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2 ***Haloarcula sebkhae* sp. nov., an extremely halophilic archaeon**
3 **from algerian hypersaline environment**

4

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18

19 **Keywords:** Haloarchaea; *Haloarcula*; halophilic archaeon; hypersaline environments; salt
20 lake; sebkha.

21 The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA *rrnA* and *rpoB*' gene
22 sequences of strain SWO25^T are HQ844527 and KJ644779, respectively.

23 One supplementary figure and one supplementary table are available with the online
24 Supplementary Material.

25 Abbreviations: PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PGS,
26 phosphatidylglycerol sulfate; TGD-2, triglycosyl diether; DGD, diglycosyl diether

27 **Abstract**

28 One halophilic organism, SWO25 was isolated from salted water sampled in Algeria in the
29 salt lake (sebkha) of Ouargla. The novel strain stained Gram-negative, and cells were
30 pleomorphic with a red pigmentation. Strain SWO25 grew optimally at 35-45°C, at pH 6.0-
31 8.0 and 0.05-0.25 M MgCl₂ concentrations. Cells were extremely halophilic, with an optimal
32 growth at 4.3-5.1 M NaCl. The predominant membrane polar lipids were C20C20 glycerol
33 diether derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate,
34 phosphatidylglycerol sulfate, and triglycosyl diether and diglycosyl diether. The major
35 respiratory menaquinone component was MK-8. Cells were highly tolerant to the presence of
36 decane and isooctane in the growth medium. Chemotaxonomic properties supported the
37 assignment of strain SWO25 to the genus *Haloarcula*. The DNA G+C content was 61.1 mol%.
38 DNA-DNA hybridization and phylogenetic analyses of the 16S rRNA and *rpoB*' genes
39 showed that strain SWO25 is distinct from known *Haloarcula* species.

40 Based on phenotypic, chemotaxonomic, genotypic and phylogenetic data, we describe a
41 novel species of the genus *Haloarcula* for which the name *Haloarcula sebkhae* sp. nov. is
42 proposed. The type strain is SWO25^T (=CIP 110583^T=JCM 19018^T).

43

44

45 Extremely halophilic archaea of the class *Halobacteria* Grant *et al.* 2002 thrive in
46 hypersaline environments worldwide, such as salt lakes or solar salterns. At the present time,
47 many of the 59 genera belonging to the *Halobacteria* (*Haloadaptatus*, *Halapricum*,
48 *Haloarchaeobius*, *Haloarcula*, *Halobaculum*, *Haloferax*, *Halomicroarcula*, *Halomicrobium*,
49 *Halopiger*, *Haloquadratum*, *Halorubellus*, *Halorubrum*, *Halorussus*, *Halosimplex*,
50 *Halostella*, *Haloterrigena*, *Natrialba*, *Natrinema*, *Natronoarchaeum*) have been reported to
51 harbour multiple copies of 16S rRNA genes [1-7]. This feature both impairs phylogenetic
52 studies and introduces a bias in molecular-based diversity evaluation of environmental
53 samples, as the 16S rRNA gene is considered the standard marker for these applications [8].
54 While 1-1.3% nucleotide substitution between the 16S rRNA genes from two strains is the
55 threshold generally accepted for distinguishing species [9], one study indicates that
56 intragenomic heterogeneity remains below 1% for 87.5% of the 952 prokaryotic genomes
57 displaying such feature [8]. A few genera belonging to the family *Haloarculaceae* Gupta *et al.*
58 *al.* 2016 (*Haloarcula* Torreblanca *et al.* 1986 and the closely related genera *Halomicroarcula*
59 Zhang and Cui 2014, *Halomicrobium* Oren *et al.* 2002 and *Halosimplex* Vreeland *et al.* 2003)
60 [10, 11], display several copies of the 16S rRNA gene, with a heterogeneity of 3-9.4% [12-
61 14].

62 Intragenomic 16S rRNA gene heterogeneity was first detected in *Haloarcula marismortui*
63 Oren *et al.* 1990 [15, 16], and then shown as a characteristic common to all *Haloarcula*
64 strains, some displaying up to four copies of the gene [17]. In fact, sequencing of the genome
65 of *Har. marismortui* ATCC 43049 has revealed the presence of three copies, named *rrnA*,
66 *rrnB*, and *rrnC* [18]. A phylogenetic analysis of *Haloarcula* and *Halomicrobium* 16S rRNA
67 genes has demonstrated that two monophyletic clusters could be defined, type I including
68 *Har. marismortui rrnA* gene, and type II, corresponding to *Har. marismortui rrnB* and *rrnC*

69 [12]. Moreover, the finding that the *pyrD* gene encoding dihydroorotate oxidase was found
70 immediately upstream all type I sequences, led to the hypothesis that type I sequences
71 represented the orthologous gene derived from the ancestral gene, while paralogous type II
72 sequences could have arisen through gene duplication [4]. The use of the orthologous 16S
73 rRNA type I *rrnA* gene may resolve the problem in the phylogenetic analyses of *Haloarcula*
74 species.

75 In a previous study on extremely halophilic archaea from Algerian hypersaline
76 environments, we isolated thirteen strains of *Haloarcula* spp. A phylogenetic analysis of the
77 two copies of the 16S rRNA gene showed they form a distinct cluster from the currently nine
78 validated species of *Haloarcula*. Moreover, sequence alignments revealed nucleotide
79 substitutions very specific to this cluster, which includes the two solvent-tolerant strains OHF-
80 1 and OHF-2 isolated from French commercial salt [19-21]. This suggests that these
81 *Haloarcula* strains may constitute a new species. The aim of this study is a description of an
82 Algerian strain belonging to this new species, *Haloarcula sebkhae* sp. nov, which represents
83 a novel species within the family *Haloarculaceae*. The characterization of this strain was
84 achieved by following a polyphasic approach according to the minimal standards for the
85 description of new taxa in the order *Halobacteriales* Grant and Larsen 1989 [22].

86

87 A new strain named SWO25 was isolated from salted water (pH = 8.1, 4.8 M NaCl)
88 sampled in 1997 in the sebkha of Ouargla, a salt lake located in the Algerian Sahara (31°57'N,
89 5°20'E) [19]. Strain SWO25 was isolated in 1% (w/v) peptone (Merck), 0.1% (w/v) meat
90 extract (Merck), 0.2% (w/v) yeast extract (Merck), 4.3 M NaCl, pH 7.2, following incubation
91 at 37°C. The medium was solidified with 2% agar. Strain SWO25 as well as *Har. quadrata*
92 JCM11927^T, *Har. argentinensis* DSM 12282^T, *Har. salaria* JCM15759^T, *Har. amylolytica*
93 JCM 13557^T and *Har. tradensis* JCM15760^T, were routinely grown aerobically in DSMZ
94 *Halobacteria* medium 372 (<http://www.dsmz.de>) at 37°C [19].

95 Cell morphology and motility were examined by phase-contrast microscopy in
96 exponentially growing liquid cultures. Gram staining was performed according to Dussault
97 [23], and pigmentation was determined by observing colonies on medium 372 agar plates after
98 incubation at 37°C for 1-2 weeks. Growth range and optima for NaCl and Mg²⁺
99 concentrations, temperature, and pH were examined in medium 372. Anaerobic growth in the
100 presence of L-arginine, NaNO₃ or DMSO (each at 5 g L⁻¹) was tested for 15 days in the dark
101 in completely filled 20 mL tubes containing medium 372, using a BBL Gaspak anaerobic jar
102 (Becton, Dickinson and Cie, Sparks, MD, USA). Single-carbon source utilization tests were
103 performed in BC medium and BC medium with low yeast extract (1 and 0.1 g L⁻¹ respectively)
104 [24] supplemented with appropriate carbon source (500 µg mL⁻¹) in microtiter plates.
105 Following incubation for 10 days, phenol red (85 µg mL⁻¹) was added to the medium to detect
106 the production of acid from the different carbon sources. Growth was determined by
107 monitoring the turbidity at 600 nm of 10 mL-liquid cultures incubated at 37°C. Growth in the
108 presence of organic solvents (isooctane, cyclooctane, decane) was tested at 30°C for 13 days
109 as described by Usami *et al.* [20, 21] in tubes containing 2 mL medium 372 and 1 mL solvent
110 or sterile paraffin as control.

111 Colonies formed on agar plates were red-pigmented with a circular regular shape (diameter
112 2-6 mm) after 1-2 weeks of growth. The cells of strain SWO25 were stained Gram-negative
113 and pleomorphic with small coccoid, triangular, and short rod-like morphotypes (1-3 μm)
114 when grown in liquid medium. Growth occurred from 1.7-5.1 M NaCl, with optimal growth
115 at highest NaCl concentrations (4.3-5.1 M). The organisms had an optimal growth temperature
116 between 35 and 45°C, and could not grow below 25°C or above 55°C. Mg^{2+} was not required
117 for growth, but optimal growth occurred at 0.05-0.25 M MgCl_2 concentrations. The optimum
118 pH for growth was between 6.0 and 8.0 with growth occurring in the range 5.0 to 9.0.
119 Anaerobic growth was possible in the presence of nitrate and DMSO but not L-arginine.
120 Growth occurred in the presence of isooctane and decane, but not with cyclooctane in a growth
121 medium containing 3.4 or 5.1 M NaCl. High tolerance to decane was previously observed by
122 Usami and co-workers for *Haloarcula* strains OHF-1, OHF-2 and *Har. argentinensis* [20,21].
123 Strain SWO25, like OHF-1 and OHF-2, was more tolerant to isooctane than *Har.*
124 *argentinensis*, whatever the NaCl concentration. Therefore, tolerance to the presence of
125 isooctane in the growth medium may constitute a differential characteristic of the novel
126 species.

127 All biochemical tests were performed aerobically. Assays for catalase and oxidase
128 activities, nitrate reduction with gas production, formation of indole, and hydrolysis of gelatin,
129 Tween-80, starch, and casein, were performed in 372 agar medium according to standard or
130 modified procedures previously described by Oren *et al.* [22, 25]. Oxidase and catalase tests
131 were positive. Gelatin, starch and Tween 80 were hydrolyzed, but not casein. Nitrate reduction
132 occurred with gas production; no indole formation was detected. Utilization of D-glucose, D-
133 galactose, D-xylose, D-ribose, glycerol and sorbitol occurred with acid production, but
134 utilization of sucrose, D-trehalose, D-fructose, cellobiose and L-lysine resulted in growth
135 without acid production. Growth with mannose, D-lactose, L-aspartic acid or D-raffinose as

136 sole source of carbon was not possible. Sensitivity to antimicrobial agents was determined on
137 372 agar plates by using filter-sterilized antibiotics. Strain SWO25 was found to be sensitive
138 to novobiocin (5 µg) and anisomycin (50 µg) but resistant to ampicillin (30 µg), bacitracin
139 (10 µg), erythromycin (15 µg), neomycin (30 µg), chloramphenicol (30 µg), and rifampicin
140 (5 µg). Detailed results of the phenotypic tests and the nutritional features of strain SWO25
141 are given in the species description. Table 1 shows the differential characteristics of strain
142 SWO25 in comparison with those of type strains belonging to closely related species of the
143 *Haloarcula* genus, *Har. argentinensis* [24], *Haloarcula japonica* Takashina *et al.* 1991 [26],
144 *Har. salaria* and *Har. tradensis* [27].

145 Polar lipids were extracted from 40 mL culture pellets, using methanol/chloroform/aqueous
146 phase (1:1:0.9, v/v) as described by Bligh and Dyer [28]. Dried lipids extracts were dissolved
147 in methanol/chloroform (2:1, v/v). Polar lipids were separated by one- and two-dimensional
148 TLC by using silica gel plates (Merck silica gel 60 F₂₅₄) and visualized as described [29],
149 except for glycerol-containing lipids which were revealed with anthrone [30]. The major polar
150 lipids as determined by one and two-dimensional TLC (Supplementary Fig. S1 and
151 Supplementary Fig. S2) were the C20C20 glycerol diether derivatives of
152 phosphatidylglycerol, methyl-phosphatidylglycerol phosphate, phosphatidylglycerol sulfate,
153 triglycosyl diether, diglycosyl diether. Analysis of respiratory quinones was carried out by the
154 DSMZ Identification Service, (Braunschweig, Germany). Respiratory lipoquinones were
155 extracted from freeze dried cells as described by Tindall *et al.* [31], purified by TLC and
156 analysed by reversed-phase HPLC. The major respiratory lipoquinone was the menaquinone
157 MK-8 (92%), while other lipoquinones were found less abundantly (MK-7: 5% and MK-
158 8(H₂): 3%). These results are in accordance with those previously published for other
159 *Haloarcula* species [32].

160 For DNA G+C content and DNA–DNA hybridization experiments, the DNA was purified
161 by chromatography on hydroxyapatite according to Cashion *et al.* [33]. The DNA G+C
162 content and DNA–DNA hybridization were carried out by the DSMZ Identification Service.
163 The DNA G+C content for strain SWO25, as determined by HPLC according to Mesbah *et*
164 *al.* [34] was 61.1 mol%. This value is in the range of those encountered for *Haloarcula* species
165 (60.1-64.7 mol%) [27]. DNA–DNA hybridizations were carried out according to the thermal
166 denaturation and renaturation method [35, 36] using a Cary spectrophotometer equipped with
167 a Peltier temperature controller (Varian). The DNA-DNA hybridization values between strain
168 SWO25 and the type strains of other species of *Haloarcula* are indicated in Table S1. Strain
169 SWO25 presented the highest DNA-DNA hybridization percentage with *Har. japonica* JCM
170 7785^T (68.0-67.2%), *Har. tradensis* JCM 15760^T (68.8-66.0%), and *Har. salaria* JCM 15759^T
171 (68.0-66.0%). These values are consistent with the 65%-75% range generally accepted for
172 belonging to the same species. DNA-DNA hybridization values with all other *Haloarcula*
173 species were much lower (Table S1), clearly indicating that strain SWO25 does not belong to
174 any of these species.

175 Genomic DNA was extracted from 20 mL exponential phase culture and purified according
176 to the Qiagen genomic DNA extraction procedure (Qiagen, Germany). The 16S rRNA genes
177 were amplified using specific primers, cloned and sequenced as previously described [19].
178 PCR-mediated amplification and sequencing of the RNA polymerase subunit B (*rpoB*') genes
179 were performed as described by Minegishi *et al.* [37]. The phylogenetic analyses were
180 performed on the following website: <http://www.phylogeny.fr> [38], as previously described
181 [19]. Strain SWO25 harbors two copies of the 16S rRNA gene, belonging to type I and type
182 II sequences [19]. All the sequences used to construct the phylogenetic tree were designated
183 *rrnA* for type I sequences, and *rrnB* for type II sequences, as defined in [19]. Phylogenetic
184 analysis indicated that strain SWO25 is distinct from known species among the *Haloarcula*

185 genus (Fig.1). Regarding the orthologous *rrnA* gene; the closest strains were *Har.*
186 *argentinensis* JCM 9737^T (98.8% identity), *Har. tradensis* JCM 15760^T (97.6% identity),
187 and *Har. salaria* JCM 15759^T (95.1% identity). Similar observations can be inferred from
188 phylogenetic analysis using the paralogous (*rrnB*) 16S rRNA genes. The *rrnB* phylogenetic
189 analysis supports clustering of strain SWO25 with strains OHF-1 and OHF-2 (Fig.1). The
190 phylogenetic analysis of the *rpoB*' gene (Fig. 2) confirmed these observations (95.6% identity
191 with the *rpoB*' sequence of the three strains), but the *rpoB*' sequence of *Har. japonica* JCM
192 7785^T was also closely related to that of SWO25 (95.7 % identity). Finally, DNA-DNA
193 hybridization analysis supports that strain SWO25 is closely related to the species *Har.*
194 *japonica*, *Har. tradensis* and *Har. salaria*, while the phylogenetic data support that strain
195 SWO25 is more closely related to the species *Har. argentinensis*, *Har. tradensis* and *Har.*
196 *salaria*. However, biochemical data (Table 1) do not support that SWO25 is likely to belong
197 to any of these species. Therefore taken together, chemotaxonomic (Table 1) and phylogenetic
198 (Fig. 1) analyses indicate that strain SWO25 is distinct from other *Haloarcula* species and
199 constitutes a novel species.

200 Based on the phenotypic, chemotaxonomic, genotypic and phylogenetic data presented in
201 this paper, strain SWO25 is considered to represent a novel species of the genus *Haloarcula*
202 into the family *Haloarculaceae* for which the name *Haloarcula sebkhae* sp. nov. is proposed.

203

204 **Description of *Haloarcula sebkhae* Barreteau *et al.*, 2018 sp. nov.**

205 *Haloarcula sebkhae* (N.L. gen. n. *sebkhae* of a *sebkha*, the Arabic name for a salt pan,
206 defined geologically as flat expanses of ground covered with salt and other minerals, usually
207 found in hot deserts).

208 Cells Gram-stain-negative, non-motile are pleomorphic with coccoid, triangular, and short
209 rod-like morphotypes (1-3 μm), with no observable mobility. Colonies are circular with a 2-
210 6 mm diameter and entire margin, smooth, shiny, red pigmented on *Halobacteria* DSMZ
211 medium 372 after 1-2 weeks of incubation at 37°C. Growth occurs with 1.7-5.1 M NaCl
212 (optimum 4.3-5.1 M), at pH 5.0-9.0 (optimum pH 7.0-8.0) and at 25-55°C (optimum 35-
213 45°C). Mg^{2+} is not required, but optimum growth occurs with 0.05-0.25 M. Anaerobic growth
214 was observed in the presence of nitrate and DMSO, but not in the presence of L-arginine.
215 Nitrate is reduced to nitrite, and gas production is observed from nitrate. The major polar
216 lipids are C20C20 glycerol diether derivatives of phosphatidylglycerol, phosphatidylglycerol
217 phosphate, phosphatidylglycerol sulfate, triglycosyl diether, diglycosyl diether. The major
218 respiratory lipoquinone is MK-8 along with two minor quinones: MK-7 and MK-8(H₂). Indole
219 formation is negative. Gelatin, starch, and Tween 80 are hydrolysed, but not casein. Catalase-
220 and oxidase-positive. D-glucose, D-galactose, D-xylose, D-ribose, D-fructose, sucrose, D-
221 trehalose, cellobiose, glycerol, sorbitol, and L-lysine are used as single carbon and energy
222 source for growth. No growth occurs on D-mannose, D-lactose, L-aspartic acid, or D-raffinose
223 as single carbon sources. Acid is produced from D-glucose, D-galactose, D-xylose, D-ribose,
224 glycerol and sorbitol, but not from D-fructose, sucrose and D-trehalose. The organisms are
225 susceptible to anisomycin and novobiocin, and resistant to aphidicoline, bacitracin,
226 erythromycin, rifampicin, neomycin, ampicillin, and chloramphenicol. The cells are highly
227 tolerant to the presence of decane and isooctane in a growth medium containing 3.4 or 5.1 M
228 NaCl, but do not grow in the presence of cyclooctane. The DNA of strain SWO25^T has a G+C
229 content of 61.1 mol%.

230 The type strain SWO25^T (=CIP 110583^T=JCM 19018^T) was isolated from salted water
231 sampled in the sebkhah of Ouargla in the Sahara (Algeria).

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238 **Conflicts of interest**

239 The authors declare no conflicts of interest.

240

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344
345

346 **Figure legends :**

347 **Figure 1:**

348 Maximum Likelihood tree based on the 16S rRNA genes showing the relationships between
349 strain SWO25^T and other members of the genus *Haloarcula*. All the sequences used to
350 construct the phylogenetic tree were designated *rrnA* for type I sequences, and *rrnB* for type
351 II sequences, as defined in [19]. The sequences of *Halobacterium salinarum* corrig. (Harrison
352 and Kennedy 1922) Elazari-Volcani 1957 (Approved Lists 1980) (JCM 8978^T) were used as
353 an outgroup. GenBank accession numbers are indicated in parentheses. Bootstrap values
354 (expressed as percentages of 1000 replications) higher than 80% are indicated. Bars represent
355 expected numbers of substitutions per nucleotide position.

356

357 **Figure 2:**

358 Maximum Likelihood tree based on *rpoB*' genes showing the relationships between strain
359 SWO25^T and other members of the genus *Haloarcula*. The sequences of *Hbt. salinarum* were
360 used as an outgroup. GenBank accession numbers are indicated in parentheses. Bootstrap
361 values (expressed as percentages of 1000 replications) higher than 80% are indicated. Bars
362 represent expected numbers of substitutions per nucleotide position.

363

364 **Supplementary Figure S1:**

365 One-dimensional thin-layer chromatogram on silica gel were performed for total polar lipids
366 extracted from strain SWO25^T (a), *Har. tradensis* (b), *Har. salaria* (c), *Har. argentinensis* (d).
367 The plates were migrated using a solvent system composed of chloroform: methanol: water
368 (65:25:4, v/v). Total polar lipids were detected by spraying the plate with 5%
369 phosphomolybdic acid in ethanol, followed by heating the chromatogram at 120°C (A).
370 Phosphate-containing lipids appeared as blue spots after spraying a second plate with

371 molybdenum blue solution (B). A third plate was sprayed with 0.2% anthrone in sulfuric acid,
372 then heated at 120°C, to reveal glycolipids and glycerol containing lipids (C).PG:
373 phosphatidylglycerol; PGP: phosphatidylglycerol phosphate; PGS: phosphatidylglycerol
374 sulfate; TGD-2: triglycosyl diether; DGD: diglycosyl diether.

375

376 **Supplementary Figure S2:**

377 Two-dimensional thin-layer chromatogram on silica gel of total polar lipids extracted from
378 strain SWO25^T (a), *Har. tradensis* (b), *Har. salaria* (c), *Har. argentinensis* (d). The plates
379 were migrated using a solvent system composed of chloroform: methanol: water (65:25:4,
380 v/v) in the first dimension and (chloroform: methanol: acetic acid: water (80:12:15:4, v/v) in
381 the second dimension. Total polar lipids were detected by spraying the plate with 5%
382 phosphomolybdic acid in ethanol, followed by heating the chromatogram at 120°C (A).
383 Phosphate-containing lipids appeared as blue spots after spraying a second plate with
384 molybdenum blue solution (B). A third plate was sprayed with 0.2% anthrone in sulfuric acid,
385 then heated at 120°C, to reveal glycolipids and glycerol containing lipids (C). PG:
386 phosphatidylglycerol; PGP: phosphatidylglycerol phosphate; PGS: phosphatidylglycerol
387 sulfate.

388

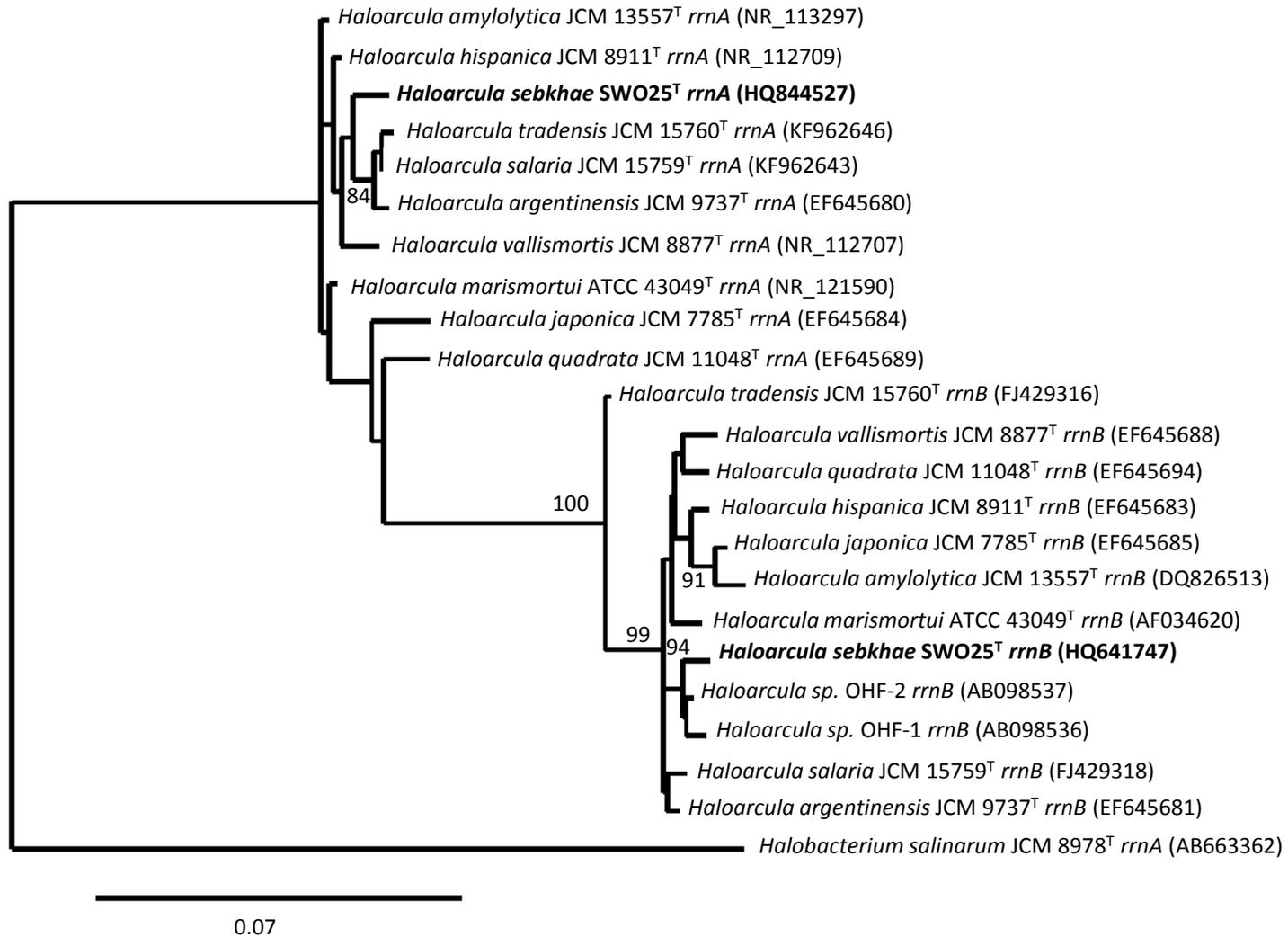
Figure 1

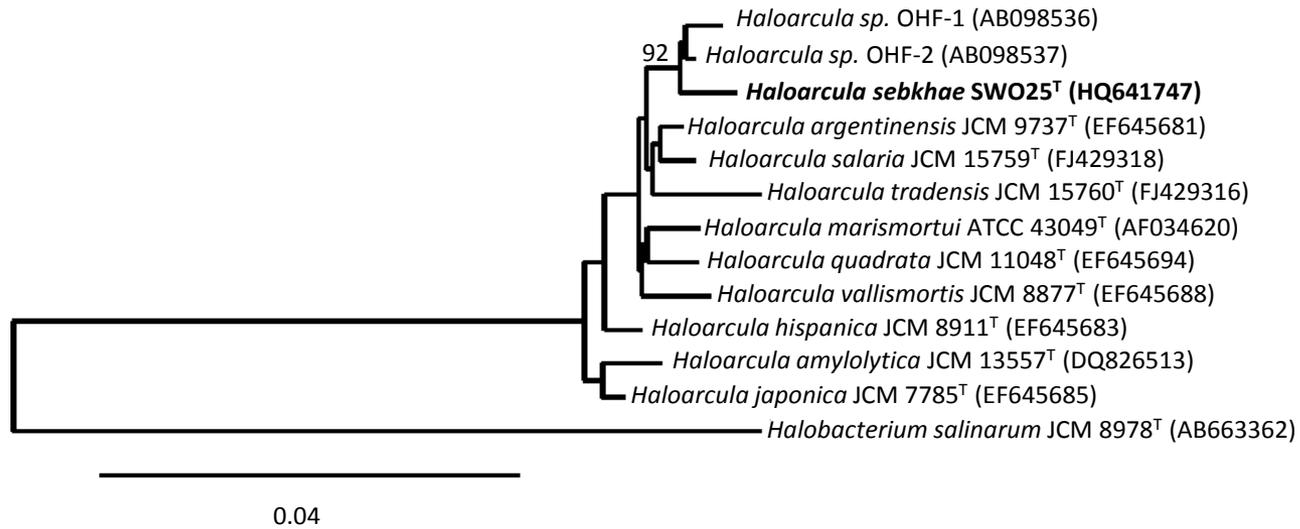
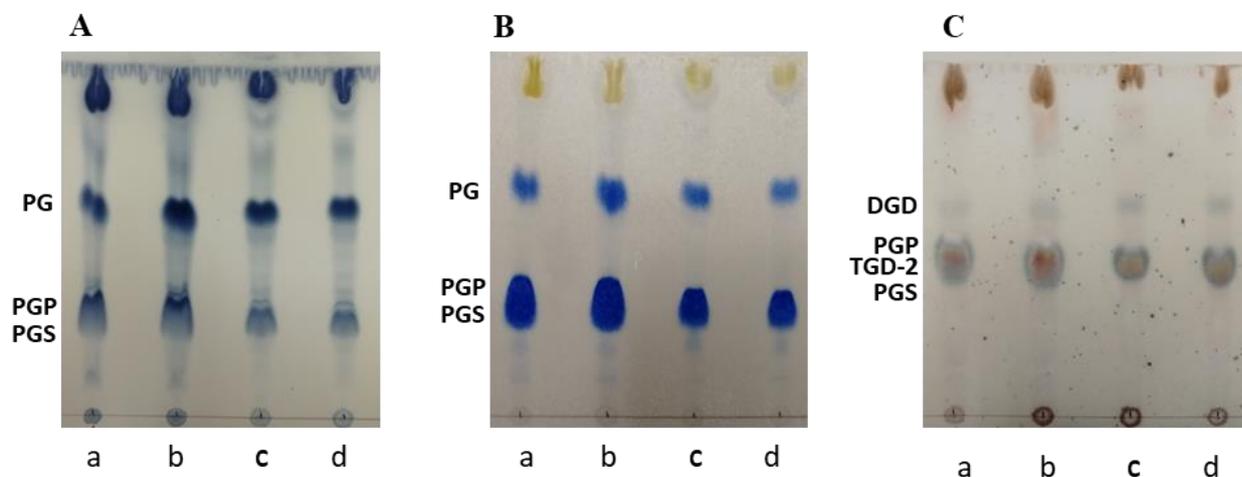
Figure 2

Table S1. DNA-DNA relatedness of strain SWO25^T with other species of the genus *Haloarcula*

	% DNA-DNA similarity with strain SWO25^T
<i>Har. japonica</i> DSM6131 ^T	68.8-67.2
<i>Har. tradensis</i> JCM15760 ^T	68.8-66.0
<i>Har. salaria</i> JCM15759 ^T	68.0-66.0
<i>Har. argentinensis</i> DSM12282 ^T	48.2-54.7
<i>Har. marismortui</i> DSM3752 ^T	51.2-49.8
<i>Har. amylolytica</i> JCM 13557 ^T	50.2-48.5
<i>Har. hispanica</i> DSM4426 ^T	47.5-46.1
<i>Har. vallismortis</i> DSM3756 ^T	43.4-47.3
<i>Har. quadrata</i> DSM11927 ^T	16.5-15.2

The given values are results of measurements in duplicate.

Supplementary Figure S1

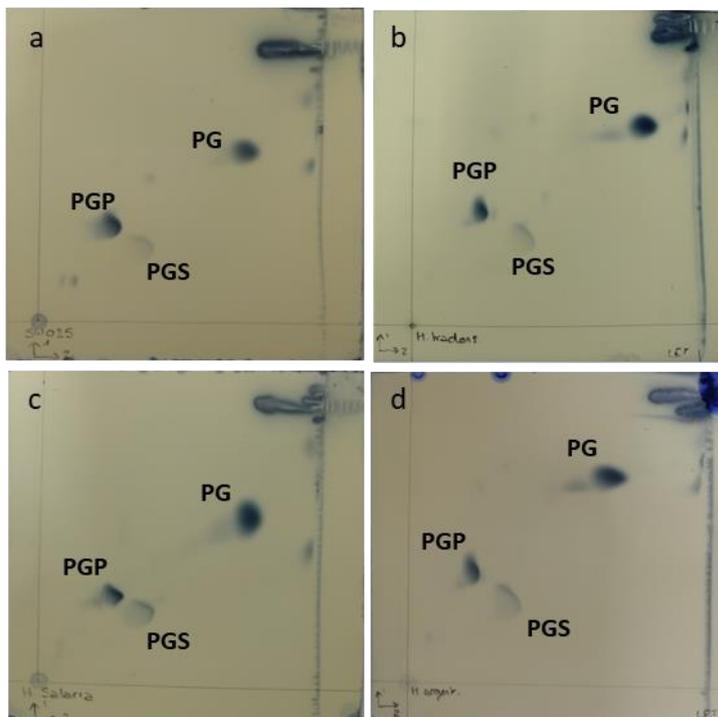


Supplementary Figure S1:

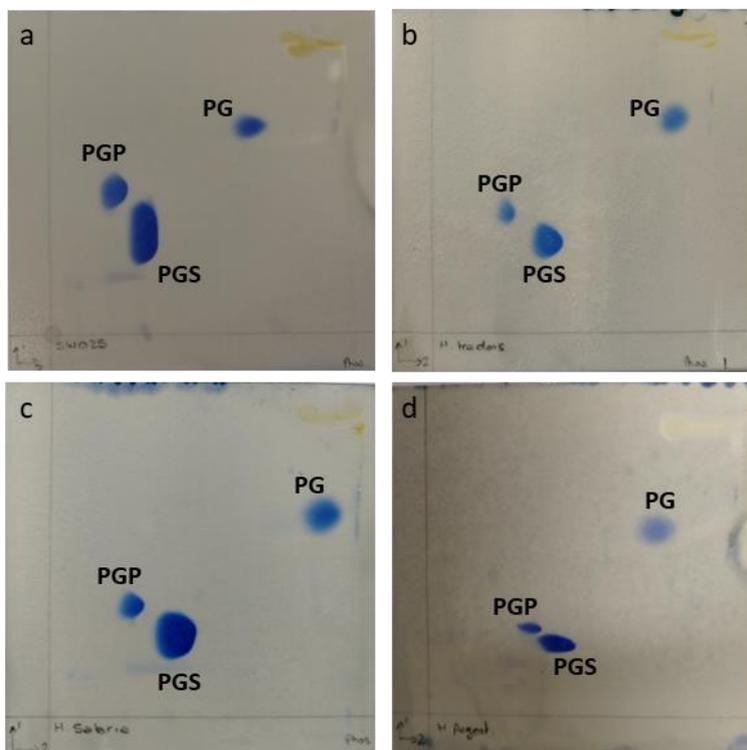
One-dimensional thin-layer chromatogram on silica gel were performed for total polar lipids extracted from strain SWO25^T (a), *Har. tradensis* (b), *Har. salaria* (c), *Har. argentinensis* (d).. The plates were migrated using a solvent system composed of chloroform: methanol: water (65:25:4, v/v). Total polar lipids were detected by spraying the plate with 5% phosphomolybdic acid in ethanol, followed by heating the chromatogram at 120°C (A). Phosphate-containing lipids appeared as blue spots after spraying a second plate with molybdenum blue solution (B). A third plate was sprayed with 0.2% anthrone in sulfuric acid, then heated at 120°C, to reveal glycolipids and glycerol containing lipids (C). PG: phosphatidylglycerol; PGP: phosphatidylglycerol phosphate; PGS: phosphatidylglycerol sulfate; TGD-2: triglycosyl diether; DGD: diglycosyl diether.

Supplementary Figure S2

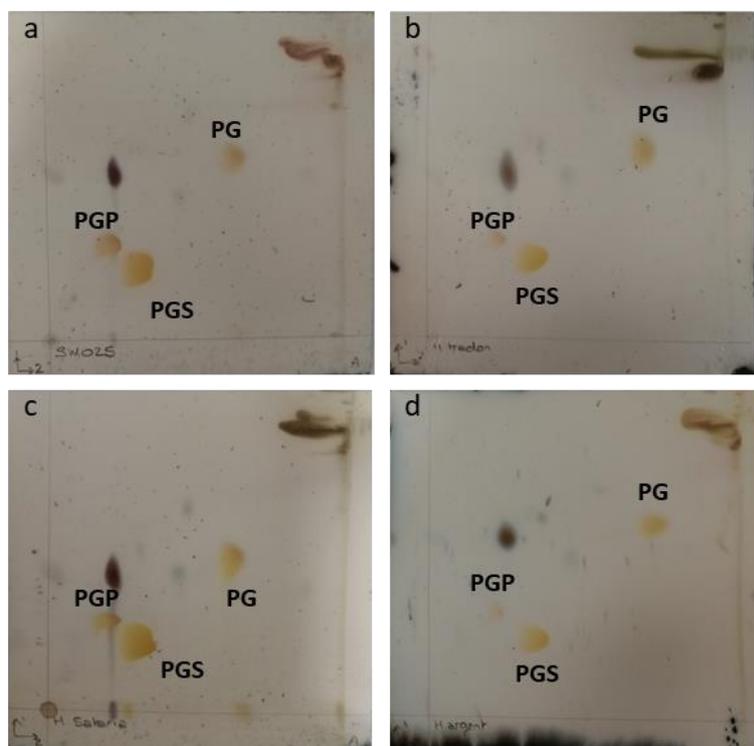
A



B



C



Supplementary Figure S2:

Two-dimensional thin-layer chromatogram on silica gel of total polar lipids extracted from strain SWO25^T (a), *Har. tradensis* (b), *Har. salaria* (c), *Har. argentinensis* (d). The plates were migrated using a solvent system composed of chloroform: methanol: water (65:25:4, v/v) in the first dimension and (chloroform: methanol: acetic acid: water (80:12:15:4, v/v) in the second dimension. Total polar lipids were detected by spraying the plate with 5% phosphomolybdic acid in ethanol, followed by heating the chromatogram at 120°C (A). Phosphate-containing lipids appeared as blue spots after spraying a second plate with molybdenum blue solution (B). A third plate was sprayed with 0.2% anthrone in sulfuric acid, then heated at 120°C, to reveal glycolipids and glycerol containing lipids (C). PG: phosphatidylglycerol; PGP: phosphatidylglycerol phosphate; PGS: phosphatidylglycerol sulfate.

Table 1

Differential characteristics between strains SWO25^T and the type strains of closely related species of the genus *Haloarcula*.

Strains: 1, SWO25^T (this study); 2, *Har. japonica* TR-1^T [26]; 3, *Har. salaria* HST03^T [27]; 4, *Har. tradensis* HST01-2R^T [27]; 5, *Har. argentinensis* arg-1^T [24].

+, positive; -, negative; R, resistant; S, sensitive. ND, No data available.

Characteristic	1	2	3	4	5
Cell morphology	Pleomorphic	Pleomorphic	Pleomorphic	Pleomorphic	Pleomorphic
Pigmentation	Red	Red-orange	Red	Red	Orange-red
Range (optimum) growth:					
NaCl (M)	1.7-5.1 (4.3-5.1)	2.5-5.1 (3.4)	2.6-5.1 (3.4-4.3)	2.6-5.1 (3.4-4.3)	2-4.5 (3-3.5)
Mg ²⁺ (M)	0-0.3 (0.05-0.25)	0.04-0.3 (0.08)	0.08-0.4 (0.16-0.24)	0.08-0.4 (0.16-0.24)	0.03-0.15 (0.1)
Temperature (°C)	25-55 (35-45)	24-45 (42-45)	15-45 (37)	15-45 (37)	25-55* (35-40)*
pH	5-9 (6-8)	6-8 (7-7.5)	6-8 (7)	6-8 (7)	5-9* (6-7)*
Sensitivity to:					
Bacitracin	R	S	S	S	R*
Rifampicin	R	R	R	R	R*
Ampicillin	R	R	R	R	R*
Chloramphenicol	R	R	R	R	R
Novobiocin	S	S	R	S	S
Hydrolysis of:					
Gelatin	+	-	-	-	+*
Starch	+	-	+	+	+*
Casein	-	-	-	-	-*
Tween 80	+	ND	+	+	+*
Nitrate reduction	+	+	ND	ND	+*
Gas production from NaNO ₃	+	+	-	-	+*
Indole formation	-	+	-	-	-*
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Utilization of:					
D-Glucose	+	+	+	+	+
D-Galactose	+	+	-	-	+
Glycerol	+	+	+	+	+
Sorbitol	+	+	-	-	+*
L-aspartic acid	-	ND	+	+	-*
L-Lysine	+	ND	-	+	-*
Acid production from:					
D-Glucose	+	+	-	-	+
D-Galactose	+	+	-	-	+
DNA G+C content (mol%)	61.1	63.3	61.6	62.2	62.0

* Determined in this study