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Halapricum hydrolyticum sp. nov., a beta-1,3-glucan utilizing haloarchaeon from hypersaline lakes

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ABSTRACT

Two strains of neutrophilic haloaloarchaea were selectively enriched from hypersaline lakes in southwestern Siberia using β-1,3-glucans as a substrate. The strains were nearly identical in their phenotypes and according to phylogenomic analysis, and represent a distant novel species group in the genus *Halapricum* of the family *Haloarculaceae*. The main phenotypic property of the novel isolates is the ability to hydrolyze and grow with the polysaccharides curdlan and pachyman. Such potential has, to date, not been seen in any other haloarchaea in pure cultures. The strains are obligately aerobic saccharolytics. Apart from the insoluble β-1,3-glucans, they utilized soluble α-glucans (starch, pullulan and glycogen) and a limited number of sugars. The major ether-bound polar phospholipids include PGP-Me and PG. The glyco- and sulfolipids were absent. The major respiratory menaquinone is MK-8:8. On the basis of their unique physiological properties and the results of phylogenomic analysis, the isolates are suggested to be classified into a novel species *Halapricum hydrolyticum* sp. nov. (type strain HArc-curdl5-1^T = DSM 114193^T = UQM 41587^T).

Introduction

Most species of haloarchaea, extremely halophilic members of the phylum *Halobacteriota*, known in pure culture have been retrieved and further cultivated using simple soluble organic compounds, such as sugars or organic acids or rich complex media based on amino acid mixtures. However, our recent exploration of the polysaccharide-utilizing potential of halo(natrono)archaea living in hypersaline salt and soda lakes using a targeted selective enrichment strategy allowed us to obtain a broad range of new isolates. These were both from already known genera and from several novel genus lineages, and were capable of hydrolysis and further utilization as a growth substrate of a wide spectrum of polysaccharides, including cellulose, chitin and various alpha–beta-linked glucans (Sorokin et al., 2015; 2018; 2019; 2022). Two of the most difficult to utilize polysaccharides under hypersaline conditions were curdlan and pachyman, both insoluble beta-1,3-backbone-glucans from bacteria (the former) and fungi (the latter) with a helix-like secondary structure (Zhan et al., 2012; Zhang and Edgar, 2014). They are known substrates for fungal and bacterial glycoside hydrolases, belonging to the GH families 64, 81 and 128 (Pluvinage et al., 2017; Hettle et al., 2017). Some of those (according to the CAZy database (https://www.cazy.org)) are encoded in a few haloarchaeal genomes, such as the GH81 and 128 homologs. However, until now there was no clear evidence of the utilization of curdlan as a substrate and curdlan/pachyman-hydrolyzing enzymes have not been identified in the Archaeal domain.

Our recent targeted enrichment cultivation approach allowed us, for the first time, to obtain in pure culture several strains of haloarchaea from hypersaline lakes which utilize curdlan and pachyman as growth substrates (Sorokin et al., 2022). This paper presents the results of the taxonomy characterization and functional genome analysis for two closely related neutrophilic salt lake isolates forming a distinct novel species in the genus *Halapricum*.

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Materials and methods

Media and cultivation conditions

Surface sediment and near-bottom brines from several inland (athalassic) hypersaline lakes with neutral pH in Kulunda Steppe (Altai, Russia) served as the source for enrichment and further isolation of pure cultures as described previously (Sorokin et al., 2022). The brines of the lakes belong to the chloride-sulfate types with pH from 7.8 to 8.2, total salt from 220 to 280 g l^{-1} and low Mg content (below 5 mM). Fine sediment particles containing most of the microbial cells were separated from the bulk brine-sediment suspension (1:10, v:v) by 30 min gravity sedimentation in 50 ml Falcon tubes. The top 20 ml was placed into 50 ml screw cap bottles with rubber septa and preincubated for 2 days at 30 °C on a rotary shaker with addition of 100 mg l^{-1} each of ampicillin and streptomycin to depress the bacterial population. This was followed by a centrifugation step and the resulting pellets were resuspended into the base mineral medium containing the following (g l^{-1}): NaCl, 240, K_2HPO_4 2.5; NH_4Cl 0.5, KCl 5 and 20 mg l^{-1} yeast extract. The final pH was adjusted to 7 with 0.5 M KH_2PO_4. After sterilization and cooling, 1 ml each of acidic trace metal and vitamin solutions (Pfennig and Lippert, 1966); 1 mM $MgSO_4$ and 50 mg l^{-1} of $CaCl_2$ (from 25 and 10 % sterilized stock solutions, respectively) were added to the base medium. Curdlan or pachyman (Megazyme, Ireland) were added from a concentrated suspension in sterile distilled water to a final concentration of 1 g l^{-1}). Cultivation was carried out in 30 ml glass screw-cap bottles with a rubber septa to prevent evaporation on a rotary shaker at 35 °C and 150 rpm. Growth was followed by the cell turbidity increase and pink color formation after allowing the residual solid phase polysaccharides to sediment. The isolation of pure cultures was achieved by a serial dilution to extinction, followed by plating on the same medium as described previously (Sorokin et al., 2022). Individual colonies were placed into 12 ml serum bottles with 3 ml of liquid medium containing original substrate and those showing growth were repeatedly plated to check for colony uniformity and the final purity was confirmed by microscopy and genome sequencing. In total, two pure cultures were isolated: strain HArc-curdl5-1^T and HArc-curdl7 using either curdlan or pachyman as substrates, respectively.

Pure culture characterization

Life cell microscopy (Zeiss Axioplane Imaging 2, Germany) was performed in two modes: in phase contrast and after staining cells attached to curdlan particles with SYTO9 (Invitrogen kit L7012). Growth in liquid cultures was monitored spectrophotometrically (OD_{600}) after forceful passing of a 2 ml portion through a thin syringe needle several times through the culture followed by 10 min sedimentation of insoluble polysaccharide particles, or directly in the case of cultures grown with soluble substrates. Anaerobic growth was investigated using 12 ml serum bottles sealed with butyl rubber stoppers after 3 cycles of evacuation-flushing with sterile argon either by fermentation or in the presence of elemental sulfur (1 g l^{-1}), thiosulfate, DMSO (10 mM), nitrate (5 mM) and nitrite (2 mM). Utilization of single organic carbon substrates and various polysaccharides (from Megazyme or Sigma-Aldrich) was studied using the same bottles but with 3 ml medium (1 g l^{-1} substrates) under air incubated on a rotary shaker at 35 °C and 150 rpm. The effect of salinity on growth was examined with cellobiose as substrate within a range of NaCl from 1 to 5 M at pH 7.0. For the pH profiling (also with cellobiose) the unsterile base medium containing 4 M NaCl was prepared with the following buffer systems: 0.05 M HEPES adjusted to the pH range 6–8 with 0.5 M K_2HPO_4 and 0.05 M Tris-HCl for pH 8–9. Actual pH was taken into account at the end of experiment. Sulfide formation during anaerobic growth was measured by the methylene blue method and the standard tests (urease, oxidase, catalase, protease, lipase and tryptophanase activities) were performed as described previously (Sorokin et al., 2021). Nitrite formation from nitrate was measured spectrophotometrically (Eck, 1966).

Lipids and quinones

Intact polar lipids (IPLs) and quinones were extracted using a modified Bligh-Dyer procedure from freeze-dried biomass and analyzed by Ultra High Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS), as described previously (Bale et al., 2021; Sorokin et al., 2022).

Genome sequencing

The genomes of HArc-curdl5-1^T and HArc-curdl7 were sequenced using the MiSeq Illumina platform and assembled as described previously (Sorokin et al., 2022). The details of genome statistics are given in the **Supplementary Table S1**. The draft assemblies are available in the GenBank under the numbers GCA_025517535 and GCA_025517565.

Genomic and phylogenomic analyses

Phylogenomic analysis based on the "ar122" set of conserved single copy archaeal proteins (Rinke et al., 2021) was performed as follows: the protein sequences were identified in *in silico* proteomes of type species of all genera within *Haloarculaceae* family and species belonging to *Salinirussus*; *Halovenus*, *Halocatena*, *Halomarina*, *Salella* (genera which should be affiliated to *Haloarculaceae* family according GTDB taxonomy) and aligned using the GTDB-tk v.1.7.0 with reference data v.202 (Chaumeil et al., 2019). The phylogenomic tree was constructed in the RAxML v.8.2.12 (Stamatakis, 2014) with the PROTGAMMAILG model of amino acid substitution and 1000 rapid bootstrap replications as local support values. The tree was visualized using iTOL v.6.5.2 (Letunic and Bork, 2019).

Several whole genome-based comparisons were done: average nucleotide identity (ANI) was calculated using pyani v0.2.12 (Pritchard et al., 2016); average amino acid identity (AAI) – using aai.rb script (Rodriguez-R and Konstantinidis, 2016) with Diamond program (Buchfink et al., 2015); percentage of conserved proteins (POCP) – using following script (https://github.com/hoelzer/pocp); and digital DNA-DNA hydridisation (dDDH) by the Genome-to-Genome Distance Calculator 2.1 online tool (https://ggdc.dsmz.de/ggdc.php) (Rodriguez-R and Konstantinidis, 2016; Buchfink et al., 2015).

For advanced analysis of glycoside hydrolases of GH81 family in halophilic archaea, CAZy domain search and phylogenetic analysis were performed. All proteins belonging to GH81 family with evidence at protein level from Uniprot, two GH81 enzymes encoded in genomes of strains HArc-curld5-1 and HArc-curld7, as well as proteins which were found during BLAST with GH81 enzymes as queries against NCBI non-redundant protein database (organism list was limited to *Archaea*; e-value threshold was 0.05) were analyzed. Further all proteins were analyzed using dbCAN v.4 (Zhang et al., 2018) with Hmmer tool to detect target domains: only proteins with GH81 (minimal coverage 0.7) and some additional carbohydrate-binding domains (minimal coverage 0.5) were kept in local protein set. These sequences were aligned using Mafft v.7 (Katoh et al., 2017) with E-INS-i method. Obtained alignment was treated using trimAL v1.4.1 with -gt 0.95 (Capella-Gutiérrez et al., 2009). The phylogenetic tree for GH81 glycosidases was constructed and decorated as described for "ar122"-based phylogenomic tree (see above).

Results and discussion

Enrichment and isolation of pure cultures

A primary enrichment with curdlan and pachyman were long in development, starting to show a visible growth and substrate

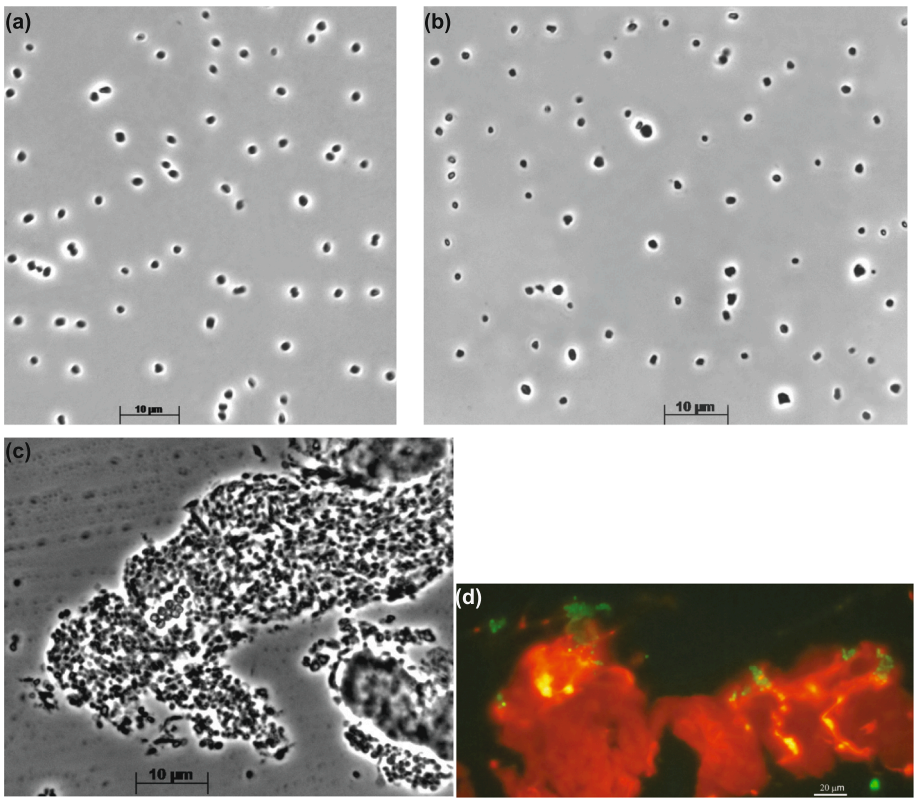

Fig. 1. Cell morphology of the beta-1,3-glucan-utilizing haloarchaea growing aerobically with at 4 M NaCl, pH 7 (**a-b**), phase contrast microscopy of strains HArc-curdl5-1^T and HArc-curdl7, respectively, growing with cellobiose. (**c-d**), colonization of curdlan by cells of strain Harc-curdl5-1^T, demonstrated by the phase contrast and fluorescent microscopy (life staining by SYTO-9) respectively.

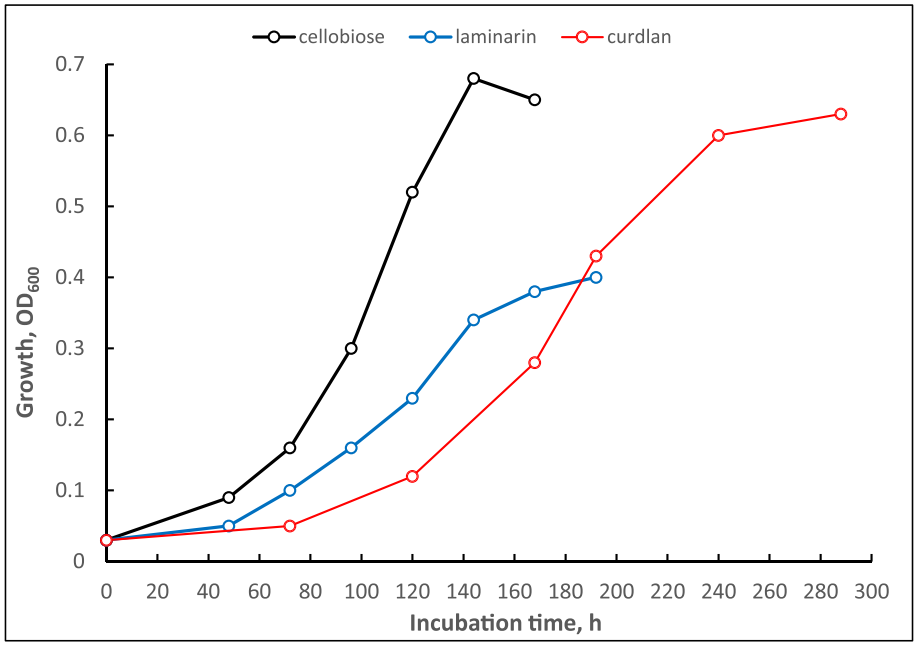

Fig. 2. Comparative growth dynamics of strain HAcurdl5-1^T at 4 M NaCl (pH 7) with three different substrates. Mean results from a duplicate experiment.

degradation only after a month time incubation. Both enrichments were dominated by coccoid cells of variable size. Growth in serial dilutions was achieved up to (10^{-8}), however ordinary surface plating gave extremely poor growth unable to produce separate colonies. In contrast, the soft agar-shake technique was much more successful, resulting in well separated red colonies inside the agar up to 10^{-6} dilutions. Finally, from those two pure cultures were isolated with stable growth in liquid media: strain HArc-curdl5-1^T from the curdlan enrichments and HArc-curdl7 – from the pachyman enrichments.

Table 1
Composition of intact polar lipids identified in strain HArc-curdl5[T].

Polar head group	Core	[M + H]$^+$	Assigned elemental composition	Percent
PGP-Me	EXT-AR	971.7449	$C_{52}H_{109}O_{11}P_2$	26
	Uns(1)-EXT-AR	969.7286	$C_{52}H_{107}O_{11}P_2$	7.6
	Uns(2)-EXT-AR	967.7138	$C_{52}H_{105}O_{11}P_2$	7.6
	Uns(3)-EXT-AR	965.6976	$C_{52}H_{103}O_{11}P_2$	22
	Lyso-AR	621.3527	$C_{27}H_{59}O_{11}P_2$	2.8
	AR	901.6658	$C_{47}H_{99}O_{11}P_2$	22
	Total			**88**
PG	EXT-AR	877.7623	$C_{51}H_{106}O_8P$	5.7
	Uns(1)-EXT-AR	875.7461	$C_{51}H_{104}O_8P$	0.9
	AR	807.6837	$C_{46}H_{96}O_8P$	4.8
	Lyso-AR	527.3704	$C_{26}H_{56}O_8P$	0.5
	Total			**12**
	Sum AR			**27**
	Sum EXT-AR			**32**
	Sum *uns*-EXT-AR			**38**
	Sum lyso-AR			**3.3**

PGP-Me = phosphatidylglycerolphosphate methyl ester; PG = phosphatidylglycerol;
AR = archaeol (C_{20}-C_{20}); EXT-AR = extended archaeol (C_{20}-C_{25}); lyso = one alkyl.
chain is absent; uns = unsaturated.

Phenotypic properties

The cells of both isolates were nonmotile, true to ellipsoid cocci and red pigmented, as is typical for aerobic haloarchaea. During the initial growth phase with curdlan or pachyman, most of the cells were associated with the polyshaccharide phase, forming microcolonies on the aggregate surface (Fig. 1), but later on the polysaccharide degradation was accompanied by a massive release of the free cells.

The isolates belong to the saccharolytic type, utilizing a few poly- and oligo-sugars. The polysaccharide spectrum included: 2 insoluble beta-1,3-glucans, curdlan and pachyman; soluble beta-1,3/beta1,6-glucan laminarin; soluble beta-fructan inulin and soluble alpha-glucans, including starch and glycogen. The utilized sugars included glucose, fructose, sucrose, trehalose (best growth), raffinose, maltose, cellobiose, melezitose, melibiose and galactose (weak growth). The comparative growth dynamics of strain HAcurdl5-1 with 3 different substrates is shown in Fig. 2.

Anaerobic growth was possible in fermentative mode (tested with cellobiose and trehalose). Oxidized sulfur compounds (sulfur, thiosulfate, DMSO) were not uitilized as the electron donor. Likewise, nitrate nor nitrite did not stimulate anaerobic growth with trehalose, although up to 3 mM nitrate was reduced to nitrite. When trehalose was used at a 10 times reduced concentration (0.1 g l^{-1}) and H_2 was added to the gas phase, reduction of nitrate to nitrite did result in an increase in the growth rate and two times higher nitrite formation in comparison to the fermentative conditions, indicating that the organism can perform dissimilatory nitrate respiration with low-potential electron donors, such as H_2, but the final growth yield was not much higher in presence of nitrate. When nitrite was used instead of nitrate at mixotrophic conditions (trehalose + H_2), the fermentative growth was definitely inhibited, despite the organism being able to slowly reduce nitrite (**Supplementary Fig. S1**).

Both isolates were extremely halophilic (optimal growth at 4 M NaCl, no growth below 2.5 M), neutrophilic (optimum at pH 7.5) and mesophilic (optimum at 35–40 °C) and they did not demand a high Mg concentration, with the optimal growth already at 1 mM. Ammonium, but not urea or nitrate, served as the nitrogen source. Catalase activity was absent, oxidase activity test showed weak positive results and the indole formation from tryptophane (by the Kovach test) was negative. Lipase and protease activities were negative and amylase was positive in plate tests with emulsified olive oil, casein and soluble starch, respectively. HArc-curdl5-1 growing with cellobiose was sensitive to rifampicin at 25 and chloramphenicol at 50 mg l^{-1}, but resistant to streptomycin, ampicillin, kanamycin and vancomycin up to 100 mg l^{-1}.

Chemotaxonomy

The core of the membrane phospholipids HArc-curdl5-1 were represented by three varieties of archaeol: C_{20}-C_{20} archaeol, C_{20}-C_{25} extended archaeol and unsaturated extended archaeols in roughly equal proportions, amounting for 97 % of the total membrane lipids. In addition, lyso-archaeol (whereby one of the isoprenoid chains is absent) was detected as a minor component (3 %). The polar head of the intact ether lipids was dominated by phosphatidylglycerolphosphate methyl ester (PGP-Me), with the phosphatidylglycerol (PG) second in abundance (Table 1**).** The glyco- and sulfo-lipids commonly found in neutrophilic haloarchaea were not detected. The dominant respiratory lipoquinone was MK-8:8 (94.3 % from the total), with a minor proportion of MK-8:7 (4.4 %) and MK7:7 (1.2 %).

Phylogenetic analysis

Both isolates had two greatly dissimilar *rrn* operons in the genomes (nearly identical in the pairwise comparison, indicating that they belonged to single species), with a 16S rRNA gene identity to each other of only 91.6 % (**Fig. S2**). The sequences of the *rrn* operon A clustered with the members of genus *Halapricum* and mostly related to *Halapricum desulfuricans* HSR12-2^T *rrn* operon A (Sorokin et al., 2021) (99.18 % identity). The 16S *rrn* operon B genes of HArc-curdl strains were 96 % identical to the 16S *rrn* operon B of *Halapricum desulfuricans* HSR12-2^T and together they were forming a separated genus lineage within the family *Haloarculaceae*. This indicated that the novel isolates may belong to a new species of the genus *Halapricum* (Song et al., 2014). Indeed, a phylogenomic analysis based on 122 archaeal conserved single-copy protein markers revealed that the strains HArc-curdl5-1^T and HArc-curdl7 formed a distinct species-level branch in the *Halapricum* genus (Fig. 3**; Fig. S3**). The separate species status of novel isolates was also supported by the whole genome comparison indexes, ANI, AAI, POCP and DDH (**Supplementary Table S2**). ANI values between strains HArc-curdl5-1^T or HArc-curdl7 and *Halapricum desulfuricans* HSR12-2^T (GCF_017094525.1), *Halapricum salinum* CBA1105^T (GCF_004799665.1) were 87.46 % and 84.35 %. These values are far lower than the 95 ~ 96 % threshold proposed for species delineation (Kim et al., 2014; Richter and Rosselló-Móra, 2009). Calculated AAI values between strains HArc-curdl5-1^T or HArc-curdl7 and *Halapricum desulfuricans*; *Halapricum salinum* were 85 % and 73.4 % respectively, higher than the proposed threshold for novel genus of 65 % (Konstantinidis et al., 2017), and lower than the 95 % threshold for the same species (Konstantinidis and Tiedje, 2005). Also POCP values between strains HArc-curdl5-1^T/HArc-curdl7 and *H. desulfuricans*; *H salinum* were 76.8 %/76.7 % and 68 %/67.9 %, while the genus-level threshold is 50 % (Qin et al., 2014). According to Genome-to-Genome Distance Calculator (Meier-Kolthoff et al., 2022) DNA-DNA hybridization estimate between HArc-curdl5-1^T/HArc-curdl7 and *H. desulfuricans* is 31 %; probability that DDH > 70 % (i.e., same species): 0.16 %.

To assess the ecological significance of the described strains, we have tried to find closely related MAGs. For this purpose, we analyzed all 126 MAGs in the NCBI database that belong to the order *Halobacteriales*. The analysis was conducted by assessing the ANI level by using pyani (Pritchard et al., 2016). None of these MAGs had ANI levels with the described strains greater than 90 %. Moreover, the genus *Halapricum*

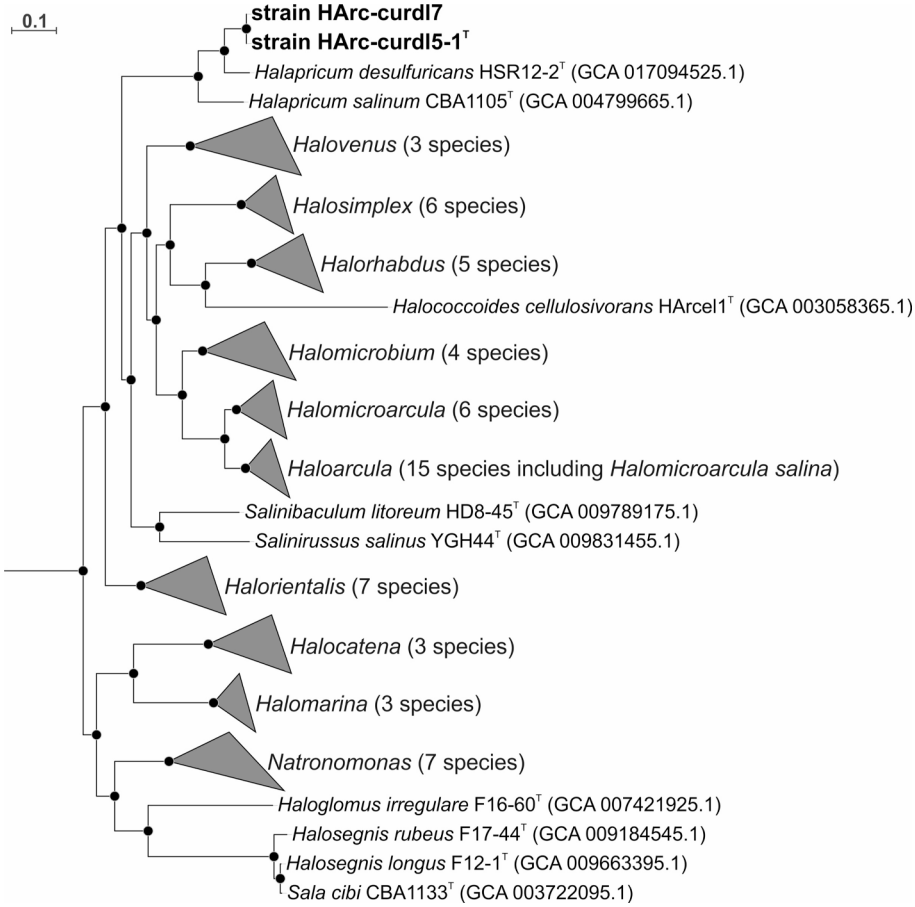

Fig. 3. Maximum likelihood phylogenetic tree based on concatenated alignment of 122 conserved archaeal proteins and showing position of strains HArc-curdl5-1^T and HArc-curdl7 (in bold) within the family *Haloarculaceae*. The branch lengths correspond to the number of substitutions per site with corrections associated with the models. The black circles at nodes indicate that the percentage of corresponding support values was above 50. Species of some genera are collapsed and numbers of species are given in brackets. *Archaeoglobus fulgidus* VC-16^T, *Methanocella paludicola* SANAE^T and *Methanothermobacter thermautotrophicus* Delta H^T were used as an outgroup (not shown).

does not contain any MAGs in the GTDB. In this situation, the only possibility to estimate the distribution of the described strains from public databases is 16S rRNA gene-based analysis. For this purpose, 14,765 partial or complete 16S rRNA gene sequences belonging to *Halobacterales* were obtained from the SILVA database. We found only 7 sequences that had a level of identity greater than 98.7 % with the 16S rRNA genes of the HArc-curdl isolates. In all cases, the sequences were similar to genes from the rrn operon A (unrelated to *Halomicrobium;* see **Suppl. Fig.S2**). The most related sequences (99.4 %-99.6 %) were found in the Dabancheng salt lake in China (KM496561), in sediments of Tunisian multipond solar saltern (Baati et al., 2010) and in hypersaline microbial mats of Oman (Vogt et al., 2018). Less related sequences (98.8 % to 99.0 %) were also found in hypersaline ecotopes, such as solar salterns and sediments of hypersaline lakes.

Functional genome analysis

As mentioned above, the unique property of the novel isolates is their ability to utilize insoluble beta-1,3-glucans. The results of screening for the presence of genes encoding potential polysaccharidolytic enzymes in these archaea has been published previously (Sorokin et al., 2022) and in particular indicated a presence in both genomes of a gene encoding a large translocated protein containing two functional domains: a GH81 hydrolytic domain specific for the beta-1,3-endoglucanases and a carbohydrate-binding domain CBM6, also specific for this GH family. Only a few haloarchaeal genomes encode homologous proteins, in particular a very similar one is present in the genome of one of the two described *Halapricum* species – *H. desulfuricans*. Accordingly, we tested *H. desulfuricans* strain HSR12-1 for the ability to grow aerobically with curdlan, pachyman and laminarin after adaptation to aerobic growth on cellobiose from a primary anaerobic DMSO-respiring culture. The results were strongly positive for laminarin, while only a slight growth (in comparison with the HArc-curdl strains) was observed with curdlan (Supplementary **Fig. S4**) and no growth at all with pachyman.

Overall, at the moment only a few enzymes belonging to the GH81 family have been characterized from eukaryotes (fungi and plants) and bacteria but none from archaea. All studied proteins are active against laminarin and some of them against pachyman or curdlan. Very weak activities are observed against beta-glucans with mixed beta-1,3- and beta-1,4-linkages (McGrath and Wilson, 2006; Martín-Cuadrado et al., 2008). Only two characterized GH81 enzymes, Q9KG76 and Q9UT45, contain carbohydrate-binding modules, and deletions of them decreased activity of the enzymes with the insoluble substrates (Hettle et al., 2017; Kumar et al., 2018). Phylogenetic analysis of the GH81 proteins from the haloarchaeal strains HArc-curdl5-1 and HArc-curdl7 demonstrated their close relation with the homologues from *Halapricum desulfuricans* HSR12-1 and *Halorhabdus* strains which also possess a CBM6 domain (Fig. 4), except for a *Halosolutus gelatinilyticus* SQT-29–1 one containing CBM6 and CBM56 and a cluster containing the CBM56 domain. In general, the haloarchaeal GH81 proteins formed a compact cluster with two different CBM-containing subclusters, although, both subclusters also included multiple cases devoid of any CBM domains. Most probably such proteins belong to laminarinases.

Other functionally important proteins encoded in the HArc-curdl5-1

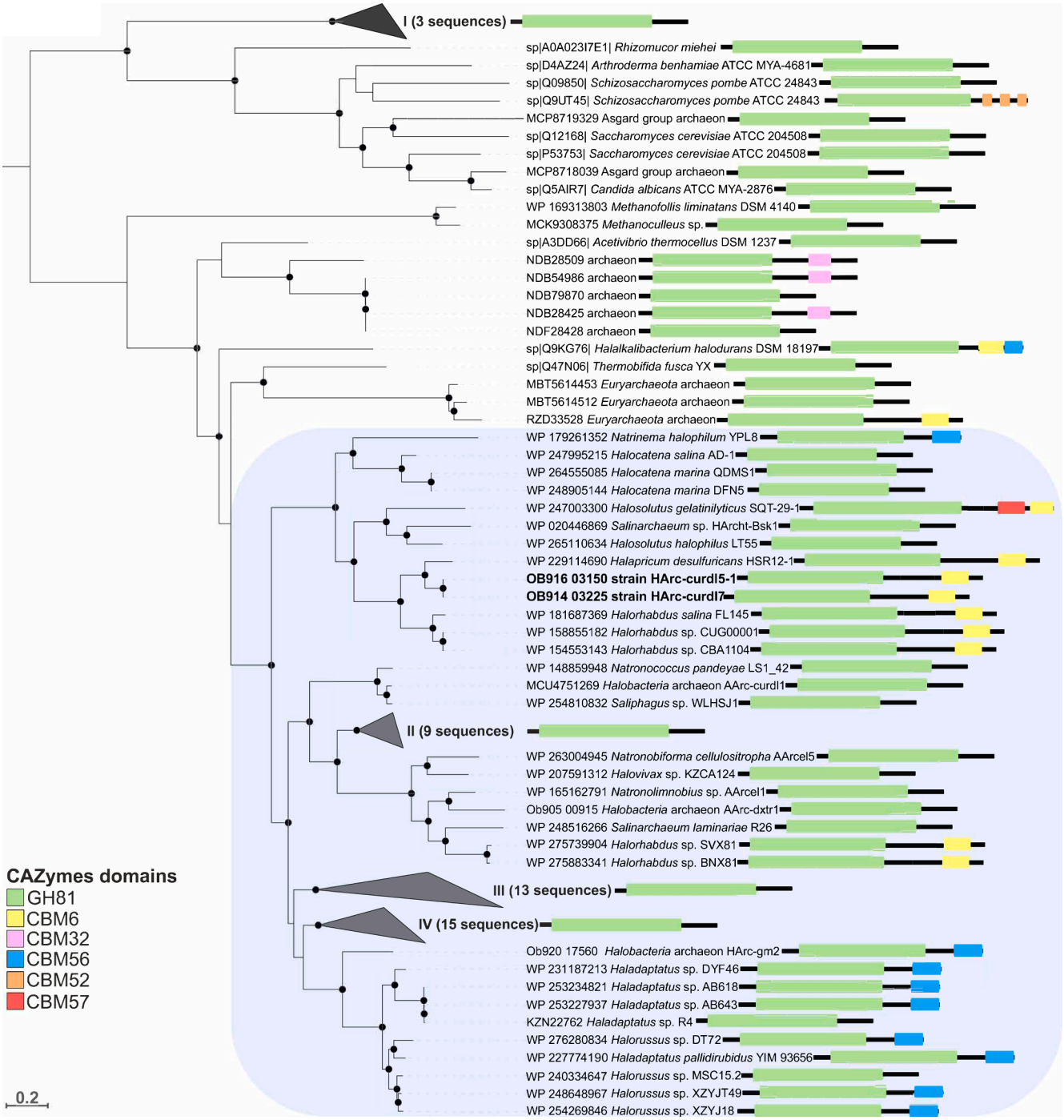

Fig. 4. Comparative phylogenetic analysis of *endo*-beta-1,3 glucanase from the GH81 family found among haloarchaea (in a blue-shaded box). Domain architectures are given near each protein. The branch lengths correspond to the number of substitutions per site with corrections associated with the models. The black circles at nodes indicate that the percentage of corresponding support values was above 50. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

genome are listed in **Supplementary Table S3**. They include the salt-pH homeostasis, energy-related complexes and oxidative stress response systems. Interestingly, both HArc-curdl genomes coded for the similar array of the [Ni,Fe] hydrogenase complexes found in *H. desulfuricans*, including an extracellular membrane-bound uptake hydrogenase type 1a and three cytoplasmic complexes, probably functioning in fermentative H_2 formation (Sorokin et al., 2021). The presence in the genomes of loci encoding a membrane-bound uptake hydrogenase, a membrane-bound respiratory nitrate-reductase of the Nar-type and a respiratory copper nitrite reductase NirK might explain the ability of HArc-curdla5-1^T to use H_2 as the electron donor for anaerobic nitrate/nitrite respiration (Supplementary **Fig.S1**). However, the N_2O reductase encoding gene markers were not present.

In contrast to the multiple *H. desulfuricans* isolates, the curdlan-utilizing isolates had no capacity for sulfur-dependent anaerobic respiration (tested with cellobiose, trehalose or trehalose + H_2) despite their genomes containing two loci coding for putative thiosulfate-reductase (PhsABC) and DMSO-reductase (DmsABC), similar to the ones present in the *H. desulfuricans* strains.

Comparative properties of the novel isolates with the species of the

Table 2
Comparative properties of the β-1,3-glucan utilizing isolates with the related species from the the genus *Halapricum* [10; 23].

Property	***Halapricum hydrolyticum* HArc-curdl5-1^T**	*Halapricum desulfuricans* HSR12-1; HSR12-2^T	*Halapricum salinum* JCM 19729^T
Number of isolates	2	9	1
Cell morphology	nonmotile coccoids	nonmotile coccoids	nonmotile rods
Pigmentation	red	red	pink
PHA accumulation	–	+	–
Anaerobic growth	+	+	–
by:	–	+	w+
sugar fermentation	–	+	w+
sulfur respiration			
thiosulfate respiration			
sulfoxide respiration (DMSO)			
nitrate to nitrite respiration	+ (H_2)	+ (H_2)	–
nitrite respiration	+(H_2)	–	–
Psr operons in genomes	0	3	1
e-donors for anaerobic growth	sugars, H_2	sugars, glycerol, starch, H_2 (v; w)	glucose
Substrates for aerobic growth	sugars, starch, curdlan, pachyman, laminarin	sugars, starch, glycerol, laminarin (1 strain)	sugars, glutamate
Amylase	+	+	–
Esterase/lipase	– (tributyrin/olive oil)	– (tributyrin/ olive oil)	– (Tweens 20, 40, 80)
Protease	– (casein; gelatin)	– (casein; gelatin)	– (gelatin)
Catalase/oxidase	–/w+	–/w+	–/+
Indole from tryptophane	–	–	–
Salinity range (opt.) M Na^+	2.5–5.0 (4.0)	3.0–5.0 (4.0)	2.5–5.0 (3.2–4.6)
Mg requirement (optimum)	low (1 mM)	low (1 mM)	moderate (5–200 mM)
pH range (opt.)	6.8–7.8 (7.5)	6.5–7.8 (7.0–7.2)	7.0–8.0 (7.0–7.2)
Temperature max (^oC)	48	50	45
Core lipids (archaeols)	C_{20}-C_{20}, C_{20}-C_{25} DGE	C_{20}-C_{20}, C_{20}-C_{25} DGE	C_{20}-C_{20}, C_{20}-C_{25} DGE
Intact membrane polar lipids: phospholipids	PG, PGP-Me	PG, PGP-Me	PG, PGP-Me
glycolipids	–	MG, DG	MG, DG
Respiratory lipoquinones	MK-8:8 (major) MK-8:7 (minor) MK-7:7 (minor)	MK-8:7 (major) MK-8:8 (minor) MMK-8:7; MMK-8:8 (minor)	MK-8:7; MK-8:8
DNA G + C (%, genomic)	63.0–63.1	63.7–64.6 (4 strains)	63.7
Isolation source	nland hypersaline lakes	nland hypersaline lakes	sea solar saltern

NR, not reported; (v) - variable property in different strains; w (weak); Psr - polysulfide reductase.
Lipids: (PG) phosphatidylglycerol, phosphatidylglycerophosphate (PGP), (PGP-Me) phosphatidyl-glycerophosphate methyl ester, monoglycosyl ether (MG), diglycosyl (DG), (DGE) - dialkyl glycerol ether. Quinones: MK, menaquinone; MMK, methyl-menaquinone.

genus *Halapricum* are shown in Table 2**.** The main difference of the new isolates from the described *Halapricum species* is their ability to utilize insoluble beta-glucans as growth substrates and the absence of glycolipids among the membrane polar lipids. Furthermore, despite an

Table 3
Halapricum hydrolyticum: protologue.

Parameter	Species: *Halapricum hydrolyticum* sp. nov.
Species name	*hydrolyticum*
Genus name	*Halapricum*
Species status	sp. nov.
Etymology	hyd.ro.ly'ti.cum Gr. neut. n. *hydor*, water; Gr. adj. *lytikos*, dissolving, splitting; N.L. neut. adj. *hydrolyticum*, polymer dissolving
Description of the new taxon	The cells are nonmotile coccoids 1–2 μm producing red pigments. The cells lyze in distilled water. The core membrane diether lipids are dominatedby C_{20}-C_{20} DGE (archaeol) and C_{20}-C_{25} DGE (extended archaeol) with 0–3 double bonds. The polar lipid head groups include phosphatidylglycerolphosphate methyl ester (PGP-Me) as a major component and less abundant phosphatidylglycerol (PG). The dominant respiratory quinone is MK-8:8 with the MK-8:7 s in abundance and a minor fraction of MK7:7. It is a saccharolytic and facultatively anaerobic heterotroph. Capable of anaerobic growth either by sugar fermentation or by anaerobic nitrate/nitrite respiration with H_2 as the electron donor presumably to the level of N_2O. Do not grow by anaerobic respiration with sulfur compounds as acceptors. Represents first example of haloarchaea capable of utilizing insoluble beta-1,3-glucans (curdlan and pachyman) for growth. Also can grow with soluble beta-1,3/1,6-glucan laminarin, beta-fructan inulin and alpha-glucans starch and glycogen. The spectrum of utilized sugars include hexoses glucose, fructose, raffinose, trehalose, maltose, sucrose, melezitose and melibiose. Ammonium, but not nitrate or urea, serves as the N-source. Oxidase is weakly positive, catalase is negative. Maximum growth temperature is 48 °C. It is a low Mg-demanding, extreme halophile, with a range of NaCl for growth from 2.5 to 5 M (optimum at 4 M) and a neutrophile, with a pH range for growth from 6.8 to 7.8 (optimum at 7.5). The G + C content of the DNA is 63.0–63.1 % (two genomes). Habitat - hypersaline salt lakes. The type strain (HArc-curdl5-1^T = DSM 114193^T = UQM 41587^T) was isolated from sediments of hypersaline salt lakes in Kulunda Steppe (Altai, Russia). The species also includes a second, closely related strain HArc-curdl7.
Authors	Dimitry Y. Sorokin, Alexander G. Elcheninov, Alexander Y. Merkel, Michel Koenen, Nicole J. Bale and Ilya V. Kublanov
Title	*Halapricum hydrolyticum* sp. nov., a beta-1,3-glucan utilizing haloarchaeon from hypersaline lakes
Journal	Systematic and Applied Microbiology
Corresponding author	Dimitry Y. Sorokin
E-mail of corresponding author	soroc@inmi.ru; d.sorokin@tudelft
Designation of the type strain	HArc-curdl5-1
Strain collection numbers	DSM 114193; UQ 41587
16S rRNA gene accession numbers	Genomic locus tags for HArc-curdl5-1^T: OB916_11930; OB916_16655
Genome assembly accession numbers	GCA_025517535; GCA_025517565
Genome status	Drafts
G + C, %	63.0–63.1 (genomes of 2 strains)
Country of origin	Russian Federation
Region of origin	Altai region
Date of isolation	2015–11-15
Source of isolation	Surface sediments from hypersaline salt lakes
Sampling dates	2015–08-05
Geographic location	south-western Siberia, Kulunda Steppe
Latitude	51°39′ N; 49°10′ N; 48°14′ N
Longtitude	79°48′ E; 46°39′ E; 46°35′ E
Depth	0.05 *m*
Temperature of the sample	20 °C
pH of the sample	7.5–8.0
Salinity of the sample	22–24 %
Number of strains in study	2

(*continued on next page*)

Table 3 (*continued*)

Parameter	Species: *Halapricum hydrolyticum* sp. nov.
Source of isolation of non-type strains	Same as for the type strain
Growth medium, incubation conditions	4 M total NaCl, pH 7; incubation – 35–37 °C; shaker 150 rpm
Conditions of preservation	Deep freezing in 15 % glycerol (v/v)
Gram stain	Negative
Cell shape	Coccoids
Cell size	1–2 μm in diameter
Motility	Nonmotile
Sporulation	None
Colony morphology	Red, convex, smooth, up to 2 mm
Temperature range for growth	nd
Lowest temperature for growth	nd
Highest temperature for growth	45
Optimal temperature for growth	35–40
Lowest pH for growth	6.8
Highest pH for growth	7.8
Optimum pH for growth	7.5
pH category	Neutrophilic
Lowest NaCl concentration for growth	2.5 M
Highest NaCl concentration for growth	5.0 M
Optimum salt concentration for growth	4.0 M
Other salts important for growth	KCl (5 mM); $MaSO_4$ (1–5 mM)
Salinity category	Extremely halophilic
Relation to oxygen	Facultative anaerobe
O_2 conditions for strain testing	Fully aerobic
Carbon source used (class)	Carbohydrates
Specific compounds	beta-1,3-glucans curdlan and pachyman
Nitrogen source	Ammonium
Terminal electron acceptor	O_2, NO_3^- and NO_2^-
Energy metabolism	Chemoorganotrophic
Phospholipids	Core membrane lipids are C_{20}-C_{20} DGE (archaeol) and C_{20}-C_{25} DGE (extended archaeol). Polar head groups are phosphatidylglycerophosphate methylester (PGP-Me) and phosphatidylglycerol (PG)
Respiratory lipoquinones	MK-8:8 (major); MK-8:7 and MK7:7 (minor)
Glycolipids and sulfolipids	Absent
Habitat	Hypersaline lakes
Extraordinary feautures	Utilization of insoluble beta-1,3-glucans for growth

obviously higher phylogenetic proximity of the HArc-curdl isolates to *Halapricum desulfuricans*, the novel glucan-utilizing strains lack the major phenotypic property of the latter, namely its potential for anaerobic sulfur-dependent respiration. Also, methylmenaquinones (known as thermoplasmata quinones) have, so far, been detected only in *H. desulfuricans* among the whole *Halobacteria* class.

Overall, on the basis of distinct phenotypic and genomic features, the two beta-1,3-utilizing isolates from hypersaline salt lakes, HArc-curdl5-1^T and HArc-curdl7, are proposed to be classified in a novel species *Halapricum hydrolyticum* sp. nov. The species protologue is presented in Table 3.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2023.126471.

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