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Halanaerobaculum

Hedi, Fardeau, Sadfi, Boudabous, Ollivier and Cayol 2009, 317 ^{VP}

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Hal.an.ae.ro.ba'cu.lum. Gr. masc. n. *hals*, salt; Gr. pref. *an*, not; Gr. masc. or fem. n. *aer*, air; L. neut. n. *baculum*, stick; N.L. neut. n. *Halanaerobaculum*, salt stick not living in air.

Abstract

The genus *Halanaerobaculum* comprises one species with validly published name, *Halanaerobaculum tunisiense*, which was isolated from hypersaline surface sediments of El-Djerid Chott, Tunisia. The genus is halophilic, strict anaerobic and chemoorganotrophic. The genus *Halanaerobaculum* belongs to the family *Halobacteroidaceae*; order *Halanaerobiales*; class *Clostridia*.

Keywords: Bacteria, Extreme halophile, mesophile, anaerobe, saline sediment.

Cells are mesophilic, **halophilic, strictly anaerobic rod-shaped bacteria** occurring singly and in pairs, or occasionally as long chains in young cultures, with size ranging from 0.7-1 µm to 4-13µm. **Electron microscopy of ultrathin sections revealed a Gram-negative-like**

thin, and wavy cell wall, but cells stain Gram-positive. Spores are not observed. The major cellular fatty acids are C_{16:1} and C_{16:0}.

DNA G+C content (mol %): 34.3 (HPLC).

Type species: **Halanaerobaculum tunisiense** Hedi, Fardeau, Sadfi, Boudabous, Ollivier and Cayol 2009, 317 ^{VP}

Number of species with validated names: 1.

Family classification: The genus *Halanaerobaculum* is classified within the family *Halobacteroidaceae*; order *Halanaerobiales*; class *Clostridia*.

Further descriptive information

The genus *Halanaerobaculum*, at the time of writing, is comprised of one species with a validly published name, *H. tunisiense*, isolated from the sediment sample containing 20% NaCl (w/w) of the hypersaline Lake El-Djerid located in southern Tunisia (Hedi et al., 2009). *Halanaerobaculum tunisiense* is a strictly anaerobic rod-shaped bacterium occurring singly and in pairs, or occasionally as long chains in young cultures with cell size ranging from 0.7-1 µm to 4-13 µm. Electron microscopy of ultrathin sections of *Halanaerobaculum tunisiense* revealed a Gram-negative-like thin, and wavy cell wall, with an irregular electron-density and a clear periplasm but stained Gram-positive. Spores were not observed, and no growth was obtained after pasteurization at 80°C for 10 min.

Properties of the species of the genus *Halanaerobaculum* are described in Table 1. The optimal growth temperature of *Halanaerobaculum* is 42°C (range 30-50°C). It is a halophilic bacterium growing optimally at 20-22% NaCl with growth occurring in the presence of NaCl

concentrations between 14% and 30% NaCl. The optimum pH for growth is 7.2-7.4 (range 5.9-8.4) (Table 1).

Yeast extract or bio-trypticase is required for growth and can also be used as sole energy source. *Halanaerobaculum tunisiense* ferments carbohydrates *i.e.* glucose, sucrose, galactose, mannose, maltose, cellobiose, pyruvate and starch.

The doubling time in the presence of glucose and yeast extract under optimal conditions is 2.1 h (Table 1). Thiosulfate, sulfate, sulfite, sulfur, nitrate and nitrite are not used as electron acceptors.

The cell membrane fatty acid composition of *Halanaerobaculum tunisiense* is shown in Table 2. Cellular fatty acids of *Halobacteroides halobius* (MD-1) and *Halanaerobacter lacunarum* (DSM 6640^T) are also reported for comparison. Similarly to *Halobacteroides halobius*, its closest phylogenetic relative, the major membrane fatty acids present in *Halanaerobaculum tunisiense* are C_{16:1} and C_{16:0} acids; minor fatty acids are C_{14:0} and C_{10:0} acids.

Enrichment and isolation procedures

Halanaerobaculum tunisiense was isolated in saline medium described by Hedi et al. (2009). The pH was adjusted to 8.3 with 10 M KOH. The medium was boiled under a stream of O₂-free N₂ gas and cooled to room temperature; 5 ml was then dispensed into Hungate tubes, under a stream of N₂-CO₂ (80:20) gas mixture. Before inoculation, 0.1 ml 10% NaHCO₃ and 0.1 ml 10% MgCl₂.6H₂O were injected from sterile stock solutions. Enrichments were performed in Hungate tubes inoculated with 10 % of sample and incubated at 37°C. The culture was purified by repeated use of the Hungate roll tube method (Hungate 1969).

Enrichment cultures were positive after incubation at 37°C for 2 weeks. Colonies in roll tubes were round with entire edges, smooth, flat, opaque and yellowish-cream. They were 0.5 to 1 mm in diameter after 3 weeks of incubation. Single colonies were picked, and serial dilution

in roll tubes were repeated at least twice before the culture was considered pure. Several pure cultures similar in morphology and with the same profile for glucose metabolism were obtained.

Maintenances procedures

Stock cultures can be maintained in culture medium and can be kept in the refrigerator for 1 month. Liquid cultures retain viability for at least one year after storage at -80°C in the basal medium containing 20% glycerol (v/v). Culture collections supply lyophilized cultures.

Taxonomic comments

Phylogenetic analyses using 16S rRNA gene sequence analysis indicated that *Halanaerobaculum tunisiense* was a member of the low G+C (34.3 %) containing Gram-positive phylum. The phylogenetic analysis indicated that *Halanaerobaculum tunisiense* belonged to a subdivision of the family *Halobacteroidaceae*, forming a separate lineage in a cluster that also includes other genera of halophilic anaerobes, such as species of genera *Halobacteroides* and *Halanaerobacter*. The degree of similarity of the 16S rRNA of *Halanaerobaculum tunisiense* and *Halobacteroides halobius*, its closest relative, is 92%. Furthermore, no halophilic representative of family *Halobacteroidaceae* has been reported to grow at NaCl concentration equal to or higher than 20 % (w/v). Here it was described as the first strictly anaerobic, halophilic, fermentative bacterium isolated from sediments of a hypersaline lake in Tunisia (El-Djerid Chott) belonging to this family which grows optimally at 20% NaCl. As the phenotypic and phylogenetic characteristics of this bacterium are different from those of all previously described halophilic anaerobes, it was classified as a novel species of a novel genus in this family.

105

106 ***List of species of the genus Halanaerobaculum***

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108 *Halanaerobaculum tunisiense*

109 Hedi, Fardeau, Sadfi, Boudabous, Ollivier and Cayol 2009, 317 ^{VP}

110

111 tu.ni.si.en'se. N.L. Neut. adj. *tunisiense*, referring to Tunisia, the country where the bacterium
112 was first recovered.

113 Cells have a Gram-negative wall structure but stain Gram-positive. They are non-motile, non-
114 sporulating rods appearing singly, in pairs, or occasionally as long chains. Rods are 0.7-1 µm
115 by 4-13 µm. Growth is possible at NaCl concentrations ranging from 14 to 30%, with an
116 optimum at 20-22%. Optimal growth occurs at pH values between 7.2 and 7.4. Optimum
117 temperature for growth is 42°C (range 30°C to 50°C). Yeast extract is required for growth on
118 sugars, e.g. galactose, D-glucose, maltose, D-mannose, sucrose, starch, and cellobiose.
119 Pyruvate is also fermented. It cannot utilize DL-fructose, mannitol, D-ribose, raffinose,
120 succinate, D-xylose, fumarate, casamino acids, acetate or lactate. Thiosulfate, sulfate, sulfite,
121 sulfur, nitrate and nitrite were not used as electron acceptors. The end products of glucose
122 fermentation were butyrate, lactate, acetate, CO₂, and H₂.

123 16S rRNA gene sequence similarity values with members of the genus *Halobacteroides* is
124 92%, *H. halobius* being its closest phylogenetic relative.

125 *DNA G+C content (mol %):* 34.3 (HPLC).

126 *Type strain:* 6SANG (DSM 19997, JCM 15060).

127 *EMBL/GenBank accession number (16S rRNA gene):* EU327343.

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130 **References**

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FIGURE CAPTIONS

Figure 1 Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of *Halanaerobaculum tunisiense* with the type strains of related genus of the family *Halobacteroidaceae*. The consensus tree was constructed with mega 6 software (Tamura et al., 2013) using the Neighbor-Joining, Maximum-Evolution and Maximum-Likelihood methods. The numbers at nodes represent bootstrap values (>70%) based on 2000 replicates. There were a total of 1264 positions in the final dataset. GenBank accession numbers are given in parentheses. *Halanaerobium praevalens* DSM 2228^T (GenBank accession no. CP002175) was used as the outgroup. Bar, 2 substitutions per nucleotide position.

183

184 **Table 1:** Characteristics differentiating the genus *Halanaerobaculum tunisiense* from its
 185 closest relative.

186

Characteristics	<i>Halanaerobaculum tunisiense</i> ^a	<i>Halobacteroides halobius</i> ^b	<i>Halanaerobacter lacunarum</i> ^c
Morphology	Rods	Flexible rods	Flexible rods
Cell size (µm)	0.7-1 x 4-13	0.5 x 10-20	0.7-1 x 0.5-6
Gram stain reaction	Positive	Negative	Negative
Motility	-	+	+
NaCl range (%)	17.5-35	5.8-17.5	10-30
Optimum NaCl (%)	25	8.8-14.6	15-18
Temp range (°C)	30-50	30-47	25-52
Optimum temp (°C)	42	37-42	35-40
pH range	5.9-8.4	ND	6.0-8.0
Optimum pH	7.2-7.4	6.5	6.5-7.0
Doubling time (h)	2.1		
G + C content (mol%)	34.3	30.7	32.4
Use of substrates ^d			
Casamino acids	-	ND	ND
Cellobiose	+	-	±

Fructose	-	+	+
Galactose	+	+	<u>±</u>
Lactate	-	-	-
Mannitol	-	-	+
Maltose	+	+	+
Pyruvate	+	+	<u>±</u>
Raffinose	-	+	-
D-ribose	-	-	<u>±</u>
D-xylose	-	ND	ND
Fermentation products from glucose	Butyrate, lactate, acetate, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂
Reduction of S ^o	-	ND	+

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188 ^a Data from Hedi et al. 2009

189 ^b Data from Oren et al. 1984

190 ^c Data from Zhilina et al. 1991

191 ^d +, supported growth; -, did not support growth

192 ND, not determined

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200
201
202 Table 2: Cellular fatty acid compositions (%) of *Halanaerobaculum tunisiense* and members
203 of phylogenetically related species.

Fatty Acid	<i>Halanaerobaculum tunisiense</i> ^a	<i>Halobacteroides halobius</i> ^b	<i>Halanaerobacter lacunarum</i> ^c
Major			
C ₁₆ :1	62.3	54	73.2
C ₁₆ :0	19.3	25.6	18.7
Minor			
C ₁₄ :0	8.1	11.9	4.6
C ₁₄ :1	ND	4.3	0.8
C ₁₈ :0	ND	0.4	1.0
C ₁₈ :1	ND	2.5	0.6
C ₁₂ :0 3-OH	7.5	ND	ND
C ₁₀ :0	2.6	ND	ND

204
205 ND : not detected

206 ^a Data from Hedi et al. 2009

207 ^b Data from Oren et al. 1984

208 ^c Data from Zhilina et al. 1991

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