Pseudorhodobacter psychrotolerans sp. nov., a psychrotolerant bacterium isolated from terrestrial soil, and emended description of the genus *Pseudorhodobacter*

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A Gram-stain-negative, facultatively aerobic, cream-coloured, ovoid-shaped, non-motile and psychrotolerant bacterial strain, PAMC 27389^T, was isolated from terrestrial soil collected on King George Island, Antarctica. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain PAMC 27389^T belongs to the genus *Pseudorhodobacter*, sharing highest similarities with the type strains of *Pseudorhodobacter wandonensis* (96.9 %), *Pseudorhodobacter antarcticus* (96.8 %), *Pseudorhodobacter ferrugineus* (96.5 %) and *Pseudorhodobacter aquimaris* (95.4 %). Average nucleotide identity values between strain PAMC 27389^T and the type strains of *P. wandonensis*, *P. antarcticus*, *P. ferrugineus* and *P. aquimaris* were 70.8, 70.9, 71.0 and 70.5 %, respectively and the genome-to-genome distances were 18.4–19.1 %, indicating PAMC 27389^T is clearly distinguished from the most closely related *Pseudorhodobacter* species. The genomic DNA G+C content was 60.1 mol%. Strain PAMC 27389^T grew at 0–37 °C (optimally at 15–20 °C), at pH 5.5–9.0 (optimally at pH 6.5–7.0) and in the presence of 0.5–3.0 % (w/v) sea salt (optimally with 0.5 %). It lacked bacteriochlorophyll *a*. The major fatty acids (>5 %) were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{18:1}\omega7c$ 11-methyl. The major polar lipids were phosphatidylcholine, phosphatidylglycerol, an unidentified phospholipid, an unidentified aminolipid, an unidentified lipid and three unidentified aminophospholipids. The major respiratory quinone was Q-10. Based on the phenotypic, chemotaxonomic and genomic data presented, we propose the name *Pseudorhodobacter psychrotolerans* sp. nov. with the type strain PAMC 27389^T (=KCTC 42640^T=JCM 30764^T).

The genus *Pseudorhodobacter*, a member of the *Alphaproteobacteria*, was first proposed by Uchino *et al.* (2002) with the reclassification of *Agrobacterium ferrugineum*, which was isolated from seawater of the Baltic Sea (Rüger & Höfle, 1992), as *Pseudorhodobacter ferrugineus*. At the time of writing, the genus *Pseudorhodobacter* comprised four validly published species names in the List of Prokaryotic Names with Standing in Nomenclature (Parte, 2014). Members of the genus *Pseudorhodobacter* have been isolated from intertidal sandy sediments, seawater and wood falls (Uchino *et al.*, 2002; Jung *et al.*, 2012; Chen *et al.*, 2013; Lee *et al.*, 2013). The genomic G+C contents of the type strains of species of the genus *Pseudorhodobacter* range from 57.1 to 61.6 mol%. In this study, a bacterial strain, PAMC 27389^T, isolated from Antarctic terrestrial soil was subjected to a polyphasic taxonomic analysis and allocated to the genus *Pseudorhodobacter*.

A terrestrial soil sample was collected from King George Island, Antarctica (62° 12.37′ S 58° 47.40′ W), on 12 February 2011. The terrestrial soil sample was preserved at −80 °C until use. For cultivation, a serially diluted aliquot (100 µl) of the sample in 0.85 % (w/v) NaCl was spread on R2A agar (BD Difco) plates and incubated at

Abbreviation: ANI, average nucleotide identity.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PAMC 27389^T is KT163920.

Two supplementary tables and three supplementary figures are available with the online Supplementary Material.

15 °C for 11 days. Pure cultures of the bacterial isolates were deposited with the Polar and Alpine Microbial Collection (PAMC; Lee *et al.*, 2012) and preserved as glycerol suspensions (20 %, v/v, in distilled water) at −80 °C. In this study, one of these strains, PAMC 27389^T, was routinely cultured on modified TYS agar medium [0.5 % tryptone (Oxoid), 0.1 % yeast extract and 0.5 % sea salt], with a reduced concentration of sea salt compared with standard TYS medium [0.5 % tryptone (Oxoid), 0.1 % yeast extract and 3 % sea salt (Chen *et al.*, 2013)] at 15 °C after determination of the optimal temperature for growth. For comparative analyses with strain PAMC 27389^T, four type strains of *Pseudorhodobacter* species, *Pseudorhodobacter wandonensis* KCTC 23672^T, *Pseudorhabdobacter antarcticus* KCTC 23700^T, *Pseudorhabdobacter aquimaris* KCTC 23043^T and *P. ferrugineus* LMG 22047^T, were purchased from Korean Collection of type Cultures (KCTC) and Laboratory of Microbiology Gent Bacteria Collection (LMG) and used as reference strains following cultivation under comparable conditions as for PAMC 27389^T.

Genome relatedness was investigated by whole genome sequencing of strain PAMC 27389^T and the four reference type strains. Genomic DNAs were extracted using a DNeasy Blood & Tissue kit (Qiagen) and genome sequencing was performed using the MiSeq sequencer system (Illumina) at Chun Lab (Seoul, Korea). Celera Assembler (version 7.0) was used for assembly (Myers *et al.*, 2000). The degree of pairwise genome-based relatedness was estimated by both the 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 method described by Auch *et al.* (2010). The genomic DNA G+C content was calculated directly from the genome sequence. The details of draft genomes for the five strains, PAMC 27389^T and the four reference strains, are summarized in Table S1 (available in the online Supplementary Material). The ANI values calculated for estimation of the degree of pairwise genome-based relatedness between strain PAMC 27389^T and *P. wandonensis* KCTC 23672^T, *P. antarcticus* KCTC 23700^T, *P. ferrugineus* LMG 22047^T and *P. aquimaris* KCTC 23043^T were 70.8, 70.9, 71.0 and 70.5 %, respectively (Table S2) and this level is well below the ANI cut-off values (95–96 %) that have been proposed for delineating bacterial species (Goris *et al.*, 2007; Richter & Rosselló-Móra, 2009). Mean DNA–DNA hybridization values between strain PAMC 27389^T and the other type strains estimated by genome-to-genome distance calculation were 18.4–19.1 % (Table S2), indicating that strain PAMC 27389^T is distinguishable from other *Pseudorhodobacter* species (Rosselló-Mora and Amann, 2001). The genomic DNA G+C content of strain PAMC 27389^T was determined to be 60.1 mol% from the draft genome sequence (Table S1), a value within the range for the genus *Pseudorhodobacter* (57.1–61.6 mol%) determined by HPLC analysis.

The 16S rRNA gene sequence (1464 nt) retrieved from whole genome sequencing was compared with those of all type strains in the EzTaxon-e database (Kim *et al.*, 2012), aligned with those of type strains showing high similarities and phylogenetic trees were reconstructed using three tree-making algorithms, the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods using the program MEGA 6 (Tamura *et al.*, 2013). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications of the sequences. Comparison of 16S rRNA gene sequences showed that strain PAMC 27389^T is closely related to the type strains of *Pseudorhodobacter* species at more than 95 % similarity. In phylogenetic trees inferred from the three tree-making algorithms, PAMC 27389^T formed a monophyletic clade with the type strains of species of the genus *Pseudorhodobacter* and was separated from other genera in the family *Rhodobacteraceae*, indicating that strain PAMC 27389^T is a member of the genus *Pseudorhodobacter* (Fig. 1).

Tests to assess the phenotypic characteristics of strain PAMC 27389^T were performed along with the four reference *Pseudorhodobacter* strains. As a preliminary experiment for determination of the optimal medium for growth, the five strains were incubated on R2A agar, TYS agar and modified TYS agar medium at different temperatures (0, 4, 10, 15, 20, 25, 30, and 37 °C) and we found that overall growth was best on modified TYS medium. Thus, modified TYS medium was determined as the basal medium for further tests. The temperature range and optimum for growth were determined by culturing strains in modified TYS broth [0.5 % tryptone (Oxoid), 0.1 % yeast extract and 0.5 % sea salt] at different temperatures (0, 4, 10, 15, 17, 20, 25, 30 and 37 °C) for 12 days. The pH range (pH 5.0–10.0 at intervals of 0.5 pH units) and optimal pH for growth were determined by assessing changes in OD_{600} (EnVision plate reader; PerkinElmer) in pH-buffered modified TYS broth using MES for pH 5.0–6.0, MOPS for pH 6.5–7.0, HEPES for pH 7.5–8.0, Tris for pH 8.5–9.0 and CHES for pH 9.5–10.0. Salt tolerance was determined by measuring OD_{600} at 15 °C using synthetic TYS broth without sea salt supplemented with 0–3 % (w/v) sea salt (at intervals of 0.5 %), and with 4, 5, 7.5, 10 and 15 % (w/v) sea salt. Cell size and morphology were examined by transmission electron microscopy (CM200; Philips). Cell motility was investigated by using the hanging-drop method described by Bernardet *et al.* (2002). Growth under anaerobic conditions was observed using the MGC anaerobic system (Mitsubishi Gas Chemical) after 2 weeks of incubation. Catalase activity was tested with 3 % H_2O_2 and oxidase activity was determined using tetramethyl-*p*-phenylenediamine according to the methods described by Kovacs (1956). Hydrolysis was tested on modified TYS solid medium supplemented with Tweens 20, 40, 60 and 80 [each at 1 % (v/v)], starch, skimmed milk, xanthine and hypoxanthine [each at 1 % (w/v)]. Other biochemical activities of PAMC 27389^T were determined by using API 20NE, API ZYM and API 50CH kits (bioMérieux) according to the manufacturer's instructions except that bacterial strains were suspended in 0.5 % (w/v) sea salt. For spectral analysis of pigment absorption, the culture

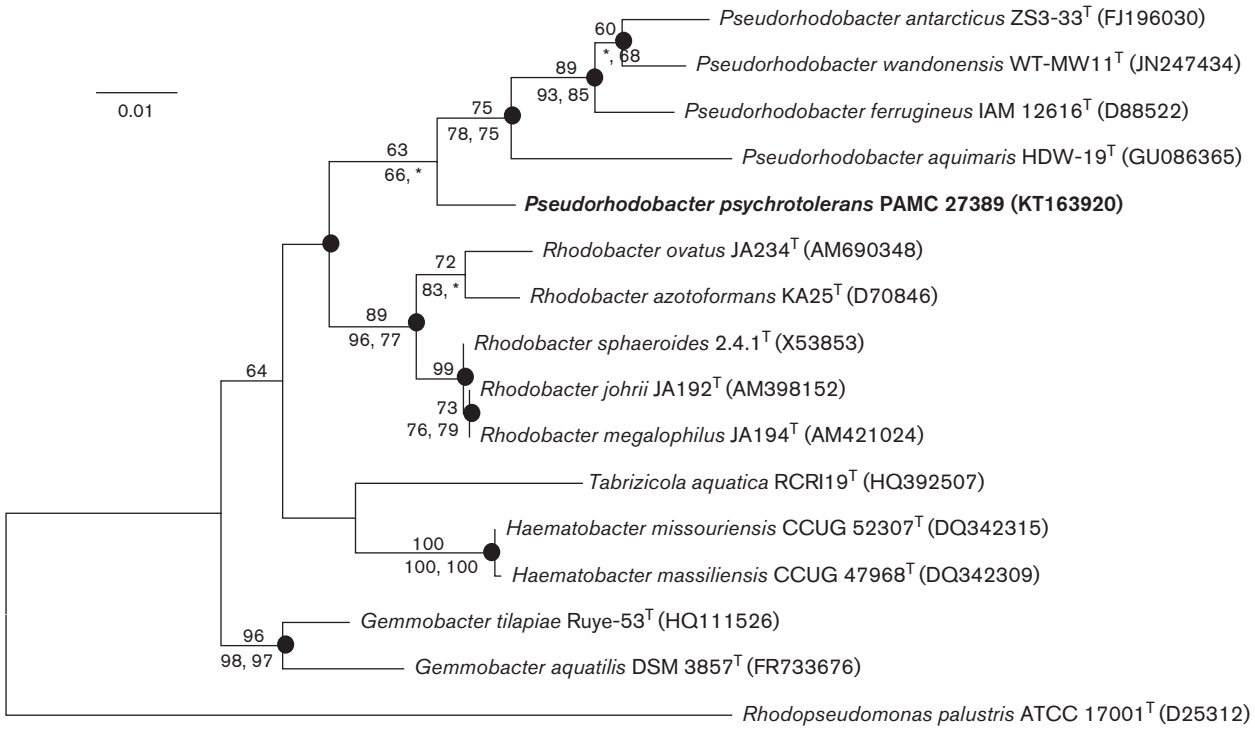

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences showing the relationships of strain PAMC 27389^T with species of the genus *Pseudorhodobacter* and other closely related members of the family *Rhodobacteraceae*. Filled circles indicate that the corresponding nodes were recovered by all treeing methods. Bootstrap values (≥60 %) based on 1000 resamplings are shown above nodes for the maximum-likelihood method and below nodes for the neighbour-joining and maximum-parsimony methods, respectively. *Rhodopseudomonas palustris* ATCC 17001^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

was washed twice via centrifugation with a MOPS buffer adjusted to pH 7.5 and disrupted using sonication (VCX-500; Vibra). After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was scanned using a spectrophotometer (S-3100; SCINCO). No peaks above 600 nm were detected (Fig. S1), indicating that the strain did not contain bacteriochlorophyll *a*. The morphological, physiological and biochemical characteristics of strain PAMC 27389^T are described in Figs S2 and S3, Table 1 and the species description. Physiological characteristics such as hydrolysis of macromolecules, enzyme activities and acid production distinguished strain PAMC 27389^T from other species of the genus *Pseudorhodobacter*.

The fatty acid methyl esters of strain PAMC 27389^T and the four type strains of *Pseudorhodobacter* species grown on modified TYS solid medium at 15 °C for 7 days were extracted as described by Sasser (1990) and analysed using GC (Agilent technologies 6890) according to the instructions of the Microbial Identification System (MIDI; version 6.2) with the TSBA6 database. 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).

Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). The major fatty acids (>5 %) were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 84.7 %) and $C_{18:1}\omega7c$ 11-methyl (5.3 %) (Table 2). The overall profiles of fatty acids of the five strains were similar to each other in that summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) is predominant, comprising 82 % or more of the total fatty acid methyl esters. However, $C_{18:1}\omega7c$ 11-methyl comprised 5.3 % in strain PAMC 27389^T but 0.8 % or less in the other type strains. Q-10 was the only respiratory quinone detected in strain PAMC 27389^T and Q-10 was also found in the other four reference strains of *Pseudorhodobacter* as a sole quinone. The polar lipids found in PAMC 27389^T were phosphatidylcholine, phosphatidylglycerol, an unidentified phospholipid, an unidentified aminolipid, an unidentified lipid and three unidentified aminophospholipids (Fig. S3). The presence of phosphatidylcholine and phosphatidylglycerol was common to PAMC 27389^T, *P. antarcticus* KCTC 23700^T, *P. wandonensis* KCTC 23672^T, *P. ferrugineus* LMG 22047^T and *P. aquimaris* KCTC 23043^T. However, the composition or presence of phospholipids, aminolipids, aminophospholipids and

Table 1. Major characteristics that differentiate strain PAMC 27389^T from closely related type strains of the genus *Pseudorhodobacter*

Strains: 1, PAMC 27389^T; 2, *P. wandonensis* KCTC 23672^T; 3, *P. antarcticus* KCTC 23700^T; 4, *P. ferrugineus* LMG 22047^T; 5, *P. aquimaris* KCTC 23043^T. Data were obtained in this study, unless otherwise indicated. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2	3	4	5
Isolation source	Terrestrial soil	Wood falls	Intertidal sandy sediments	Seawater	Seawater
Temperature range for growth (°C) (optimum)	0–37 (15–20)	0–37 (25–30)	0–30 (15)	0–37 (15–25)	0–30 (25–30)
Sea salt tolerance range for growth (%, w/v) (optimum)	0.5–3.0 (0.5)	0.5–7.5 (1.5)	0–10 (1.0)*	0.5–7.5 (1.0–2.5)	0.5–12.5 (0.5–1.5)
pH range for growth (optimum)	5.5–9.0 (6.5–7.0)	5.5–9.5 (7.0–7.5)	5.0–10.0 (7.5)*	6.0–9.5 (7.5)	6.0–10.0 (8.0–9.0)
API ZYM test					
Alkaline phosphatase	+	+	+	+	−
Valine arylamidase, cystine arylamidase	+	−	w	−	−
Trypsin, α-chymotrypsin	+	−	−	−	−
Acid phosphatase	+	+	+	w	−
α-Galactosidase	−	+	−	−	−
β-Glucuronidase, β-galactosidase	+	w	−	−	−
α-Glucosidase	+	+	−	−	−
API 20NE test					
Aesculin hydrolysis	+	+	+	+	−
Gelatin hydrolysis	+	−	−	−	−
4-Nitrophenyl-β-D-galactopyranoside, glucose fermentation	+	+	−	+	−
API 50CH test					
Erythritol	w	−	−	−	−
D-Arabinose	w	w	−	w	−
L-Arabinose	w	−	−	w	−
D-Ribose, L-rhamnose, salicin	+	−	−	−	−
D-Xylose	w	+	w	w	−
D-Galactose, D-mannose, *N*-acetylglucosamine	−	+	−	−	−
D-Glucose	−	w	w	−	−
D-Fructose	w	+	−	−	−
Inositol, D-sorbitol	−	−	−	−	w
D-Mannitol	−	+	−	−	w
Aesculin ferric citrate	+	+	+	−	−
Cellobiose, D-arabitol	−	+	−	−	−
Sucrose, trehalose	−	w	−	−	−
Xylitol, D-lyxose	w	−	−	−	−
D-Fucose	+	+	−	w	−
L-Fucose	+	+	−	+	−
Hydrolysis of:					
Xanthine	−	w	−	−	−
Hypoxanthine	+	+	−	−	+
Tween 20	+	+	w	−	−
Tween 40	−	w	−	−	−
Tween 60	w	+	−	−	−
Tween 80	−	+	−	−	−

*NaCl tolerance and PH range for growth data were taken from Chen *et al.* (2013).

lipids among PAMC 27389^T and the type strains of *Pseudorhodobacter* species was different from each other.

Overall, strain PAMC 27389^T formed a phylogenetic clade with the four type strains of the genus *Pseudorhodobacter* (Fig. 1). However, physiological characteristics such as hydrolysis of macromolecules, enzyme activities and acid production, genomic data compared based on calculation of ANI values and genome-to-genome distances, and the different composition of polar lipids distinguished strain PAMC 27389^T from other type strains of the genus

Table 2. Cellular fatty acid composition of strain PAMC 27389^T and the type strains of species of the genus *Pseudorhodobacter*

Strains: 1, PAMC 27389^T; 2, *P. wandonensis* KCTC 23672^T; 3, *P. antarcticus* KCTC 23700^T; 4, *P. ferrugineus* LMG 22047^T; 5, *P. aquimaris* KCTC 23043^T. All data are from the present study. −, Not detected.

Fatty acids	1	2	3	4	5
Straight-chain					
$C_{16:0}$	0.8	2.0	0.6	1.3	0.7
$C_{17:0}$	–	–	–	0.7	–
$C_{18:0}$	1.8	3.2	2.8	3.7	1.4
Unsaturated					
$C_{14:1}\omega5c$	–	0.4	0.5	0.3	–
$C_{18:1}\omega9c$	–	0.7	1.9	1.4	–
$C_{19:1}\omega6c$	–	–	1.6	–	–
iso-$C_{17:1}\omega5c$	–	0.4	–	–	–
anteiso-$C_{17:1}\omega9c$	1.7	–	–	–	–
$C_{18:1}\omega7c$ 11-methyl	5.3	–	–	0.8	–
10-Methyl					
$C_{19:0}$ 10-methyl	1.2	–	–	–	–
Hydroxy					
$C_{10:0}$ 3-OH	–	4.1	–	2.9	2.5
$C_{18:0}$ 3-OH	3.1	–	–	–	–
Summed features					
3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$)	–	3.1	–	1.3	4.0
8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$)	84.7	82.0	89.6	84.3	88.2
Unknown					
ECL 11.799	–	4.1	2.9	3.4	3.1
ECL 14.959	1.5	–	–	–	–

Pseudorhodobacter. In conclusion, based on the genomic, physiological and chemotaxonomic data described above, we suggest that strain PAMC 27389^T represents a novel species of the genus *Pseudorhodobacter*, for which the name *Pseudorhodobacter psychrotolerans* sp. nov. is proposed.

Emended description of the genus *Pseudorhodobacter*

The description is as given by Uchino *et al.* (2002), Jung *et al.* (2012), Chen *et al.* (2013) and Lee *et al.* (2013) with the following amendment. Some species grow facultatively aerobically.

Description of *Pseudorhodobacter psychrotolerans* sp. nov.

Pseudorhodobacter psychrotolerans (psy.chro.to′ler.ans. Gr. adj. *psychros* cold; L. pres. part. *tolerans* tolerating; N.L. part. adj. *psychrotolerans* cold-tolerating).

Cells are Gram-stain-negative, facultatively aerobic, cream-coloured, oxidase- and catalase-positive, non-motile and ovoid-shaped, approximately 0.5–1.0 μm in diameter and 1.0–2.0 μm in length. Colonies are 1.0–2.0 mm in diameter after incubation for 7 days at 15 °C on modified TYS agar medium. Growth occurs at 0–37 °C (optimally at 15–20 °C), at pH 5.5–9.0 (optimally at pH 6.5–7.0) and in the presence of 0.5–3.0 % (w/v) sea salt (optimally at 0.5 %). Bacteriochlorophyll *a* is not produced. Positive for oxidase and catalase acitivity. Hypoxanthine and Tweens 20 and 60 (weakly) are hydrolysed, but starch, skimmed milk, xanthine and Tweens 40 and 80 are not. According to the API 20NE test, positive for aesculin and gelatin hydrolysis, glucose fermentation, and assimilation of 4-nitrophenyl-β-D-galactopyranoside, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine and maltose, but negative for nitrate reduction, production of indole, and assimilation of D-glucose, L-arginine, urea, potassium gluconate, caprate, adipate, malate, citrate and phenyl acetate. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucuronidase, α-glucosidase and β-galactosidase, but negative for lipase (C14), α-galactosidase, β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase. In the API 50CH system, acid is produced from erythritol (weakly positive), D-arabinose (weakly positive), L-arabinose (weakly positive), D-ribose, D-xylose (weakly positive), D-fructose (weakly positive), L-rhamnose, aesculin ferric citrate, salicin, xylitol (weakly positive), D-lyxose (weakly positive), D-fucose and L-fucose, but negative for glycerol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-mannose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, turanose, D-tagatose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The major fatty acids (>5 %) are summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{18:1}\omega7c$ 11-methyl. The major isoprenoid quinone is Q-10. The polar lipids are phosphatidylcholine, phosphatidylglycerol, an unidentified phospholipid, an unidentified aminolipid, an unidentified lipid and three unidentified aminophospholipids.

The type strain, PAMC 27389^T (=KCTC 42640^T=JCM 30764^T), was isolated from a terrestrial soil sample collected from King George Island, Antarctica.

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