Approximately 2 g of sample was used to inoculate 20 ml enrichment medium (EX-medium) containing the following (g l^{-1}): NaCl, 220; NaBr, 0·1; $MgSO_4 . 7H_2O$, 20; KCl, 5; NH_4Cl, 2; $NaHCO_3$, 0·2; KH_2PO_4, 0·5; yeast extract (Difco), 2; trypticase peptone (BBL), 1; and trace-metal solution (TMS 3), 2 ml (Ingvorsen & Jørgensen, 1984). The pH was adjusted to 7·6 with 5 M NaOH and the medium was autoclaved for 20 min at 121 °C. After sterilization and cooling to 5 °C, 5 ml sterile $CaCl_2$ solution ($CaCl_2 . 6H_2O$, 100 g l^{-1}) and 2 ml sterile $FeCl_2$/$MnCl_2$ solution ($FeCl_2 . 4H_2O$, 20 g l^{-1} + $MnCl_2 . 4H_2O$, 20 g l^{-1}) were added. Sterile solutions of streptomycin, tetracycline and chloramphenicol were also added at final concentrations of 100, 20 and 20 mg l^{-1}, respectively. Glucose was added to EX-medium as a carbon substrate at a final concentration of 2 g l^{-1}. A 0·1 ml aliquot from a serial dilution of the enrichment culture was spread on solid EX-medium (15 g l^{-1} agar) containing 2 g glucose l^{-1}. After 3 weeks incubation at 30 °C, several red-pigmented colonies appeared on the agar plates. Pure cultures were obtained by restreaking several times; one colony was picked to serve as the strain for characterization.

Growth media. The culture medium (27% Tris 10) used for maintenance of strain AX-2 contained the following (g l^{-1}): NaCl, 270; NaBr, 0·1; $MgSO_4 . 7H_2O$, 20; KCl, 5; NH_4Cl, 2; Tris/HCl, 12; yeast extract (Difco), 1; and trace-metal solution (TMS 3), 2 ml. The pH was adjusted to 7·6 with 5 M NaOH and the medium was autoclaved for 20 min at 121 °C. After sterilization and cooling to 5 °C, 2·5 ml sterile KH_2PO_4 solution (50 g l^{-1}), 0·5 ml sterile $CaCl_2 . 6H_2O$ solution (100 g l^{-1}) and 0·25 ml sterile $FeCl_2 . 4H_2O$/$MnCl_2 . 4H_2O$ solution (20 g l^{-1} each) were added. Agar plates were prepared by using Tris 10 medium containing 20% NaCl and 15 g agar l^{-1}. Glucose (2 g l^{-1}) served as the carbon substrate in both liquid and solid medium. Cell material for lipid analysis was grown on GSL-2 medium containing 20% (w/v) NaCl (Wainø *et al.*, 1999) at 37 °C with shaking. Cells were harvested by centrifugation, freeze-dried and stored at −20 °C before analysis.

Culture maintenance and morphological tests. The isolate was grown at 30 °C and routinely transferred to fresh medium or kept on agar plates at 5 °C. Staining of flagella and endospores was performed according to the methods of Leiffson and Schaeffer-Fulton, respectively (Gerhardt *et al.*, 1981). The morphology of colonies was observed on 20% Tris 10 agar plates after 3–6 weeks growth.

Physiological and biochemical tests. Growth in a defined medium containing 20% (w/v) NaCl was tested in Tris 9 medium. This medium was prepared by replacing the yeast extract from the Tris 10 medium with 1 ml vitamin solution (Balch *et al.*, 1979). The effect of NaCl was determined in both Tris 9 (0–30% NaCl) and Tris 10 (6–30% NaCl) medium at 30 °C using 0·2% (w/v) glucose as substrate. The effect of $MgSO_4$ was tested in 27% Tris 10 medium at 30 °C using 0·4% (w/v) glucose as substrate. Growth at various temperatures and pH values was also determined in 27% Tris 10 containing 0·2% (w/v) and 0·1% (w/v) glucose, respectively. Anaerobic growth was tested in both complex (AM) and defined medium (AM 2). AM and AM 2 media were prepared by replacing the $MgSO_4 . 7H_2O$ content of the Tris 10 and Tris 9 media, respectively, with an equimolar amount of $MgCl_2 . 6H_2O$. Glucose, acetate or ethanol was added as a potential substrate, while S°, SO_4^{2-}, $S_2O_3^{2-}$ or NO_3^- was added as a potential electron acceptor. Fermentation of glucose was tested in AM 2 medium without added electron acceptors. Growth on the following substrates was tested at 0·2% and 0·5% (w/v) substrate concentrations in 20% Tris 10 medium: glucose, xylose, fructose, amylose, arabinose, galactose, lactose, maltose, sucrose, Na-acetate, Na-citrate, Na-formiate, Na-glucuronate, Na-lactate, Na-pyruvate, *N*-acetyl-glucosamine, L-alanine, betaine, L-lysine, phenylalanine, L-proline, L-serine, acetamide, ethanol, glycerol, methanol, sorbitol, glycogen, peptone, yeast extract, starch, casein, gelatin and Tween 80. All growth tests were performed at 30 °C, except for temperature tests, and growth was determined by measuring the optical density of the culture at 600 nm.

Gram staining was performed using the method of Dussault (1955). The presence of catalase and of oxidase were tested according to Gerhardt *et al.* (1981). Methyl red and Voges–Proskauer tests were carried out as described by Gerhardt *et al.* (1981) but modified by adding 27% (w/v) NaCl, 2% (w/v) $MgSO_4 . 7H_2O$, 0·5% (w/v) KCl and 0·25% yeast extract to the test medium. Arginine dihydrolase, lysine and ornithine decarboxylase were tested as described by Skerman (1967) but modified by adding 27% (w/v) NaCl, 2% (w/v) $MgCl_2 . 6H_2O$ and 0·5% (w/v) KCl to the test medium. The production of indole, urease and tryptophan deaminase, as well as the utilization of citrate, were tested using an API-20 E test (20100; bioMérieux). Hydrolysis of aesculin and gelatin were tested using an API-20 NE test (20050; bioMérieux). Reduction of NO_3^- to NO_2^- or N_2 and gas production from sugars were tested according to Gerhardt *et al.* (1981). Reduction of NO_3^- was tested using 20% Tris 10 medium containing 0·1 % (w/v) KNO_3 and 0·1% (w/v) glucose, while gas production from sugars was tested using 20% Tris 9 medium containing 0·5% (w/v) glucose. In both cases, test tubes contained inverted Durham vials and the medium was overlaid with mineral oil. The production of hydrogen sulfide was tested as described by Cord-Ruwisch (1985) on aliquots from anaerobic growth tests. The determination of antibiotic susceptibility was performed by spreading bacterial suspensions on solid EX-medium containing 2 g l^{-1} glucose and applying Neo-Sensitabs (DK; Rosco Diagnostica). Inhibition zones were measured after 18 d growth and interpreted according to the manufacturer's manual. The production of poly-β-hydroxybutyrate (PHB) was examined by growing bacteria in a C-rich, N- and P-limited medium as recommended by Fernandez-Castillo *et al.* (1986) and Lillo & Rodriguez-Valera (1990). The PHB-medium was prepared by omitting KH_2PO_4 from the 20% Tris 10 medium and reducing the NH_4Cl content to 0·05 g l^{-1}. Furthermore, 1% (w/v) glucose was added as substrate. The presence of PHB was tested for according to Gerhardt *et al.* (1981).

Electron microscopy. Exponentially growing cells were harvested and fixed in cold 2·5% glutaraldehyde (0·1 M cacodylate buffer, pH 7·2). After 1 h fixation, the cells were washed three times in cacodylate buffer and subsequently postfixed for 1 h in 1% osmium tetroxide buffered with veronal acetate buffer (pH 7·2). The pellet was dehydrated with propanol and embedded in TAAB 812 resin (TAAB Laboratories equipment). Thin sections were prepared with an Ultramicrotome (LKB Instruments) and poststained with uranyl acetate and lead citrate. Finally, the sections were examined in a Phillips CM-20 electron microscope operated at 120 kV.

Detection of diether lipids. Diether lipids were released from

100 mg freeze-dried cells using comparatively mild hydrolytic methods that did not lead to significant cleavage of hydroxylated isoprenoid ether lipids (B. J. Tindall, unpublished results; Eikel & Sprott, 1992). The ether lipids were analysed using silica-gel TLC (art. no. 818135; Macherey-Nagel) with either double development in hexane/*tert*-butylmethylether (4:1, v/v) (detection of diether lipids) or in hexane/*tert*-butylmethylether/acetic acid (25:25:1, by vol.) (detection of diether and tetraether lipids). Lipid material was visualized using dodecamolybdophosphoric acid.

Extraction of respiratory lipoquinones and polar lipids. Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cell material using the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol/hexane (Tindall, 1990a, b) and the polar lipids were extracted by adjusting the remaining methanol/0·3 % aqueous NaCl phase (containing the cell debris) to give a choroform/methanol/0·3 % aqueous NaCl mixture (1:2:0·8, by vol.). The extraction solvent was stirred overnight and the cell debris pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0·3 % aqueous NaCl mixture to a ratio of 1:1:0·9 (by vol.).

Analysis of respiratory lipoquinones. Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (art. no. 805023; Macherey-Nagel), using hexane/*tert*-butylmethylether (9:1, v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out with LDC analytical HPLC equipment (Thermo Separation Products) fitted with a reverse-phase column (2 mm × 125 mm, 3 μm, RP_{18}; Macherey-Nagel), using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

Analysis of polar lipids. Polar lipids were separated by two-dimensional, silica-gel TLC (art. no. 818135; Macherey-Nagel). The first direction was developed in chloroform/methanol/water (65:25:4, by vol.) and the second in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate Schiff (α-glycols), Dragendorff (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids).

DNA base composition and 16S rDNA sequence analysis. DNA base composition and 16S rDNA sequence analysis was carried out at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The G+C content of the DNA was determined by HPLC according to Mesbah *et al*. (1989). Genomic DNA extraction, PCR-mediated amplification and purification of the PCR products were carried out as described previously (Rainey *et al*., 1996). Purified PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the Applied Biosystems 373A DNA sequencer. Sequences were aligned taking secondary structure into consideration. Sequence similarities were calculated using the maximum number of positions possible and covered the region at positions 23–1482 (*Escherichia coli* numbering), which coincided with positions 18–1415 when *Halobacterium halobium* sequence X03407 was used as the reference secondary structure. Sequences were not masked. The 16S rDNA dendrogram was inferred using programs from the PHYLIP package. Similarity values were used to calculate distances using DNADIST; the programs FITCH and DNAML were used to infer the 16S rDNA dendrogram. No attempt was made to test the significance of the branching order (based on the sequence data alone) of all taxa present, because of the presence of long pendant edges and several short internal edges in the dendrogram. Under such conditions it is not always possible to resolve the branching order unambiguously.

Sequences used for the analysis were as follows: strain AX-2^T, AF071880; *Halobacterium cutirubrum*, K02971; *Halococcus morrhuae* (ATCC 17082^T) X72588; *Halococcus salifodinae* (B1p^T), Z28387; *Haloarcula vallismortis* (IFO 14741^T), D50851; *Haloarcula mukohataei* (arg-2^T = JCM 9738^T), D50850; *Natronomonas pharaonis* (JCM 8858^T), D87971; *Halorubrum saccharovorum* (JCM 8865^T), U17364; *Halorubrum vacuolatum* (JCM 9060^T), D87972; *Natronobacterium gregoryi* (NCIMB 2189^T), D87970; *Natronococcus amylolyticus* (Ah-36^T), D43628; *Natronococcus occultus* (NCIMB 2192^T), Z28378; *Natrialba magadii* (NCIMB 2190^T), X72495; *Natrialba asiatica* (172P1^T), D14123; and *Haloferax volcanii*, K00421.

RESULTS

Morphology

The cells exhibited anything from irregular coccoid or ellipsoid to apparently triangular, club-shaped or rod-shaped forms (Fig. 1). Hence, they were extremely pleomorphic. However, the proportion of rod-shaped cells was highest in younger cultures. Rod-shaped cells were 2–10 × 0·5–1 μm in size, ellipsoid cells were 1–2 × 1 μm and spherical cells had a diameter of approximately 1 μm. The organisms were motile and had a single flagellum 3–5 μm long. The cells stained Gram-negative and endospores were not produced. Red colonies were formed on agar plates. The colonies were circular, with diameters ranging from 0·5 to 3·5 mm, depending on age. They were convex with an entire margin and a shiny surface.

Physiological and biochemical characterization

Strain AX-2^T was capable of growing over a wide range of salinities in both complex (Tris 10) and defined (Tris 9) medium. Growth was possible at NaCl

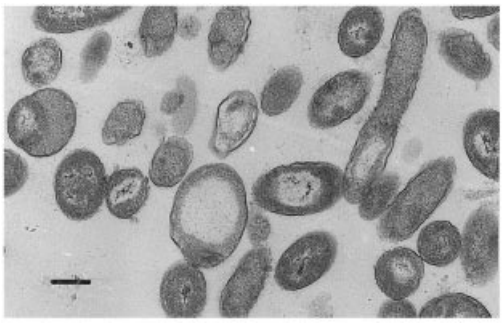

Fig. 1. Transmission electron micrograph showing pleomorphic cells of strain AX-2^T. Bar, 1 μm.

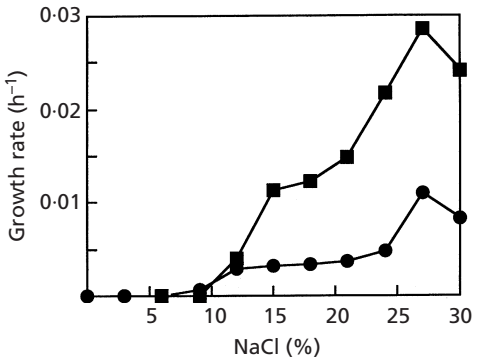

Fig. 2. Growth of strain AX-2^T at different NaCl concentrations. The organisms were grown at 30 °C in either complex medium (Tris 10, ■) or defined medium (Tris 9, ●) containing 0·2 % (w/v) glucose.

concentrations ranging from 9 % in defined medium and 12 % in complex medium to the point of saturation (approx. 30 % NaCl) at 30 °C (Fig. 2). In both media, optimal growth was at 27 % NaCl. However, because of evaporation the salinity of the cultures increased 1–2 % during growth. When grown at 30 °C and in complex medium containing 27 % NaCl, the $MgSO_4$ range supporting growth was 0·05–20 % (2–800 mM). There was no growth at 25 % $MgSO_4$. The $MgSO_4$ content of the medium had only minor effects on the growth rates of the cultures, but the lag phase was extended for several weeks at high $MgSO_4$ concentrations. In the presence of 27 % NaCl, the organisms grew at temperatures between 17 and 55 °C, with optimal growth occurring at 50 °C. The strain could grow over a pH range of 5·5–8·5 at 30 °C in presence of 27 % NaCl; the optimal pH for growth was between 6·7 and 7·1.

The isolate was tested for growth on various carbon sources in both complex and defined medium. Only a very few sugars, such as glucose, xylose and fructose, supported growth. None of the tested organic acids, amino acids, alcohols, glycogen, peptone, yeast extract or starch stimulated growth. Acid was produced when the strain was grown on sugars. The strain grew in defined anaerobic medium (AM-2) by fermenting glucose. However, in the presence of sulfur, growth was stimulated, with concomitant production of hydrogen sulfide, perhaps in a facilitated fermentation manner like that suggested by Widdel & Pfennig (1992). No clear evidence of growth was found when either acetate or ethanol was used as a non-fermentable substrate in combination with S^o, SO_4 or S_2O_3 as the potential electron acceptor. Nitrate, however, was unambiguously reduced to nitrite in anaerobic incubations with glucose, but was not observed to stimulate growth.

The isolate was catalase- and oxidase-positive, lysed in distilled water and did not require amino acids for growth. The strain was susceptible to bacitracin and novobiocin but resistant to ampicillin, carbenicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, penicillin, polymyxins, rifampicin, streptomycin and tetracycline. Several aerobic, extremely halophilic members of the *Archaea* have been shown to produce high amounts of PHB when grown under C-rich and N-limited conditions (Fernandez-Castillo *et al.*, 1986). Therefore, strain AX-2^T was tested and found positive for PHB production when cultured in PHB medium. The organism was tested for the production of various enzymes. Arginine dihydrolase was negative. In the absence of this enzyme, the mechanism supporting anaerobic growth on arginine, as described by Hartmann *et al.* (1980), cannot operate. An API ZYM test (25200; bioMérieux) showed that the strain was positive for esterase, lipase and glucosidase. (See Table 1 for details of additional biochemical characteristics.)

Table 1. Biochemical and physiological features of strain AX-2^T

All tests were performed at salinities of at least 20 % NaCl. +, Positive; −, negative; PHB, poly-β-hydroxybutyrate.

Characteristic	AX-2^T
Gram reaction	−
Fermentative growth	+
Amino acid requirement	−
Mg^{2+} requirement (mM)	2
Temp. range for growth (°C)	17–55 (optimum 50)
pH range for growth	5·5–8·5 (optimum 6·7–7·1)
NaCl range for growth (%)	9–30 (optimum 27)
$MgSO_4$ range for growth (%)	0·05–20
Lysis in water	+
Catalase	+
Oxidase	+
Urease	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Tryptophan deaminase	−
Utilization of citrate (Simmons' citrate)	−
Hydrolysis of:	
Aesculin	+
Gelatin	−
Starch	−
Carbohydrates	+
Methyl red	−
Voges–Proskauer	−
Acid production from carbohydrates	+
Gas production from carbohydrates	−
PHB production	+
Indole production	−
NO_3^- reduction	+
H_2S production	+

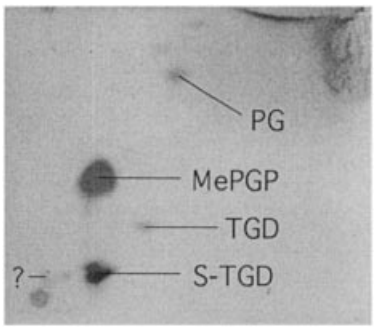

Fig. 3. Polar lipid composition of *Halorhabdus utahensis* separated by two-dimensional TLC (for conditions, see Methods). PG, phosphatidyl glycerol; MePGP, methylated-phosphatidyl glycerophosphate; TGD, triglycosyl glycolipid; S-TGD, sulfated triglycosyl glycolipid. Direction of development: first dimension, horizontal; second dimension, vertical. The origin is in the bottom left-hand corner.

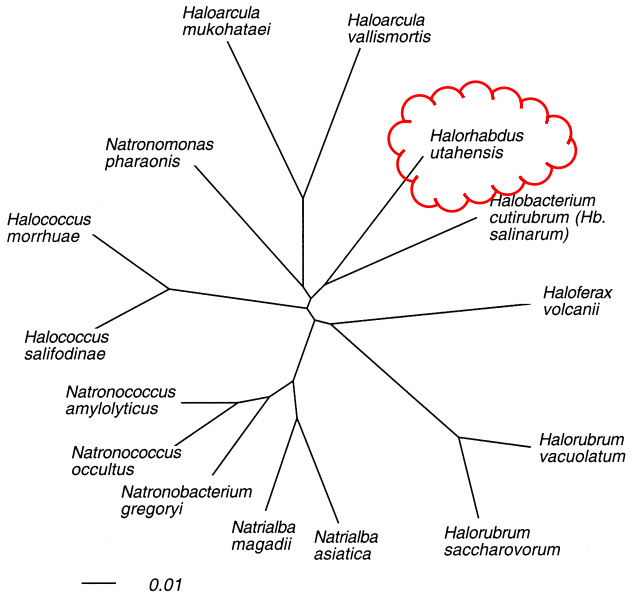

Fig. 4. Unrooted phylogenetic tree showing the relationship between strain AX-2^T and various members of the family *Halobacteriaceae*. Bar, 0·01 changes per nucleotide.

Ether lipids, quinones and polar lipids

Examination of the lipid fraction after hydrolysis of intact cells indicated that isoprenoid ether lipids were present, a feature unique to members of the *Archaea*. The ether lipids comprised only diether lipids, tetraether lipids being absent. The major diether lipids present were the diphytanyl derivatives (C_{20},C_{20}). Menaquinones were the only respiratory lipoquinones present and comprised MK-8 and MK-8(VIII-H_2). As shown in Fig. 3, the major polar lipids present were PG, MePGP, a TGD and an S-TGD. Furthermore, small amounts of an unknown polar lipid were produced. Nevertheless, the presence of diether lipids, MK-8 and MK-8(VIII-H_2) coupled with the presence of PG and Me-PGP are characteristic features of the monophyletic taxon defined by the family *Halobacteriaceae*.

DNA base composition and phylogeny

The G+C content of the DNA of strain AX-2^T was 64 mol %. Approximately 95 % (1421 bases) of the 16S rRNA sequence was determined. Comparison of the sequence with members of the family *Halobacteriaceae* revealed that strain AX-2^T was distantly related to the other genera investigated. Apparently, *Halobacterium cutirubrum* (*Halobacterium salinarum*) is the closest relative of strain AX-2^T, having a sequence similarity value of 90·5 %. However, the similarity values were almost identical for several aerobic, extremely halophilic members of the *Archaea* representing various genera, e.g. *Natronomonas pharaonis* (89·7 %), *Natronobacterium gregoryi* (88·8 %) and *Natrialba asiatica* (88·3 %). Thus, strain AX-2^T represents a new species within a new genus of the family *Halobacteriaceae*. The unrooted phylogenetic tree in Fig. 4 shows the relationship between strain AX-2^T and the representatives of the family *Halobacteriaceae*.

DISCUSSION

Strain AX-2^T was isolated from sediment of the hypersaline Great Salt Lake, UT, USA. On the basis of its pigmentation, pleomorphic nature, NaCl-dependent growth, antibiotic susceptibility, chemical composition and 16S rDNA sequence, strain AX-2^T was identified as a member of the family *Halobacteriaceae*. Developments within the taxonomy of the family *Halobacteriaceae* have seen a change from an emphasis on morphology and physiology (1930–1975) to the distinction of various genera on the basis of lipid composition (1978–1992). Since 1992 the inclusion of sequence data, particularly that from the 16S rDNA, has become of prime importance in modern taxonomy. Fry *et al.* (1991) and Devereux *et al.* (1990) have tried to lay down guidelines for the delineation of genera, using 16S rDNA similarity values. In practice, however, delineation based on 16S rDNA sequences alone has ranged from setting fixed similarity values to taking different evolutionary depths and evolutionary rates into consideration. Therefore, it seems beneficial to adopt a polythetic system in which the resolution of various data sets (phenotypic and genotypic) is assessed when the taxonomic status of a strain is inferred. The 16S rDNA similarity values of strain AX-2^T indicate that this is a new species within a new genus. However, it is also possible to distinguish this genus from all other described genera within the family *Halobacteriaceae* by evaluating the phenotypic data; however, because the species is monotypic, little can be said about the future features differentiating species within the genus. Strain AX-2^T is unique within the family *Halobacteriaceae*, in terms of its inability to utilize complex substrates for growth (i.e. yeast extract or peptone) and its lipid composition. Thus, the

Table 2. Distinction of genera belonging to the family *Halobacteriaceae*, based on phenotypic properties

Data were obtained from Torreblanca *et al*. (1986), Ihara *et al*. (1997), Emerson *et al*. (1994), Grant & Larsen (1989), Tindall *et al*. (1984), Kamekura & Dyall-Smith (1995), Oren *et al*. (1995), McGenity & Grant (1995) and Soliman & Trüper (1982). Abbreviations: Pleom., pleomorphic; PG, phosphatidyl glycerol; PGP, phosphatidyl glycerophosphate; Me-PGP, methylated-phosphatidyl glycerophosphate; PGS, phosphatidyl glycerosulfate; DGD, diglycosyl diether; S-DGD, sulfated diglycosyl diether; S_2-DGD, bis-sulfated diglycosyl diether; TGD, triglycosyl diether; S-TGD, sulfated triglycosyl diether; S-TeGD, sulfated tetraglycosyl diether. No attempt has been made to differentiate between the different structural forms of S-DGD or TGD because this is not significant in the differentiation of strain AX-2^T, which represents the genus *Halorhabdus*.

Phenotypic property	*Halorhabdus*	*Haloarcula*	*Halobacterium*	*Halococcus*	*Haloferax*	*Halorubrum*	*Halobaculum*	*Natrialba*	*Natronobacterium*	*Natronococcus*	*Natronomonas*
Cell shape	Pleom. rods	Pleom. rods	Rods	Cocci	Pleom. rods	Pleom. rods or rods	Rods	Rods	Rods	Cocci	Rods
Mg^{2+} required for growth (mM)*	2	3	5	<1	5	5	approx. 100	0	<10	<1	<10
Polar lipids:											
PG	+	+	+	+	+	+	+	+	+	+	+
Me-PGP†	+	+	+	+	+	+	+	+	+	+	+
Cyclic-PGP	−	−	−	−	−	−	−	−	−	+	−
PGS	−	+	+	−	−	+	−	−	−	−	−
DGD	−	+	−	−	+	−	−	−	−	−	−
S-DGD	−	−	−	+	+	+	+	−	−	−	−
S_2-DGD	−	−	−	−	−	−	−	+	−	−	−
TGD	+	+	+	+	−	−	−	−	−	−	−
S-TGD	+	−	+	−	−	−	−	−	−	−	−
S-TeGD	−	−	+	−	−	−	−	−	−	−	−

* The amount of Mg^{2+} required for growth represents the lowest value found for a member of the genus.

† Although the older literature refers to the presence of PGP in all members of the family *Halobacteriaceae*, it is now known that this compound is in fact methylated (Me-PGP).

creation of a new genus, *Halorhabdus*, within which *Halorhabdus utahensis* is the type species (of which strain AX-2^T is the type strain), can be justified.

Physiology

Strain AX-2^T had an extremely high salinity optimum of 27% (w/v) NaCl for growth. Although it is not unusually high – most extremely halophilic archaea are reported to grow optimally at 20–26% (w/v) NaCl (Grant & Larsen, 1989) – it seems to be the highest reported salinity optimum of any living organism. However, it is pertinent to be cautious concerning conclusions based on these data since the range of salinity supporting growth, as well as the optimum salinity, is highly dependent on other growth parameters, most importantly temperature and nutrition (Forsyth & Kushner, 1970).

Chemotaxonomy

The ether lipid, respiratory lipoquinone and polar lipid composition of strain AX-2^T clearly indicated that it was a member of the monophyletic group within the *Archaea* defined by the family *Halobacteriaceae*. However, a number of features distinguished strain AX-2^T from other, currently known taxa within this family (see Table 2). In particular, the presence of S-TGD and the absence of PGS were unique features of strain AX-2^T. Members of the family *Halobacteriaceae* can be divided into a number of chemical groups. These include one group in which only phospholipids are present and another in which phospholipids and glycolipids are present. The second of these groups may be further divided into a group in which PGS is present and another in which PGS is absent. Furthermore, a distinction may be made between those organisms in which the predominant glycolipids are either TGDs, S-DGDs or S-TGDs. To date, members of the genus *Halobacterium* constitute the only known group of aerobic, extremely halophilic micro-organisms within the *Archaea* which produce an S-TGD, like strain AX-2^T. However, members of the genus *Halobacterium* also produce a TGD (and/or its sulfated derivative) and PGS, unlike strain AX-2^T (which synthesizes neither of these compounds). Thus, strain AX-2^T may be distinguished unambiguously from all other known members of the family *Halobacteriaceae*.

Taxonomy

On the basis of the phenotypic (biochemical, physiological, morphological and chemical) and genotypic (16S rDNA sequence and G+C) data, strain AX-2^T may be considered to be a member of a new taxon within the family *Halobacteriaceae* for which we propose the name *Halorhabdus utahensis* gen. nov., sp. nov.

Description of *Halorhabdus* Wainø, Tindall and Ingvorsen gen. nov.

Halorhabdus [Ha.lo.rhab′dus. Gr. n. *hals* salt; Gr. n. *rhabdos* rod or stick; M.L. masc. n. *Halorhabdus* salt (-loving) rod].

Cells are extremely pleomorphic although most are rod-shaped. Rod-shaped cells are 0·5–1 μm wide and 2–10 μm long. Motile by a single flagellum. Colonies are red and circular with an entire margin and a shiny surface. Extremely halophilic and lyse in water. Grows at 9–30 % (1·5–5·1 M) NaCl; optimum growth at 27 % (4·6 M) NaCl. Grows between 0·05 and 20 % $MgSO_4$ (2–800 mM) in the presence of 27 % NaCl. Grows at temperatures from 17 to 55 °C, with an optimum at 50 °C. The pH range for growth is 5·5–8·5, with an optimum between 6·7 and 7·1. Ferments glucose. Sulfur stimulates fermentative growth. Amino acids are not required for growth; grows under aerobic or anaerobic conditions in defined media. Gram-negative cell wall. Catalase- and oxidase-positive. Amino acids are not decarboxylated or deaminated. Produces β-glucosidase and lipase. Does not produce β-galactosidase. Hydrogen sulfide is produced. Methyl red and Voges–Proskauer tests are negative. No utilization of citrate (Simmons' citrate). No production of indole. PHB is produced. Acid is produced from carbohydrates. Cells are resistant to ampicillin, carbenicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, penicillin, polymyxins, rifampicin, streptomycin and tetracycline but susceptible to bacitracin and novobiocin. A limited number of organic substrates are used for growth. The G+C content of the only species in the genus is 64 mol %. Ether lipids are diphytanyl derivatives. MK-8 and MK-8(VIII-H_2) are the only respiratory lipoquinones present. The polar lipids present are PG, Me-PGP, TGD, S-TGD and an unknown component. PGS is absent. The type species of the genus is *Halorhabdus utahensis* sp. nov.

Description of *Halorhabdus utahensis* Wainø, Tindall and Ingvorsen sp. nov.

Halorhabdus utahensis (u′tah.ensis. M.L. n. pertaining to the state of Utah, USA, where the strain was isolated).

The description of *Halorhabdus utahensis* is identical to that given above for the genus, with the following additions. The following substrates (0·2 %, w/v) are utilized for growth: glucose, xylose and fructose. No growth on amylose, arginine, D-arabinose, D-galactose, lactose, maltose, sucrose, Na-acetate, Na-citrate, Na-formiate, Na-D-glucuronate, Na-lactate, Na-pyruvate, *N*-acetyl-glucosamine, L-alanine, betaine, L-lysine, phenylalanine, L-proline, L-serine, acetamide, ethanol, glycerol, methanol, D-sorbitol, glycogen, peptone, yeast extract, starch, casein, gelatin or Tween 80. Aesculin is hydrolysed. No hydrolysis of gelatin or starch. Does not produce protease or urease. Nitrate is reduced to nitrite. Does not produce arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase or tryptophan deaminase. The G+C content of the DNA of the only strain known in the species is 64 mol %. The type strain, strain AX-2^T, isolated from Great Salt Lake, UT, USA, has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) as strain DSM 12940^T.

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