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ORIGINAL PAPER

Description of *Wenzhouxiangella salilacus* sp. nov., a moderate halophilic bacterium isolated from a salt lake in Xinjiang Province, China

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Abstract A Gram-stain negative, non-motile, strictly aerobic and rod-shaped bacterium, designated as 15181^T, was isolated from a salt lake in Xinjiang Province, China. Strain 15181^T was able to grow at 10–40 °C (optimum 37 °C), pH 6.0–8.5 (optimum 7.0) and with 1–14% NaCl (optimum 4%, w/v). According to phylogenetic analysis based on 16S rRNA gene sequences, strain 15181^T was assigned to the genus *Wenzhouxiangella* with high 16S rRNA gene sequence similarity of 97.4% to *Wenzhouxiangella sediminis* XDB06^T, followed by *Wenzhouxiangella marina* KCTC 42284^T (95.9%). Strain 15181^T exhibited ANI values of 80.0% and 72.0% to *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T, respectively. The in silico DDH analysis revealed that strain 15181^T shared 19.1% and 18.7% DNA relatedness with *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T, respectively. Chemotaxonomic analysis showed that the sole respiratory quinone was ubiquinone-8, the major fatty acids included iso-$C_{15:0}$, iso-$C_{16:0}$ and summed feature 9 ($C_{16:0}$ 10-methyl and/or iso-$C_{17:1}\omega9c$). The major polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified glycolipids, two unidentified phospholipids, two unidentified aminophospholipids and an unidentified lipid. On the basis of phenotypic, genotypic and chemotaxonomic characteristics presented in this study, strain 15181^T is concluded to represent a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 15181^T is MF618256. The GenBank accession numbers for the whole genome sequences of strain 15181^T and *Wenzhouxiangella sediminis* XDB06^T are QUZL00000000 and QUZK00000000, respectively.

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novel species in the genus *Wenzhouxiangella*, for which the name *Wenzhouxiangella salilacus* sp. nov. is proposed. The type strain is 15181^T (=KCTC 62172^T=MCCC 1K03442^T).

Keywords *Wenzhouxiangella salilacus* · Salt lake · Polyphasic taxonomy

Abbreviations

ANI Average nucleotide identity
DDH DNA–DNA hybridization

Introduction

The genus *Wenzhouxiangella* was first proposed by Wang et al. (2015) with the description of *Wenzhouxiangella marina*. A novel family *Wenzhouxiangellaceae* belonging to the order *Chromatiales* was also proposed at the same time. The genus *Wenzhouxiangella* currently comprises only two species, *W. marina* KCTC 42284^T and *Wenzhouxiangella sediminis* XDB06^T (Guo et al. 2016), and both of them were isolated from marine environments. Members of the genus *Wenzhouxiangella* are Gram-staining negative, non-motile, rod-shaped and catalase-positive. Members of the genus *Wenzhouxiangella* possesses ubiquinone 8 (Q-8) as predominant respiratory quinone, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as the major polar lipids, iso-$C_{15:0}$ and iso-$C_{16:0}$ as the major fatty acids. During the recent survey of a archaeal and bacterial diversity of the Barkol Lake, a bacterial strain designated as 15181^T was obtained. Strain 15181^T was able to grow in high-salinity (14%, w/v) and low-temperature (10 °C), which indicate that the strain 15181^T may possess potentials for biotechnological applications. Strain 15181^T is distinct from all hitherto known species and therefore may represent a novel species. Based on polyphasic taxonomic approaches, strain 15181^T was found to represent a novel species of the genus *Wenzhouxiangella*, for which the name *Wenzhouxiangella salilacus* sp. nov. is proposed.

Materials and methods

Isolation and cultivation

Strain 15181^T was isolated from a water sample obtained from Barkol Lake (43°37′9.68″N, 92°46′20.72″E) which is located in the northwest of Xinjiang Uyghur Autonomous Region of China. The pH of the lake water is 7.0 and the salinity is 22.6% (w/v). The sample was diluted and spread onto marine agar 2216 (BD, USA) using a tenfold dilution series method and incubated at 30 °C. After 7 days of incubation, a yellow-colored colony was picked and named as 15181^T. The colony was purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. The isolate was routinely cultured in marine broth 2216 (BD, USA) medium. For long-term preservation, purified strains were preserved at − 80 °C with 25% (v/v) glycerol and also by lyophilization with 20% (w/v) skimmed milk. The reference type strains *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T were obtained from College of Marine Science, Shandong University, China and Korean Collection for Type Cultures (KCTC; Korea) and cultured under the same conditions for comparative analysis.

Phenotypic and biochemical analysis

Cell morphology was determined by using optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) as described by Huo et al. (2010). Exponentially growing cells incubated on MA plates were suspended and stained with uranyl acetate and then fixed on the copper mesh before observed with transmission electron microscopy. Motility was determined in semi-solid medium. Unless otherwise stated, strains investigated in all the following tests were cultured at the optimal temperature.

Salt tolerance was investigated in modified MB medium (without Na^+) at various NaCl concentrations (0, 0.5 and 1.0–15.0%, at increments of 1%, w/v). The growth temperature was respectively tested at 4, 10, 20, 25, 30, 37, 40, 45, 50 and 55 °C. The pH range (from pH 5.5 to 10.0, at intervals of 0.5 pH units) was determined in MB medium with the addition of 30 mM buffering agents (Zhang et al. 2018) including MES (pH 5.5–6.5), PIPES (pH 6.5–7.5), Tricine buffer

(pH 7.5–8.5) and CAPSO (pH 9.0–10.0). Gram staining was performed by following the method outlined by Claus (1992). Catalase and oxidase activities, PHB production, H_2S production from thiosulfate and L-cysteine, hydrolysis of starch, casein, L-tyrosine and cellulose were tested according to Zhu (2011). Hydrolysis of Tweens 20, 40 and 80 were examined as described by Sun et al. (2015). Other biochemical tests were performed using the methods described by Mata et al. (2002).

The utilization of single carbon sources was performed using Biolog GEN III identification system according to the manufacturer's instructions. Acid production was tested by using API 50CH (bioMérieux) strips. Leifson modified O/F medium (MOF; Leifson, 1963) was used to suspend the cells for the inoculation of API 50CH tests. API 50CH strips were read after 24 h and 48 h. Additional enzyme activities and biochemical characteristics were tested by API 20NE and API ZYM (bioMérieux) kits, and they were read after 24 h and 4 h, respectively. Susceptibility to antibiotics was determined on MA plates for 7 days at 37 °C using antibiotic discs (Hangzhou Microbial Reagent Co. Ltd, Hangwei^{TM}), and considered positive when the radius of the inhibition zone was over 1.5 cm. The antibiotics tested were (μg per disc, unless indicated): amikacin (30), amoxicillin (10), bacitracin (0.04 IU), cephalothin (30), chloramphenicol (30), clindamycin (2), doxycycline (30), erythromycin (15), gentamicin (10), (2), kanamycin (30), lincomycin (2), mefoxin (30), nalidixic acid (30), norfloxacin (10), novobiocin (30), nystatin (100), ofloxacin (5), penicillin G (10 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30). Anaerobic growth was determined in an anaerobic jar (MGC) with AnaeroPack (MGC) using modified MA, to which 20 mM sodium thiosulfate, 5 mM sodium sulfite, 20 mM sodium sulfate, 5 mM sodium nitrite and 20 mM sodium nitrate were respectively added as electron acceptors for 30 days incubation.

Molecular studies and phylogenetic analysis

A quick bacteria genomic DNA extraction kit was used (DongSheng Biotech) to obtain high quality PCR template. An almost complete 16S rRNA gene sequence of strain 15181^T was obtained by PCR using the primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′)/1492R (5′-GGTTACCTTGTTACGACTT-3′) and the PCR products were cloned into pMD19-T vector (Takara) for sequencing (Xu et al. 2007). The sequence was compared with the closely related taxa provided by the EzTaxon-e service (Kim et al. 2012). Multiple sequences were aligned with clustal W1.8 (Thompson et al. 1994). Phylogenetic trees were reconstructed using the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and the maximum-parsimony (Fitch 1971) methods with the MEGA 7 program package (Kumar et al. 2016). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura 1980) for the neighbor-joining method. All bootstrap analyses were based on 1000 replications.

The genomes of 15181^T and *W. sediminis* XDB06^T were sequenced using Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA) at the Beijing Genomics Institute (Shenzhen, China). The output reads were assembled using ABySS software (Simpson et al. 2009). The quality of microbial genomes was assessed using the bioinformatics tool CheckM 1.0.8 (Parks et al. 2015). The open reading frames (ORFs) were predicted and annotated by Glimmer v.3.0 (Delcher et al. 2007) and Rapid Annotation using Subsystem Technology (RAST) server online (Overbeek et al. 2014). The genome sequence of *W. marina* KCTC 42284^T (CP012154) was retrieved from the GenBank database. The average nucleotide identity (ANI) was calculated using the OrthoANIu algorithm of the Chun lab's online Average Nucleotide Identity calculator (Lee et al. 2016). In silico DNA–DNA hybridization (DDH) values were calculated by genome-to-genome distance calculator (GGDC) (Meier-Kolthoff et al. 2013). The genomic DNA G+C content of strain 15181^T, *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T was calculated from genome sequence.

Chemotaxonomic characterization

For the preparation of cellular fatty acid methyl esters (FAMEs), cells of strain 15181^T, *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T were harvested and lyophiled at the exponential stage of growth according to Kuykendall et al. (1988). Identification and quantification of the FAMEs were performed by the Sherlock Microbial Identification System (MIDI) with the standard MIS Library Generation software version 4.5 (Microbial ID). Cells of

the three strains which grown on MB medium for 5 days at 37 °C were used for isoprenoid quinone and polar lipids analysis. Isoprenoid quinones were extracted from lyophilised cells (200 mg) with chloroform/methanol (2:1, v/v.) and were analysed by using HPLC–MS system (Agilent). Polar lipids were analysed by two-dimensional TLC with silica gel 60 F254 plates (Merck) according to Han et al. (2016).

Results and conclusion

Phenotypic and biochemical characteristics

Cells of strain 15181^T were observed to be Gram-stain negative and rod-shaped, measuring 0.4–0.6 μm wide and 2.0–8.0 μm long (Supplementary Fig. S1). The morphological characteristics between strain 15181^T and the two reference strains were similar. Strain 15181^T was found to grow optimally at pH 7.0, 37 °C and in the presence of 4.0% (w/v) NaCl. The following compounds are utilized as sole carbon sources according to the Biolog GEN III identification system: D-arabitol, γ-amino-butryric acid, dextrin, D-fructose, L-fucose, L-galactonic acid lactone, α-D-glucose, glucuronamide, D-glucuronic acid, L-glutamic acid, α-hydroxy-butyric acid, *p*-hydroxy-phenylacetic acid, α-D-lactose, D-mannitol, D-mannose, mucic acid, L-rhamnose and D-saccharic acid. Other substrates in the GEN III MicroPlates are not utilized as sole carbon source. No single substrate of the API 50 CH system is converted to acid. With API ZYM systems, alkaline phosphatase, leucine arylamidase, trypsin and α-chymotrypsin are positive; esterase (C4), esterase lipase (C8), valine arylamidase, cysteine arylamidase, acid phosphohydrolase and naphtol-AS-BI-phosphohydrolase are weakly positive; β-glucosidase, β-glucuronidase, lipase(C14), α-galactosidase, *β*-galactosidase, α-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase are negative. Strain 15181^T was sensitive to amoxicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, mefoxin, norfloxacin, ofloxacin, penicillin G, rifampicin and vancomycin, but resistant to amikacin, bacitracin, doxycycline, lincomycin, nalidixic acid, novobiocin, nystatin, streptomycin and tetracycline.

There are several significant physiological and biochemical differences between strain 15181^T and its relatives. Strain 15181^T was able to grow at relatively low temperature (10 °C), whereas the lowest growth temperature of *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T was 25 °C and 20 °C, respectively. Strain 15181^T was unable to hydrolyze casein, gelatin and Tween 40, while *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T both are positive for hydrolysis of those substrates. Furthermore, strain 15181^T showed negative in acid production from all the substrates provided by the kit, but two reference strains were able to produce acid from D-ribose, D-tagatose and potassium 5-ketogluconate. Additionally, according to API ZYM results, strain *W. marina* KCTC 42284^T showed positive in esterase lipase (C8), valine arylamidase, acid phosphohydrolase and naphtol-AS-BI-phosphohydrolase, while those enzymes were weakly positive in strain 15181^T and *W. sediminis* XDB06^T. A detailed comparison of the physiological and biochemical characteristics of strain 15181^T and its reference strains is summarized in Table 1. Other results of physiological and biochemical tests are given in the species description.

Molecular characterization and phylogenetic analysis

The almost-complete 16S rRNA gene sequence (1524 nt) of strain 15181^T was obtained. The analysis of 16S rRNA gene sequence similarity between strain 15181^T and other representative type strains revealed that the novel isolate is closely related to the members of the genus *Wenzhouxiangella*. Strain 15181^T shared the highest sequence similarity of 97.4% with *W. sediminis* XDB06^T. Strain 15181^T was also closely related to *W. marina* KCTC 42284^T (95.9%, sequence similarity) but shared low sequence similarity (< 92.0%) with all other currently described species. Phylogenetic analysis based on the multiple sequences alignment indicated that strain 15181^T formed a distinct clade with *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T, and the three strains shared a branch with a high bootstrap value of 100% in the neighbor-joining tree (Fig. 1). Topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to the neighbour-joining tree.

Table 1 Differential characteristics of strain 15181^T and closely related type strains of the genus *Wenzhouxiangella*

Characteristic	1	2	3
Cell size (μm)	0.4–0.6 × 2.0–8.0	0.3–0.5 × 1.3–5.9	0.5–0.6 × 2.5–3.2
Temperature range (optimum) (°C)	10–40 (37)	25–45(37)*	20–45(37)†
NaCl range (optimum) (%, w/v)	1–14(4)	1.0–12(4)*	0.5–13(3–5)†
Relationship with O_2	Strictly aerobic	Facultatively anaerobic	Strictly aerobic
Hydrolysis of:			
Casein	–	+	+
Gelatin	–	+	+
Tween 40	–	+	+
Tween 80	–	–	+
Utilization of:			
γ-Amino-butryric acid	+	+	–
D-Fructose-6-PO4	–	–	+
D-Fucose	–	–	+
L-Galactonic acid lactone	+	–	+
D-Galactose	–	–	+
D-Galacturonic acid	–	–	+
Gentiobiose	–	–	+
L-Glutamic acid	+	+	–
α-Hydroxy-butyric acid	+	+	–
p-Hydroxy-phenylacetic acid	+	–	–
D-Mannitol	+	+	–
D-Melibiose	–	+	–
Mucic acid	+	+	–
D-Saccharic acid	+	+	–
D-Turanose	–	–	+
Acid production from:			
Potassium 5-ketogluconate	–	+	+
D-Ribose	–	+	+
D-Tagatose	–	+	+
Antibiotics susceptibility:			
Doxycycline	–	+	–
Gentamicin	+	–	+
Kanamycin	+	–	–
Nalidixic acid	–	+	+
Novobiocin	–	+	+
Tetracycline	–	+	–
API ZYM			
Acid phosphohydrolase	w	w	+
Esterase lipase (C8)	w	w	+
Naphtol-AS-BI-phosphohydrolase	w	w	+

Table 1 continued

Characteristic	1	2	3
Valine arylamidase	w	w	+
DNA G+C content (mol%)	62.9%	65.4%	65.3%
Polar lipids	DPG, PG, PE, GL1, GL2, PL1, PL2, APL1, APL2, L1	DPG, PG, PE, GL1, GL2, PL1, PL2, PL3, APL1, L1	DPG, PG, PE, GL1, GL2, PL1, PL2, PL4, APL1, L1

Taxa: 1, strain 15181^T; 2, *W. sediminis* XDB06^T; 3, *W. marina* KCTC 42284^T. Data were from this study unless otherwise indicated. All strains were positive for catalase activities. All strains were negative in indole production, glucose fermentation, arginine dihydrolase, Voges–Proskauer reaction, hydrolysis of β-galactosidase, aesculin, starch and CM-cellulose. The following substrates: dextrin, α-D-glucose, D-mannose, D-fructose, L-fucose, L-rhamnose, D-glucuronic acid and glucuronamide could be used by all strains as the sole carbon sources. All strains were sensitive to amoxicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, mefoxin, norfloxacin, ofloxacin, penicillin G, rifampicin and vancomycin, but resistant to amikacin, bacitracin, lincomycin, nystatin and streptomycin. –, negative; +, positive; w, weakly positive

*Data was taken from Guo et al. (2016)

†Data was taken from Wang et al. (2015)

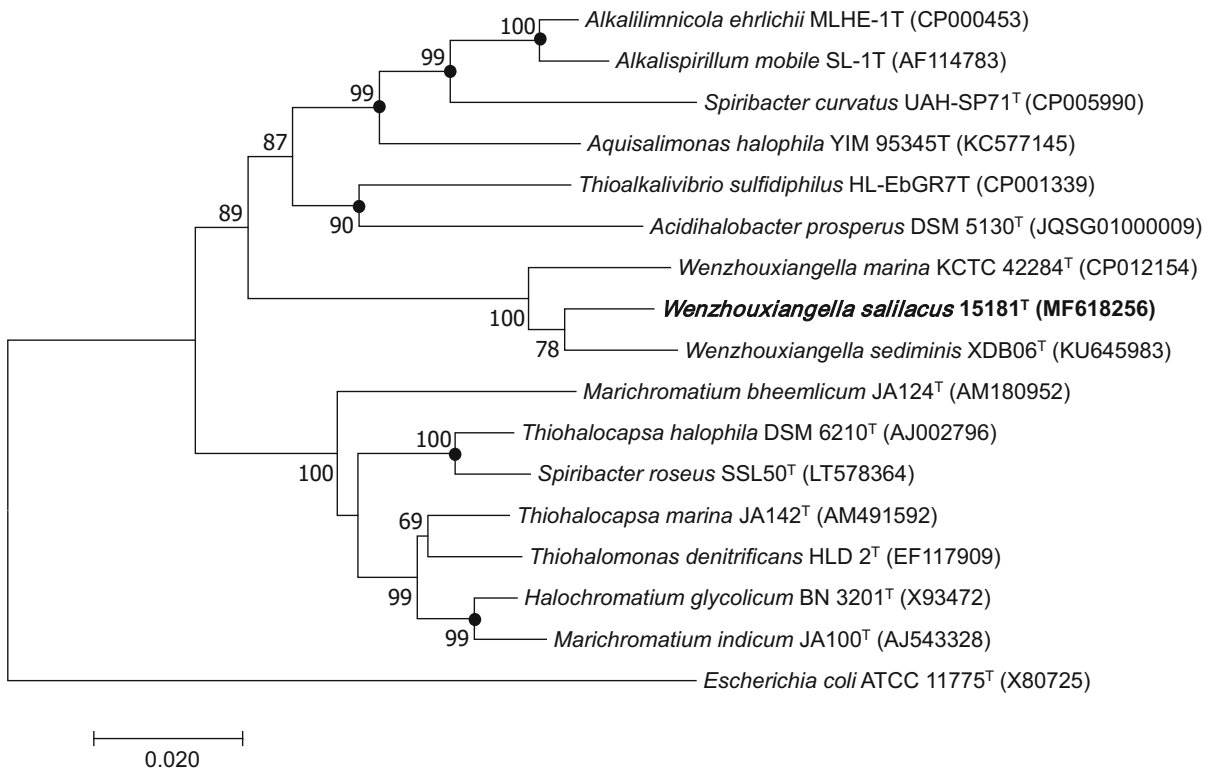

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain 15181^T and related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values above 50% are shown. Filled circles indicate that the corresponding nodes were also recovered in both maximum-likelihood and maximum-parsimony trees. *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725) was used as outgroup. Bar, 0.02 substitutions per nucleotide position

The draft genomes of strain 15181^T and *W. sediminis* XDB06^T are 3,128,413 bp and 3,506,010 bp in length, assembled into 55 and 54 contigs, respectively. The genome completeness of strain 15181^T and *W. sediminis* XDB06^T was 97.1% and 97.8%, with a contamination percentage of 2.0% and 1.2% respectively. Genome sequences estimated to be $\geq$ 95% completeness, with $\leq$ 5% contamination, are considered as excellent reference genomes for deeper analyses (Parks et al. 2015). The genomic DNA G+C content of strain 15181^T calculated from the draft genome sequence is 62.9 mol% which is slightly lower than that of *W. sediminis* XDB06^T (65.4%) and *W. marina* KCTC 42284^T (65.3%). Strain 15181^T showed ANI values of 80.0% and 72.0% to *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T respectively. The in silico DDH analysis (the recommended results from formula 2) indicated that strain 15181^T shared 19.1% DNA relatedness with *W. sediminis* XDB06^T and 18.7% with *W. marina* KCTC 42284^T, respectively. The ANI values and in silico DDH values were significantly lower than the threshold values of the prokaryotic species boundary (ANI 94–96% and in silico DDH 70%) (Richter and Rossello-Mora 2009; Wayne et al. 1987), indicating a low genotypic relatedness between strain 15181^T and the two reference species of the genus *Wenzhouxiangella*.

Chemotaxonomic results

The main cellular fatty acids of strain 15181^T were found to be iso-$C_{15:0}$ (33.3%), iso-$C_{16:0}$ (21.2%) and summed feature 9 ($C_{16:0}$ 10-methyl and/or iso-$C_{17:1}\omega$9c, 13.9%). Iso-$C_{15:0}$ and iso-$C_{16:0}$ are predominant fatty acids of the genus *Wenzhouxiangella* (Wang et al. 2015), as well as strain 15181^T, *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T. The complete fatty acid profiles of strain 15181^T and the reference type strains are summarized in Table 2. The sole respiratory quinone of strain 15181^T was identified as Q-8 (100%), which is one of the typical characteristics of the genus *Wenzhouxiangella*. The polar lipids of strain 15181^T were found to consist of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), two uncharacterized glycolipids (GL1, GL2), two uncharacterized phospholipids (PL1, PL2), two uncharacterized aminophospholipids (APL1, APL2) and an uncharacterized lipid (L1). The polar lipids profile of strain 15181^T was similar to the two type strains of the genus *Wenzhouxiangella*, except some minor

Table 2 Cellular fatty acids for strain 15181^T together with type strains of related species

Fatty acid	1	2	3
Straight-chain			
$C_{14:0}$	0.3	0.3	TR
$C_{16:0}$	1.1	2.6	1.6
$C_{17:0}$	–	0.6	TR
Unsaturated			
iso-$C_{15:1}$ F	–	TR	0.4
$C_{15:1}\omega 6c$	0.7	0.8	0.3
iso-$C_{16:1}$	0.3	0.5	1.0
$C_{20:2}\omega$6,9 *c*	0.7	0.1	–
Branched			
iso-$C_{10:0}$			0.3
iso-$C_{11:0}$	4.2	2.2	3.0
iso-$C_{12:0}$	2.2	2.8	2.5
iso-$C_{13:0}$	2.4	0.8	1.1
Anteiso-$C_{13:0}$	0.3	0.3	TR
iso-$C_{14:0}$	2.8	1.3	2.5
iso-$C_{15:0}$	**32.3**	**18.6**	**24.6**
Anteiso-$C_{15:0}$	3.1	2.1	0.5
iso-$C_{16:0}$	**21.2**	**22.6**	**38.5**
iso-$C_{17:0}$	5.1	19.7	5.6
Anteiso-$C_{17:0}$	0.6	1.5	0.2
iso-$C_{18:0}$	0.4	2.3	1.1
iso-$C_{19:0}$	–	1.1	TR
Hydroxy			
iso-$C_{13:0}$ 3-OH	4.9	5.0	4.2
Summed feature*			
1	0.8	0.5	2.4
3	1.3	2.4	0.8
5	0.3	–	–
9	13.9	10.2	8.1

Taxa: 1, strain 15181^T; 2, *W. sediminis* XDB06^T; 3, *W. marina* KCTC 42284^T. Data were obtained in this study. Values are the percentage of total fatty acids; fatty acids amounting to < 0.3% of the total fatty acids in all strains listed are omitted. –, Not detected; TR, traces (< 0.3%). Major components ($\geq$ 10%) are highlighted in bold

*Summed features are groups of two or three fatty acids that could not be separated by GLC with the MIDI System. Summed feature 1 contains iso-$C_{15:1}$ and/or $C_{13:0}$ 3-OH; summed feature 3 contains $C_{16:1}\omega$7c and/or $C_{16:1}\omega$6c; summed feature 5 contains $C_{18:0}$ ante and/or $C_{18:2}\omega$6, 9c; summed feature 9 contains $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}\omega$9c

differences. APL2, which is present in strain 15181^T, was not detected in *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T. Moreover, PL3 and PL4 were found in *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T, respectively, but the two spots were not detected in strain 15181^T (Supplementary Fig. S2).

Taxonomic conclusion

On the basis of phenotypic, molecular phylogenetic and chemotaxonomic results presented above, strain 15181^T exhibits many typical characteristics of the genus *Wenzhouxiangella*, such as being catalase and oxidase positive, moderately halophilic, having Q-8 as the predominant respiratory quinone and having diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids. However, the phylogenetic distinctiveness, as well as the phenotypic and chemotaxonomic differences (Table 1) clearly shows differences between strain 15181^T and the reference strains. Additionally, the low ANI and in silico DDH relatedness values to the closely related species *W. sediminis* XDB06^T and *W. marina* KCTC 42284^T confirmed that strain 15181^T represents a species distinct from the recognized *Wenzhouxiangella* species. Therefore, strain 15181^T represents a novel species of the genus *Wenzhouxiangella*, for which the name *Wenzhouxiangella salilacus* sp. nov. is proposed. The taxonumber of strain 15181^T is TA00684 in the online digital protologue.

Description of *Wenzhouxiangella salilacus* sp. nov.

Wenzhouxiangella salilacus (sa.li.la'cus. L. neut. n. *sal* salt; L. masc. n. *lacus* a lake; N.L. gen. n. *salilacus* of a salt lake).

Cells are Gram-stain negative, non-motile, rod-shaped, approximately 0.4–0.6 μm in width and 2.0–8.0 μm in length. Colonies are 0.5–1.0 mm in diameter, convex, smooth, circular, and yellow-colored on MA after 5 days incubation at 37 °C. Growth occurs at 10–40 °C (optimum 37 °C), 1–14% (w/v) NaCl (optimum 4%, w/v) and pH 6.0–8.5 (optimum 7.0). No growth is observed in anaerobic conditions by anaerobic respiration with $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, NO_2^- or NO_3^- as electron acceptors. Negative in oxidase, methyl red and Voges–Proskauer reaction, nitrate or nitrite reduction, indole production and arginine dihydrolase. Positive in catalase and H_2S production (from thiosulfate and L-cysteine). No hydrolysis of aesculin, urea, casein, gelatin, tyrosine, starch and CM-cellulose. The sole respiratory quinone is ubiquinone-8. The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and summed feature 9 ($C_{16:0}$ 10-methyl and/or iso-$C_{17:1}\omega9c$). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two uncharacterized glycolipids, two uncharacterized phospholipids, two uncharacterized aminophospholipids and an uncharacterized lipid.

The type strain 15181^T (=KCTC 62172^T=MCCC 1K03442^T) was isolated from a salt lake, Xinjiang province, China. The DNA G+C content of the type strain is 62.9 mol% (as determined from the draft genome).

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Author's contribution W.Y.Z. and M.W. conceived the study. S.B.H., X.J.H., Y.H.Y. and Z.J. performed the research. R.Z., R.J.W. and Y.H.Y. analyzed data. S.B.H. and Y.H.R. wrote the paper. All authors read and approved the manuscript.

Compliance with ethical standards

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

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

References

Claus D (1992) A standardized gram staining procedure. World J Microbiol Biotechnol 8:451–452

Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Guo LY, Dunlap CA, Rooney AP, Chen GJ, Du ZJ (2016) *Wenzhouxiangella sediminis* sp. nov., isolated from coastal sediment. Int J Syst Evol Microbiol 66:4575–4579

Han SB, SuY HuJ, Wang RJ, Sun C et al (2016) *Terasakiella brassicae* sp. nov., isolated from the wastewater of a pickle-processing factory, and emended descriptions of *Terasakiella pusilla* and the genus *Terasakiella*. Int J Syst Evol Microbiol 66:1807–1812

Huo YY, Xu XW, Cui HL, Wu M (2010) *Gracilibacillus ureilyticus* sp nov., a halotolerant bacterium from a saline–alkaline soil. Int J Syst Evol Microbiol 60:1383–1386

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J Mol Evol 16:111–120

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Kuykendall LD, Roy MA, Oneill JJ, Devine TE (1988) Fatty-acids, antibiotic-resistance, and deoxyribonucleic-acid homology groups of *Bradyrhizobium-japonicum*. Int J Syst Bacteriol 38:358–361

Lee I, Kim YO, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103

Mata JA, Martinez-Canovas J, Quesada E, Bejar V (2002) A detailed phenotypic characterisation of the type strains of *Halomonas* species. Syst Appl Microbiol 25:360–375

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ et al (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42:D206–D214

Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055

Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131

Saitou N, Nei M (1987) The neighbor-joining method—a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM et al (2009) ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123

Sun C, Pan J, Zhang XQ, Su Y, Wu M (2015) *Pseudoroseovarius zhejiangensis* gen. nov., sp nov., a novel alphaproteobacterium isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae* comb. nov. Antonie Van Leeuwenhoek 108:291–299

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal-W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Wang GH, Tang MX, Li T, Dai SK, Wu HL et al (2015) *Wenzhouxiangella marina* gen. nov, sp. nov, a marine bacterium from the culture broth of *Picochlorum* sp. 122, and proposal of *Wenzhouxiangellaceae* fam. nov. in the order *Chromatiales*. Antonie Van Leeuwenhoek 107:1625–1632

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O et al (1987) Report of the ad-hoc-committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Xu XW, Wu YH, Zhou Z, Wang CS, Zhou YG et al (2007) *Halomonas saccharevitans* sp nov., *Halomonas arcis* sp nov and *Halomonas subterranea* sp nov., halophilic bacteria isolated from hypersaline environments of China. Int J Syst Evol Microbiol 57:1619–1624

Zhang WY, Yuan Y, Su DQ, He XP, Han SB et al (2018) *Gallaecimonas mangrovi* sp. nov., a novel bacterium isolated from mangrove sediment. Antonie Van Leeuwenhoek 111:1855–1862

Zhu XF (2011) Modern experimental technique of microbiology. Zhejiang University Press, Hangzhou **(English translation)**