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Haloarcula sebkhae sp. nov., an extremely halophilic archaeon from Algerian hypersaline environment

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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA rrmA and rpoB’ gene sequences of strain SWO25ᵀ are HQ844527 and KJ644779, respectively.

One supplementary figure and one supplementary table are available with the online Supplementary Material.
Abbreviations: PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PGS, phosphatidylglycerol sulfate; TGD-2, triglycosyl diether; DGD, diglycosyl diether

Abstract

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

Based on phenotypic, chemotaxonomic, genotypic and phylogenetic data, we describe a novel species of the genus *Haloarcula* for which the name *Haloarcula sebkhae* sp. nov. is proposed. The type strain is SWO25ᵀ (=CIP 110583ᵀ=JCM 19018ᵀ).
Extremely halophilic archaea of the class *Halobacteria* Grant et al. 2002 thrive in hypersaline environments worldwide, such as salt lakes or solar salterns. At the present time, many of the 59 genera belonging to the *Halobacteria* (Haloadaptatus, Halapricum, Haloarchaeobius, Haloarcula, Halobaculum, Halofex, Halomicroarcula, Halomicrobium, Halopiger, Haloquadratum, Halorubellus, Halorubrum, Halorussus, Halosimplex, Halostella, Haloterrigena, Natrialba, Natrinema, Natronoarchaeum) have been reported to harbour multiple copies of 16S rRNA genes [1-7]. This feature both impairs phylogenetic studies and introduces a bias in molecular-based diversity evaluation of environmental samples, as the 16S rRNA gene is considered the standard marker for these applications [8]. While 1-1.3% nucleotide substitution between the 16S rRNA genes from two strains is the threshold generally accepted for distinguishing species [9], one study indicates that intragenomic heterogeneity remains below 1% for 87.5% of the 952 prokaryotic genomes displaying such feature [8]. A few genera belonging to the family *Haloarculaceae* Gupta et al. 2016 (*Haloarcula* Torreblanca et al. 1986 and the closely related genera *Halomicroarcula* Zhang and Cui 2014, *Halomicrobium* Oren et al. 2002 and *Halosimplex* Vreeland et al. 2003) [10, 11], display several copies of the 16S rRNA gene, with a heterogeneity of 3-9.4% [12-14].

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


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

In a previous study on extremely halophilic archaea from Algerian hypersaline environments, we isolated thirteen strains of \textit{Haloarcula} spp. A phylogenetic analysis of the two copies of the 16S rRNA gene showed they form a distinct cluster from the currently nine validated species of \textit{Haloarcula}. Moreover, sequence alignments revealed nucleotide substitutions very specific to this cluster, which includes the two solvent-tolerant strains OHF-1 and OHF-2 isolated from French commercial salt [19-21]. This suggests that these \textit{Haloarcula} strains may constitute a new species. The aim of this study is a description of an Algerian strain belonging to this new species, \textit{Haloarcula sebkhae} sp. nov, which represents a novel species within the family \textit{Haloarculaceae}. The characterization of this strain was achieved by following a polyphasic approach according to the minimal standards for the description of new taxa in the order \textit{Halobacteriales} Grant and Larsen 1989 [22].
A new strain named SWO25 was isolated from salted water (pH = 8.1, 4.8 M NaCl) sampled in 1997 in the sebkha of Ouargla, a salt lake located in the Algerian Sahara (31°57’N, 5°20’E) [19]. Strain SWO25 was isolated in 1% (w/v) peptone (Merck), 0.1% (w/v) meat extract (Merck), 0.2% (w/v) yeast extract (Merck), 4.3 M NaCl, pH 7.2, following incubation at 37°C. The medium was solidified with 2% agar. Strain SWO25 as well as *Har. quadrata* JCM11927T, *Har. argentinensis* DSM 12282T, *Har. salaria* JCM15759T, *Har. amylolytica* JCM 13557T and *Har. tradensis* JCM15760T, were routinely grown aerobically in DSMZ Halobacteria medium 372 (http://www.dsmz.de) at 37°C [19].

Cell morphology and motility were examined by phase-contrast microscopy in exponentially growing liquid cultures. Gram staining was performed according to Dussault [23], and pigmentation was determined by observing colonies on medium 372 agar plates after incubation at 37°C for 1-2 weeks. Growth range and optima for NaCl and Mg²⁺ concentrations, temperature, and pH were examined in medium 372. Anaerobic growth in the presence of L-arginine, NaNO₃ or DMSO (each at 5 g L⁻¹) was tested for 15 days in the dark in completely filled 20 mL tubes containing medium 372, using a BBL Gaspak anaerobic jar (Becton, Dickinson and Cie, Sparks, MD, USA). Single-carbon source utilization tests were performed in BC medium and BC medium with low yeast extract (1 and 0.1 g L⁻¹ respectively) [24] supplemented with appropriate carbon source (500 µg mL⁻¹) in microtiter plates. Following incubation for 10 days, phenol red (85 µg mL⁻¹) was added to the medium to detect the production of acid from the different carbon sources. Growth was determined by monitoring the turbidity at 600 nm of 10 mL-liquid cultures incubated at 37°C. Growth in the presence of organic solvents (isooctane, cyclooctane, decane) was tested at 30°C for 13 days as described by Usami *et al.* [20, 21] in tubes containing 2 mL medium 372 and 1 mL solvent or sterile paraffin as control.
Colonies formed on agar plates were red-pigmented with a circular regular shape (diameter 2-6 mm) after 1-2 weeks of growth. The cells of strain SWO25 were stained Gram-negative and pleomorphic with small coccoid, triangular, and short rod-like morphotypes (1-3 µm) when grown in liquid medium. Growth occurred from 1.7-5.1 M NaCl, with optimal growth at highest NaCl concentrations (4.3-5.1 M). The organisms had an optimal growth temperature between 35 and 45°C, and could not grow below 25°C or above 55°C. Mg²⁺ was not required for growth, but optimal growth occurred at 0.05-0.25 M MgCl₂ concentrations. The optimum pH for growth was between 6.0 and 8.0 with growth occurring in the range 5.0 to 9.0. Anaerobic growth was possible in the presence of nitrate and DMSO but not L-arginine. Growth occurred in the presence of isooctane and decane, but not with cyclooctane in a growth medium containing 3.4 or 5.1 M NaCl. High tolerance to decane was previously observed by Usami and co-workers for Haloarcula strains OHF-1, OHF-2 and Har. argentinensis [20,21]. Strain SWO25, like OHF-1 and OHF-2, was more tolerant to isooctane than Har. argentinensis, whatever the NaCl concentration. Therefore, tolerance to the presence of isooctane in the growth medium may constitute a differential characteristic of the novel species.

All biochemical tests were performed aerobically. Assays for catalase and oxidase activities, nitrate reduction with gas production, formation of indole, and hydrolysis of gelatin, Tween-80, starch, and casein, were performed in 372 agar medium according to standard or modified procedures previously described by Oren et al. [22, 25]. Oxidase and catalase tests were positive. Gelatin, starch and Tween 80 were hydrolyzed, but not casein. Nitrate reduction occurred with gas production; no indole formation was detected. Utilization of D-glucose, D-galactose, D-xylose, D-ribose, glycerol and sorbitol occurred with acid production, but utilization of sucrose, D-trehalose, D-fructose, cellobiose and L-lysine resulted in growth without acid production. Growth with mannose, D-lactose, L-aspartic acid or D-raffinose as
sole source of carbon was not possible. Sensitivity to antimicrobial agents was determined on 372 agar plates by using filter-sterilized antibiotics. Strain SWO25 was found to be sensitive to novobiocin (5 µg) and anisomycin (50 µg) but resistant to ampicillin (30 µg), bacitracin (10 µg), erythromycin (15 µg), neomycin (30 µg), chloramphenicol (30 µg), and rifampicin (5 µg). Detailed results of the phenotypic tests and the nutritional features of strain SWO25 are given in the species description. Table 1 shows the differential characteristics of strain SWO25 in comparison with those of type strains belonging to closely related species of the *Haloarcula* genus, *Har. argentinensis* [24], *Haloarcula japonica* Takashina et al. 1991 [26], *Har. salaria* and *Har. tradensis* [27].

Polar lipids were extracted from 40 mL culture pellets, using methanol/chloroform/aqueous phase (1:1:0.9, v/v) as described by Bligh and Dyer [28]. Dried lipids extracts were dissolved in methanol/chloroform (2:1, v/v). Polar lipids were separated by one- and two-dimensional TLC by using silica gel plates (Merck silica gel 60 F_{254}) and visualized as described [29], except for glycerol-containing lipids which were revealed with anthrone [30]. The major polar lipids as determined by one and two-dimensional TLC (Supplementary Fig. S1 and Supplementary Fig. S2) were the C20C20 glycerol diether derivatives of phosphatidylglycerol, methyl-phosphatidylglycerol phosphate, phosphatidylglycerol sulfate, triglycosyl diether, diglycosyl diether. Analysis of respiratory quinones was carried out by the DSMZ Identification Service, (Braunschweig, Germany). Respiratory lipoquinones were extracted from freeze dried cells as described by Tindall et al. [31], purified by TLC and analysed by reversed-phase HPLC. The major respiratory lipoquinone was the menaquinone MK-8 (92%), while other lipoquinones were found less abundantly (MK-7: 5% and MK-8(H2): 3%). These results are in accordance with those previously published for other *Haloarcula* species [32].
For DNA G+C content and DNA–DNA hybridization experiments, the DNA was purified by chromatography on hydroxyapatite according to Cashion et al. [33]. The DNA G+C content and DNA–DNA hybridization were carried out by the DSMZ Identification Service. The DNA G+C content for strain SWO25, as determined by HPLC according to Mesbah et al. [34] was 61.1 mol%. This value is in the range of those encountered for Haloarcula species (60.1-64.7 mol%) [27]. DNA–DNA hybridizations were carried out according to the thermal denaturation and renaturation method [35, 36] using a Cary spectrophotometer equipped with a Peltier temperature controller (Varian). The DNA-DNA hybridization values between strain SWO25 and the type strains of other species of Haloarcula are indicated in Table S1. Strain SWO25 presented the highest DNA-DNA hybridization percentage with Har. japonica JCM 7785T (68.0-67.2%), Har. tradensis JCM 15760T (68.8-66.0%), and Har. salaria JCM 15759T (68.0-66.0%). These values are consistent with the 65%-75% range generally accepted for belonging to the same species. DNA-DNA hybridization values with all other Haloarcula species were much lower (Table S1), clearly indicating that strain SWO25 does not belong to any of these species.

Genomic DNA was extracted from 20 mL exponential phase culture and purified according to the Qiagen genomic DNA extraction procedure (Qiagen, Germany). The 16S rRNA genes were amplified using specific primers, cloned and sequenced as previously described [19]. PCR-mediated amplification and sequencing of the RNA polymerase subunit B (rpoB') genes were performed as described by Minegishi et al. [37]. The phylogenetic analyses were performed on the following website: http://www.phylogeny.fr [38], as previously described [19]. Strain SWO25 harbors two copies of the 16S rRNA gene, belonging to type I and type II sequences [19]. All the sequences used to construct the phylogenetic tree were designated rrnA for type I sequences, and rrnB for type II sequences, as defined in [19]. Phylogenetic analysis indicated that strain SWO25 is distinct from known species among the Haloarcula
genus (Fig. 1). Regarding the orthologous rrnA gene; the closest strains were Har. argentinensis JCM 9737T (98.8% identity), Har. tradensis JCM 15760T (97.6% identity), and Har. salaria JCM 15759T (95.1% identity). Similar observations can be inferred from phylogenetic analysis using the paralogous (rrnB) 16S rRNA genes. The rrnB phylogenetic analysis supports clustering of strain SWO25 with strains OHF-1 and OHF-2 (Fig. 1). The phylogenetic analysis of the rpoB’ gene (Fig. 2) confirmed these observations (95.6% identity with the rpoB’ sequence of Har. japonica JCM 7785T) was also closely related to that of SWO25 (95.7% identity). Finally, DNA-DNA hybridization analysis supports that strain SWO25 is closely related to the species Har. japonica, Har. tradensis and Har. salaria, while the phylogenetic data support that strain SWO25 is more closely related to the species Har. argentinensis, Har. tradensis and Har. salaria. However, biochemical data (Table 1) do not support that SWO25 is likely to belong to any of these species. Therefore taken together, chemotaxonomic (Table 1) and phylogenetic (Fig. 1) analyses indicate that strain SWO25 is distinct from other Haloarcula species and constitutes a novel species.

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

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

*Haloarcula sebkhae* (N.L. gen. n. *sebkhae* of a sebkha, the Arabic name for a salt pan, defined geologically as flat expanses of ground covered with salt and other minerals, usually found in hot deserts).
Cells Gram-stain-negative, non-motile are pleomorphic with coccoid, triangular, and short rod-like morphotypes (1-3 µm), with no observable mobility. Colonies are circular with a 2-6 mm diameter and entire margin, smooth, shiny, red pigmented on Halobacteria DSMZ medium 372 after 1-2 weeks of incubation at 37°C. Growth occurs with 1.7-5.1 M NaCl (optimum 4.3-5.1 M), at pH 5.0-9.0 (optimum pH 7.0-8.0) and at 25-55°C (optimum 35-45°C). Mg²⁺ is not required, but optimum growth occurs with 0.05-0.25 M. Anaerobic growth was observed in the presence of nitrate and DMSO, but not in the presence of L-arginine. Nitrate is reduced to nitrite, and gas production is observed from nitrate. The major polar lipids are C20C20 glycerol diether derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate, phosphatidylglycerol sulfate, triglycosyl diether, diglycosyl diether. The major respiratory lipoquinone is MK-8 along with two minor quinones: MK-7 and MK-8(H₂). Indole formation is negative. Gelatin, starch, and Tween 80 are hydrolysed, but not casein. Catalase-and oxidase-positive. D-glucose, D-galactose, D-xylose, D-ribose, D-fructose, sucrose, D-trehalose, cellobiose, glycerol, sorbitol, and L-lysine are used as single carbon and energy source for growth. No growth occurs on D-mannose, D-lactose, L-aspartic acid, or D-raffinose as single carbon sources. Acid is produced from D-glucose, D-galactose, D-xylose, D-ribose, glycerol and sorbitol, but not from D-fructose, sucrose and D-trehalose. The organisms are susceptible to anisomycin and novobiocin, and resistant to aphidicoline, bacitracin, erythromycin, rifampicin, neomycin, ampicillin, and chloramphenicol. The cells are highly tolerant to the presence of decane and isoctane in a growth medium containing 3.4 or 5.1 M NaCl, but do not grow in the presence of cyclooctane. The DNA of strain SWO25ᵀ has a G+C content of 61.1 mol%.

The type strain SWO25ᵀ (=CIP 110583ᵀ=JCM 19018ᵀ) was isolated from salted water sampled in the sebkha of Ouargla in the Sahara (Algeria).

Acknowledgements
This work was supported by the ATM Microorganisms of the National Museum of Natural History, Paris, France. We are grateful to Nacera Imadalou-Idres from the University of Bejaia for providing Haloarcula sp. SWO25, Anissa Tazrart for technical help with the culture optimization and growth characteristics determination, and to Soizic Prado for lending material for TLC.

Conflicts of interest

The authors declare no conflicts of interest.
REFERENCES


**Figure legends:**

**Figure 1:**
Maximum Likelihood tree based on the 16S rRNA genes showing the relationships between strain SWO25\(^{T}\) and other members of the genus *Haloarcula*. All the sequences used to construct the phylogenetic tree were designated *rrnA* for type I sequences, and *rrnB* for type II sequences, as defined in [19]. The sequences of *Halobacterium salinarum* corrig. (Harrison and Kennedy 1922) Elazari-Volcani 1957 (Approved Lists 1980) (JCM 8978\(^{T}\)) were used as an outgroup. GenBank accession numbers are indicated in parentheses. Bootstrap values (expressed as percentages of 1000 replications) higher than 80% are indicated. Bars represent expected numbers of substitutions per nucleotide position.

**Figure 2:**
Maximum Likelihood tree based on *rpoB*’ genes showing the relationships between strain SWO25\(^{T}\) and other members of the genus *Haloarcula*. The sequences of *Hbt. salinarum* were used as an outgroup. GenBank accession numbers are indicated in parentheses. Bootstrap values (expressed as percentages of 1000 replications) higher than 80% are indicated. Bars represent expected numbers of substitutions per nucleotide position.

**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\(^{\circ}\)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:**

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.
Figure 1

Haloarcula amylolytica JCM 13557" rRNA (NR_113297)
Haloarcula hispanica JCM 8911" rRNA (NR_112709)
**Haloarcula sebkhae SWO25" rRNA (HQ844527)**
Haloarcula tradens JCM 15760" rRNA (KF962646)
Haloarcula salaria JCM 15759" rRNA (KF962643)
Haloarcula argentinensis JCM 9737" rRNA (EF645680)
Haloarcula vallismortis JCM 8877" rRNA (EF645684)
Haloarcula marismortui ATCC 43049" rRNA (NR_121590)
Haloarcula japonica JCM 7785" rRNA (EF645684)
Haloarcula quadrata JCM 11048" rRNA (EF645689)
Haloarcula tradens JCM 15760" rRNA (FJ429316)

Haloarcula vallismortis JCM 8877" rRNA (EF645688)
Haloarcula quadrata JCM 11048" rRNA (EF645694)
Haloarcula hispanica JCM 8911" rRNA (EF645683)
Haloarcula japonica JCM 7785" rRNA (EF645685)
Haloarcula marismortui ATCC 43049" rRNA (AF034620)
**Haloarcula sebkhae SWO25" rRNA (HQ641747)**
Haloarcula sp. OHF-2 rRNA (AB098537)
Haloarcula sp. OHF-1 rRNA (AB098536)
Haloarcula salaria JCM 15759" rRNA (FJ429318)
Haloarcula argentinensis JCM 9737" rRNA (EF645681)
Halobacterium salinarum JCM 8978" rRNA (AB663362)

Click here to access/download: Figure; Fig.1-Barreteau et al-Vmars18.pptx
Figure 2

- *Haloarcula sebkhae SWO25* (HQ641747)
- *Haloarcula argentinensis* JCM 9737<sup>T</sup> (EF645681)
- *Haloarcula salaria* JCM 15759<sup>T</sup> (FJ429318)
- *Haloarcula tradensis* JCM 15760<sup>T</sup> (FJ429316)
- *Haloarcula marismortui* ATCC 43049<sup>T</sup> (AF034620)
- *Haloarcula quadrata* JCM 11048<sup>T</sup> (EF645694)
- *Haloarcula vallismortis* JCM 8877<sup>T</sup> (EF645688)
- *Haloarcula hispanica* JCM 8911<sup>T</sup> (EF645683)
- *Haloarcula amylolytica* JCM 13557<sup>T</sup> (DQ826513)
- *Haloarcula japonica* JCM 7785<sup>T</sup> (EF645685)
- *Halobacterium salinarum* JCM 8978<sup>T</sup> (AB663362)

0.04
Table S1. DNA-DNA relatedness of strain SWO25<sup>T</sup> with other species of the genus *Haloarcula*

<table>
<thead>
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<th>Species</th>
<th>% DNA-DNA similarity with strain SWO25&lt;sup&gt;T&lt;/sup&gt;</th>
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<tbody>
<tr>
<td><em>Har. japonica</em> DSM6131&lt;sup&gt;T&lt;/sup&gt;</td>
<td>68.8-67.2</td>
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<tr>
<td><em>Har. tradensis</em> JCM15760&lt;sup&gt;T&lt;/sup&gt;</td>
<td>68.8-66.0</td>
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<tr>
<td><em>Har. salaria</em> JCM15759&lt;sup&gt;T&lt;/sup&gt;</td>
<td>68.0-66.0</td>
</tr>
<tr>
<td><em>Har. argentinensis</em> DSM12282&lt;sup&gt;T&lt;/sup&gt;</td>
<td>48.2-54.7</td>
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<td><em>Har. marismortui</em> DSM3752&lt;sup&gt;T&lt;/sup&gt;</td>
<td>51.2-49.8</td>
</tr>
<tr>
<td><em>Har. amylolytica</em> JCM 13557&lt;sup&gt;T&lt;/sup&gt;</td>
<td>50.2-48.5</td>
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<td>43.4-47.3</td>
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<tr>
<td><em>Har. quadrata</em> DSM11927&lt;sup&gt;T&lt;/sup&gt;</td>
<td>16.5-15.2</td>
</tr>
</tbody>
</table>

The given values are results of measurements in duplicate.
Supplementary Figure S1:

One-dimensional thin-layer chromatogram on silica gel were performed for total polar lipids extracted from strain SWO25\textsuperscript{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
**Supplementary Figure S2:**

Two-dimensional thin-layer chromatogram on silica gel of total polar lipids extracted from strain SWO25\textsuperscript{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<sup>T</sup> and the type strains of closely related species of the genus *Haloarcula*.

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

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

<table>
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* Determined in this study