Psychroserpens jangbogonensis sp. nov., a psychrophilic bacterium isolated from Antarctic marine sediment

Kiwoon Baek,[1] Yung Mi Lee,[1,2] Chung Yeon Hwang,[1] Hyun Park,[1] You-Jung Jung,[1] Mi-Kyeong Kim,[1] Soon Gyu Hong,[1] Ji Hee Kim[1] and Hong Kum Lee[1]

Correspondence
Hong Kum Lee
hklee@kopri.re.kr

[1]Division of Polar Life Sciences, Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon 406-840, Republic of Korea

[2]School of Biological Sciences, College of Natural Science, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-747, Republic of Korea

A Gram-staining-negative, yellow-pigmented, aerobic, rod-shaped and non-motile bacterium, PAMC 27130^T, was isolated from the marine sediment of the Ross Sea, Antarctica. The temperature, pH and NaCl tolerance ranges for growth were 4–20 °C, pH 6.0–9.0 and 0.5–5.0 % (w/v) NaCl, respectively. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain PAMC 27130^T belonged to the genus *Psychroserpens* and was closely related to *Psychroserpens mesophilus, Psychroserpens damuponensis* and *Psychroserpens burtonensis* with 97.2, 94.7 and 94.2 % sequence similarities, respectively. Genomic relatedness analyses based on average nucleotide identity and genome-to-genome distance showed that strain PAMC 27130^T could be clearly distinguished from other species of the genus *Psychroserpens* . The genomic DNA G+C content was 32.7 mol%. The major fatty acids (>10 %) were $C_{20:4}\omega6c$ (13.2 %), iso-$C_{15:0}$ (12.3 %), iso-$C_{15:1}$ G (11.7 %) and iso-$C_{15:0}$ 3-OH (10.0 %). The major respiratory isoprenoid quinone was menaquinone-6 (MK-6) and the polar lipids were phosphatidylethanolamine, two unidentified aminolipids, an unidentified phospholipid, an unidentified aminophospholipid and three unidentified lipids. On the basis of genotypic and phenotypic data collected in this study, it is proposed that strain PAMC 27130^T represents a novel species of the genus *Psychroserpens*, for which the name *Psychroserpens jangbogonensis* sp. nov. is proposed. The type strain is PAMC 27130^T (=KCTC 42128^T=JCM 30228^T).

The genus *Psychroserpens* is a member of the family *Flavobacteriaceae* (Bowman *et al.*, 1997; Bernardet *et al.*, 2002; Bernardet & Nakagawa, 2006) in the phylum *Bacteroidetes*. At the time of writing, the genus *Psychroserpens* consisted of three species with validly published names. The genus *Psychroserpens* was first proposed with a description of *Psychroserpens burtonensis* that was isolated from Burton Lake, Antarctica (Bowman *et al.*, 1997). Two other species, *Psychroserpens mesophilus* and *Psychroserpens damuponensis*, were isolated from a young marine biofilm formed on an acrylic surface (Kwon *et al.*, 2006) and from a seawater sample collected from the coast at Damupo beach, Republic of Korea (Lee *et al.*, 2013), respectively. Members of the genus *Psychroserpens* are characterized as being Gram-staining-negative, yellow to yellowish-orange, oxidase-positive and obligately aerobic with rod-shaped cells and MK-6 as the major respiratory quinone. In the present study, strain PAMC 27130^T, isolated from Antarctic marine sediment, is described as a representative of a novel species of the genus *Psychroserpens* based on the results of a polyphasic taxonomic approach.

Sediment samples were collected from Ross Sea in the Southern Ocean (74° 37′ 30.61″ S 164° 14′ 56″ E) on 11 February 2011 by using a grap sampler. The sediment samples were suspended in 20 % (v/v) glycerol and preserved at −80 °C until use. Strain PAMC 27130^T was isolated by using the standard dilution plating method on marine agar 2216 (MA; BD Difco) and incubating the plate at 10 °C for 12 days. A total of 14 colonies with different morphologies were picked and streaked on MA plates three

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PAMC 27130^T is KJ475160. The NCBI BioProject accession numbers for the draft genome sequences of strain PAMC 27130^T, *Psychroserpens mesophilus* JCM 13413^T and *Psychroserpens damuponensis* JCM 17632^T are PRJNA255842, PRJNA255843 and PRJNA255844, respectively.

One supplementary table and two supplementary figures are available with the online Supplementary Material.

or more times to obtain pure cultures. Among the 14 strains isolated from Antarctic sediment and identified by 16S rRNA gene sequencing, only one strain, designated PAMC 27130^T, was affiliated with the genus *Psychroserpens*, while the others were affiliated with five other genera, including *Flavobacterium*, *Loktanella*, *Polaribacter*, *Roseovarius* and *Winogradskyella* (Lee *et al.*, 2014). All strains were deposited at the Polar and Alpine Microbial Collection (PAMC; Lee *et al.*, 2012) of Korea Polar Research Institute and the strains were preserved as glycerol suspensions (20 %, v/v, in distilled water) at −80 °C. Reference strains *Psychroserpens mesophilus* JCM 13413^T and *Psychroserpens damuponensis* JCM 17632^T were purchased from the Japan Collection of Microorganisms (JCM) and *Psychroserpens burtonensis* CIP 105822^T was from the Collection of Institut Pasteur (CIP) for comparisons. After the optimal growth conditions of the three reference strains along with strain PAMC 27130^T were determined in this study, all strains were routinely cultured on MA at 15 °C.

For 16S rRNA gene sequencing, genomic DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's instructions. The 16S rRNA gene was amplified and sequenced as described by Cho & Giovannoni (2004). The resulting 16S rRNA gene sequence (1455 nt) of strain PAMC 27130^T was compared with those of all type strains in the EzTaxon-e database (Kim *et al.*, 2012). The 16S rRNA gene sequence of strain PAMC 27130^T was aligned with those of its closest relatives using the RDPII online aligner (Cole *et al.*, 2014). Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987) with the Jukes–Cantor distance (Jukes & Cantor, 1969), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using the MEGA6 program (Tamura *et al.*, 2013). The robustness of the phylogenetic trees generated by the three tree-making algorithms was confirmed by bootstrap analyses based on 1000 random resamplings of the sequences. Comparison of 16S rRNA gene sequences showed that strain PAMC 27130^T was closely related to *Psychroserpens mesophilus* (97.2 % sequence similarity), *Psychroserpens damuponensis* (94.7 % sequence similarity) and *Psychroserpens burtonensis* (94.2 % sequence similarity). In all phylogenetic trees generated in this study, strain PAMC 27130^T and the three species of the genus *Psychroserpens* with validly published names constituted a robust clade and separated well from other genera in the family *Flavobacteriaceae*, indicating that strain PAMC 27130^T belonged to genus *Psychroserpens* (Fig. 1).

For investigation of genome relatedness, whole genome sequencing was attempted for strain PAMC 27130^T, *Psychroserpens mesophilus* JCM 13413^T and *Psychroserpens damuponensis* JCM 17632^T. The details of draft genomes for the three strains, PAMC 27130^T and the two reference strains, are summarized in Table S1 (available in the online Supplementary Material). The draft genome sequence of *Psychroserpens burtonensis* DSM 12212^T (=CIP 105822^T) was retrieved from the Shotgun Assembly Sequences (GenBank accession number AUDE01000000) in NCBI for genomic comparison. Genomic DNA samples were extracted using a DNeasy Blood & Tissue kit (Qiagen) and genome sequencing was performed using the MiSeq sequencer system (Illumina). The degree of pairwise genome-based relatedness was estimated by both an average nucleotide identity (ANI) value following the BLAST-based ANI calculation method described by Goris *et al.* (2007), and the genome-to-genome distance calculation (GGDC) method described by Auch *et al.* (2010). The ANI values calculated for estimation of the degree of pairwise genome-based relatedness between strain PAMC 27130^T and the type strains of *Psychroserpens mesophilus*, *Psychroserpens damuponensis* and *Psychroserpens burtonensis* were 83.9, 81.6 and 81.9 %, respectively (Table 1) and this level is well below the ANI cut-off values (95–96 %) proposed for delineating bacterial species (Goris *et al.*, 2007; Richter & Rosselló-Móra, 2009). Consistently, DNA–DNA hybridization values estimated by GGDC were 20.3–23.9 % between strain PAMC 27130^T and the other type strains (Table 1), indicating that strain PAMC 27130^T represents a distinctive species of the genus *Psychroserpens* (Rosselló-Mora & Amann, 2001). The genomic DNA G+C content was calculated directly from the genome sequence. The genomic DNA G+C content of strain PAMC 27130^T was determined to be 32.7 mol%.

The temperature range and optimal temperature for growth were determined by culturing strain PAMC 27130^T and the three reference strains on MA at different temperatures (4, 10, 15, 20, 25, 30 and 37 °C) for 7 days. The pH range and optimal pH for growth were determined in artificial seawater (ASW) medium (Choo *et al.*, 2007) supplemented with 0.5 % (w/v) peptone and 0.1 % (w/v) yeast extract at pH 5.0–10.0 (at intervals of 0.5 pH unit). The pH was adjusted using the following buffering system: MES pH 5.0–6.0, MOPS pH 6.5–7.0, HEPES pH 7.5–8.0, Tris pH 8.5–9.0 and CHES pH 9.5–10.0. NaCl requirement and tolerance were determined on ASW medium supplemented with 0–3 % (at intervals of 0.5 %), 4, 5, 7.5, 10 and 15 % (w/v) NaCl. The growth of each culture was monitored by measuring optical density at 600 nm (EnVision plate reader; PerkinElmer) every day for up to 7 days. The presence of flagella, cell morphology and cell size were examined using transmission electron microscopy (TEM) (CM200; Phillips). For TEM examination, cells were negatively stained with 2.0 % (w/v) uranyl acetate on a carbon-coated copper grid. Cellular motility was observed in fresh wet mounts using the hanging drop method (Bernardet *et al.*, 2002). Anaerobic growth was examined using the MGC anaerobic system (Mitsubishi Gas Chemical) after 2 weeks of incubation. Gram staining was performed using a Gram-staining kit (Sigma). The presence of flexirubin-type pigments was determined by the bathochromatic shift test with 20 % (w/v) KOH solution. Cellular pigments were extracted with acetone/methanol (1 : 1, v/v) and the absorption spectra were examined by using a UV–visible spectrophotometer

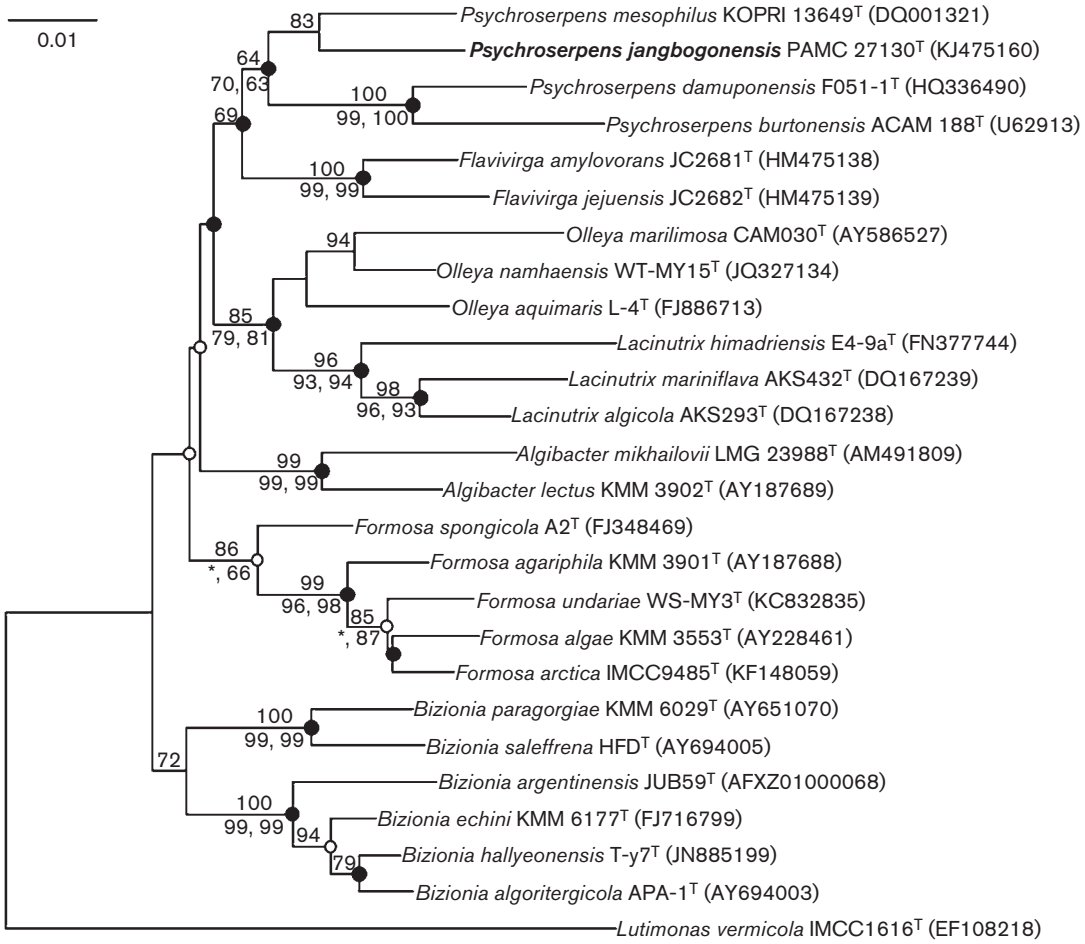

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain PAMC 27130^T and other representatives of the family *Flavobacteriaceae*. Bootstrap values (>60 %) based on 1000 resamplings are shown above nodes for the neighbour-joining and below nodes for the maximum-likelihood and maximum-parsimony methods, respectively. Filled circles indicate that the corresponding nodes were recovered by all treeing methods. *Bootstrap value obtained from maximum-likelihood. Bar, 0.01 substitutions per nucleotide position. *Lutimonas vermicola* IMCC1616^T was used as an outgroup.

(Optizen 2120UV; Mechasis). The catalase test was performed by observation of bubbles after the addition of 3 % (v/v) hydrogen peroxide to the cell suspensions. Hydrolysis of Tweens 20, 40, 60 and 80 (each 1 %, v/v) and starch (1 %, w/v) was tested using MA supplemented with each component according to the method of Smibert & Krieg (1994). Decomposition of hypoxanthine and xanthine (each 1 %, w/v) was tested using MA supplemented with

Table 1. Results of genomic relatedness analyses based on the average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (DDH) inferred by the genome-to-genome distance

	ANI (%)				DDH (%)			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1. PAMC 27130^T	–	83.9	81.6	81.9	–	23.9±2.4	20.3±2.3	20.8±2.3
2. *Psychroserpens mesophilus* JCM 13413^T		–	81.7	81.4		–	19.9±2.3	19.9±2.3
3. *Psychroserpens damuponensis* JCM 17632^T			–	83.3			–	23.3±2.4
4. *Psychroserpens burtonensis* DSM 12212^T				–				–

Table 2. Major characteristics that differentiate strain PAMC 27130^T from the type strains of species of the genus *Psychroserpens*

Strains: 1, *Psychroserpens jangbogonensis* sp. nov. PAMC 27130^T; 2, *Psychroserpens mesophilus* JCM 13413^T; 3, *Psychroserpens damuponensis* JCM 17632^T; 4, *Psychroserpens burtonensis* CIP 105822^T. All data were obtained in this study. +, Positive; −, negative; R, resistant; S, susceptible. All strains are positive for catalase, activity of alkaline phosphatase, valine arylamidase and acid phosphatase, and acid production from D-glucose and maltose. All strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase (PNPG), lipase (C14), α-galactosidase, β-galactosidase, α-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, hydrolysis of hypoxanthine, xanthine and Tween 60, and acid production from glycerol, erythritol, D-ribose, D-xylose, L-xylose, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, *N*-acetylglucosamine, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, turanose, L-fucose and potassium gluconate.

Characteristic	1	2	3	4
Temperature range for growth (optimum) (°C)	4–20 (15)	4–40 (25)	4–37 (30)	0–20 (10–15)
Oxidase	+	+	+	−
Gelatin liquefaction	−	+	+	+
Hydrolysis of:				
Aesculin	−	−	+	−
Starch	+	+	+	−
Tween 40	−	+	+	+
Skimmed milk, Tween 20, Tween 80	−	−	+	−
Enzyme activities (API ZYM)				
Leucine arylamidase	+	+	−	+
Esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase	−	+	+	−
α-Chymotrypsin	−	+	−	+
Trypsin	−	−	+	−
Cystine arylamidase	−	−	−	+
Acid production from (API 50CH):				
D-Lyxose	+	+	−	+
Glycogen	+	−	+	−
Methyl α-D-glucopyranoside, potassium 2-ketogluconate	+	−	−	−
D-Mannose	−	+	+	+
D-Fructose, potassium 5-ketogluconate	−	+	+	−
Cellobiose	−	+	−	+
D-Arabinose, D-adonitol, D-galactose, methyl α-D-mannopyranoside, lactose, xylitol, D-tagatose, D-arabitol, L-arabitol	−	+	−	−
Aesculin ferric citrate	−	−	+	−
L-Arabinose, amygdalin, arbutin, salicin, gentiobiose, D-fucose	−	−	−	+
Antibiotic susceptibility				
Rifampicin (30 μg)	S	R	S	R
Chloramphenicol (30 μg)	S	R	R	S
Nalidixic acid (30 μg), tetracycline (30 μg)	R	R	R	S
DNA G+C content (mol%)	32.7	33.4	32.5	33.4*

*The genomic DNA G+C content was calculated from the draft genome available under the accession number AUDE01000000.

each component according to the protocol of Gordon *et al.* (1974). Other biochemical tests were performed using API 20NE, API ZYM and API 50CH test kits (bioMérieux) according to the manufacturer's instructions except that bacterial strains were suspended in ASW medium. Susceptibility to antibiotics was analysed using the disc diffusion method. The following antibiotic discs (Oxoid) were used (μg per disc): ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), rifampicin (30), tetracycline (30) and vancomycin (30). The morphological, physiological and biochemical characteristics of strain PAMC 27130^T are given in Table 2 and the species description. A number of phenotypic characteristics, including temperature range and optimal temperature for growth, hydrolysis of macromolecules, enzyme activities, acid production from a variety of substrates, and susceptibility to antibiotics differentiated strain PAMC 27130^T from *Psychroserpens mesophilus* JCM 13413^T, *Psychroserpens damuponensis* JCM 17632^T and *Psychroserpens burtonensis* CIP 105822^T.

The fatty acid methyl esters of strain PAMC 27130^T and the three reference strains were extracted from cultures grown

on MA at 15 °C for 5 days and were analysed according to the instruction of the Sherlock Microbial Identification System version 6.1 (MIDI) using the TSBA6.1 database. The major fatty acids (>10 %) of strain PAMC 27130^T were $C_{20:4}\omega6c$ (13.2 %), iso-$C_{15:0}$ (12.3 %), iso-$C_{15:1}$ G (11.7 %) and iso-$C_{15:0}$ 3-OH (10.0 %) (Table 3). It was notable that a significant amount (13.2 %) of $C_{20:4}\omega6c$ (6,9,12,15) was found in strain PAMC 27130^T but not in the other type strains. The isoprenoid quinones extracted according to the method described by Minnikin *et al.* (1984) were separated by TLC and analysed by using HPLC (Collins, 1985). The respiratory quinone detected in strain PAMC 27130^T was menaquinone-6 (MK-6), which is generally found in members of the family *Flavobacteriaceae*. Polar lipids of strain PAMC 27130^T were extracted from lyophilized bacterial cells and examined using two-dimensional TLC followed by detection with the reagents molybdatophosphoric acid, ninhydrin, molybdenum blue, α-naphthol, Dragendorff's solution and Schiff's solution (Minnikin *et al.*, 1984). The polar lipids found in strain PAMC 27130^T were phosphatidylethanolamine, two unidentified aminolipids, an unidentified phospholipid, an unidentified aminophospholipid and three unidentified lipids (Fig. S1). This composition was similar to that of type strains of species of the genus *Psychroserpens* with respect to the presence of phosphatidylethanolamine, unidentified aminolipids, an unidentified phospholipid and unidentified lipids (Bowman *et al.*, 1997; Kwon *et al.*, 2006; Lee *et al.*, 2013). However, the presence of an unidentified aminophospholipid in PAMC 27130^T distinguished it from the reference strains.

Overall, strain PAMC 27130^T formed a phylogenetic clade with the three type strains of species of the genus *Psychroserpens* (Fig. 1). The chemotaxonomic profiles of strain PAMC 27130^T were generally similar to those of members of the genus *Psychroserpens*. However, phenotypic characteristics, including temperature range and optimal temperature for growth, hydrolysis of macromolecules and enzyme activities differentiated strain PAMC 27130^T from other species of the genus *Psychroserpens*. Therefore, strain PAMC 27130^T represents a novel species of the genus *Psychroserpens*, for which the name *Psychroserpens jangbogonensis* sp. nov. is proposed.

Table 3. Cellular fatty acid composition of strain PAMC 27130^T and members of the genus *Psychroserpens*

Strains: 1, *Psychroserpens jangbogonensis* sp. nov. PAMC 27130^T; 2, *Psychroserpens mesophilus* JCM 13413^T; 3, *Psychroserpens damuponensis* JCM 17632^T; 4, *Psychroserpens burtonensis* CIP 105822^T. All data were taken from this study. Values represent percentages of total fatty acids; those that represented <1.0 % in all strains are not shown. tr, Trace (<1.0 %); –, not detected.

Fatty acid	1	2	3	4
$C_{15:0}$	–	1.9	3.4	4.8
$C_{16:0}$	1.6	–	tr	1.0
$C_{15:0}$ 2-OH	6.7	–	2.6	6.0
$C_{17:0}$ 2-OH	4.2	–	4.0	7.8
$C_{18:1}\omega5c$	–	–	3.0	–
$C_{20:4}\omega6c$ (6,9,12,15)	13.2	–	–	–
anteiso-$C_{17:1}\omega9c$	–	–	–	1.7
iso-$C_{14:0}$	–	1.6	–	0.5
iso-$C_{15:0}$	12.3	14.3	15.2	10.3
iso-$C_{16:0}$	1.0	2.3	2.0	3.3
iso-$C_{15:0}$ 3-OH	10.0	6.5	9.3	4.0
iso-$C_{16:0}$ 3-OH	9.7	20.0	10.3	8.1
iso-$C_{17:0}$ 3-OH	8.5	12.7	11.5	10.9
iso-$C_{15:1}$ G	11.7	23.0	12.7	9.5
iso-$C_{16:1}$ G	2.0	6.6	2.9	5.3
anteiso-$C_{15:0}$	3.3	2.0	4.4	13.7
anteiso-$C_{15:1}$ A	7.3	3.8	4.3	6.7
Summed features*				
3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$)	8.4	5.3	5.4	2.2
9 (iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$)	–	–	8.1	4.3

*Summed features represent groups of two or more fatty acids that could not be separated with the MIDI system.

Description of *Psychroserpens jangbogonensis* sp. nov.

Psychroserpens jangbogonensis (jang.bo.go.nen′sis. N.L. masc. adj. *jangbogonensis* of or belonging to Jangbogo, the Korean research station in Antarctica).

Cells are Gram-staining-negative, oxidase- and catalase-positive, aerobic, yellow-pigmented, non-motile, non-gliding and flexirubin-negative. Cells are rod-shaped (0.6–0.8 × 2.4–3.5 μm; Fig. S2). Colonies are circular, convex, shiny with entire margins, and approximately 1.5 mm in diameter on MA plates after 5 days of incubation at 15 °C. Growth occurs at 4–20 °C (optimum, 15 °C), pH 6.0–9.0 (optimum, pH 8.0) and in the presence of 0.5–5.0 % (w/v) NaCl (optimum, 2.5–3.0 %) after 5 days of incubation on MA at 15 °C. Starch is hydrolysed. Hypoxanthine, xanthine and Tweens 20, 40, 60 and 80 are not hydrolysed. In the API ZYM system, alkaline phosphatase, leucine arylamidase, valine arylamidase and acid phosphatase are positive, but esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative. All tests are negative in the API 20NE system. In the API 50CH system, acid is produced from D-glucose, D-mannitol, methyl α-D-glucopyranoside, maltose, starch, glycogen, D-lyxose and potassium 2-ketogluconate, but negative for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, lactose, melibiose, sucrose,

trehalose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 5-ketogluconate. Susceptible to ampicillin, chloramphenicol, erythromycin, rifampicin and vancomycin, but resistant to gentamicin, kanamycin, nalidixic acid and tetracycline. The major cellular fatty acids are $C_{20:4}\omega6c$, iso-$C_{15:0}$, iso-$C_{15:1}$ G and iso-$C_{15:0}$ 3-OH. The major isoprenoid quinone is MK-6. The polar lipids are phosphatidylethanolamine, two unidentified aminolipids, an unidentified phospholipid, an unidentified aminophospholipid and three unidentified lipids.

The type strain, PAMC 27130^T (=KCTC 42128^T=JCM 30228^T), was isolated from a sediment sample collected from the Ross Sea, Antarctica.

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