

Domibacillus tundrae sp. nov., isolated from active layer soil of tussock tundra in Council, Alaska

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ABSTRACT

A novel Gram-positive, spore-forming, aerobic, motile and rod-shaped bacterium designated as strain PAMC 80007^T was isolated from active layer soil sample of moist acidic tundra in 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 %), *D. robiginosus* (98.3 %) and *D. 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 *Domibacillus* species. The major fatty acids (>5 %) were iso-C15:0 (24.7 %), C16:1 ω11c (16.8 %), anteiso-C15:0 (16.5 %), C16:0 (15.6 %) and anteiso-C17:0 (8.7 %). The major respiratory isoprenoid quinones MK-6 and MK-7, and the polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, phospholipid and two unidentified lipids. The major whole-cell sugar was ribose with minor quantity of glucose and meso-diaminopimelic acid (type A1γ) was present in the cell-wall peptidoglycan. Results from polyphasic study suggested that strain PAMC80007^T is a novel species of the genus *Domibacillus* for which the name *Domibacillus tundrae* sp. nov. is proposed.

INTRODUCTION

The genus *Domibacillus* was a member of the family *Bacillaceae* and first proposed with a description of *Domibacillus robiginosus* (Seiler *et al.*, 2013). *Domibacillus robiginosus* was isolated from pharmaceutical clean room in eastern Germany and its morphological, chemotaxonomic and phylogenetic characteristics were different from the closely related members. Taxonomically *Domibacillus* belongs to phylum *Firmicutes*, class *Bacilli*, order *Bacillales* and family *Bacillaceae* (Parte, 2014). At the time of writing, three species, *D. robiginosus* (Seiler *et al.*, 2013), *D. indicus* (Sharma *et al.*, 2014) and *D. enclensis* (Sonalkar *et al.*, 2014) with validly published names are included in the genus *Domibacillus*. Members of genus *Domibacillus* are Gram-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. In this current study, we proposed that strain PAMC 80007^T represents a novel species of the genus *Domibacillus* based on its differences in phenotypic and genotypic characteristics.

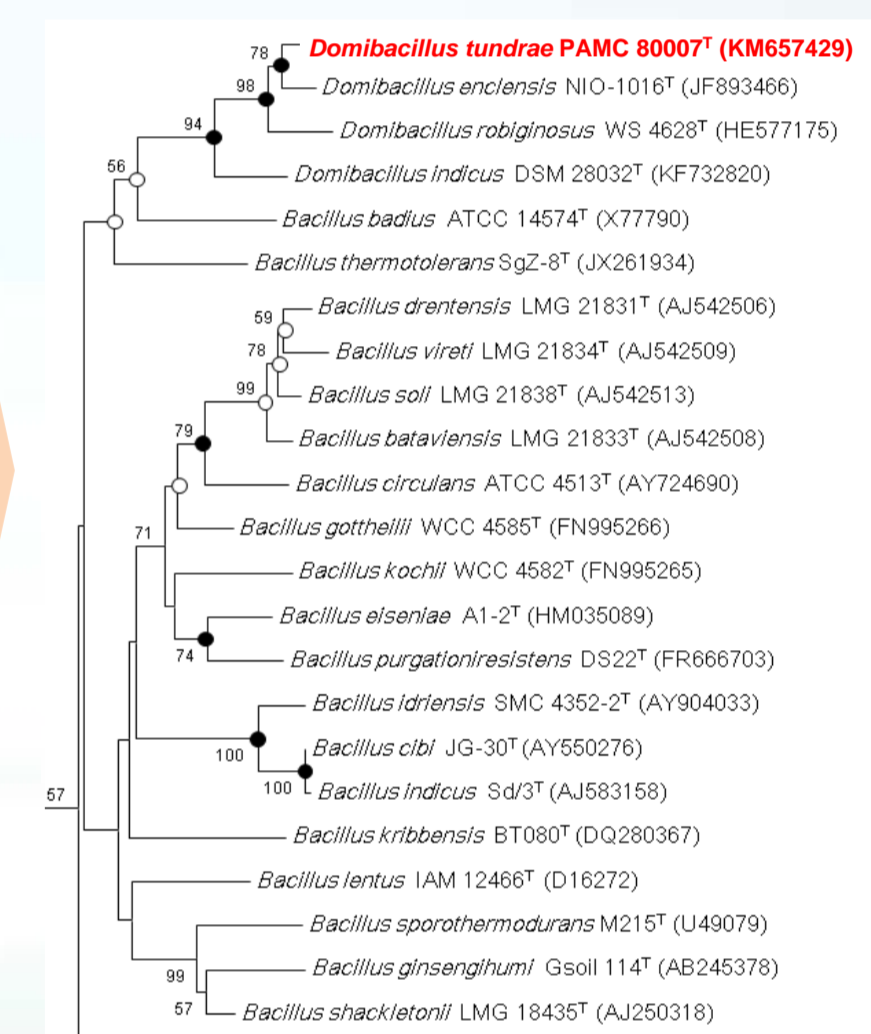
METHODS



Active layer soil collected from moist acidic tussock tundra in Council, Alaska (64.50765° N, 163.42696° W)

An aliquot of the resuspended soil sample was spread on TSA and incubated at 20 °C for 10 days.

16S rRNA gene similarity analysis and polyphasic analysis on microorganism



RESULTS

Characteristics	1	2	3	4
Spore position	C, S	T†	C, S†	C, 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	-	-	+	-
α-Galactosidase, β-galactosidase	-	+	+	-
Acid production from:				
Glycerol, D-Melezitose	+	+	-	-
Inositol	-	+	-	+
Amidon	-	-	+	+
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‡

Fatty acid	1	2	3	4
C _{14:0}	2.8	1.0	1.6	3.9
C _{16:0}	15.6	6.8	6.2	11.7
C _{16:1} ω11c	16.8	8.3	10.5	17.2
C _{18:0}	2.3	0.9	0.7	4.8
C _{18:1} ω9c	1.2	-	-	1.1
iso-C _{14:0}	0.5	-	-	1.8
iso-C _{15:0}	24.7	34.0	27.0	18.3
iso-C _{16:0}	-	-	0.6	1.7
iso-C _{17:0}	4.5	12.1	7.1	2.2
iso-C _{17:1} ω10c	2.1	4.7	2.7	-
anteiso-C _{15:0}	16.5	13.5	24.6	25.0
anteiso-C _{17:0}	8.7	12.2	14.2	7.4
C _{16:1} ω7c alcohol	-	-	-	2.0
Summed features				
3 (C _{16:1} ω7c and/or C _{16:1} ω 6c)	0.9	1.5	1.8	-
4 (C _{17:1} iso and/or C _{17:1} anteiso)	3.8	4.3	2.5	3.0

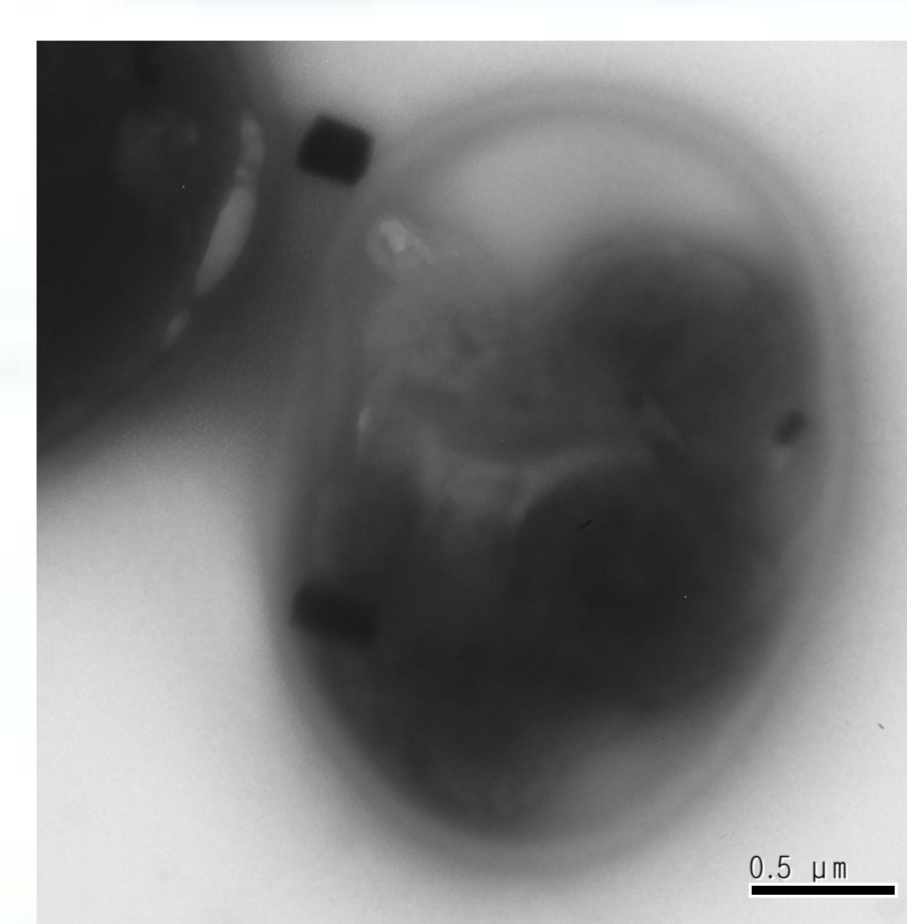
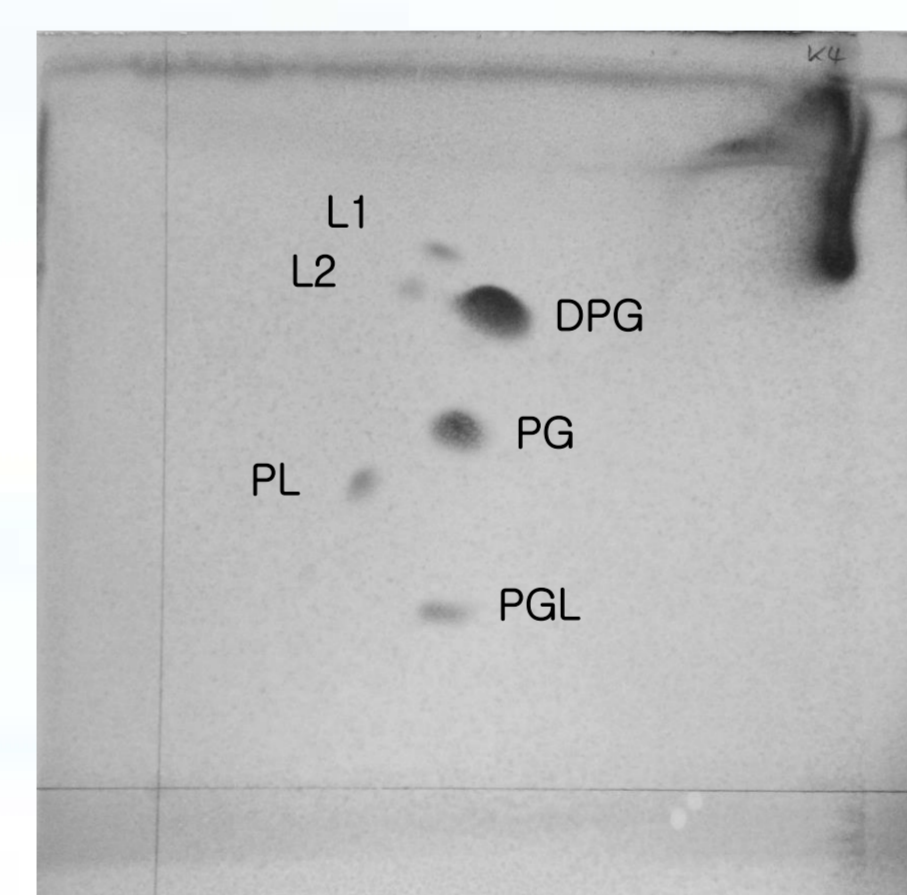


Table 2. Cellular fatty acid composition of strain PAMC 80007^T and members of the genus *Domibacillus*

Fig. 1. Two-dimensional thin-layer chromatogram of total polar lipid profile of strain PAMC 80007^T.

Fig. 2. Transmission electron micrograph of cells of strain PAMC 80007^T. Bar, 0.5 µm

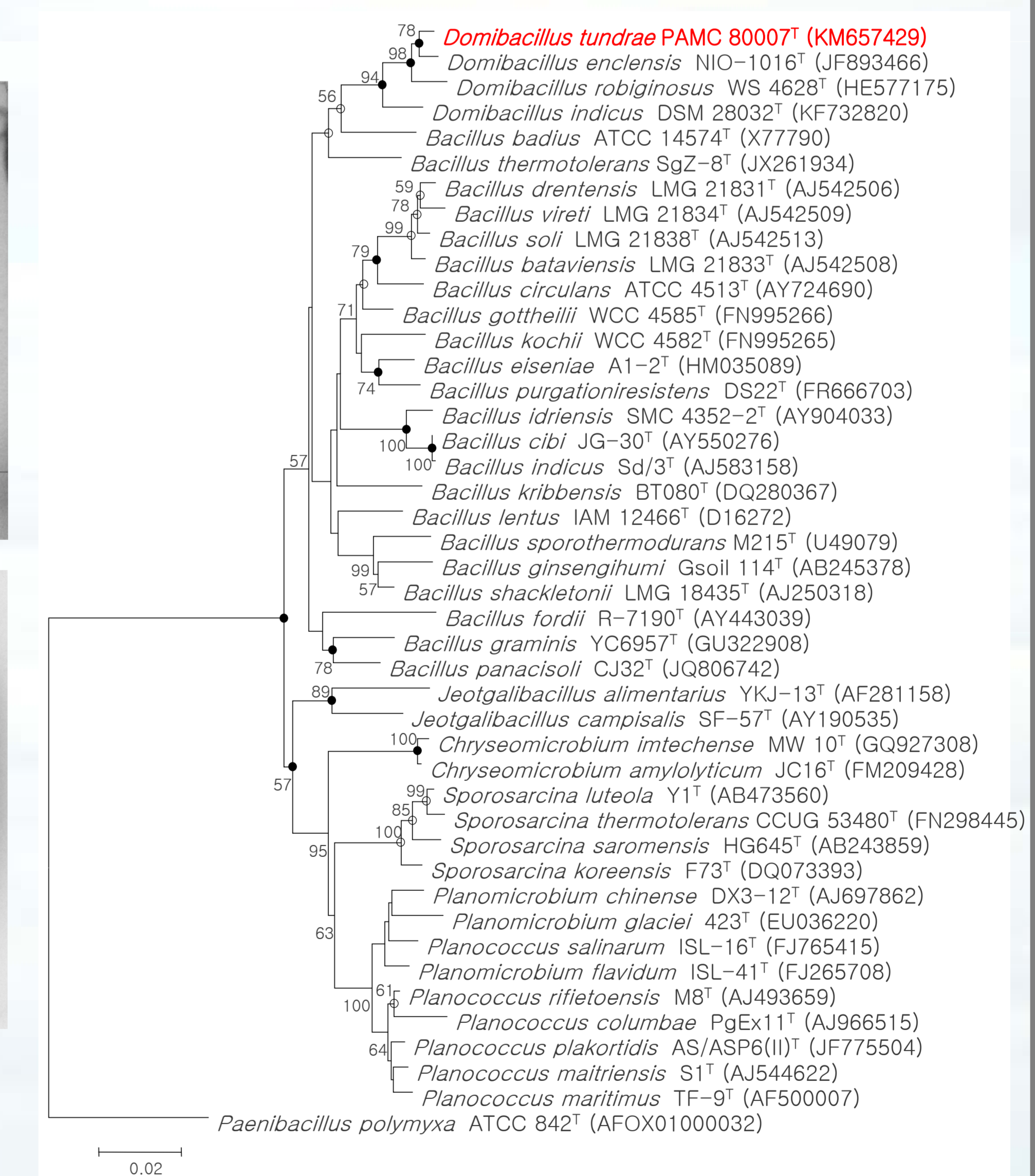


Fig. 3. Neighbor-joining tree showing the phylogenetic positions of *Domibacillus tundrae* PAMC 80007^T and related species on the basis of 16S rRNA gene sequence. Only bootstrap values above 50 % are shown (1000 resamplings) at the branching points. Solid circles indicate that the corresponding nodes were also obtained in both the maximum-likelihood and the minimum-evolution trees, while open circles in the latter tree only. Bar, 0.02 nucleotide substitution per site.

Table 1. Differential characteristics between strain PAMC 80007^T and type strains of other *Domibacillus* species 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.
† Data from Sonalkar *et al.* (2014); ‡ Data from Seiler *et al.* (2013); § Data from Sharma *et al.* (2014)

CONCLUSION

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

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REFERENCES

- [1] Minnikin, D. E. *et al.* (1984) *J Microbiol Methods* 2, 233–241.
- [2] Seiler, H., Wenning, M. & Scherer, S. (2013) *Int J Syst Evol Microbiol* 63, 2054–2061.
- [3] Sharma, A. *et al.* (2014) *Int J Syst Evol Microbiol* 64, 3010–3015.
- [4] Sonalkar, V. V. *et al.* (2014) *Int J Syst Evol Microbiol* 64, 4098–4102.