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ORIGINAL PAPER

Ancylomarina psychrotolerans sp. nov., isolated from sediments of Fildes Peninsula and emended the description of genus *Ancylomarina*

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Abstract A Gram-stain negative, obligately anaerobic, non-motile, asporogenous long rod-shaped and non-flagellated bacterial strain, designated 4SWWS2-6^T, was isolated from sediment in the intertidal zone of Fildes Peninsula, Antarctica. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 4SWWS2-6^T belongs to the genus *Ancylomarina* and showed high sequence similarity with *Ancylomarina subtilis* FA102^T (96.5%). Optimal growth occurred at pH 6.5, 16 °C and in the presence of 3% (w/v) NaCl. Strain 4SWWS2-6^T contained menaquinone-7 (MK-7) as the major respiratory quinone and held iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ 3-OH as the major cellular fatty acids. The major polar lipids were phosphatidylethanolamine, phosphatidylmonomethylethanolamine, an aminolipid, two unidentified lipids and an unidentified phospholipid. The DNA G + C content of strain 4SWWS2-6^T was 37.6 mol%. On the basis of the polyphasic analyses, strain 4SWWS2-6^T is considered to represent a novel species in the genus *Ancylomarina*, for which the name *Ancylomarina psychrotolerans* sp. nov. is proposed. The type strain is 4SWWS2-6^T (= KCTC 15504^T = MCCC 1K01618^T).

Keywords *Ancylomarina psychrotolerans* sp. nov. · Marine sediments · Fildes Peninsula · The Antarctica

The GenBank Accession Number for the 16S rRNA gene sequence of *Ancylomarina psychrotolerans* 4SWWS2-6^T (=KCTC 15504^T=MCCC 1K01618^T) is KT266597.

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Introduction

The genus *Ancylomarina*, a member of family *Marinilabiliaceae* in the phylum *Bacteroidetes*, was firstly proposed by Wu et al. (2016) with *Ancylomarina subtilis* as the type species. *A. subtilis* was isolated from marine sediment in the coastal area of Weihai, PR China. At the time of writing, the genus *Ancylomarina* contains only one species, *A. subtilis*, representing a group of Gram-stain negative, facultatively

anaerobic, oxidase and catalase are negative, filamentous and non-motile bacteria requiring NaCl for growth. The genus *Ancylomarina* is characterized by menaquinone 7 (MK-7) as the predominant respiratory quinone, iso-$C_{15:0}$ and iso-$C_{15:0}$ 3-OH as the major fatty acids. We describe here a bacterium 4SWWS2-6^T isolated from sediment in the intertidal zone of Fildes Peninsula, Antarctica. The aim of the present study was to determine the exact taxonomic position of the strain 4SWWS2-6^T by a polyphasic taxonomic approach. The resultant data showed that strain 4SWWS2-6^T represents a novel species in the genus *Ancylomarina*, for which the name *Ancylomarina psychrotolerans* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of strains

The bacterial strain, 4SWWS2-6^T, was isolated from the intertidal zone of Fildes Peninsula, King George Island, the Antarctica (Jan 11th, 2015; 62°12′S, 59°00′W), in an investigation of phylogenetic diversity and geographical distribution of bacteria in marine sediments. Samples were immediately inoculated into sterile liquid enrichment medium (Wang et al. 2015). The medium was prepared anaerobically and kept under an atmosphere of highly purified 100% nitrogen contained (concentrations in $g{\cdot}L^{-1}$, unless stated otherwise): PIPES, 6.5 g; NaCl, 25.0 g; $MgSO_4{\cdot}7H_2O$, 2.7 g; $MgCl_2{\cdot}6H_2O$, 4.3 g; NH_4Cl, 0.25 g; KCl, 0.5 g; $CaCl_2{\cdot}2H_2O$, 0.14 g; $K_2HPO_4{\cdot}3H_2O$, 0.14 g; $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, 0.002 g; casamino acid, 1.0 g; yeast extract, 0.1 g; trace elements solution, 1 mL; cysteine, 0.3 g; resazurin, 0.001 g; vitamin solution, 10 ml; distilled water, 1L. Trace elements solution and Vitamin solution were prepared according to the descriptions of Balch et al. (1979) and Steinsbu et al. (2010), respectively. Enrichment cultures were incubated at 4 °C in 10 ml vials sealed by butyl-rubber stoppers. After 30 days of cultivation, strain 4SWWS2-6^T was purified by streaking on enrichment solid medium (2% agar) in the anaerobic packet of AneroPack-Anaero (Mitsubishi Gas Chemical Co.). The strain was routinely cultured on marine agar 2216E (MA; Becton–Dickinson) at 16 °C. Stock cultures were preserved in sterile 0.85% (w/v) saline supplemented with 20% (v/v, final concentration) glycerol at − 80 °C. *A. subtilis* FA102^T, *Marinifilum fragile* JCM 15579^T (Na et al. 2009) and *Marinifilum flexuosum* DSM 21950^T (Ruvira et al. 2013), were chosen as reference strains for the phenotypic characterization and fatty acid analysis, which were cultured at the same conditions as strain 4SWWS2-6^T [MA/marine broth 2216 (MB; BD), 28 °C], unless otherwise specified. *A. subtilis* FA102^T was obtained from College of Marine Science, Shandong University at Weihai. *M. fragile* JCM 15579^T and *M. flexuosum* DSM 21950^T were obtained from the Japan Collection of Microorganisms (JCM) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), respectively.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA extraction, PCR amplification, cloning and sequencing of the 16S rRNA gene were performed according to the description of Yu et al. (2013). The almost complete 16S rRNA gene sequence of 4SWWS2-6^T (1487nt) was manually checked and submitted to GenBank. Pairwise similarity values between strain 4SWWS2-6^T and closely related type strains were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; (Kim et al. 2012). The 16S rRNA gene sequence was aligned with closely related sequences belonging to the members of the genera *Ancylomarina*, *Marinifilum*, *Saccharicrinis*, *Prolixibacter*, *Draconibacterium*, *Alkaliflexus*, *Carboxylicivirga* and Geofilum using CLUSTAL_X (Thompson et al. 1997). Phylogenetic trees based on the neighbor-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms were constructed by the software package MEGA version 7.0 (Tamura et al. 2016) and the genetic distances of the first two trees were calculated by Kimura's two-parameter model (Yoon et al. 2017). The topologies of the phylogenetic trees were evaluated by the bootstrap resampling method of Felsenstein (1981) with 1000 replicates.

Phenotypic characterization

Cell morphology of strain 4SWWS2-6^T was determined by transmission electron microscopy (JEM-1200EX; JEOL) after cells in exponential phase negatively stained with 1.0% (w/v) phosphotungstic acid. Gram-staining and flagellum staining were

performed according to Beveridge et al. (2007). For salinity and pH ranges supporting growth, strains were inoculated in 5 ml vials sealed with butyl-rubber stoppers and transferred into 96-well microplates for the optical density measurements (wavelength 590 nm). The temperature range for growth was determined on MA plates by incubating cultures at 10–42 °C (10, 16, 20, 24, 28, 32, 37 and 42 °C) for 1 week, and at 0 °C and 4 °C for 4 weeks. In the salinity experiment, artificial seawater (Lyman and Fleming 1940) with Na^+ replaced by appropriate K^+ was used to prepared synthetic marine ZoBell broth (5 g Bacto peptone, 1 g yeast extract and 0.01 g FePO4 in 1 L water); NaCl concentration was adjusted to 0–15.0% (w/v, at intervals of 1.0%). Growth was evaluated at pH 2.0–5.0 (at intervals of 1.0 pH unit) and at pH 5.5–10.0 (at intervals of 0.5 pH unit) in marine broth 2216E (MB; Becton–Dickinson) using the following buffer systems: H_3PO_4/KH_2PO_4 (pH 2.0), sodium ethanoate/ethanoic acid (pH 3.0–6.0), KH_2PO_4/NaOH (pH 7.0–8.0) and $Na_2CO_3/NaHCO_3$ (pH 9.0–10.0). To test for aerobic growth, strain 4SWWS2-6^T was cultured at 16 °C for 1 month on MA in aerobic atmosphere. Various phenotypic characterizations of 4SWWS2-6^T and related reference strains were tested according to the standard approaches (Tindall et al. 2007) with sterile seawater. The tested characteristics included: activities of catalase, oxidase and hydrolysis of starch, casein, gelatin and Tweens 20, 40 and 80 (method 2, (Tindall et al. 2007)). DNase agar (Qingdao Hope Bio-technology) prepared with sterile seawater was used to detect the DNase activity. Chitin (1.0%, w/v) and sodium alginate (2.0%, w/v) were added to MA plates to determine the degradation by the formation of clear zones around colonies directly or after flooding with appropriate solutions (Teather and Wood 1982). Activities of constitutive enzymes, the fermentation/oxidation profile, acid production, and substrate utilization as sole carbon and energy source were performed using API 20A strip and the Biolog AN MicroPlate kit according to the manufacturers' instructions except that sterile seawater was used to inoculate the strips.

Chemotaxonomic characterisation

For cellular fatty acid analysis, strain 4SWWS2-6^T and the related reference strains were grown in parallel to the exponential stage on MB medium at 28 °C for 48 h (Li et al. 2016). Fatty acid methyl esters were prepared and analyzed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.10) and identified by the TSBA 6.0 database of the Microbial Identification System (Sasser 1990). For analysis of the respiratory quinones and polar lipids, cells were harvested from MB after incubation at 16 °C shaken for 48 h and freeze-dried. Polar lipids were extracted according to the methods from Minnikin et al. (1984), separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform/methanol/water (65: 25: 4, v/v) for the first dimension and chloroform/methanol/acetic acid/water (80: 12: 15: 4, v/v) for the second dimension (Komagata and Suzuki 1988). Extracted lipids were identified by spraying the plates with appropriate detection reagents. In brief, 5% ethanolic molybdophosphoric acid was used to detect all the lipids. Ninhydrin spray was applied to determine most of the lipids with free amino groups, while spraying the plate with the lipid phosphate reagent of Dittmer and Lester after the ninhydrin spray could reveal the presence of phospholipids. The respiratory quinones of strain 4SWWS2-6^T was extracted with chloroform/methanol (2:1, v/v) and identified by HPLC as described by Xie and Yokota (2003).

Mol% G + C determination

Genomic DNA was extracted from strain 4SWWS2-6^T by standard methods (Ausubel et al. 1995). The G + C content (mol%) of the chromosomal DNA was determined according to the methods described by (Mesbah and Premachandran 1989) using a reverse-phase HPLC.

Results and discussion

Phenotypic characteristics

The strain 4SWWS2-6^T was found to be a long rod-shaped (approximately 0.4–0.7 μm in width and 2.0–5.0 μm in length) (Fig. S1), Gram-stain negative, obligate anaerobic, non-flagellated and non-motile bacterium. Colonies on MA are white, transparent, and regular margins after incubation for 72 h at 16 °C. Growth occurs at 4–37 °C (optimum 16 °C), in the

presence of 1–5% NaCl (w/v, optimum 3.0%) and at pH 5.5–8.5 (optimum pH 6.5). No growth occurred in the absence of salts. The strain was positive for hydrolysis of starch and alginate. The other cultural, physiologically and biochemical characteristics of strain 4SWWS2-6^T are given in the species description. The strain 4SWWS2-6^T is similar to the reference strain of the phylogenetically related species of the genus *Ancylomarina.* All the above characteristics demonstrated that strain 4SWWS2-6^T belongs to the genus *Ancylomarina.* Meanwhile, strain 4SWWS2-6^T could be clearly differentiated from the reference strains based on the features including the temperature and NaCl ranges that supported growth, the O_2 metabolism, enzyme activity, oxidase activity, indole production, H_2S production. A detailed list of differential properties observed in strain 4SWWS2-6^T and closely related strains are listed in Table 1.

Phylogenetic analysis based on 16S rRNA gene sequences

Pairwise alignment of the 16S rRNA gene sequence of strain 4SWWS2-6^T showed high sequence similarity with *A. subtilis* FA102^T (96.5%), followed by *M. albidiflavum* FB208^T (Xu et al. 2016) (93.5%), *M. fragile* JCM 15579^T (93.1%) and *Marinifilum flexuosum* DSM 21950^T (92.9%). The phylogenetic trees obtained by neighbour-joining, maximum-likelihood and maximum-parsimony methods revealed that strain 4SWWS2-6^T formed the closest relationship with *A. subtilis* FA102^T and located in the same cluster with three species of the genus *Marinifilum* (Fig. 1). Phylogenetic analyses and low 16S rRNA gene similarity to the type species of the genus *Ancylomarina* indicated that strain 4SWWS2-6^T represents a novel species in the genus *Ancylomarina.* The *Asinibacterium lactis* LCJ02^T, belonging to the class of *Sphingobacteriales* (Kämpfer 2011) was chosen as outgroup.

Table 1 Differential characteristics between strain 4SWWS2-6^T and three reference strains

Characteristic	1	2	3	4
Colony color	White	Beige[a]	Ivory[b]	Beige[c]
Cell size (μm)				
Width	0.4–0.7	0.3–0.4[a]	0.5[b]	0.3–0.4[c]
Length	2.0–5.0	2.9–30[a]	0.3–3.8[b]	2.6–30[c]
Range for growth(optimum)				
Temperature (°C) (optimum temperature)	4.0–37.0 (16.0)	8.0–33.0[a] (28.0–30.0)	20.0–37.4[b] (33)	20.0–30.0[c]
NaCl (%) (optimum NaCl%)	1.0–5.0 (3.0)	0.5–5.0[a] (2.0)	1.0–7.0[b] (3.0)	2.0–5.0[c]
pH (optimum pH)	5.5–8.5 (6.5)	6.0–8.5[a] (7.0)	6.0–8.0[b] (7.0)	ND[c]
Enzyme activity				
Alginase	–	+	+	+
Gelatinase	–	+	+	+
Oxidase	–	+	+[b]	+[c]
Indole production	–	+	+	+
H_2S prodution	+	–	–	–
O_2 Metabolism	o	f[a]	f[b]	f[c]
DNA G+C content (mol%)	37.6	36.5[a]	35.7[b]	35.8[c]

Strains: 1 4SWWS2-6^T; 2 *A. subtilis* FA102^T (Wu et al. 2016); 3 *M. fragile* JCM 15579^T (Na et al. 2009); 4 *M. flexuosum* DSM 21950^T (Ruvira et al. 2013). All data are from this study except for DNA G+C content, cell size, temperature (°C), NaCl (%), pH range, O_2 metabolism, catalase and oxidase for growth, which are from the original species descriptions

+ positive reaction, − negative reaction, *ND* not detected, *o* obligately anaerobic, *f* facultatively anaerobic

[†]Data taken from [a]Wu et al. (2016), [b]Na et al. (2009), [c]Ruvira et al. (2013)

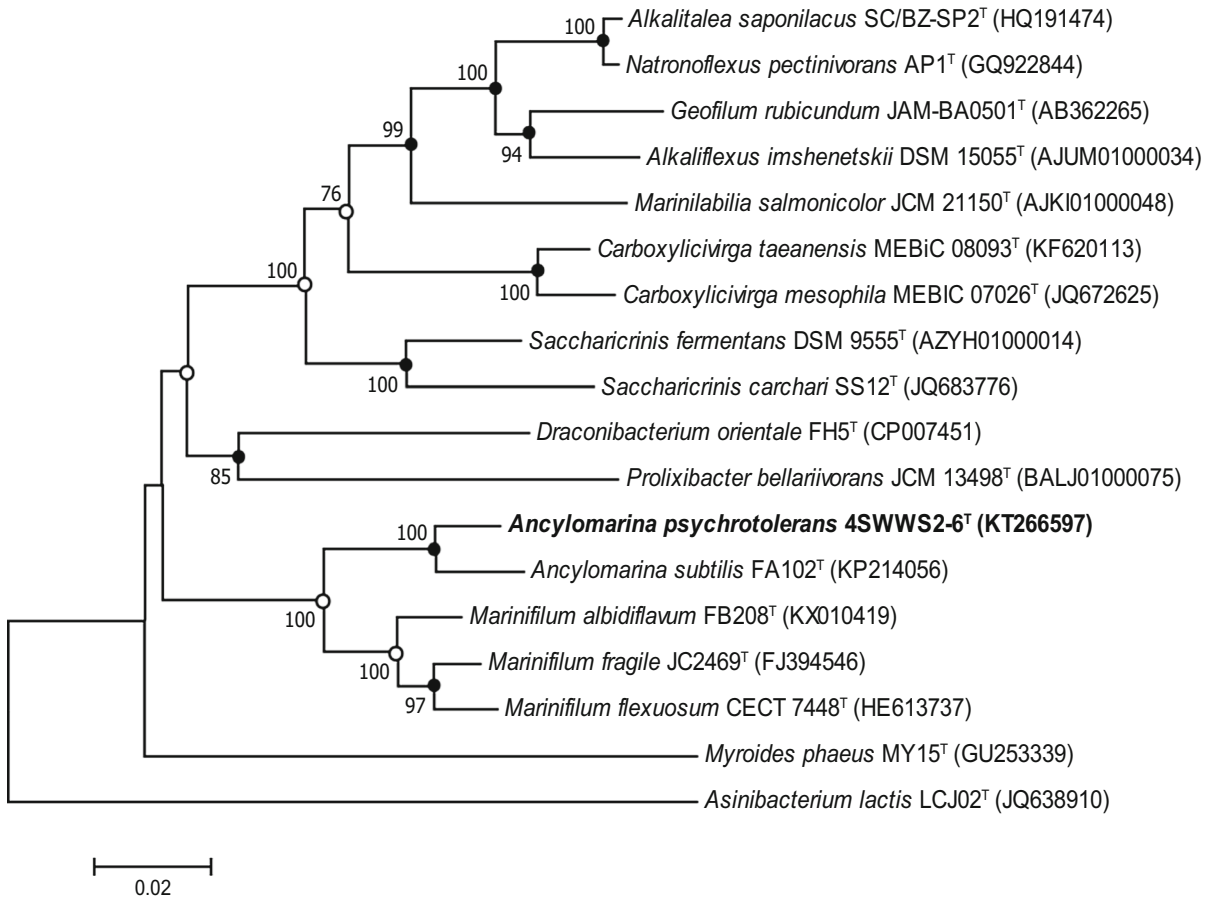

Fig. 1 Phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship between 4SWWS2-6^T and members of the genera *Ancylomarina*, *Marinifilum*, *Saccharicrinis*, *Prolixibacter*, and *Draconibacterium*. *Asinibacterium lactis* LCJ02^T was chosen as outgroup. The tree is based on the Kimura's two-state parameter model (Tamura et al. 2016) and neighbour-joining algorithm. Bootstrap values (> 70%) based on 1000 replicates are shown at branch points. Filled circles indicate nodes recovered in trees created with all three algorithms and open circles nodes recovered in trees created with two of the algorithms. Bar, 0.02 nt substitutions per nucleotide position

Chemotaxonomic characteristics

The cellular fatty acid profiles of strain 4SWWS2-6^T and the reference strains are shown in Table 2. The major cellular fatty acids of strain 4SWWS2-6^T (> 10% of the total fatty acids) were iso-$C_{15:0}$ (27.4%), anteiso-$C_{15:0}$ (19.0%) and iso-$C_{15:0}$ 3-OH (17.9%). The iso-$C_{15:0}$ in strain 4SWWS2-6^T was less than *A. subtilis* FA102^T, *M. fragile* JCM 15579^T and *M. flexuosum* DSM 21950^T. Strain 4SWWS2-6^T contained more anteiso-$C_{15:0}$ than all reference strains. Besides, the proportion of iso-$C_{15:0}$ 3-OH and iso-$C_{17:0}$ 3-OH in 4SWWS2-6^T was similar to *A. subtilis* FA102^T, which were significantly different from *M. fragile* JCM 15579^T and *M. flexuosum* DSM 21950^T. The predominant respiratory quinone detected in strain 4SWWS2-6^T was menaquinone-7 (MK-7). The major polar lipids detected in strain 4SWWS2-6^T was phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), aminolipid (AL), unidentified phospholipid (PL) and unidentified polar lipid (L1-2) (Fig. S2). The DNA G + C content of strain 4SWWS2-6^T was 37.6 mol%, which is similar to the other species in the genus *Ancylomarina* (36.5 mol%). The major features of strain 4SWWS2-6^T, including the major respiratory quinone and the predominant cellular fatty acids (Table 2), were similar to the reference strain of the genus *Ancylomarina*.

Table 2 Cellular fatty acid compositions between the novel isolate 4SWWS2-6^T, *A. subtilis* FA102^T, *M. fragile* JCM 15579^T and *M. flexuosum* DSM 21950^T

Fatty acid	1	2	3	4
Straight-chain				
$C_{15:0}$	tr	–	tr	1.6
$C_{16:0}$	1.8	tr	2.0	2.0
Branched				
anteiso-$C_{13:0}$	1.3	tr	–	–
iso-$C_{13:0}$	3.8	3.5	tr	1.4
iso-$C_{15:0}$	**27.4**	**47.0**	**49.9**	**54.7**
anteiso-$C_{15:0}$	**19.0**	8.3	2.4	1.9
iso-$C_{17:0}$	–	tr	1.6	1.8
iso-$C_{17:1}\omega 9c$	tr	1.7	7.4	3.2
Hydroxy				
$C_{15:0}$ 2-OH	2.0	1.3	tr	–
iso-$C_{15:0}$ 3-OH	**17.9**	**19.5**	7.0	6.5
$C_{17:0}$ 2-OH	3.3	1.5	tr	tr
iso-$C_{17:0}$ 3-OH	2.5	5.5	**15.3**	**15.2**

Strains: 1, 4SWWS2-6^T; 2, *A. subtilis* FA102^T; 3, *M. fragile* JCM 15579^T; 4, *M. flexuosum* DSM 21950^T. All data are from this study. tr, Traces (< 1%); – not detected. Values are percentages of the total fatty acids. Fatty acids amounting to < 1% of t he total in all strains are not shown. Bold type indicates major fatty acids (> 10%)

On the basis of phenotypic characteristics and phylogenetic provements, strain 4SWWS2-6^T is considered to represent a novel species in the genus *Ancylomarina*, for which the name *Ancylomarina psychrotolerans* sp. nov. is proposed. The Digital Protologue database TaxonNumber for strain 4SWWS2-6^T is TA00344.

Emended the description of genus *Ancylomarina*

The description is the same as that provided by Wu et al. (2016) with the following modification: members of genus *Ancylomarina* are facultative anaerobes or obligate anaerobes.

Description of *Ancylomarina psychrotolerans* sp. nov.

Ancylomarina psychrotolerans (psy.chro.to'le.rans. Gr. adj. *psychros*, cold; L. pres. part. *tolerans*, tolerating; N.L. part. adj. *psychrotolerans*, tolerating cold temperature).

Cells are obligate anaerobic, Gram-stain negative, long rod-shaped (approximately 2.0–5.0 μm in length and 0.4–0.7 μm in width), non-flagellated and non-motile. Colonies on MA are white, transparent, and regular margins after incubation for 72 h at 16 °C. Growth occurs at 4–37 °C (optimum 16 °C), in the presence of 1–5% NaCl (w/v, optimum 3.0%) and at pH 5.5–8.5 (optimum pH 6.5). Oxidase and catalase are negative. Starch and alginate can be hydrolyzed, but cellulose, agar, Tweens 20, 40, 80 and casein cannot be hydrolyzed. In the API 20A strip, acid is produced from glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, glycerol, cellose, mannose, melezitose, raffinose, sorbitol, rhamnose trehalose and esculin. H_2S is produced, while indole is not. The following substrates are oxidized in the Biolog AN MicroPlate: α-Cyclodextrin, β-Cyclodextrin, dextrin, dulcitol, D-fructose, L-fucose, D, L-α-Glycerol Phosphate, m-inositol, β-methyl-D-glucoside, L-rhamnose, acetic acid, glyoxylic acid, α-hydroxybutyric acid, α-ketobutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, D-malic acid, pyruvic acid, succinamic acid, succinic acid, succinic acid mono-methyl ester, m-tartaric acid, L-alanyl-L-glutamine, L-alanyl-L-threonine, L-glutamic acid, L-threonine, L-valine, L-valine plus L-aspartic acid, thymidine and uridine-5'-monophosphate, the other substrates are not oxidized. The dominant fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ 3-OH. The major respiratory quinone is MK-7. The major polar lipids comprise one phosphatidylethanolamine, one phosphatidylmonomethylethanolamine, one aminolipid, two unidentified lipids and an unidentified phospholipid. The DNA G+C content of the type strain is 37.6 mol%.

The type strain, 4SWWS2-6^T (= KCTC 15504^T = MCCC 1K01618^T), was isolated from sediments in the intertidal zone of Fildes Peninsula, Antarctica.

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Conflict of interest The authors declare no conflict of interest.

References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1995) Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology, 3rd edn. Wiley, New York

Balch W, Fox G, Magrum L, Woese C, Wolfe R (1979) *Methanogens*: reevaluation of a unique biological group. Microbiol Rev 43:260

Beveridge TJ, Lawrence JR, Murray RG (2007) Sampling and staining for lightmicroscopy. In: Schmidt TM, Snyder LR, Reddy CA, Beveridge TJ, Breznak JA, Marzluf G (eds) American Society for Microbiology Methods for General and Molecular Microbiology. American Society for Microbiology, WashingtonDC, pp 19–33

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

Kämpfer P (2011) Order I. Sphingobacteriales ord. nov. Bergey's manual of systematic bacteriology vol 4, p 330

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

Komagata K, Suzuki KI (1988) 4 Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207

Li G, Zeng X, Liu X, Zhang X, Shao Z (2016) *Wukongibacter baidiensis* gen. nov., sp. nov., an anaerobic bacterium isolated from hydrothermal sulfides, and proposal for the reclassification of the closely related *Clostridium halophilum* and *Clostridium caminithermale* within *Maledivibacter* gen. nov., and *Paramaledivibacter* gen. nov., respectively. Int J Syst Evol Microbiol 66:4355

Lyman J, Fleming RH (1940) Composition of sea water. J Mar Res 3:134–146

Mesbah M, Premachandran U, Whitman W-B (1989) Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. J Chromatogr 479:297–306

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M et al (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Na H, Kim S, Moon EY, Chun J (2009) *Marinifilum fragile* gen. nov., sp. nov., isolated from tidal flat sediment. Int J Syst Evol Microbiol 59:2241–2246

Ruvira MA, Lucena T, Pujalte MJ, Arahal DR, Macian MC (2013) *Marinifilum flexuosum* sp. nov., a new bacteroidetes isolated from coastal Mediterranean sea water and emended description of the genus *Marinifilum* Na et al. 2009. Syst Appl Microbiol 36:155–159

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, technical note 101. MIDI Inc, Newark

Steinsbu BO, Thorseth IH, Nakagawa S, Inagaki F, Lever MA et al (2010) *Archaeoglobus sulfaticallidus* sp. nov., a thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. Int J Syst Evol Microbiol 60:2745–2752

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

Teather RM, Wood PJ (1982) Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl environ microbiol 43:777–780

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tindall BJ, Sikorski J, Smibert RA, Krieg NR, Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (2007) Phenotypic characterization and the principles of comparative systematics. Methods for general and molecular microbiology. American Society for Microbiology, Washington DC, pp 330–393

Wang YP, Shi XC, Yu SL, Zhang XH (2015) Diversity of culturable anaerobic bacteria isolated from intertidal sediments of Qingdao. Mar Sci 39:92–99

Wu WJ, Zhao JX, Chen GJ, Du ZJ (2016) Description of *Ancylomarina subtilis* gen. nov., sp. nov., isolated from coastal sediment, proposal of *Marinilabiliales* ord. nov. and transfer of *Marinilabiliacea*e, *Prolixibacteraceae* and *Marinifilacea*e to the order *Marinilabiliales*. Int J Syst Evol Microbiol 66:4243–4249

Xie CH, Yokota A (2003) Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. J Gen Appl Microbiol 49:345–349

Xu ZX, Mu X, Zhang HX, Chen GJ, Du ZJ (2016) *Marinifilum albidiflavum* sp. nov., isolated from coastal sediment. Int J Syst Evol Microbiol 66(11):4589–4593

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617

Yu T, Yin Q, Song X, Zhao R, Shi X, Zhang XH (2013) *Aquimarina longa* sp. nov., isolated from seawater, and emended description of *Aquimarina muelleri*. Int J Syst Evol Microbiol 63:1235–1240