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Domibacillus tundrae sp. nov., isolated from active layer soil of tussock tundra in Alaska, and emended description of the genus *Domibacillus*

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A novel Gram-stain-positive, spore-forming, aerobic, motile and rod-shaped bacterium designated strain PAMC 80007^T was isolated from an active layer soil sample of Council, Alaska. Optimal growth of strain PAMC 80007^T was observed at 30 °C, pH 7.0 and in the presence of 2 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain PAMC 80007^T belonged to the genus *Domibacillus*. This strain was closely related to *Domibacillus enclensis* (98.3 %), *Domibacillus robiginosus* (98.3 %) and *Domibacillus indicus* (97.2 %). Genomic DNA G+C content was 43.5 mol% and genomic relatedness analyses based on the average nucleotide identity and the genome-to-genome distance showed that strain PAMC 80007^T is clearly distinguished from the closely related species of the genus *Domibacillus*. The major fatty acids (>5 %) were iso-$C_{15:0}$ (24.7 %), $C_{16:1}\omega11c$ (16.8 %), anteiso-$C_{15:0}$ (16.5 %), $C_{16:0}$ (15.6 %) and anteiso-$C_{17:0}$ (8.7 %). The major respiratory isoprenoid quinones were menaquinone-6 (MK-6) and menaquinone-7 (MK-7), and the polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, phospholipid and two unidentified lipids. *meso*-Diaminopimelic acid (type A1γ) was present in the cell-wall peptidoglycan, and the major whole-cell sugar was ribose with a minor quantity of glucose. Results from a polyphasic study suggested that strain PAMC 80007^T represents a novel species of the genus *Domibacillus* for which the name *Domibacillus tundrae* sp. nov. is proposed. The type strain is PAMC 80007^T (=JCM 30371^T=KCTC 33549^T=DSM 29572^T). An emended description of the genus *Domibacillus* is also provided.

The genus *Domibacillus* is a member of the family *Bacillaceae* and was first proposed with a description of *Domibacillus robiginosus*, which forms red-pigmented colonies (Seiler *et al.*, 2013). *Domibacillus robiginosus* was isolated from a pharmaceutical clean room in eastern Germany and its morphological, chemotaxonomic and phylogenetic characteristics were different from the closely related members of the genera *Bacillus*, *Jeotgalibacillus* and *Planococcus* (Seiler *et al.*, 2013). Taxonomically, *Domibacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Bacillales* and family *Bacillaceae* (Parte, 2014). At the time of writing, three species, *D. robiginosus* (Seiler *et al.*, 2013), *Domibacillus indicus* (Sharma *et al.*, 2014) and *Domibacillus enclensis* (Sonalkar *et al.*, 2014), with validly published names are included in the genus *Domibacillus*. Members of genus *Domibacillus* are Gram-stain-positive, spore-forming, oxidative and aerobic rods. MK-6 and MK-7 are the dominant quinones and the cell-wall peptidoglycan contains *meso*-diaminopimelic acid (type A1γ). The major whole cell sugars are glucose and ribose. The reddish colonies are one of the characteristic features of the genus. In this current study, we proposed that strain PAMC 80007^T represents a novel species of the genus *Domibacillus* based on differences in phenotypic and genotypic characteristics.

Strain PAMC 80007^T was isolated from the active layer soil collected from moist acidic tussock tundra in Council, Alaska (64.50765° N 163.42696° W) in August 2011. The soil samples were suspended in 40 % glycerol and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PAMC 80007^T is KM657429. NCBI BioProject accession numbers for the draft genome sequences of strain PAMC 80007^T, *Domibacillus enclensis* DSM 25145^T, *Domibacillus robiginosus* DMS 25058^T and *Domibacillus indicus* DSM 28032^T are PRJNA278101, PRJNA278100, PRJNA278098 and PRJNA278099, respectively.

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

preserved at −80 °C. An aliquot of the resuspended soil sample was spread on tryptic soy agar (TSA; Difco) at 20 °C for 10 days. Colonies were picked and streaked on TSA, and then single colonies of the culture were purified by repeated subculture. Among those isolates, strain PAMC 80007^T was found to form creamy-yellow, circular colonies. This strain was deposited at the Polar and Alpine Microbial Collection (PAMC; Lee *et al.*, 2012) of Korea Polar Research Institute and preserved as glycerol suspensions (20 %, v/v) at −80 °C. For physiological and chemotaxonomic comparison between strain PAMC 80007^T and the three recognized species of the genus *Domibacillus*, *D. robiginosus* DSM 25058^T, *D. indicus* DSM 28032^T and *D. enclensis* DSM 25145^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). After determination of the optimal growth conditions of the three reference type strains along with strain PAMC 80007^T, all strains were routinely cultured on marine agar (MA; Difco) at 30 °C.

For 16S rRNA gene sequence analysis, 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, and the almost-complete 16S rRNA gene sequence (1482 bp) of strain PAMC 80007^T was obtained and used as a query to search for similar sequences in the EzTaxon-e database (Kim *et al.*, 2012). The 16S rRNA gene sequence of strain PAMC 80007^T was aligned with those of its closest relatives using the jPHYDIT program (Jeon *et al.*, 2005). Phylogenetic analysis was performed using the MEGA 6 program (Tamura *et al.*, 2013). Neighbour-joining (NJ; Saitou and Nei, 1987) trees were reconstructed using the Kimura two-parameter model (Kimura, 1980) with the gamma-distributed and the pairwise deletion options. Maximum-parsimony (MP; Fitch, 1971) trees were reconstructed using the Tree-Bisection-Reconnection (Swofford *et al.*, 1996) heuristic search method with the number of initial trees (random addition) as 10 and partial deletion options. Maximum-likelihood (ML) (Felsenstein, 1981) trees were reconstructed using the Kimura two-parameter model (Kimura, 1980) with gamma-distributed with invariant sites (G+I) and partial deletion options, based on the result of the best-fit model. The robustness of the phylogenetic trees reconstructed by NJ, MP and ML methods was confirmed by bootstrap analyses based on 1000 replications.

Comparison of 16S rRNA gene sequences showed that strain PAMC 80007^T was most closely related to *D. enclensis* (98.3 %), *D. robiginosus* (98.3 %) and *D. indicus* (97.2 %). Evaluation of tree topology revealed that strain PAMC 80007^T, *D. enclensis*, *D. robiginosus*, and *D. indicus* formed a robust clade and separated well from other genera in the family *Bacillaceae*, indicating strain PAMC 80007^T belonged to the genus *Domibacillus* (Fig. 1). In order to investigate genome relatedness, whole genome sequencing was attempted for strain PAMC 80007^T, *D. robiginosus* DSM 25058^T, *D. indicus* DSM 28032^T and *D. enclensis* DSM 25145^T. The details of draft genomes for the four strains, PAMC 80007^T and the three reference strains, are summarized in Table S1 (available in the online Supplementary Material). Genomic DNAs were extracted using a DNeasy Blood & Tissue kit (Qiagen) and genome sequencing was performed using the MiSeq sequencer system (Illumina) at ChunLab (Seoul, Korea). 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 the estimation of the degree of pairwise genome-based relatedness between strain PAMC 80007^T and the type strains of *D. enclensis*, *D. robiginosus* and *D. indicus* were 78.6, 78.5 and 80.3 %, respectively (Table S1), and this level is well below the ANI cut-off values (95–96 %) proposed for delineating bacterial species (Goris *et al.*, 2007; Richter and Rosselló-Móra, 2009). Consistently, DNA–DNA hybridization values estimated by GGDC were 22.1–23.9 % between strain PAMC 80007^T and the other type strains (Table S1), indicating that strain PAMC 80007^T represents a distinctive species of the genus *Domibacillus* (Rosselló-Mora and Amann, 2001). The genomic DNA G+C content of strain PAMC 80007^T, directly calculated from its genome sequence, was determined to be 43.5 mol%.

The fatty acid methyl esters in whole cells of strain PAMC 80007^T and the three reference strains were extracted from cultures grown on TSA at 30 °C for 3 days and were analysed as described by the Sherlock Microbial Identification System version 6.1 (MIDI) using the TSBA6.1 database. The analysis of isoprenoid quinones was carried out by the identification service of the Korean Culture Center of Microorganisms (KCCM). Polar lipids were extracted from lyophilized bacterial cells and examined using two-dimensional TLC followed by detection with the reagents molybdatophosphoric acid for total lipids, ninhydrin for free amino groups, molybdenum blue for phosphorus-containing lipids, α-naphthol for sugars, Dragendorff's solution for quaternary nitrogen, and Schiff's solution for α-glycols (Minnikin *et al.*, 1984). Cell-wall sugar and peptidoglycan analyses of the whole-cell hydrolysate were carried out by identification service of the DSMZ according to the methods of Schumann (2011).

Tests for phenotypic characteristics were performed on strain PAMC 80007^T along with the three reference strains of species of the genus *Domibacillus*. Growth of strain PAMC 80007^T was checked on nutrient agar (NA; Difco), TSA, R2A (Difco) agar and MA plates and better growth was obtained on MA and TSA. In order to monitor colour changes of the colonies, strain PAMC 80007^T was incubated on TSA, MA, NA and R2A at 30 °C for 7 days and checked every day. The temperature range for growth was tested on MA at different temperatures (0, 4, 10, 15, 20,

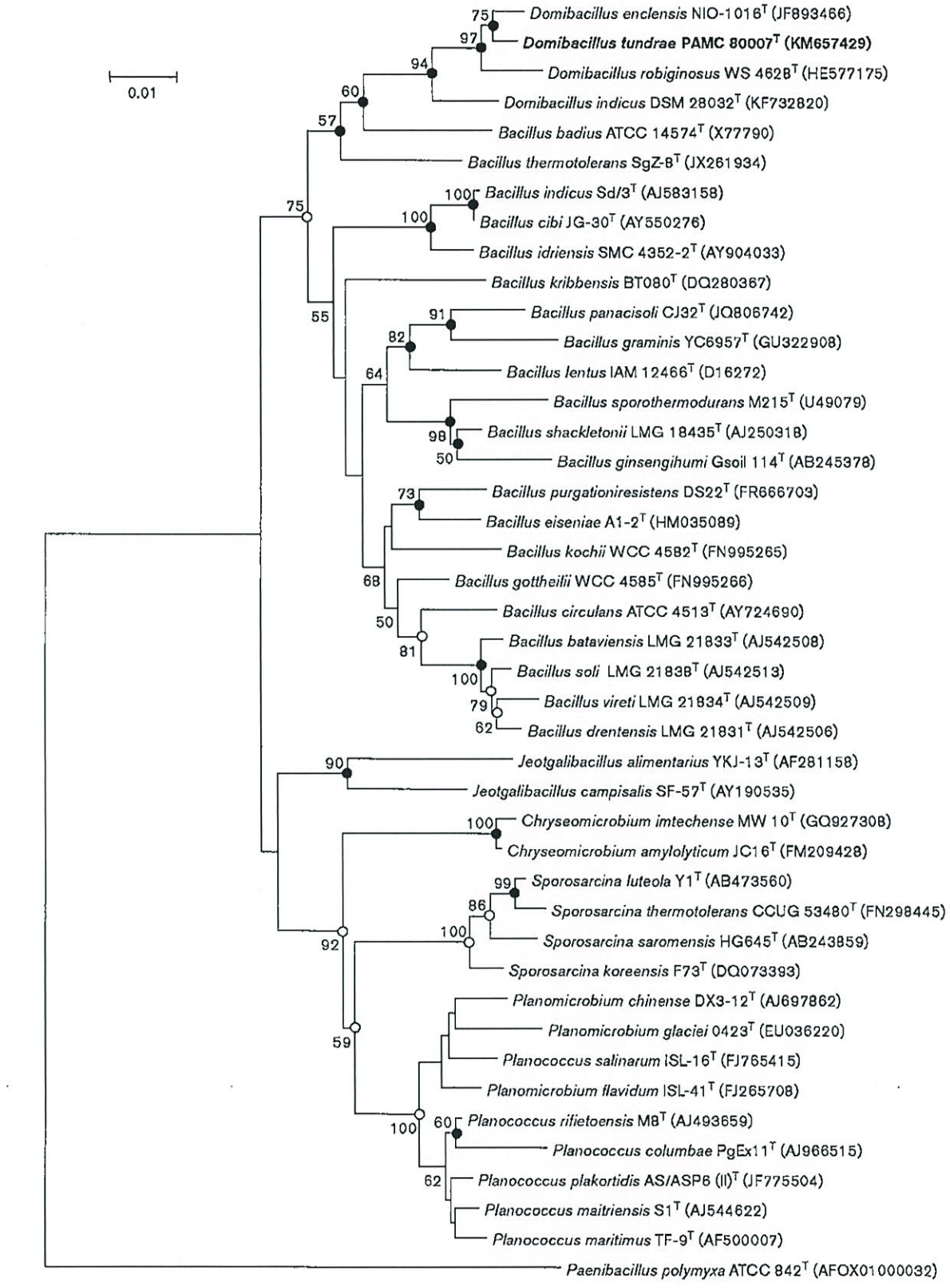

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain PAMC 80007^T and related species on the basis of 16S rRNA gene sequence. Only bootstrap values above 50 % are shown (1000 resamplings) at the branching points. Filled circles at nodes indicate that these were also obtained in both the maximum-likelihood and the maximum-parsimony trees, while open circles indicate that these nodes were obtained in the former tree only. *Paenibacillus polymyxa* ATCC 842^T was used as an outgroup. Bar, 0.01 nucleotide substitutions per site.

25, 30, 37, 42 and 45 °C) for 7 days. The pH range and optimal pH for growth were tested in pH-buffered marine broth (MB; Difco) at pH 5.5–10.5 (at intervals of 0.5 pH unit), using the following buffering system: MES for pH 5.5–6.5, MOPS for pH 7.0–7.5, 2-amino-2-methyl-1,3-propanediol (AMPD) for pH 8.0–9.5 and CAPS for pH 10.0–10.5. NaCl tolerance was determined on artificial seawater (ASW) medium (Choo *et al.*, 2007) supplemented with 0.5 % peptone and 0.1 % yeast extract with 0, 0.5, 1–10 % (at intervals of 1 %), 12, 15 and 20 % (w/v) NaCl. The growth of each culture was monitored by assessing changes in OD_{600} (EnVision plate reader; PerkinElmer). Cell size and morphology were examined by transmission electron microscopy (TEM) (CM200; Philips). Cells used for TEM were negatively stained with 2 % (w/v) uranyl acetate on a carbon-coated copper grid. Cell motility was investigated by using the hanging-drop method described by Bernardet *et al.* (2002). Growth under anaerobic conditions was tested 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 endospores was assessed by malachite green staining (Smibert and Krieg, 1994). Oxidase and catalase tests were performed according to the methods described by Smibert & Krieg (1994) and Cappuccino & Sherman (2002), respectively. Hydrolysis of Tweens 20, 40, 60 and 80 (each 1 %, v/v), starch (1 %, w/v) and casein (2 % skimmed milk, w/v) was tested using MA containing each component according to the method of Smibert & Krieg (1994) by incubating at 30 °C. The enzyme activities and acid production tests were performed using API ZYM, API 20E and API 50 CH kits (bioMérieux) in triplet according to the manufacturer's instructions. Antibiotic susceptibility was evaluated using the disc diffusion method and the following antibiotic discs were used (μg per disc; Oxoid): ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), rifampicin (30), tetracycline (30) and vancomycin (30). The physiological, morphological and biochemical characteristics of strain PAMC 80007^T are listed in Table 1 and in the species description. Differential characteristics of the four strains representing the genus *Domibacillus* are presented in Table 1. A transmission electron micrograph is shown in Fig. S1. Cells of strain PAMC 80007^T were Gram-stain-positive, aerobic, motile, spore-forming and rod-shaped. Strain PAMC 80007^T formed creamy-white colonies, non-reddish pigmented, and the colour of colonies did not change to be reddish.

The major fatty acids (>5 %) of strain PAMC 80007^T were iso-$C_{15:0}$ (24.7 %), $C_{16:1}\omega11c$ (16.8 %), anteiso-$C_{15:0}$ (16.5 %), $C_{16:0}$ (15.6 %) and anteiso-$C_{17:0}$ (8.7 %) (Table S2). The respiratory quinones detected in strain PAMC 80007^T were MK-6 (84.3 %) and MK-7 (15.7 %) as major and minor menaquinones, respectively, generally found in members of the genus *Domibacillus*. The cell wall peptidoglycan was A1γ type with *meso*-diaminopimelic acid. The major whole cell-sugar of strain PAMC 80007^T was ribose and a minor quantity of glucose was also detected. The polar lipids found in PAMC 80007^T were diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, phospholipid and two unidentified lipids (Fig. S2).

Overall, strain PAMC 80007^T formed a robust clade with the three type strains of the genus *Domibacillus* (Fig. 1). The chemotaxonomic profiles of strain PAMC 80007^T were generally similar to those of members of the genus *Domibacillus*. However, phenotypic characteristics, including hydrolysis of some macromolecules, and enzyme activities differentiated strain PAMC 80007^T from other species of the genus *Domibacillus*. Therefore, strain PAMC 80007^T represents a novel species of the genus *Domibacillus*, for which the name Domibacillus *tundrae* sp. nov. is proposed.

Emended description of the genus *Domibacillus* Seiler *et al.* 2013

The description of genus *Domibacillus* is as given previously (Seiler *et al.*, 2013; Sharma *et al.*, 2014; Sonalkar *et al.*, 2014), with the following characteristics from the present study. Oxidase activity and colony colour are species-dependent.

Description of *Domibacillus tundrae* sp. nov.

Domibacillus tundrae (tun'drae. N.L. gen. fem. n. *tundrae* from the tundra, the place of isolation of the type strain).

Forms cream-yellow, circular colonies of approximately 2.3 mm in diameter on MA plates after 72 h of incubation at 30 °C. Cells are Gram-stain-positive, motile, aerobic and short rod-shaped (1.4–1.8 × 2–2.6 μm) with spherical to slightly ellipsoidal spores located centrally and subcentrally in swollen sporangia. Temperature and pH ranges for growth are 10–42 °C (optimum 30 °C) and pH 6.0–8.5 (optimum pH 7), respectively. Tolerates up to 0–8 % (w/v) NaCl in ASW medium and grows well with 2 % NaCl. Hydrolyses aesculin, Tweens 40, 60 and 80 and starch, but not casein, L-tyrosine or Tween 20. Catalase, oxidase, Voges–Proskauer reaction, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase are positive. Acid is produced from glycerol, D-xylose, D-glucose, D-fructose, D-mannitol, aesculin, salicin, maltose, sucrose, trehalose, melezitose, raffinose, D-arabitol, glycogen, xylitol, gentiobiose and potassium gluconate.

Table 1. Major characteristics that differentiate strain PAMC 80007^T from the type strains of species of the genus *Domibacillus*

Strains: 1, Domibacillus *tundrae* sp. nov. PAMC 80007^T; 2, *D. enclensis* DSM 25145^T; 3, *D. robiginosus* DSM 25058^T; 4, *D. indicus* DSM 28032^T. All data were obtained in this study unless indicated. +, Positive; −, negative. T, terminal; C-S, central to/or subterminal; s, sporangia swollen; S, spherical; S-E, spherical to/or ellipsoidal; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PL, phospholipid; L, unidentified lipid. All strains are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, hydrolysis of aesculin, Tweens 40 and 60, and acid production from aesculin, salicin and raffinose. All strains are negative for lipase (C14), valine arylamidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, protease and acid production from erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, D-xylose, D-tagatose, D-fucose, L-fucose, L-arabitol and potassium 2-ketogluconate.

Characteristic	1	2	3	4
Spore position	C-S	C-S*	C-S†	T‡
Spore/sporangium shape	S, S-E, s	S-E, s*	S, S-E, s†	S, S-E, s*
Temperature range for growth (°C)	10–42	25–45*	13–45*	10–40*
Motility	+	+	+	−
NaCl tolerance (%, w/v)	8	12*	8.5†	6‡
Oxidase	+	−*	−†	−‡
Hydrolysis of:				
Starch	+	−	+	+
Tween 20	−	−	+	+
Tween 80	+	+	−	+
Production of:				
Acid phosphatase, α-glucosidase, β-glucosidase	−	+	+	+
Trypsin	−	−	+	−
Cystine arylamidase, β-glucuronidase	−	+	−	−
α-Galactosidase, β-galactosidase	−	+	+	−
Acid production from:				
Glycerol, melezitose	+	+	−	−
D-Xylose, D-mannitol, maltose, D-glucose, D-fructose, sucrose, trehalose, gentiobiose, potassium gluconate	+	+	+	−
Methyl β-D-xylopyranoside, D-mannose, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, inulin	−	+	−	−
L-Arabinose, L-rhamnose, *N*-acetylglucosamine, melibiose, turanose	−	+	+	+
D-Galactose, D-sorbitol, amygdalin, cellobiose, lactose	−	+	+	−
Inositol	−	+	−	+
Starch	−	−	+	+
Glycogen	+	−	+	+
D-Arabitol, xylitol	+	−	−	−
Potassium 5-ketogluconate	−	−	+	−
Antibiotic susceptibility				
Nalidixic acid (30 μg)	−	+	+	+
DNA G+C content (mol%)	43.5	46.5	42.8	44.6
Major menaquinone	MK-6, MK-7	MK-6, MK-7*	MK-6†	MK-6‡
Polar lipids	DPG, PG, PGL, PL, L1, L2	DPG, PG, PGL*	DPG, PG, PGL, PL†	DPG, PG, PL1, PL2‡

*Data from Sonalkar *et al.* (2014).
†Data from Seiler *et al.* (2013).
‡Data from Sharma *et al.* (2014).

Susceptible to ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, rifampicin, tetracycline and vancomycin but resistant to nalidixic acid. MK-6 and MK-7 are predominant respiratory quinones. Polar lipids of the strain are diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, phospholipid and two unidentified lipids. Cell-wall peptidoglycan is A1γ type with *meso*-diaminopimelic acid, and ribose is the predominant cell-wall sugar with a minor quantity

of glucose. Major fatty acids are iso-$C_{15:0}$, $C_{16:1}\omega11c$, anteiso-$C_{15:0}$, $C_{16:0}$ and anteiso-$C_{17:0}$ while minor fatty acids are iso-$C_{17:0}$, iso-$C_{17:1}$/anteiso-$C_{17:1}$, $C_{14:0}$, $C_{18:0}$, iso-$C_{17:1}\omega10c$, $C_{18:1}\omega9c$, $C_{16:1}\omega7c$/ $C_{16:1}\omega6c$ and iso-$C_{14:0}$.

The type strain, PAMC 80007^T (=JCM 30371^T=KCTC 33549^T=DSM 29572^T), was isolated from a soil sample collected from tussock tundra in Alaska, USA. The genomic DNA G+C content of the type strain is 43.5 mol%.

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