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Halanaerobaculum

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3 **Halanaerobaculum**

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

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12 Hal.an.ae.ro.ba'cu.lum. Gr. masc. n. *hals*, salt; Gr. pref. *an*, not; Gr. masc. or fem. n. *aer*, air;

13 L. neut. n. *baculum*, stick; N.L. neut. n. *Halanaerobaculum*, salt stick not living in air.

14

15 **Abstract**

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

21

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

23

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

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

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

30

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

33

34 Number of species with validated names: 1.

35

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

38

39 **Further descriptive information**

40

41 The genus *Halanaerobaculum*, at the time of writing, is comprised of one species with a
42 validly published name, *H. tunisiense*, isolated from the sediment sample containing 20%
43 NaCl (w/w) of the hypersaline Lake El-Djerid located in southern Tunisia (Hedi et al., 2009).

44 *Halanaerobaculum tunisiense* is a strictly anaerobic rod-shaped bacterium occurring singly
45 and in pairs, or occasionally as long chains in young cultures with cell size ranging from 0.7-
46 1 µm to 4-13 µm. Electron microscopy of ultrathin sections of *Halanaerobaculum tunisiense*
47 revealed a Gram-negative-like thin, and wavy cell wall, with an irregular electron-density and
48 a clear periplasm but stained Gram-positive. Spores were not observed, and no growth was
49 obtained after pasteurization at 80°C for 10 min.

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

53 concentrations between 14% and 30% NaCl. The optimum pH for growth is 7.2-7.4 (range
54 5.9-8.4) (Table 1).

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

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

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

66

67 **Enrichment and isolation procedures**

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69 *Halanaerobaculum tunisiense* was isolated in saline medium described by Hedi et al. (2009).
70 The pH was adjusted to 8.3 with 10 M KOH. The medium was boiled under a stream of O₂-
71 free N₂ gas and cooled to room temperature; 5 ml was then dispensed into Hungate tubes,
72 under a stream of N₂-CO₂ (80:20) gas mixture. Before inoculation, 0.1 ml 10% NaHCO₃ and
73 0.1 ml 10% MgCl₂.6H₂O were injected from sterile stock solutions. Enrichments were
74 performed in Hungate tubes inoculated with 10 % of sample and incubated at 37°C. The
75 culture was purified by repeated use of the Hungate roll tube method (Hungate 1969).

76 Enrichment cultures were positive after incubation at 37°C for 2 weeks. Colonies in roll tubes
77 were round with entire edges, smooth, flat, opaque and yellowish-cream. They were 0.5 to
78 1 mm in diameter after 3 weeks of incubation. Single colonies were picked, and serial dilution

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

82

83 **Maintenances procedures**

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

88

89 **Taxonomic comments**

90

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

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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}

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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, sulfite, 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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157 **FIGURE CAPTIONS**

158

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

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

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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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Table 2: Cellular fatty acid compositions (%) of *Halanaerobaculum tunisiense* and members 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

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ND : not detected

^a Data from Hedi et al. 2009

^b Data from Oren et al. 1984

^c Data from Zhilina et al. 1991