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ORIGINAL PAPER

Saccharopolyspora qinghaiensis sp. nov., a novel actinobacterium isolated from a salt lake

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Abstract A novel halophilic, Gram-positive and aerobic actinobacterium, designated strain AFM 20147^T, was isolated from a sediment sample collected from Xiaochaidan Salt Lake of Qinghai, China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain AFM 20147^T belongs to the genus *Saccharopolyspora*, shows high sequence similarities to *Saccharopolyspora griseoalba* AFM 10238^T (99.41%) and *Saccharopolyspora halophila* YIM 90500^T (98.20%), and has low similarities (below 98.0%) with other members of the genus. The DNA–DNA relatedness values of strain AFM 20147^T with *S. griseoalba* AFM 10238^T and *S. halophila* YIM 90500^T were 40 ± 1.7% and 37 ± 2.3%, respectively. Optimal growth was found to occur at 28 °C, pH 7.5 and in the presence of 7.5% (w/v) NaCl. Strain AFM 20147^T was found to contain *meso*-diaminopimelic acid as the cell wall diamino acid, and galactose and arabinose as the whole cell sugars. The major fatty acids were identified as iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The major polar lipids were identified as diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylinositol and phosphatidylcholine. MK-9(H_4) was found to be the predominant menaquinone and the DNA G+C content was determined to be 67.8 mol%. DNA–DNA relatedness data, together with phenotypic and chemotaxonomic differences, clearly distinguish the isolate from its close neighbours. On the basis of the data from this polyphasic analysis, a novel species *Saccharopolyspora qinghaiensis* sp. nov. is proposed. The type strain is *S. qinghaiensis* AFM 20147^T (=KCTC 49190^T =CGMCC 4.7556^T).

Keywords Halophilic actinobacteria · *Saccharopolyspora qinghaiensis* · Polyphasic taxonomy

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Introduction

The genus *Saccharopolyspora* was first established by Lacey and Goodfellow (1975) with the description of *Saccharopolyspora hirsuta* as the type species, and

emended subsequently by Warwick et al. (1994). The genus was assigned to the family *Pseudonocardiaceae* (Embley et al. 1988; Stackebrandt et al. 1997; Zhi et al. 2009). The genus currently encompasses 31 species and 3 subspecies with validly published names (http://www.bacterio.net/saccharopolyspora.html). Members of the genus *Saccharopolyspora* are aerobic, Gram-positive actinobacteria. The substrate hyphae fragment into rod-shaped elements and the aerial hyphae segment into bead-like chains of spores. The cell wall composition is *meso*-diaminopimelic acid, arabinose and galactose as the characteristic sugars in whole cell hydrolysates, and also iso- and anteiso-branched fatty acids. The predominant menaquinone type is MK-9(H_4) (Embley et al. 1987; Goodfellow et al. 1989). Their DNA G+C contents are in the range 66–77 mol% (Goodfellow et al. 1989). Members of the genus *Saccharopolyspora* have been isolated from a broad range of habitats, including plant materials, soil, marine sponge, saline lake, compost, manure, mouldy hay and a clinical sample (Yang et al. 2018). The primary reservoir of *Saccharopolyspora* is the soil (Veyisoglu et al. 2017).

The present polyphasic study was designed to establish the taxonomic status of a putatively novel *Saccharopolyspora* strain, AFM 20147^T, isolated from a soil sample collected from Xiaochaidan salt lake of Qinghai Province, China. The data show that isolate AFM 20147^T represents a new *Saccharopolyspora* species for which the name *Saccharopolyspora qinghaiensis* is proposed.

Materials and methods

Isolation and preservation

The actinobacterial strain, AFM 20147^T, was isolated from Xiaochaidan salt lake of Qinghai Province (July 27th, 2017; 37.522494°N, 95.514014°E), in an investigation of the phylogenetic diversity of bacteria in salt soil. The strain was isolated using the standard dilution plate method and grew on ISP 5 medium (Shirling and Gottlieb 1966) with 10% NaCl, after 15 days of aerobic incubation at 28 °C. The ISP 5 medium contained (per liter) L-asparaginic acid 1.0 g, glycerin 10.0 g, K_2HPO_4 1.0 g, microelement solution 1 mL, 20 g agar. The strain was maintained on modified Tryptic Soy Agar (TSA; Pankreatisch abgebautes Casein 15.0 g, Papainisch abgebautes Soja 5.0 g, NaCl 5.0 g, 18 g agar, 1 L distilled water) medium slants containing 5% (w/v) NaCl at 4 °C and as suspensions of mycelial fragments in glycerol (20%, v/v) at − 80 °C.

Phenotypic, physiological and biochemical characteristics

Morphological characteristics of strain AFM 20147^T were determined by scanning electron microscopy (S-4800; Hitachi) after the culture was grown on TSA medium containing 5% (w/v) NaCl at 28 °C for 7 days. Cultural characteristics were determined after incubation for 2–3 weeks on Czapek's agar (Waksman 1967), nutrient agar, potato dextrose agar and ISP 2-5 (Shirling and Gottlieb 1966) media supplemented with 5% (w/v) NaCl at 28 °C. The colours of substrate and aerial mycelia and any soluble pigments were determined by comparison with chips from the ISCC-NBS colour charts (Kelly 1964). Growth at different temperatures was tested at 10, 15, 20, 28, 35, 40, 45 and 50 °C in TSA medium with 5% (w/v) NaCl for a week. The pH tolerance was tested at pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0 at 28 °C in TSB medium with 5% (w/v) NaCl for a week. Tolerance of salt was tested by supplementing TSA medium with various concentrations of NaCl (0, 1.0, 3.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, 10.0, 13.0, 15.0, 17.0,17.5, 18.0, 19.0 and 20.0%, w/v) (Xu et al. 2005). Gram staining was carried out by using the standard Gram reaction (Murray et al. 1994). Carbon source and nitrogen source utilisation were determined according to the methods described by Smibert (1994) with various substrates and 5% NaCl. Catalase activity was tested using 3% (w/v) H_2O_2 by assessing bubble production as the positive result, according to the methods used by Smibert (1994). Other biochemical characteristics including hydrolysis of aesculin, casein, chitin, gelatin and Tweens (20 and 80), H_2S production and nitrate reduction were observed as previously described (MacFaddin 1976; Gonzalez et al. 1978; Smibert 1994). Antibiotic susceptibility tests were performed by the agar-diffusion method on TSA agar (28 °C, 3 days) after plating with bacterial suspensions equivalent to 0.5 McFarland standards.

Chemotaxonomic characterisation

Biomass used for chemical studies was obtained from cultures grown on TSB liquid medium with 5% (w/v) NaCl for 5 days at 28 °C. Whole cell sugars and cell wall amino acids were detected by HPLC after precolumn derivatisation with 1-phenyl-3-methyl-5-pyrazolone (PMP) (Tang et al. 2009a). Polar lipids were extracted, separated by two-dimensional TLC and identified using previously described procedures (Minnikin et al. 1984). Menaquinones were isolated according to Collins (1994) and were analysed by HPLC (Kroppenstedt 1982). Extraction and analysis of fatty acids were performed as described by Sasser (1990) by using the microbial identification system (MIDI) (Sherlock version 6.1; MIDIdatabase TSB A6). The G+C content of the DNA was determined according to the method of Marmur (1961) and were determined by HPLC after enzymatic degradation (Mesbah et al. 1989) using *Escherichia coli* strain DH5α as the reference.

Molecular characterisation and DNA–DNA hybridization

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene sequence were carried out as described by Li et al. (2007). The 16S rRNA gene sequence was compared with available 16S rRNA gene sequences of previous cultured species from GenBank via the BLAST program and from the EzBioCloud server databases (http://eztaxon-e.ezbiocloud.net/; Yoon et al. 2017). Phylogenetic trees were constructed using MEGA version 5.0 software package (Tamura et al. 2011) with three tree-making algorithms: neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) respectively. Kimura's two-parameter model (Kimura 1980) was used to calculate evolutionary distance matrices of the neighbour-joining method and maximum-likelihood method. The topologies of the phylogenetic trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. DNA–DNA hybridization experiments were carried out between strain AFM 20147^T and its near phylogenetic neighbours using the method described by Ezaki et al. (1989) and He et al. (2005). Six replications were done for each sample and the two extreme values (highest and lowest) for each sample were excluded. The relatedness values were expressed by calculating the means of the remaining values.

Results and discussion

Phenotypic characteristics

Strain AFM 20147^T was observed to be an aerobic, Gram-positive filamentous actinobacterium. It was found to show good growth on TSA, ISP 5, potato dextrose agar and nutrient agar media; weak growth on ISP 3 and ISP 4 agar media; and no growth on Czapek's agar and ISP 2. The colour of the aerial mycelia was observed to be light yellow gray on TSA, ISP 5, potato dextrose agar and nutrient agar media, light gray on ISP 3 and ISP 4. The colour of the substrate mycelia was light yellow on TSA, ISP 5 and nutrient agar media, light gray on potato dextrose agar, ISP 3 and ISP 4. No soluble pigment was observed to be produced. The substrate mycelium was observed to be branched and well developed; Sparse aerial mycelium was found to form long chains of spores that were non-motile and oval in shape with smooth surfaces (approximately 0.6 μm in width and 0.6–1.0 μm in length) (Supplementary Fig. S1). Temperature and pH ranges for growth of strain AFM 20147^T were 15–40 °C and pH 6.0–9.0, with optimal at 28 °C and pH 7.5. The NaCl concentration range for growth was found to be 3–17.5%, with optimal growth occurring at 7.5%. Strain AFM 20147^T can utilise cellobiose, fructose, D-galactose, D-glucose, D-mannose, D-sorbitol, starch, sucrose, D-mannitol and inositol, but does not utilise D-arabinose, dextrin, D-galactitol, lactose, maltose, L-raffinose, rhamnose, salicin, D-ribose, L-sorbose, D-trehalose and D-xylose as sole carbon sources. Strain AFM 20147^T can utilise L-alanine, L-arginine, glycine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-tyrosine, L-valine, ammonium acetate, ammonium nitrate, diammonium phosphate and potassium nitrate, but does not utilise L-aspartic acid, L-cysteine, L-serine, L-threonine, D-glutamic acid and ammonium citrate as sole nitrogen sources. The strain was found to be sensitive to amikacin (10 μg), erythromycin (15 μg), chloramphenicol (30 μg), norfloxacin (10 μg), cefazolin sodium (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), rifamycin (5 μg) and vancomycin

Table 1 Differential characteristics of strain AFM 20147^T and closely related members of the genus *Saccharopolyspora*

Characteristics	1*	2#	3#
Fragments of substrate mycelium	+	+	−
Colour of:			
Aerial mycelia	Light yellow gray to light gray	Light gray to white	White–yellow
Substrate mycelia	Light yellow to light gray	Light yellow to yellow white	Yellow to orange–yellow
Range for growth:			
Temperature (°C) (optimum)	15–40 (28)	15–45 (28)	10–45 (28–37)
NaCl (%, w/v) (optimum)	3–17.5 (7.5)	0–15 (10)	3–20 (10–15)
pH (optimum)	6–9 (7.5)	6–10 (7.5)	6–8.5 (7–8)
Enzyme activity:			
Urease	+	−	+
Nitratase	−	−	+
Amylase	+	+	−
Gelatin hydrolytic enzyme	+	+	+
Casein hydrolase enzyme	−	+	+
Utilisation of:			
Arabinose	−	+	+
Raffinose	−	+	+
Maltose	−	+	+
Lactose	−	+	+
Rhamnose	−	+	+
Trehalose	−	+	−
Mannitol	+	−	+
Xylose	−	+	+
Proline	+	+	−
Ornithine	+	+	−
Valine	+	−	+
Cycteine	−	+	+
Threonine	−	+	+
Phospholipids	DPG,PME,PE,PG,PC,PI,DPG	PC,PG,DPG	PC,PG,PI
Predominant menaquinone	MK-9(H_4)	MK-9(H_4)	MK-9(H_2)
	MK-7(H_2)	MK-9(H_6)	MK-9(H_4)
	MK-7(H_6)	MK-9(H_8)	MK-9(H_6)
	MK-9(H_6)	MK-8(H_4)	
	MK-8(H_4)		
	MK-9(H_2)		
Fatty acids	iso-C15:0 (26.9)	iso-$C_{15:0}$ (10.3)	iso-$C_{15:0}$ (16.9)
	iso-C16:0 (11.6)	iso-$C_{16:0}$ (25.5)	iso-$C_{16:0}$ (11.7)
	anteiso-C17:0 (26.1)	anteiso-$C_{17:0}$ (18.1)	anteiso-$C_{17:0}$ (28.7)
		iso-$C_{17:0}$ (11.4)	
		$C_{17:1}$ ω8c (11.3)	
G+C content (mol%)	67.8	72.7	66.3

1. *Saccharopolyspora qinghaiensis* AFM 20147^T; 2. *S. griseoalba* AFM 10238^T from our lab; 3. *S. halophila* YIM 90500^T was bought from KCTC. The symbols denote for negative (−) or positive (+) ability in case of given characteristic. Data are from this study and from Jiang et al. (2016) and Tang et al. (2009b)

*Data were obtained from this study

#Data were obtained from a previous study

Table 2 Cellular fatty acid composition of strain AFM 20147^T, *S. griseoalba* AFM 10238^T and *S. halophila* YIM 90500^T

Fatty acid	1	2	3
Straight-chain			
$C_{16:0}$	3.03	1.18	4.70
$C_{17:0}$	1.22	3.42	1.03
$C_{18:0}$	0.84	0.42	0.96
Branched-chain			
iso-$C_{15:0}$	26.93	10.27	16.87
anteiso-$C_{15:0}$	0.57	2.41	7.95
iso-$C_{16:0}$	11.58	25.48	11.72
iso-$C_{17:0}$	2.69	11.39	6.20
anteiso-$C_{17:0}$	26.08	18.08	28.67
$C_{17:0}$ 3-OH	–	–	1.53
iso-$C_{18:0}$	–	0.26	1.72
Unsaturated			
$C_{17:1}$ ω8c	–	11.34	1.78
10-methyl-$C_{17:0}$	1.76	2.04	1.20
$C_{18:1}$ ω9c	1.28	1.03	1.01
Summed feature			
3	1.44	2.39	4.00
9	–	3.38	4.63

1. *Saccharopolyspora qinghaiensis* AFM 20147^T; 2. *S. griseoalba* AFM 10238^T; 3. *S. halophila* YIM 90500^T. All data are from this study

(30 μg), but resistant to sulfamethoxazole (30 μg), penicillin G (10 IU), amikacin (30 μg), gentamicin (10 μg) and streptomycin (10 μg). Other detailed physiological and biochemical properties that differentiate between strain AFM 20147^T and closely related *Saccharopolyspora* species are given in the species description and also summarised in Table 1.

Chemotaxonomic characteristics

Strain AFM 20147^T was found to contain *meso*-diaminopimelic acid as the diagnostic cell wall amino acid, with galactose and arabinose as the whole cell sugars. The polar lipid profile for strains AFM 20147^T was found to consist predominantly of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and three unidentified polar lipid (Supplementary Fig. S2). The predominant menaquinone was identified as MK-9(H_4) (72.9%) and minor amounts of MK-7(H_2) (8.0%), MK-7(H_6) (6.9%), MK-9(H_6) (5.4%), MK-8(H_4) (3.7%) and MK-9(H_2) (2.0%) were also detected. The major respiratory quinone of strain AFM 20147^T is consistent with those reported for the strains of the genus *Saccharopolyspora*. The major fatty acids were identified as iso-$C_{15:0}$ (26.9%), anteiso-$C_{17:0}$ (26.1%) and iso-$C_{16:0}$ (11.6%). Detailed fatty acid profiles of strain AFM 20147^T and other type strains of the genus *Saccharopolyspora* are given in Table 2. The cellular fatty acid profile of strain AFM 20147^T mainly consisted of iso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$, this being similar to strain *S. halophila* YIM 90500^T but in different proportions; strain *S. griseoalba* AFM 10238^T contained iso-$C_{17:0}$ (11.4%) and $C_{17:1}$ ω8c (11.3%) as its major fatty acids, whereas strain AFM 20147^T contained only a little iso-$C_{17:0}$. The DNA G+C content of strain AFM 20147^T was determined to be 67.8 mol%.

Molecular characteristics and DNA–DNA hybridization

The comparison of the 16S rRNA gene sequence (1514 nucleotides, GenBank/EMBL/DDBJ Accession Number EU305728) of strain AFM 20147^T with the available 16S rRNA gene sequences from GenBank by using the EzTaxon-e database revealed that strain AFM 20147^T shows high sequence similarities with members of the genus *Saccharopolyspora*. Based on the pairwise comparison of 16S rRNA gene sequences, the closely related type strains of strain AFM 20147^T are *Saccharopolyspora griseoalba* AFM 10238^T (99.41%) and *Saccharopolyspora halophila* YIM 90500^T (98.20%), and has low similarities (below 98.0%) with the sequences of other members of the genus. The phylogenetic tree generated using the neighbour-joining method showed that strain AFM 20147^T formed a distinct phylogenetic lineage within the genus *Saccharopolyspora* (Fig. 1). It formed a close relationship with *S. griseoalba* AFM 10238^T and is located in the same cluster as *S. halophila* YIM 90500^T, which was also supported by the ML and MP algorithms (supplementary Figs. S3 and S4). Recently, it has been suggested that 98.7% 16S rRNA gene sequence similarity can be used as a guideline to avoid laborious DNA–DNA hybridization experiments in species delineation because the 16S rRNA gene sequence similarity equates to 70% DNA–DNA

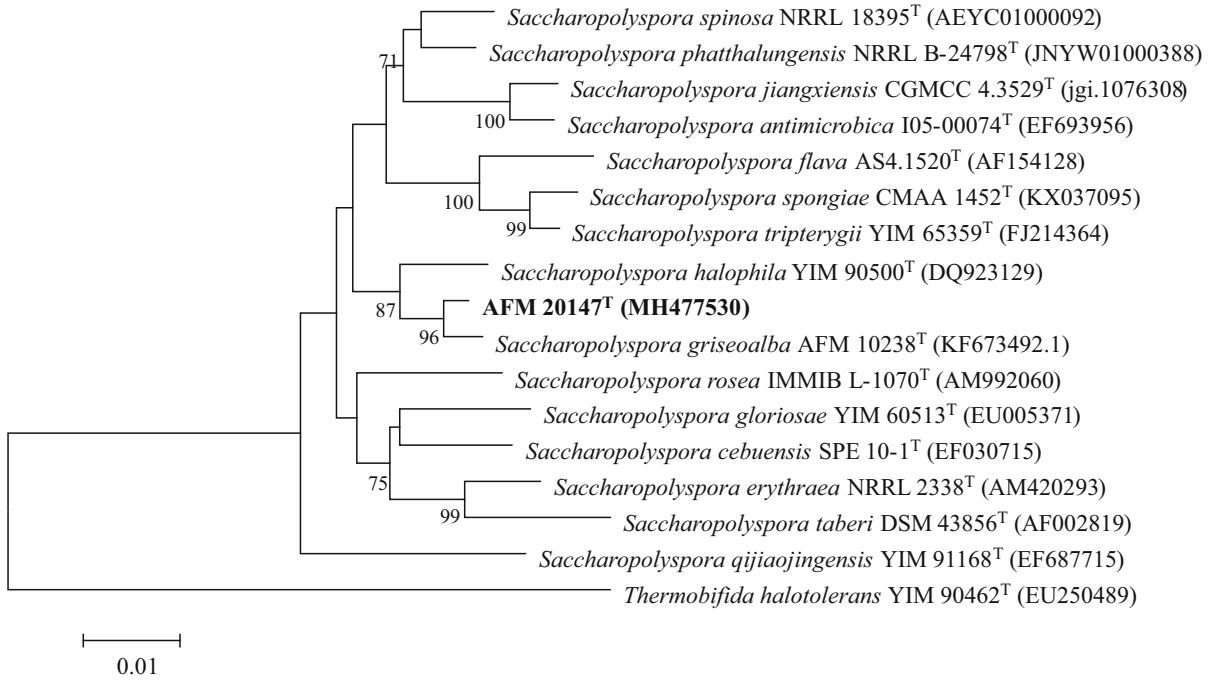

Fig. 1 Phylogenetic tree generated with the neighbor-joining algorithm based on 16S rRNA gene sequences showing the phylogenetic positions of strain AFM 20147T and related taxa. Bootstrap values with more than 70% are shown on the nodes as percentages of 1000 replicates. Thermobifida halotolerans YIM 90462T (EU250489) was used as an outgroup. The scale bar equals 0.01 changes per nucleotide position

relatedness between two strains (Vahed et al. 2018; RossellÓ-MlÓra and Amann 2015; Kim et al. 2014). Therefore, DNA–DNA hybridization experiments between strain AFM 20147^T and the type strains of *S. griseoalba* AFM 10238^T and *S. halophila* YIM 90500^T were performed. The DNA–DNA relatedness values of strains AFM 20147^T with *S. griseoalba* AFM 10238^T and *S. halophila* YIM 90500^T were determined to be 40 ± 1.7% and 37 ± 2.3%, respectively, which are below the 70% cut-off point recommended for the delineation of prokaryotic genomic species (Wayne et al. 1987). These results clearly indicate that strain AFM 20147^T represents a novel species of the genus *Saccharopolyspora*.

The morphological features, chemotaxonomic properties and the phylogenetic data clearly indicated that strain AFM 20147^T is a member of the geuns *Saccharopolyspora*. However, strain AFM 20147^T can be distinguished from its close phylogenetic neighbours by using phenotypic and chemotaxonomic characteristics (Table 1), such as differences in growth conditions (temperature and pH ranges for growth), in the hydrolysis of casein, starch and urea, in nitrate reduction, utilisation of carbon and nitrogen sources and in the profiles of menaquinones and polar lipids, as well as the proportions of some fatty acids (Table 2). In conclusion, based on the phenotypic, chemotaxonomic and phylogenetic data presented, strain AFM 20147^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora qinghaiensis* sp. nov. is proposed. The taxonumber in the Digital Protologue Database (DPD) is TA00860.

Description of *Saccharopolyspora qinghaiensis* sp. nov.

Saccharopolyspora qinghaiensis (qing.hai.en'sis.N.L. fem. adj. *qinghaiensis* referring to Qinghai Province, China, where the sample from which the type strain wad isolated was collected).

Gram-stain positive, halophilic, filamentous actinobacterium. Sparse aerial mycelium forms long chains of spores that are non-motile and oval in shape

with smooth surfaces (approximately 0.6 μm in width and 0.6–1.0 μm in length) after 7 days of growth on TSA agar. The substrate mycelia are light yellow to light gray, well developed, and fragment into rod shaped elements. No soluble pigment is produced. Temperature, pH, and NaCl ranges for growth are 15–40 °C, pH 6–9 and 3–17.5% (w/v). The optimal growth is at 28 °C, pH 7.5 and NaCl 7.5%. Cells are positive for catalase, indole production, milk peptonisation and hydrolysis of urea, starch, Tweens 20 and gelatin. Negative for nitrate reduction, methyl red and Voges–Proskauer test, H_2S production and hydrolysis of cellulose. The cell wall peptidoglycan contains *meso*-diaminopimelic acid as the principal diamino acid. Whole cell sugars are galactose and arabinose. MK-9(H_4) is the predominant menaquinone. The main cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The polar lipids are diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and three unidentified polar lipid. The G+C content of the type strain is 67.8 mol%.

The type strain, AFM 20147^T (= KCTC 49190^T = CGMCC 4.7556^T), was isolated from a soil sample collected from Xiaochaidan salt lake of Qinghai Province, China. The GenBank accession number for the 16S rRNA gene sequence of strain AFM 20147^T is MH477530.

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Author contributions YY.J, HX.L, QH.X and XM.W conceived and designed the research. YY.J, HX.L and XM.W planned experiments. YY.J, CF.M and ZH.Z performed experiments, YY.J and Q.G performed data interpretation. YY.J wrote the manuscript. QH.X and XM.W contributed substantially to the revision of the manuscript. All authors approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no direct or indirect conflict of interest.

Ethical approval This is the original work of the authors. This article does not contain any studies with human participants or animals performed by any of the authors.

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