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ORIGINAL PAPER

Aidingibacillus halophilus gen. nov., sp. nov., a novel member of the family *Bacillaceae*

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Abstract A Gram-positive, non-motile, asporogenous and aerobic bacterium, designated YIM 98012^T, was isolated from a salt lake in China. Strain YIM 98012^T was found to be catalase and oxidase positive. Optimal growth of strain YIM 98012^T was observed at 37 °C and pH 7.0 and it was found to grow in the presence of 5–20% (w/v) NaCl (optimum 10% NaCl). Phylogenetic analysis based on the 16S rRNA gene sequence indicated that the novel strain is affiliated with the family *Bacillaceae* of the phylum *Firmicutes* and that it shares high (94.7%) sequence similarity with *Alteribacillus persepolensis* DSM 21632^T and does not show sequence similarities of more than 94.0% to known members of other related genera. The major fatty acids (> 10%) were identified as anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{16:0}$. The genomic DNA G+C content was determined to be 41.0 mol% and the dominated respiratory quinone was identified as MK-7. The cell wall peptidoglycan of strain YIM 98012^T was found to contain *meso*-diaminopimelic acid, while the polar lipids profile was found to include diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. Based on physiological and chemotaxonomic characteristics, strain YIM 98012^T is concluded to be the type strain of the type species of a novel genus in the family *Bacillaceae* for which the name *Aidingibacillus halophilus* gen. nov., sp. nov. is

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proposed. The type strain is YIM 98012^T (= KCTC 33868^T = DSM 104332^T).

Keywords *Bacillaceae* · *Aidingibacillus halophilus* gen. nov., sp. nov. · Salt lake · Polyphasic taxonomy

Introduction

The family *Bacillaceae* in the order *Bacillales* is a large taxonomic group currently containing more than 55 genera with many different physiological features (http://www.bacterio.net/). Based on 16S rRNA gene sequence and chemotaxonomic analyses, many bacteria which were originally placed in the genus *Bacillus* have been reclassified as members of novel genera or transferred to other genera (Claus and Berkeley 1986). Halophilic microorganisms are also often found in the family *Bacillaceae*, such as members of the genera *Halobacillus* (Spring et al. 1996), *Oceanobacillus* (Lu et al. 2001), *Ornithinibacillus* (Mayr et al. 2006), *Terribacillus* (An et al. 2007), *Sediminibacillus* (Carrasco et al. 2008), *Streptohalobacillus* (Wang et al. 2011), *Saliterribacillus* (Amoozegar et al. 2013), *Aquibacillus* (Amoozegar et al. 2014) and *Sinibacillus* (Yang and Zhou 2014).

In this study, we describe a novel moderately halophilic, Gram-positive, asporogenous bacterial strain, designated YIM 98012^T, isolated from sediment from Ayding Lake, a saline lake, in Xinjiang, China. The comparative analysis of 16S rRNA gene sequences showed that the strain phylogenetically belongs to the family *Bacillaceae* but does not fall into any currently defined genera. Strain YIM 98012^T was subjected to a polyphasic taxonomic characterisation based on physiological and chemotaxonomic characteristics and identified as the type strain of the type species of a novel genus, *Aidingibacillus* gen. nov.

Materials and methods

Isolation of the bacterial strain and culture conditions

Strain YIM 98012^T was isolated from sediment samples collected from Ayding Lake, which is situated in the southern part of Turpan Basin in Xinjiang Uygur Autonomous Region, north-west China. The lake is 155 m below sea level, and is the lowest place in China and the second lowest place in the world, next only to the Dead Sea (− 391 m) of Jordan. The pH of the saline soil ranges between 8.0 and 8.5 and the salinity ranges from 36 to 112 (g kg^{-1} soil). The major ion contents of the sediment samples were measured (g kg^{-1} soil): Na^+ (10.6–34.3), Cl^- (16.2–82.0), SO_4^{2-} (6.8–23.5), K^+ (0.06–0.1), Ca^{2+} (2.2–4.5), Mg^{2+} (0.2–0.8) and HCO_3^- (0.1–0.4). The novel strain was isolated by plating on the Cellulose–casein multi-salts medium described by Tang et al. (2008) and incubating at 37 °C aerobically. The strain was maintained on GTY medium slants containing 10% NaCl (w/v) at 4 °C and as 20% (w/v) glycerol suspensions at − 20 °C. The GTY medium contained [(l distilled water)$^{-1}$]: 0.5 g tryptone, 2 g yeast extract, 1 g glucose, 0.5 g $CaCO_3$, 100 g NaCl and 15 g agar. The pH of the medium was adjusted to pH 7.5 (Tang et al. 2009).

Morphological, physiological, biochemical and chemotaxonomic characterisation

For phenotypic characterisation of strain YIM 98012^T, standard tests were performed. The recommended minimal standards for describing new taxa of aerobic, endospore-forming bacteria were followed (Logan et al. 2009). Cell morphology was examined with a microscope equipped with phase-contrast optics (Olympus) and transmission electron microscopy (JEM2100; JEOL), using cells from exponentially growing cultures. Gram staining was carried out by the standard Gram reaction and was confirmed using the KOH lysis test method (Cerny 1978). Gliding motility was tested in 0.2% GTY medium by using a hanging-drop technique (Bernardet et al. 2002). Motility was confirmed by puncture inoculation in semisolid agar medium. Spore formation was tested by staining with malachite green.

The temperature range (0, 4, 10, 15, 20, 28, 30, 37, 40, 45, 50 and 55 °C) and pH range (4.0–10.0, at intervals of 1 pH unit) for colony growth were determined by incubating the isolate for 2 weeks on GTY medium containing 10% NaCl. The buffers for pH tests were prepared according to the method described previously by Tang et al. (2009). The salt concentration (NaCl) range from 0 to 30% (w/v) at intervals of 1% were tested by using GTY agar without

adding any salts as the basal medium. Catalase activity was determined by production of bubbles after the addition of a drop of 3% (v/v) H_2O_2. Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Reduction of nitrate, the methyl red and Voges–Proskauer tests, hydrolysis of aesculin, gelatin, casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Dong and Cai (2001). Enzyme activities and acid production from carbohydrates were determined by API 20NE, API ZYM and API 50CH strips (bioMérieux) according to the instructions of the manufacturer. Strains were prepared using pre-warmed sterile saline medium (10% NaCl), within the density range specified by the manufacturer. Carbon-source utilisation tests were performed according to the methods described by Shirling and Gottlieb (1966). Nitrogen-source utilisation tests were analysed as described by Williams et al. (1983). Antibiotic sensitivity was explored by placing commercial antibiotic discs (OXOID) on GTY agar plates that had been spread with the isolates and then incubated at 37 °C for 7 days. Anaerobic growth was tested for up to 2 weeks on GTY medium containing 10% NaCl in a jar containing AnaeroPack-Anaero (Mitsubishi Gas Chemical Co, Inc), which works as an O_2 absorber and CO_2 generator.

Diaminopimelic acid isomers were analysed using the method of Komagata and Suzuki (1988). For the analysis of fatty acids, strains YIM 98012^T was cultured on tryptic soy agar (TSA; Difco) containing 10% NaCl at 37 °C for 72 h and fatty acid methyl esters were extracted and gas chromatography was performed as described by Sasser (1990) using the microbial identification system (MIDI) according to standard protocols. Isoprenoid quinones were extracted and purified as described by Collins et al. (1977). The purified menaquinones were dissolved in acetone and separated by reverse-phase HPLC. Polar lipids were extracted and identified by two-dimensional TLC (Minnikin and Goodfellow 1979).

Phylogenetic analysis and DNA G+C content analysis

Genomic DNA was prepared by using a commercial DNA extraction kit (Quick-DNA^{TM} Bacterial Miniprep Kit; Zymo research). The G+C contents of the DNAs were determined by reverse-phase HPLC (Mesbah et al. 1989). An approximately 1500 bp long fragment of the 16S rRNA gene was amplified from the extracted DNA by using bacterial universal primers specific to the 16S rRNA gene: 27F and 1492R (*Escherichia coli* numbering system, Weisburg et al. 1991). The obtained sequence was compared with 16S rRNA reference gene sequences retrieved from the GenBank and EMBL database by BLAST search and similarity searches were performed using the EzBioCloud Database (http://eztaxon-e.ezbiocloud.net/) (Yoon et al. 2016); phylogenetically closely related sequences belonging to the family *Bacillaceae* were downloaded from the NCBI database. These sequences were aligned using the ClustalW program integrated in the MEGA 6.0 software (Tamura et al. 2013). Alignment gaps and ambiguous bases were not taken into consideration when 1524 bases of the 16S rRNA gene were compared. Evolutionary distances (distance options according to Kimura's two-parameter model) were calculated. Phylogenetic analyses were performed using three tree-making algorithms that were the neighbour-joining, maximum-likelihood and maximum-parsimony methods. A phylogenetic tree was constructed using the neighbour-joining method using MEGA version 6.0. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein 1985).

Results and discussion

Morphological, physiological and biochemical characteristics

Cells of strain YIM 98012^T grown on GTY medium containing 10% NaCl were observed to be straight rods with dimensions 0.4–1.0 μm in width and 2.4–5.2 μm in length, and devoid of flagella (Supplementary Fig. S1). Spores were not observed following growth under a range of conditions. Gliding motility was not observed by light microscopy. The strain was determined to be positive for catalase, oxidase and hydrolysis of Tween 80 but negative for Voges–Proskauer and methyl red tests, casein, urea and starch hydrolysis, and hydrolysis of Tweens 20, 40, 60. In the API 20NE system, nitrate was not reduced to nitrite, gelatin was not liquefied, H_2S and indole are not produced. Arginine dihydrolase, lysine dihydrolase, lysine decarboxylase, β-galactosidase (PNPG) and

tryptophan deaminase activities were not detected. Other physiological properties are given in the species description. The following substrates were found to be utilised as sole carbon and nitrogen sources: amygdalin, L-arabinose, citrate, cellobiose, D-fructose, D-galactose, glycerol, D-lactose, D-mannitol, D-mannose, D-maltose, L-rhamnose, D-ribose, D-sorbitol, L-salicin, strach, D-trehalose, D-xylose, L-asparagine, L-cysteine, glycine, L-glutamic acid, L-histidine, L-leucine, methionine, L-proline, L-tyrosine and L-valine, but not D-melibiose, D-raffinose, D-saccharose, xylitol, L-alanine or L-phenylalanine. In the API ZYM system, strain YIM 98012^T was found to be positive for esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase-β-glucosidase and negative for alkaline phosphatase lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, α-galactosidase, α-glucosidase β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Acid was found to be produced from D-fructose, aesculin citrate defer and D-raffinose (API 50 CHB). Strain YIM 98012^T was found to be sensitive to ampicillin (10 μg), amphozone (30 mg), azithromycin (15 μg), bacitracin (10 U), clarithromycin(15 μg), cefalotin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), novobiocin (5 μg), neomycin (30 μg), polymyxin B (300 U), penicillin G (10 U), netilmicin (10 μg), roxithromycin (15 μg), streptomycin (10 μg), compound sulphonamides (300 μg), tobramycin (10 μg), tetracycline (30 μg), vancomycin (30 μg), but resistant to amikacin (30 μg). The detailed physiological and biochemical characteristics of strain YIM 98012^T and of closely related type strains, including of two type strains of the related genus *Alteribacillus*, *Bacillus salarius* BH169^T and *Salibacterium halotolerans* KCTC 33658^T are listed in Table 1. Strain YIM 98012^T can be distinguished from *Alteribacillus bidgolensis* P4B^T and *Alteribacillus persepolensis* HS136^T by absence of spore formation, salinity range for growth and acid production. Strain YIM 98012^T differs from the *B. salarius* BH169^T by hydrolysis of aesculin and casein. Strain YIM 98012^T can be distinguished from *S. halotolerans* KCTC 33658^T by its negative Voges–Proskauer test reaction and hydrolysis Tween 80 and its acid production of D-fructose.

Phylogenetic analysis

The nearly complete sequence (1524 bp, GenBank Accession Number KY427816) of the 16S rRNA gene was obtained and this sequence was subjected to phylogenetic analysis. According to the EzBioCloud Database, strain YIM 98012^T has high 16S rRNA gene sequence similarity to members of the family *Bacillaceae* and is closely related to the members of the genus *Alteribacillus*. Strain YIM 98012^T shares high sequence similarity with *A. persepolensis* HS136^T (94.7%), *A. bidgolensis* P4B^T (94.2%), followed by *Bacillus piscicola* NR1-3-2^T (93.4%), *B. salarius* BH169^T (93.0%). Strain YIM 98012^T showed 16S rRNA gene sequence similarity of less than 93.0% with all other current members of the family *Bacillaceae* with validly published names (Fig. 1). In the neighbour-joining, maximum-likelihood and maximum-parsimony phylogenetic trees based on 16S rRNA gene sequences, strain YIM 98012^T forms a well separated branch, among the members of the genera *Alteribacillus*, *Salibacterium*, *Bacillus*, *Salipaludibacillus* and *Scopulibacillus*, which belong to the family *Bacillaceae*. Phylogenetic analysis using the neighbour-joining method showed that the isolate formed a distinct phylogenetic line, with an 83% bootstrap value, from the genera *Alteribacillus*, *Salibacterium* and *Bacillus* (Fig. 1). The phylogenetic position was confirmed from trees generated using the maximum-likelihood algorithms with high bootstrap values (51%). Phylogenetic trees based on maximum-parsimony algorithms was also constructed, and although they demonstrated a different tree topology, the relationships among the members of the family *Bacillaceae* remained similar (Supplementary Fig. S2). Yarza et al. (2014) proposed a threshold of 94.5% sequence identity for delineation of a new genus based on 16S rRNA gene analyses. Thus, strain YIM 98012^T can be considered to represent a novel genus in the family *Bacillaceae*.

Chemotaxonomic characteristics

The predominant cellular fatty acids (> 10%) of strain YIM 98012^T were identified anteiso-$C_{15:0}$ (41.6%), anteiso-$C_{17:0}$ (21.0%), iso-$C_{16:0}$ (10.9%) and $C_{16:0}$ (10.8%), which is similar to those found in other members of the family *Bacillaceae*. Anteiso-$C_{15:0}$ is usually predominant in the type strains belonging to

Table 1 Characteristics used to distinguish strain YIM 98012^T (*Aidingibacillus halophilus* gen. nov., sp. nov.) from phylogenetically closely related taxa

Characteristic	1	2	3	4	5
Spore formation	−	+	+	+	−
Facultatively anaerobic	−	−	−	−	+
Motity	−	−	+	−	−
Range for growth:					
NaCl (%, w/v)/optimum	5–20/10	0.5–12.5/5–7.5	5–20/10	3–20/10–12	2–25/12
Temperature (°C)/optimum	18–50/37	25–40/35	25–45/40	15–40/30–35	25–45/37
pH/optimum	6.0–10.0/7.0	6.5–10.0/7.0	7.0–10.0/8.0–8.5	6.8–9.5/8.0	6.5–10.5/9.0
Nitrate reduction	−	+	−	−	−
Voges–Proskauer test	−	−	−	NA	+
Hydrolysis of:					
Aesculin	−	NA	−	+	−
Casein	−	+	+	−	−
Tween 80	+	−	+	−	NA
Acid production from:					
D-fructose	+	−	−	+	−
D-raffinose	+	−	NA	−	+
DNA G+C content (mol%)	40.1	38.9	37.1	43	48.4
Quinone composition (% of total)	MK-7 (96.2%) and MK-6 (3.7%)	MK-7 (88%) and MK-8 (2%)	MK-7	MK-7	MK-7 (98.7%) and MK-6 (1.3%)
Major fatty acids (> 10%)	Anteiso-$C_{15:0}$ (41.6%), anteiso-$C_{17:0}$ (21.0%), iso-$C_{16:0}$ (10.9%), $C_{16:0}$ (10.8%)	Anteiso-$C_{15:0}$ (24.3%), iso-$C_{15:0}$ (24.4%), anteiso-$C_{17:0}$ (16.1%), iso-$C_{17:0}$ (12.5%)	Anteiso-$C_{15:0}$ (21.0%), iso-$C_{15:0}$ (62.0%)	Anteiso-$C_{15:0}$ (49.7%), anteiso-$C_{17:0}$ (18.9%)	Anteiso-$C_{15:0}$ (38.4%), anteiso-$C_{17:0}$ (16.9%) and $C_{16:0}$ (12.9%)
Polar lipids	DPG, PC, PG, PL and GL	PG, PL and AGL	PG, PL and AGL	DPG, PG and AGL	DPG, PG and PE

Taxa: 1, strain YIM 98012 ^{T} (this study); 2, *Alteribacillus bidgolensis* P4B^T (Didari et al. 2012); 3, *Alteribacillus persepolensis* HS136^T (Amoozegar et al. 2009; Didari et al. 2012); 4, *Bacillus salarius* BH169^T (Didari et al. 2012; Lim et al. 2006); 5, *Salibacterium halotolerans* KCTC 33658^T (Reddy et al. 2015). *Note* + positive, − negative, *NA* no data available. *DPG* Diphosphatidylglycerol, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *AGL* unidentified aminoglycolipid, *PL* unidentified phospholipids, *GL* unidentified glycolipid

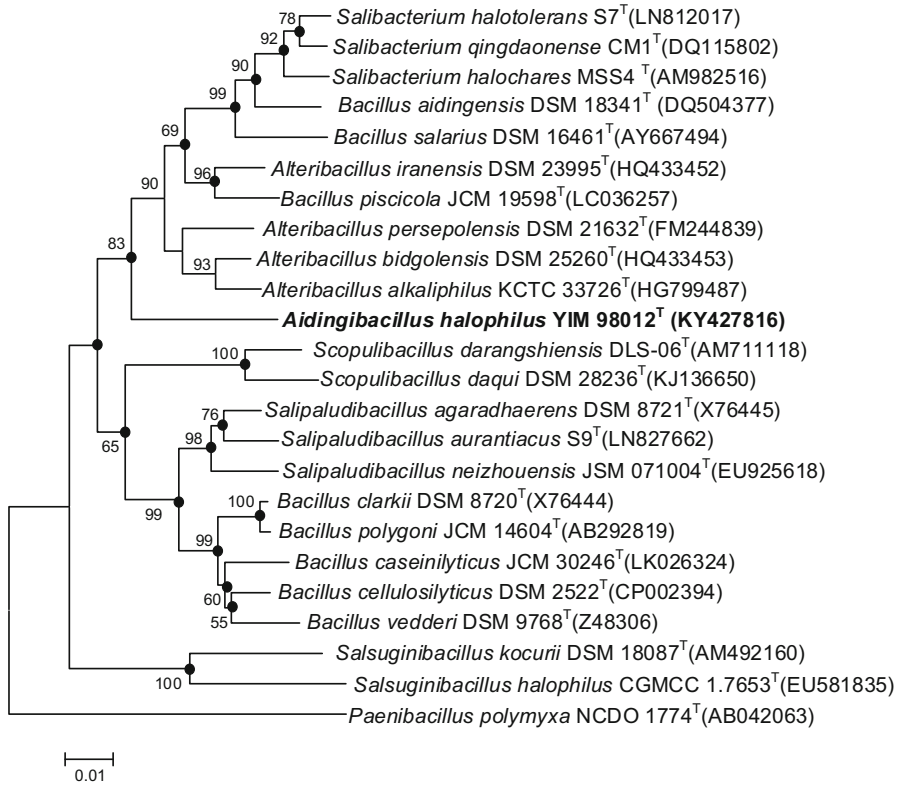

Fig. 1 Neighbor-joining phylogenetic tree showing the relationships between strain YIM 98012^T and representatives of the family *Bacillaceae*. Filled circles indicate that the corresponding nodes were also found in the tree generated with maximum-likelihood algorithm. Bootstrap percentages (based on 1000 replications) > 50% are shown at branching points. Bar, 0.01 substitutions per nucleotide position. The 16S rRNA gene sequence of *Paenibacillus polymyxa* IAM 13419^T(AB042063) was arbitrarily chosen as outgroup

the family *Bacillaceae*. Marginal quantitative differences were observed in the profile of the fatty acids compared with the type strains of *A. bidgolensis* P4B^T, *A. persepolensis* HS136^T, *B. salarius* BH169^T and *S. halotolerans* KCTC 33658^T (Table 1). However, iso-$C_{15:0}$ which is a major fatty acid in type strains of phylogenetically closely related *Alteribacillus* species, was observed to be a minor fatty acid in strain YIM 98012^T (Table 2).

The major respiratory quinone of YIM 98012^T was identified as menaquinone-7 (MK-7) with minor amounts of menaquinone-6 (MK-6), which in accordance with the characteristics of closely related genera. The polar lipids of strain YIM 98012^T were determined to be composed of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), a glycolipid (GL) and five unidentified phospholipids (PL) (Supplementary Fig. S3). The presence of DPG, PC and the presence of unidentified phospholipids in the strain YIM 98012^T lipid profile helps distinguish the strain from the type strains of the genera *Alteribacillus* (Didari et al. 2012; Amoozegar et al. 2009). The

Table 2 Cellular fatty acid composition (> 0.5%) of strain YIM 98012^T

Fatty acid	YIM 98012^T
$C_{14:0}$	0.7
iso-$C_{14:0}$	1.9
iso-$C_{15:0}$	5.2
anteiso-$C_{15:0}$	41.6
iso-$C_{16:0}$	10.9
$C_{16:0}$	10.8
iso-$C_{17:0}$	4.9
anteiso-$C_{17:0}$	21.0
$C_{17:0}$	1.0
$C_{18:0}$	0.7

presence of PC, PL and GL and the absence of unidentified aminolipids in strain YIM 98012^T can differentiate it from the members of the genus *Salibacterium* (Reddy et al. 2015). The presence of PC is a discriminative characteristic compared with the type species of closely related genera (Table 1). This characteristic supports the conclusion that strain YIM 98012^T represents a novel genus.

Strain YIM 98012^T was found to possess *meso*-diaminopimelic acid in the cell wall peptidoglycan. The DNA G+C content of strain YIM 98012^T was determined to be 41.0 mol%, which is in the range of the five phylogenetically related species, but lower than that of *Salibacterium halotolerans* (48.4 mol%).

Based on this polyphasic analysis, from the distinct phylogenetic position and combination of genotypic and phenotypic characteristics, strain YIM 98012^T cannot be assigned to any previously recognised bacterial genus and thus can be described as representing a novel species within a new genus, here named *Aidingibacillus halophilus* gen. nov., sp. nov. Characteristics that distinguish strain YIM 98012^T from the members of related genera within family *Bacillaceae* are shown in Table 1. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain YIM 98012^T is GA00042.

Description of *Aidingibacillus* gen. nov.

Aidingibacillus (Ay.ding.i.ba.cil'lus. N.L. n. Ayding, a lake, located in Xinjiang province of north-west China; L. masc. n. *bacillus* a small rod; N.L. masc. n. *Aidingibacillus* a rod-shaped microbe isolated from Ayding lake).

A member of the family *Bacillaceae*, phylum *Firmicutes*, according to 16S rRNA gene sequence analyses. Cells are rod shaped, Gram stain positive and strictly aerobic. Endospores are not formed. Moderately halophilic, growing over a wide range of NaCl concentrations with optimal growth in the presence of 10% (w/v) NaCl. Catalase and oxidase positive. The major respiratory quinone is menaquinone 7 (MK-7). The predominant cellular fatty acids are anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, a glycolipid and five unidentified phospholipids. The DNA G+C content of the type strain of the type species is 40.1 mol%. The type species is *Aidingibacillus halophilus*.

Description of *Aidingibacillus halophilus* sp. nov.

Aidingibacillus halophilus (ha.lo'phi.lus. Gr. n. *hals, halos* salt; Gr. adj. *philos loving*; N.L. masc. adj. *halophilus,* salt loving).

In addition to the characteristics given in the genus description, colonies are cream, flat and opaque, with slightly irregular edges on GTY medium. Cells are straight rods with dimensions 0.4–1.0 × 2.4–5.2 μm. Growth occurs at 18–50 °C, pH 4.0–10.0 and 5–20% (w/v) NaCl, with the optimal growth at 37 °C, pH 7.0 and 10% NaCl. Nitrate is not reduced. Urease test is negative. Voges–Proskauer and methyl red tests are negative. Indole and H_2S are not produced. Tweens 20, Tweens 40, Tweens 60, gelatin, casein or starch are not hydrolysed, while hydrolysis of Tween 80 is positive. The major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{14:0}$.

The type strain is YIM 98012^T (= KCTC 33868^T = DSM 104332^T), isolated from Ayding Lake, a salt lake, in Xinjiang, China.

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

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