Paenibacillus antarcticus sp. nov., a novel psychrotolerant organism from the Antarctic environment

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An endospore-forming strain, 20CM^T, was isolated from Antarctic sediment and identified as a member of the genus *Paenibacillus* on the basis of phenotypic and phylogenetic analyses. The organism stained Gram-variable and was facultatively anaerobic. Strain 20CM^T was psychrotolerant, growing optimally at 10–15 °C. Like other *Paenibacillus* species, it contained anteiso-$C_{15:0}$ as the major cellular fatty acid. The DNA G+C content was 40·7 mol%. 16S rRNA gene sequence analysis placed strain 20CM^T within the *Paenibacillus* cluster, with a similarity value of 99·5 % to *Paenibacillus macquariensis* DSM 2^T. DNA–DNA hybridization experiments between the Antarctic isolate and *P. macquariensis* DSM 2^T revealed a reassociation value of 47 %, indicating that strain 20CM^T and *P. macquariensis* DSM 2^T belong to different species. Based on evaluation of morphological, physiological, chemotaxonomic and phylogenetic analyses, a novel species, *Paenibacillus antarcticus* sp. nov., is proposed; the type strain is 20CM^T (=LMG 22078^T=CECT 5836^T).

Based on 16S rRNA analysis, Ash *et al.* (1993) proposed the genus *Paenibacillus* to accommodate a group of aerobic or facultatively anaerobic rod-shaped, endospore-forming bacteria. Many *Bacillus* species were transferred to the genus *Paenibacillus* based on a comparison of their 16S rRNA gene sequences with other members of the *Bacillaceae* (Ash *et al.*, 1993; Heyndrickx *et al.*, 1995, 1996a, b; Shida *et al.*, 1997a; Pettersson *et al.*, 1999). Traditionally, Gram-positive, rod-shaped, endospore-forming bacteria have been classified in the genus *Bacillus* (Claus & Berkeley, 1986). However, in recent years, the genus *Bacillus* has been separated into several distinct genera, such as *Alicyclobacillus* (Wisotzkey *et al.*, 1992), *Aneurinibacillus* and *Brevibacillus* (Shida *et al.*, 1996), *Halobacillus* (Spring *et al.*, 1996), *Paenibacillus* (Ash *et al.*, 1993), *Amphibacillus* (Niimura *et al.*, 1990), *Filobacillus* (Schlesner *et al.*, 2001), *Geobacillus* (Nazina *et al.*, 2001), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* and *Salibacillus* (Wainø *et al.*, 1999) and *Ureibacillus* (Fortina *et al.*, 2001). Antarctica is a source of novel *Bacillus* species (Logan *et al.*, 2000, 2002, 2004a); novel members of the genus *Paenibacillus* have also been described recently in this environment (Logan *et al.*, 2004b).

According to Ash *et al.* (1993), members of the genus *Paenibacillus* produce ellipsoidal endospores in swollen sporangia. The cell wall shows structures typical of Gram-positive bacteria, but usually stains negatively. The DNA G+C contents range from 40 to 54 mol% and anteiso-$C_{15:0}$ is the major cellular fatty acid. Some members of the genus produce antibacterial compounds (Slepecky & Hemphill, 1991) and iturin-like antifungal antibiotics (Chung *et al.*, 2000). One distinctive characteristic of the genus *Paenibacillus* is the ability to excrete a wide variety of enzymes that degrade natural biopolymers such as alginate, chondroitin, chitin, curdlan, starch (Kanzawa *et al.*, 1995; Nakamura, 1987; Chung *et al.*, 2000; van der Maarel *et al.*, 2000) and other polysaccharides (Priest *et al.*, 1988).

In this study, the taxonomic status of strain 20CM^T was investigated using a combination of phenotypic characterization, sequencing of the 16S rRNA gene, DNA base composition, DNA–DNA hybridization and cellular fatty acid composition analysis. Strain 20CM^T is proposed as a representative of a novel species, *Paenibacillus antarcticus* sp. nov.

Strain 20CM^T was isolated from sediment collected in Chlorite Lake on the Byers Peninsula of Livingston Island (South Shetland Islands, Antarctica). Sample aliquots were

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The GenBank/EMBL/DDBJ accession number for the16S rRNA gene sequence of *Paenibacillus antarcticus* 20CM^T is AJ605292.

A table giving fatty acid composition (Table A) and figures showing electron micrographs and a phylogenetic tree (Figs A and B, respectively) are available as supplementary material in IJSEM Online.

removed with a platinum loop and diluted in a saline solution containing (g l^{-1}, pH 7): NaCl, 0·56; KCl, 0·27; $CaCl_2$, 0·03; and $NaHCO_3$, 0·01. Trypticase soy agar (TSA; ADSA) plates were inoculated with loopfuls of several sample dilutions using the streak-plate method to obtain well-isolated colonies. Plates were incubated for 4 days at 15 °C. Isolates were maintained aerobically on TSA slopes at 4 °C and also at −80 °C on cryo-beads.

Morphology, cell size and shape of spores were determined by scanning (Hitachi model H 2300) and transmission (Hitachi model H 600AB) electron microscope observations of cells grown in trypticase soy broth (TSB; ADSA) at 15 °C. Motility was determined by phase-contrast microscopy (Olympus model CHS). Gram staining was performed according to Hucker & Conn (1923). Two alternative methods, the KOH test and the L-alanine aminopeptidase assay (Manafi & Kneifel, 1990), were also used. Oxidase, catalase and urease activities, methyl red reaction, Voges–Proskauer, nitrate reduction, indole production, citrate utilization, and hydrolyses of casein, lecithin, gelatin, DNA, tyrosine, starch and Tween 80 were determined following Cowan & Steel (1993). Dihydroxyacetone production, phenylalanine deamination and growth in the presence of lysozyme (0·1 and 0·001 %, w/v) were determined as described by Claus & Berkeley (1986). Acid production from carbohydrates and additional tests were determined using the API 50CH and API 20E system (bioMérieux). Tolerance to NaCl was measured on nutrient agar (ADSA) containing 0–10 % (w/v) NaCl. Plates were incubated at 15 °C for 30 days. The temperature range for growth was determined on TSA incubated for 14 days at 4, 10, 15, 20, 25, 30, 31, 32, 33 and 37 °C. Anaerobic growth was determined on TSB plus agar-agar (1·5 %; ADSA) by incubation in an anaerobic chamber at 15 °C for 5 days.

Cells were Gram-variable, rod-shaped (0·7 × 2·5 μm) and motile by means of peritrichous flagella (see Fig. Aa, b; available as supplementary material in IJSEM Online). Strain 20CM^T produced ellipsoidal spores in swollen sporangia in the subterminal or terminal region of the cell (see Fig. Ac, d, e; available as supplementary material in IJSEM Online). Colonies grown on TSA at 15 °C were non-pigmented, circular, slightly convex, bright and cream coloured with a diameter of 1·0–1·5 mm. Cell wall structure was Gram-positive, as demonstrated by electron microscope examinations of ultra-thin sections (see Fig. Af, available as supplementary material in IJSEM Online), although results of the KOH test and the L-alanine aminopeptidase assay indicated a Gram-negative character. The isolate was facultatively anaerobic and grew at 4–31 °C. Growth was optimal at 10–15 °C. It grew in the presence of 4 % (w/v) NaCl and in 0·001 % (w/v) lysozyme, but not in 0·1 % (w/v) lysozyme. The final pH in Voges–Proskauer broth after 7 days incubation at 15 °C was less than pH 6. The isolate was negative for production of acetylmethylcarbinol, but positive for the methyl red reaction. Strain 20CM^T did not decompose tyrosine. Phenotypic characteristics of the Antarctic isolate and the closest phylogenetic relatives were compared (Table 1). Of the organisms compared, only strain 20CM^T was positive for oxidase production. Phenotypic studies showed that the Antarctic isolate displayed characteristics consistent with those of the genus *Paenibacillus* (Ash *et al.*, 1993).

Fatty acids were prepared from 40 mg wet cell material harvested from a TSB agar (30 g TSB l^{-1}, 15 g agar l^{-1}; BBL) culture incubated for 4 days at 15 °C. The whole-cell fatty acids were determined as described previously by Bozal *et al.* (2002). Fatty acid analysis of 20CM^T (Table A, available as supplementary material in IJSEM Online) revealed that anteiso-$C_{15:0}$ (55·32 %), iso-$C_{15:0}$ (15·04 %) and $C_{16:1}\omega11c$ (7·72 %) were predominant. This fatty acid profile was in accordance with that given in the description of the genus *Paenibacillus* (Ash *et al.*, 1993). anteiso-Branched saturated $C_{15:0}$ is the predominant fatty acid found in all members of the genus *Paenibacillus* (Shida *et al.*, 1997a).

Genomic DNA was prepared according to the method of Gevers *et al.* (2001). The G+C content was determined by HPLC as described by Mesbah *et al.* (1989). DNA–DNA relatedness was measured fluorometrically using the microplate hybridization method described by Ezaki *et al.* (1989). Determination of the 16S rRNA gene sequence of strain 20CM^T and phylogenetic analyses were carried out as described previously by Bozal *et al.* (2002).

Phylogenetic studies based on 16S rRNA gene sequences confirmed that strain 20CM^T is a member of the genus *Paenibacillus* (Fig. 1; Fig. B, available as supplementary material in IJSEM Online). The 16S rRNA gene sequence of strain 20CM^T showed 99·5 % similarity to that of *Paenibacillus macquariensis* DSM 2^T, which is significant enough to suggest possible species relatedness. Stackebrandt & Goebel (1994) suggested that a sequence similarity value greater than 97 % indicated conspecificity of the strains involved. The similarity values shown by 20CM^T to other *Paenibacillus* type strains were under 97 % (*Paenibacillus borealis* DSM 13188^T, 95·5 %; *Paenibacillus odorifer* LMG 19079^T, 94·7 %). To further verify the taxonomic position of isolate 20CM^T, DNA–DNA hybridizations were performed with *P. macquariensis* LMG 6935^T (Marshall & Ohye, 1966). The low DNA–DNA reassociation value of 47 % between these two strains and the 16S rRNA gene sequence analysis confirmed the distinct position of strain 20CM^T within the genus *Paenibacillus*. The G+C content of strain 20CM^T was 40·7 mol%, which lies within the range observed for members of the genus *Paenibacillus* (Shida *et al.*, 1997a).

Morphological, physiological, chemotaxonomic and phylogenetic data showed that strain 20CM^T belongs to the genus *Paenibacillus*. DNA–DNA hybridization analysis clearly distinguished strain 20CM^T from *P. macquariensis* (Marshall & Ohye, 1966). Based on polyphasic evidence, it is proposed that strain 20CM^T be assigned as the type strain of a novel species in the genus *Paenibacillus*, *Paenibacillus antarcticus* sp. nov.

Table 1. Phenotypic characteristics that differentiate *P. antarcticus* 20CM^T from its closest relatives in the genus *Paenibacillus*

Species: 1, *P. antarcticus* (data from this study); 2, *Paenibacillus graminis* (Berge *et al.*, 2002); 3, *Paenibacillus azotofixans* (Seldin *et al.*, 1984; Seldin & Penido, 1986); 4, *P. macquariensis* (Marshall & Ohye, 1966; Shida *et al.*, 1997a, b; Elo *et al.*, 2001); 5, *P. borealis* (Elo *et al.*, 2001); 6, *P. odorifer* (Berge *et al.*, 2002). All species produced acid from galactose, D-glucose, D-fructose, amygdalin, salicin, cellobiose, maltose, melibiose, sucrose, trehalose, D-raffinose, β-gentiobiose and D-turanose. None of the species produced acid from erythritol, D-arabinose, L-xylose, adonitol, L-sorbose, rhamnose, dulcitol, inositol, L-arabitol, 2-ketogluconate or 5-ketogluconate. All species were negative for growth at 42 °C. For *P. antarcticus*, results are scored as positive or negative. For other species, results are scored as follows: +, >90 % strains positive; −, <10 % strains positive; V, 11–89 % strains positive; ND, not determined.

Characteristic	1	2	3	4	5	6
Oxidase	+	−	−	−	−	−
Voges–Proskauer test	−	ND	+	−	−	ND
Nitrate reduction	−	+	−	−	−	+
Production of dihydroxyacetone	−	ND	−	−	−	ND
Casein hydrolysis	−	ND	−	−	+	ND
Starch hydrolysis	+	ND	−	+	−	ND
Gelatin liquefaction	−	ND	−	−	−	ND
Acid production from:						
Glycerol	−	+	−	−	+	v
L-Arabinose, D-xylose, methyl β-D-xyloside, *N*-acetylglucosamine, lactose, starch	+	+	−	+	+	+
Ribose	+	−	−	+	−	+
D-Mannose	+	+	+	+	+	v
Mannitol, melezitose	−	+	+	+	+	−
Sorbitol	−	−	+	−	v	−
Methyl α-D-mannoside	−	−	−	+	−	−
Methyl α-D-glucoside	+	+	+	+	v	+
Arbutin, glycogen	−	+	−	+	+	+
Inulin	−	v	+	−	+	+
Xylitol, D-lyxose, D-tagatose	−	−	−	−	v	−
D-Fucose	−	v	−	−	−	−
L-Fucose	−	−	−	+	−	v
D-Arabitol	−	−	−	−	+	−
Gluconate	−	v	−	+	−	−
Growth at (°C):						
0	−	−	−	+	−	−
5	+	v	−	+	+	+
10	+	+	−	+	+	+
31	+	+	+	−	+	+
32	−	+	+	−	+	+
35	−	+	+	−	+	+
37	−	v	+	−	+	−
40	−	v	v	−	−	−
Growth in the presence of:						
Lysozyme (0·001 %)	+	ND	−	−	−	ND
NaCl (5 %)	−	ND	−	−	−	ND
G+C content (mol%)	40·7	52·1	51·6	39	53·6	44·0

Description of *Paenibacillus antarcticus*

Paenibacillus antarcticus (ant.arc′ti.cus. L. masc. adj. *antarcticus* of the Antarctic environment, where the organism was isolated).

Cells are rod-shaped (0·7 × 2·5 μm) and motile by means of peritrichous flagella. Subterminal or terminal ellipsoidal spores are formed in swollen sporangia. Colonies grown on TSA are non-pigmented, circular, slightly convex, bright and cream coloured. Cells are facultatively anaerobic and stain Gram-variable. Growth is not inhibited by the presence of 4 % NaCl or 0·001 % lysozyme. Growth occurs at 4 and

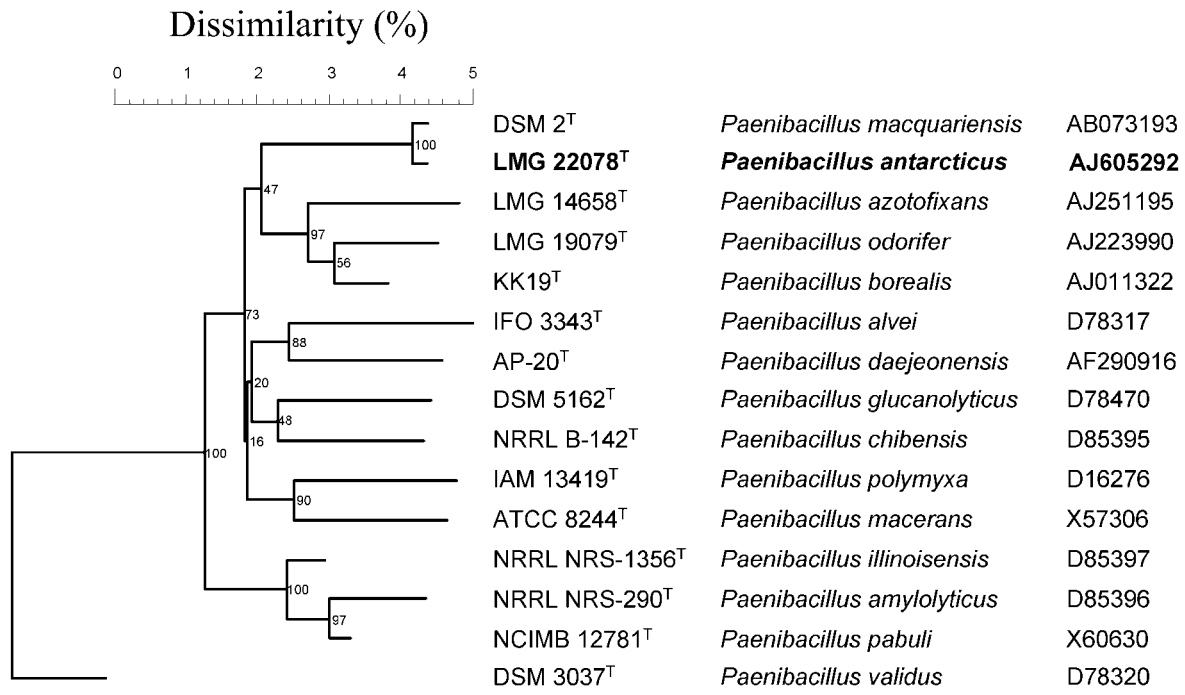

Fig. 1. Phylogenetic position of strain 20CM^T among neighbouring species of the genus *Paenibacillus*, based on 16S rRNA gene sequence analysis. Bootstrap values are indicated.

31 °C, but not at 0 or 32 °C; optimal growth occurs at 10–15 °C. Oxidase, catalase, urease and methyl red reactions are positive. Nitrate reduction, Voges–Proskauer reaction, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, dihydroxyacetone production, indole production, H_2S production, phenylalanine deamination and tryptophan deaminase are negative. Aesculin, starch and Tween 80 are hydrolysed. Does not hydrolyse casein, lecithin, gelatin, DNA or tyrosine. With API systems, acid is produced from L-arabinose, ribose, D-xylose, methyl β-D-xyloside, galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, starch, β-gentiobiose and D-turanose. Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, arbutin, inulin, melezitose, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. The predominant fatty acid is anteiso-$C_{15:0}$ (55·32 %).

The type strain is 20CM^T (=LMG 22078^T=CECT 5836^T); its G+C content is 40·7 mol%.

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