Haloarcula amylolytica sp. nov., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China

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A starch-hydrolysing and extremely halophilic archaeon (strain BD-3^T), isolated from Aibi salt lake in Xin-Jiang, China, was characterized phenotypically and genotypically in order to determine its taxonomic status. On the basis of its polar lipid composition, nucleotide sequences of its 16S rRNA genes, genomic DNA G + C content (62.4 mol%) and growth characteristics, the organism could be assigned to the genus *Haloarcula*. Phenotypic differences and low DNA–DNA hybridization values to related *Haloarcula* species distinguished strain BD-3^T from recognized *Haloarcula* species. It is therefore concluded that strain BD-3^T represents a novel species, for which the name *Haloarcula amylolytica* sp. nov. is proposed. The type strain is BD-3^T (= CGMCC 1.5335^T = JCM 13557^T).

The definition of the genus *Haloarcula* was based on the numerical taxonomy and polar lipid composition given by Torreblanca *et al.* (1986), adopting the generic name first suggested by Javor *et al.* (1982). At the time of writing, the genus *Haloarcula* comprises six recognized species (Fig. 1): *H. vallismortis* (Gonzalez *et al.*, 1978; Torreblanca *et al.*, 1986), *H. argentinensis* (Ihara *et al.*, 1997), *H. hispanica* (Juez *et al.*, 1986), *H. japonica* (Takashina *et al.*, 1990), *H. marismortui* (Elazari-Volcani, 1957; Oren *et al.*, 1990) and *H. quadrata* (Oren *et al.*, 1999). A common feature of representative strains of the genus *Haloarcula* is the presence of at least two heterogeneous 16S rRNA genes. During our surveys on halophilic archaeal diversity of the Aibi salt lake (82° 35′–83° 16′ E 44° 05′–45° 08′ N) in the Xin-Jiang region of China (Cui *et al.*, 2006), a strain that harboured three heterogeneous 16S rRNA genes and that clustered tightly with members of the genus *Haloarcula* was obtained.

Strain BD-3^T was isolated from sediment of Aibi salt lake (pH 8.1, 112.4 g salt l^{−1}), the largest salt lake in the region. The standard growth medium used for strain BD-3^T and other *Haloarcula* strains contained (g l^{−1}): Casamino acids (Difco), 7.5; yeast extract (Difco), 10; trisodium citrate, 3.0; MgCl₂·6H₂O, 10.2; KCl, 20; FeSO₄·7H₂O, 0.05; NaCl, 180

(pH adjusted to 7.2 with 1 M NaOH solution). The methods used for isolation were as described by Xin *et al.* (2000).

Phenotypic tests were performed according to the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). Cell motility and morphology were examined by phase-contrast microscopy of exponentially growing liquid cultures. Gram staining was carried out as described by Dussault (1955). Colony morphology was observed on salt-milk and the standard growth agar medium after incubation at 40 °C for 6–10 days. Anaerobic growth was tested in the presence of 5 g sodium nitrate, L-arginine or DMSO per litre in filled, stoppered tubes. To determine growth pH range (using increments of 0.5 pH units, from pH 5.0 to 10.0), 25 mM MES (pH 5.0–6.7), 25 mM PIPES (pH 6.1–7.5), 25 mM MOPS (pH 6.5–7.9), 25 mM HEPES (pH 6.8–8.2), 25 mM Tricine (pH 7.4–8.8) and 25 mM CHES (pH 8.6–10.0) were used. Tests for catalase and oxidase activities and hydrolysis of starch and of Tweens 20, 40, 60 and 80 were performed as described by Gonzalez *et al.* (1978). Nitrate reduction, H₂S formation, indole formation, and utilization of carbohydrates, sugar alcohols, amino acids and organic acids were examined as described by Oren *et al.* (1997).

Polar lipids were extracted with chloroform/methanol as described by Kamekura (1993). One- and two-dimensional TLC was performed by using silica gel plates (Kieselgel 60 F; Merck) (Kates, 1986).

DNA was extracted by using phenol/chloroform extraction followed by ethanol precipitation, as modified according to Ng *et al.* (1995). The gene encoding 16S rRNA was amplified

The GenBank/EMBL/DDBJ accession numbers for the three 16S rRNA gene sequences of strain BD-3^T are DQ826512, DQ826513 and DQ854818.

A thin-layer chromatogram of total polar lipids from strain BD-3^T and members of the genus *Haloarcula* is available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain BD-3^T and other members of the genus *Haloarcula*. Bootstrap values (expressed as percentages of 1000 replicates) greater than 49% are shown at branch points. Bar, 0.02 expected changes per site.

by PCR with the forward primer 5'-TTCCGGT-TGATCCTGCC-3' (positions 7–23, according to the *Escherichia coli* numbering scheme) and the reverse primer 5'-AAGGAGGTGATCCAGCC-3' (positions 1541–1525), modified according to Achenbach & Woese (1995). PCR was performed for 30 cycles with denaturation for 1 min at 95 °C, annealing for 1 min at 49 °C and polymerization for 90 s at 72 °C. The PCR product was cloned into T-vector, and sequenced by using an ABI BigDye3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). Phylogenetic analyses were conducted using the neighbour-joining method (MEGA version 3.1; Kumar *et al.*, 2004).

The G + C content of the DNA was determined by using the thermal denaturation method (Marmur & Doty, 1962), with a Beckman UV-Vis DU800 spectrophotometer at 260 nm.

DNA–DNA hybridization was carried out by the thermal denaturation and renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983).

Cells of strain BD-3^T were Gram-negative, motile rods, and were able to grow over a wide range of salinities (2.0–5.1 M NaCl; optimal growth at 2.9–3.2 M). Cells lysed in distilled water and 5 % brine. Colonies on salt-milk agar medium were red-pigmented. Phenotypic characteristics of strain BD-3^T are given in the species description below and Table 1. As detailed in Table 1, strain BD-3^T could be phenotypically differentiated from recognized *Haloarcula* species but had very similar phenotypic properties to *H. hispanica* ATCC 33960^T.

The polar lipid composition of strain BD-3^T was determined to be phosphatidylglycerol, phosphatidylglycerol phosphate

Table 1. Differential phenotypic characteristics between strain BD-3^T and other members of the genus *Haloarcula*

Taxa: 1, strain BD-3^T; 2, *H. marismortui* ATCC 43049^T; 3, *H. argentinensis* JCM 9737^T; 4, *H. hispanica* ATCC 33960^T; 5, *H. japonica* TR-1^T; 6, *H. vallismortis* ATCC 29715^T; 7, *H. quadrata* 801030/1^T. +, Positive; –, negative; ±, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Optimum NaCl for growth (%)	3.1	3.4–3.9	2.5–3.0	3.4–4.3	3.4	2.5	2.7–4.3
Cell shape	Rod	Pleomorphic	Triangular	Short rod	Triangular	Rod	Square
Motility	+	±	+	+	+	+	+
Hydrolysis of:							
Starch	+	–	+	+	–	–	+
Tween 80	+	–	+	+	–	–	–
Casein	–	ND	ND	±	–	–	–
Gelatin liquefaction	+	–	+	+	–	–	–
Indole formation	+	–	ND	ND	+	+	–
Utilization of:							
Sorbitol	+	+	–	+	+	–	+
Mannitol	+	+	–	+	+	–	+
D-Ribose	–	+	+	–	+	–	–
DNA G + C content (mol%)	62.4	62.0	62.0	62.7	63.3	64.7	60.1

methyl ester, phosphatidylglycerosulfate, triglycosyl diether and an unknown diglycosyl diether (see Supplementary Fig. S1 in IJSEM Online). The genomic DNA G + C content of strain BD-3^T was 62.4 mol%.

Polymorphism of 16S rRNA genes of strain BD-3^T was observed. Three 16S rRNA genes were detected, designated *rrnA* (1472 bp), *rrnB* (1472 bp) and *rrnC* (1472 bp) (Fig. 1). The *rrnA* gene sequence of BD-3^T showed high similarities to the *rrnA* gene sequence of *H. hispanica* (98.7%), the 16S rRNA gene sequence of *H. argentinensis* (98.6%), and the *rrnB* (98.6%) and *rrnC* (98.6%) gene sequences of *H. marismortui*. The *rrnB* gene sequence of BD-3^T showed highest similarity (99.5%) to the *H. marismortui* *rrnA* gene sequence. The *rrnC* gene sequence of BD-3^T showed highest similarity (98.8%) to the *H. marismortui* *rrnA* gene sequence and *H. quadrata* *rrnB* gene sequence. Levels of similarity between the three 16S rRNA genes of strain BD-3^T were 95.2% (*rrnA* and *rrnB*), 94.6% (*rrnA* and *rrnC*) and 99.0% (*rrnB* and *rrnC*). DNA–DNA hybridization values between strain BD-3^T and *H. japonica* JCM 7785^T, *H. vallismortis* ATCC 29715^T, *H. hispanica* ATCC 33960^T, *H. argentinensis* JCM 9737^T, *H. quadrata* JCM 11048^T and *H. marismortui* ATCC 43049^T were 30, 30, 34, 38, 39 and 39%, respectively.

Based on these results, it is concluded that strain BD-3^T represents a novel species of the genus *Haloarcula*, for which the name *Haloarcula amylolytica* sp. nov. is proposed.

Description of *Haloarcula amylolytica* sp. nov.

Haloarcula amylolytica (a.my.lo.ly'ti.ca. Gr. n. *amylos* starch; Gr. adj. *lytikos* dissolving; N.L. f. adj. *amylolytica* producing lysis of starch).

Cells are rod-shaped (0.6–0.8 × 2.0–2.5 µm), motile and Gram-negative. Colonies on salt-milk and standard growth agar plates containing 18% (w/v) NaCl are red, elevated and round. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 2.0–5.1 M, Mg²⁺ concentrations of 0.005–0.70 M, pH 6.5–9.0 and temperature of 20–52 °C. Optimal NaCl concentration, Mg²⁺ concentration, pH and temperature for growth are 2.9–3.2 M, 0.10–0.30 M, pH 7.0–7.5 and 41 °C, respectively. Catalase- and oxidase-positive. Anaerobic growth occurs in the presence of nitrate with formation of nitrite and gas. No anaerobic growth in the presence of arginine or DMSO. H₂S is produced from Na₂S₂O₃. Indole formation is positive. Tweens 20, 40, 60, 80 and starch are hydrolysed. Casein is not hydrolysed. Gelatin is liquefied. Glucose, sucrose, galactose, sorbitol, mannose, mannitol and maltose are utilized and acids are produced. No growth is observed on fructose, sorbose, xylose, lactose or D-ribose. Sensitive to the following antibiotics: novobiocin (30 µg per disc) and bacitracin (0.04 IU per disc). Resistant to the following antibiotics (µg per disc, unless otherwise indicated): ampicillin (10), rifampicin (5), erythromycin (15), tetracycline (30), ciprofloxacin (5), chloramphenicol (30), kanamycin (30), neomycin (30),

vancomycin (30), norfloxacin (10), streptomycin (10) and penicillin G (10 IU per disc). The molar G + C content of the DNA is 62.4% (*T_m*). The major polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerosulfate, triglycosyl diether and an unknown diglycosyl diether.

The type strain, BD-3^T (= CGMCC 1.5335^T = JCM 13557^T), was isolated from Aibi salt lake in Xin-Jiang, China.

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