Halorubrum arcis sp. nov., an extremely halophilic archaeon isolated from a saline lake on the Qinghai–Tibet Plateau, China

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A Gram-negative, aerobic, neutrophilic and extremely halophilic archaeon (strain AJ201^T), isolated from Ayakekum salt lake on the Qinghai–Tibet Plateau, was investigated by a polyphasic approach. The DNA G+C content of strain AJ201^T was 65.7 mol%. The major polar lipid profile and phylogenetic analysis based on 16S rRNA gene sequences supported the allocation of the strain to the genus *Halorubrum*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain AJ201^T from closely related species. Therefore, strain AJ201^T represents a novel species of the genus *Halorubrum*, for which the name *Halorubrum arcis* sp. nov. is proposed. The type strain is strain AJ201^T (=CGMCC 1.5343^T=JCM 13916^T).

Members of the genus *Halorubrum* (McGenity & Grant, 1995; Kamekura & Dyall-Smith, 1995) are widely distributed in hypersaline habitats (Grant & Larsen, 2001). At the time of writing, there are 16 species with validly published names in the genus *Halorubrum*, including three alkaliphilic species, *Halorubrum vacuolatum* (Mwatha & Grant, 1993; Kamekura *et al.*, 1997), *Hrr. tibetense* (Fan *et al.*, 2004) and *Hrr. alkaliphilum* (Feng *et al.*, 2005), and 13 neutrophilic species, *Halorubrum saccharovorum* (the type species; Tomlinson & Hochstein, 1976), *Hrr. sodomense* (Oren, 1983), *Hrr. lacusprofundi* (Franzmann *et al.*, 1988), *Hrr. coriense* (Nuttall & Dyall-Smith, 1993), *Hrr. trapanicum* (McGenity & Grant, 1995; Trüper, 2003), *Hrr. distributum* (Zvyagintseva & Tarasov, 1987; Oren & Ventosa, 1996), *Hrr. tebenquichense* (Lizama *et al.*, 2002), *Hrr. terrestre* (Ventosa *et al.*, 2004), *Hrr. xinjiangense* (Feng *et al.*, 2004), *Hrr. ezzemoulense* (Kharroub *et al.*, 2006), *Hrr. orientale* (Castillo *et al.*, 2006), *Hrr. lipolyticum* (Cui *et al.*, 2006) and *Hrr. aidingense* (Cui *et al.*, 2006). Here, we report the taxonomic characterization of a neutrophilic strain belonging to the genus *Halorubrum*.

Strain AJ201^T was isolated from mud of Ayakekum salt lake (37° 37′ N 89° 29′ E; 3884 m altitude) located in the Altun Mountain, in the northern part of the Qinghai–Tibet Plateau. The lake environment was described previously in detail (Xu *et al.*, 2005a). The sample was incubated with S-G medium (Sehgal & Gibbons, 1960) for approximately 1 week and the liquid was plated by using a tenfold dilution series method. After 1–2 weeks of incubation at 37 °C, a representative red colony was picked and maintained on S-G medium. The strain was purified by repeated restreaking; purity was confirmed by the uniformity of cell morphology. The optimal conditions for growth were determined in S-G medium modified with 0.85–5.1 M NaCl or 0–1.0 M Mg^{2+}.

Phenotypic tests and lipid analysis were performed according to Xu *et al.* (2005b) as mentioned previously in the minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997). Detailed results of cell morphology, physiological tests and antibiotic sensitivity are given in the species description. The major polar lipids of strain AJ201^T were determined to be $C_{20}C_{20}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl-glycerol diether (Supplementary Fig. S1 in IJSEM Online).

The 16S rRNA gene was amplified and analysed as described previously (Xu *et al.*, 2005b). The almost-complete 16S rRNA gene sequence of strain AJ201^T (1470 nt; nucleotide positions 6–1540 in the *Escherichia coli* numbering) showed 92.9–97.5 % similarity to those of the type strains of *Halorubrum* species. A phylogenetic tree was constructed by the neighbour-joining method with the MEGA3 program package (Kumar *et al.*, 2004), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). The neighbour-joining tree (Fig. 1) showed that strain AJ201^T

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AJ201^T is DQ355793.

Results of one-dimensional TLC of polar lipids of *Hrr. saccharovorum* CGMCC 1.2147^T and strain AJ201^T are available as supplementary material in IJSEM Online.

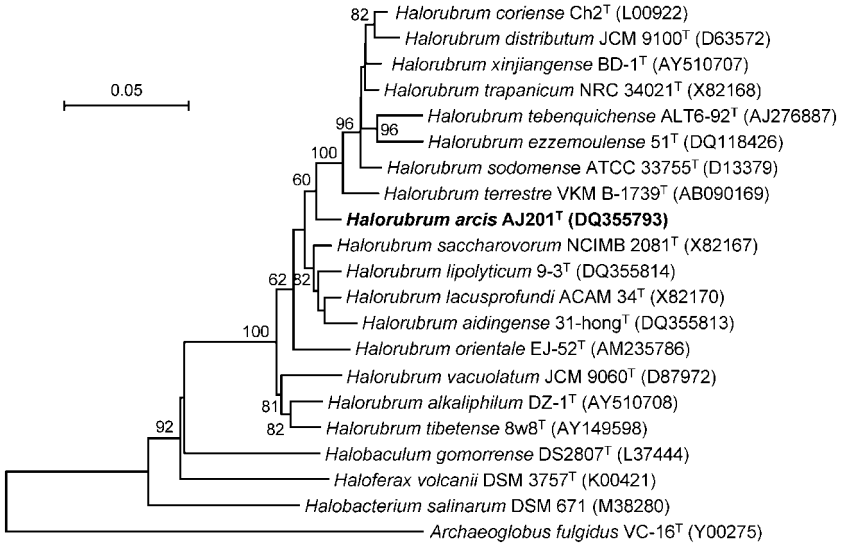

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain AJ201^T and related taxa. Bootstrap values are based on 1000 replicates; only values $\geqslant$60 % are shown. Bar, 0.05 substitutions per nucleotide position.

clustered with the neutrophilic *Halorubrum* species. The DNA G+C content of strain AJ201^T determined by thermal denaturation (T_m) (Marmur & Doty, 1962) was 65.7$\pm$0.5 mol%. DNA–DNA hybridizations were carried out in 2$\times$ SSC at 80 °C by following the procedure of De Ley *et al.* (1970), as modified by Huß *et al.* (1983), using a Beckman Coulter DU800 spectrophotometer. DNA–DNA hybridization experiments (Table 1) showed hybridization values between strain AJ201^T and closely related species of the genus *Halorubrum* ranging between 14 and 46 %, thus

Table 1. Differential phenotypic characteristics of strain AJ201^T and type strains of closely related *Halorubrum* species

Strain: 1, AJ201^T; 2, *Hrr. saccharovorum* CGMCC 1.2147^T; 3, *Hrr. lacusprofundi* CGMCC 1.3490^T; 4, *Hrr. sodomense* CGMCC 1.2206^T; 5, *Hrr. terrestre* JCM 10247^T; 6, *Hrr. coriense* CGMCC 1.3492^T; 7, *Hrr. distributum* CGMCC 1.3491^T; 8, *Hrr. tebenquichense* JCM 12290^T; 9, *Hrr. trapanicum* JCM 10477^T. +, Positive; −, negative; $\pm$, slightly positive; ND, not determined. Data were from our own comparative tests. All strains were positive for catalase and oxidase and produced acid from glycerol. They all showed negative reactions for gelatinase and indole formation.

Characteristic	1	2	3	4	5	6	7	8	9
H_2S formation from thiosulfate	+	+	−	+	−	+	+	+	+
Hydrolysis of:									
Starch	+	−	−	+	−	−	−	−	−
Tween 80	−	−	−	ND	−	−	−	−	ND
Utilization of:									
L-Aspartate	−	−	−	−	−	+	−	+	−
L-Arginine	−	−	−	−	−	−	−	$\pm$	−
Acid production from:									
Fructose	−	$\pm$	−	$\pm$	−	−	−	−	−
Glucose	+	+	−	+	−	+	+	−	+
Mannose	−	$\pm$	−	−	−	−	−	−	−
Starch	$\pm$	+	−	$\pm$	−	−	−	−	−
Arabinose	−	+	−	−	−	−	−	−	−
Maltose	+	+	−	+	−	−	+	−	+
Galactose	−	$\pm$	$\pm$	+	−	+	$\pm$	+	+
D-Ribose	−	+	−	−	−	−	−	−	−
Sucrose	−	$\pm$	−	+	−	−	−	−	$\pm$
D-Xylose	−	+	−	$\pm$	−	−	−	−	−
DNA–DNA hybridization with strain AJ201^T (%)	100	17	21	44	44	46	23	27	40

indicating that this strain represents a novel species of the genus *Halorubrum*.

The phenotypic and polar lipid characteristics suggest that strain AJ201^T is a member of the genus *Halorubrum*. Differential phenotypic properties shown in Table 1 as well as 16S rRNA gene sequence analysis and DNA–DNA hybridization data justify the creation of a novel species, for which we propose the name *Halorubrum arcis* sp. nov.

Description of *Halorubrum arcis* sp. nov.

Halorubrum arcis (ar′cis. L. gen. n. *arcis* of a height, summit or peak, referring to the isolation of the type strain from a saline lake on the Qinghai–Tibet Plateau).

Gram-negative and motile. Young cultures show rod-like cells (0.5–1 × 2–4 μm). Colonies on complex agar medium are 1–2 mm in diameter, smooth, circular, elevated and red. Halophilic. Cells lyse immediately in distilled water. NaCl concentration required for growth is 2.2–5.2 M, with an optimum at 3.4–3.9 M. Magnesium is not required for growth, and the optimum Mg^{2+} concentration is 20–200 mM. The pH and temperature for growth are pH 6.0–8.5 (optimum pH 7.5) and 25–55 °C (optimum 42 °C). Chemo-organotrophic and aerobic. Oxidase- and catalase-positive. Anaerobic growth does not occur with arginine or DMSO. Nitrate is reduced to nitrite. H_2S is produced from thiosulfate. Starch is hydrolysed. Tween 80 and casein are not hydrolysed. Gelatin is not liquefied. Casamino acids are required for growth. The following substrates are utilized for growth: glucose, maltose, glycerol and starch. No growth is observed on arabinose, fructose, galactose, lactose, mannitol, mannose, rhamnose, ribose, sorbitol, sucrose, xylose, alanine, arginine, aspartate, glycine, glutamate, lysine, ornithine, acetate, citrate, fumarate, lactate, malate, propionate, pyruvate or succinate. Acid is produced from glucose, maltose and glycerol. Sensitive to novobiocin, bacitracin, anisomycin and rifampicin, but not to ampicillin, chloramphenicol, erythromycin, neomycin or penicillin. The major polar lipids are $C_{20}C_{20}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and sulfated mannosyl-glucosyl-glycerol diether. The DNA G+C content of the type strain is 65.7 ± 0.5 mol% (T_m).

The type strain is strain AJ201^T (=CGMCC 1.5343^T=JCM 13916^T), isolated from a salt lake on the Qinghai–Tibet Plateau.

Acknowledgements

We thank Jean Euzéby for his help with the specific etymology and nomenclature. This work was supported by grants from the Major State Basic Research Development Program of China (973 Program) (grant no. 2004CB719604-3), the National Natural Science Foundation of China (grant no. 30670048) and the China Postdoctoral Science Foundation (grant no. 2005038636).

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