Paenibacillus tibetensis sp. nov., a psychrophilic bacterium isolated from alpine swamp meadow soil

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A novel psychrophilic strain, SSB001T, was isolated from an alpine swamp meadow soil in Tibet, China, and identified as a representative of a novel phylogenetic subclade in the genus Paenibacillus, with Paenibacillus antarcticus (96.2 %), Paenibacillus macquariensis (96.53 %) and Paenibacillus glacialis (96.2 %) as the most closely related species on the basis of 16S rRNA gene sequence analyses. The strain was distinguished from defined species of the genus Paenibacillus by further study of rpoB gene sequences, phenotypic characterization, cellular fatty acid composition, quinones, polar lipids and meso-diaminopimelic acid in the peptidoglycan. Based on these results, we propose the strain as a representative of a novel species named Paenibacillus tibetensis sp. nov., with SSB001T (=ACCC 19728T=DSM 29321T) as the type strain. The DNA G+C content (mol%) of strain SSB001T was 40.18 mol% (HPLC).

The Qinghai–Tibet plateau is the highest region on the Earth, known as the ‘roof of the world’, with an average altitude of more than 4500 m (http://www.china.org.cn/english/scitech/104358.htm). The climate of the area is high mountain cold zone with the mean annual temperature varying from −2 °C to 8 °C and the lowest temperature being −50 °C. The mean annual precipitation is 1947.4 mm (Falandysz et al., 2014). The air pressure and concentration of oxygen in the air are 55–70 % less than those at sea level, while the solar radiation is much stronger than in other regions (Hou et al., 2009; Ni, 2000). These extreme conditions lead to a unique composition of species in Tibet. The novel strain SSB001T was isolated from an alpine swamp meadow soil, at an altitude of 4185 m, located at 29° 36’ 21” N 94° 36’ 23” E in Shegyla Mountain, China.

According to Ash et al. (1993), members of ‘group 3’ within the genus Bacillus can be distinguished from members of other Bacillus groups using a battery of phenotypic characteristics and 16S rRNA gene sequences. A new genus accommodating these bacteria was named Paenibacillus, and Paenibacillus polymyxa was proposed as the type species (Ash et al., 1993; Judicial Commission of the International Committee for Systematics of Prokaryotes, 2005). At the time of writing, 154 species of the genus and four subspecies have been described. (http://www.bacterio.net/paenibacillus.html). Among all the species, most species are either mesophilic (Enright et al., 2003; Kim et al., 2014; Lim et al., 2006; Saha et al., 2005) or thermophilic (Li et al., 2014; Shimoyama et al., 2014; Yao et al., 2014), with the exception of three psychrophilic species, Paenibacillus macquariensis (Marshall & Ohye, 1966), Paenibacillus glacialis (Kishore et al., 2010) and Paenibacillus darwinianus (Dsouza et al., 2014). In this study, we isolated a psychrophilic strain, SSB001T, closely related to these species, which belongs to a novel species of genus Paenibacillus for which we propose the name Paenibacillus tibetensis sp. nov.

Genomic DNA was extracted from strain SSB001T using a MoBio UltraClean microbial DNA isolation kit according to the manufacturer’s protocol. For phylogenetic analysis, the 16S rRNA gene was amplified by PCR with primers 27f (5’-GAGTTTGATCCTGCTCAG-3’) and 1525r (5’-AG- AAGGAGGTGATCCAGCC-3’) (Rainey et al., 1996) and the purified PCR product was sequenced. The sequences acquired, together with related sequences obtained from the GenBank database, were aligned using the CLUSTAL W program in the MEGA 6.06 software (Tamura et al., 2013). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods based upon the model of Jukes & Cantor (1969). Phylogenetic trees were bootstrapped with 1000 replicates. Filled circles in Figs S1 and S2 (available in the online Supplementary Material) indicate the generic branches present in the phylogenetic trees generated by the neighbour-joining and maximum-likelihood methods. Both of the trees shared similar topologies with high bootstrap support, and only the smaller tree generated by neighbour-joining method was shown in Fig. 1. Sequence similarities were calculated using the database EzTaxon-e (Ezbiocloud) to compare the sequences of the 16S rRNA genes (Kim et al., 2012). The results showed that strain
SSB001\textsuperscript{T} was the closest match (96.53\% 16S rRNA gene sequence similarity) to \textit{P. macquariensis} subsp. \textit{macquariensis} NCTC 10419\textsuperscript{T}, then \textit{P. macquariensis} subsp. \textit{defensor} M4-1 (96.47\% similarity), \textit{P. glacialis} KFC91\textsuperscript{T} (96.2\%) and \textit{Paenibacillus antarcticus} LMG 22078\textsuperscript{T} (96.2\%) in that order.

To further confirm the relationship of the four strains, the \textit{rpoB} gene encoding the \(\beta\)-subunit of RNA polymerase was amplified from strain SSB001\textsuperscript{T} using primers rpoB1698f (5'-AACATCGGTTTTGATCAAC-3', corresponding to \textit{Escherichia coli} position 1643) and rpoB2041r (5'-CGTGCATGTTG- GTACCAT-3', corresponding to \textit{E. coli} position 2041) (Dahlöf \textit{et al.}, 2000) and sequenced. The phylogenetic analysis of \textit{rpoB} gene sequences showed that the strain shared 43.4\%, 44.7\% and 73.7\% similarity with \textit{P. glacialis} KFC91\textsuperscript{T}, \textit{P. antarcticus} LMG 22078\textsuperscript{T} and \textit{P. macquariensis} LMG 6935\textsuperscript{T}, respectively (Fig. S3).

According to the phenotypic characterization partially fulfilling the minimal standards of Logan \textit{et al.} (2009), a total of 153 features were analysed. \textit{P. antarcticus} LMG 22078\textsuperscript{T} and \textit{P. macquariensis} LMG 6935\textsuperscript{T}, used as the reference strains, were purchased from the Belgian Co-ordinated Collections of Micro-organisms (BCCM/LMG), and another reference strain, \textit{Paenibacillus chibensis} DSM 22343\textsuperscript{T} (=KFC91\textsuperscript{T}) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Utilization of 95 carbon sources was tested using a GP2 Microplate (Biolog) following the manufacturer’s instructions. After incubation for 24 h at 20 °C, strain SSB001\textsuperscript{T} was able to utilize dextrin, N-acetyl-D-glucosamine, L-arabinose, D-fructose, D-glucose, maltose, maltotriose, D-mannose, methyl \(\beta\)-D-glucoside, D-ribose, trehalose, D-xylene, acetic acid, succinic acid monomethyl ester, glycerol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, 3-methyl-D-glucose (weakly), palatinose (weakly), salicin (weakly), pyruvic acid methyl ester (weakly) and pyruvic acid (weakly) as sole carbon sources, but was not able to utilize \(\alpha\)-cyclodextrin, \(\beta\)-cyclodextrin, glycogen, inulin, mannann, Tween 40, Tween 80, N-acetyl-\(\beta\)-D-mannosamine, amygdalin, D-arabitol, arbutin, cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, \textit{myo}-inositol, \(\alpha\)-lactose, D-mannitol, melezitose, melibiose, methyl D-xylo-lactoside, methyl \(\beta\)-D-galactoside, methyl D-xylo-D-glucoside, methyl D-xylo-D-mannoside, D-psicose, raffinose, L-rhamnose, sedoheptulosean, D-sorbitol, stachyose, sucrose, tagatose, turanose, xylitol, \(\alpha\)-hydroxybutyric acid, \(\beta\)-hydroxybutyric acid, \(\gamma\)-hydroxybutyric acid, \(\alpha\)-hydroxyphenylacetic acid, \(\alpha\)-ketoglutaric acid, \(\alpha\)-ketolvaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, propionic acid, succinic acid, succinic acid, N-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-aspartagine, L-glutamic acid, gly cycl-L-glutamic acid, L-prolylgutamic acid, L-serine, putrescine, 2,3-butanediol, adenosine-5'-monophosphate, thymidine-5'-monophosphate, uridine-5'-monophosphate, D-fructose 6-phosphate, D-glucose 1-phosphate, D-glucose 6-phosphate or DL-\(\alpha\)-glycerol phosphate.

Acid production and additional physiological tests were performed using API 20E strips, API 50 CHB strips and API 50 CHB/E medium (BioMérieux) according to the manufacturer’s protocol over 2 days at 20 °C (Table 1). In the API 50E system, strain SSB001\textsuperscript{T} was positive for hydrolysis of arginine and starch, but negative for hydrolysis of ONPG, lysine, ornithine and tryptophan, production of indole and H\(_2\)S, reduction of nitrate and the Voges–Proskauer test. In the API 50CH system, acid was produced from ribose, D-xylose, fructose, mannose, glycogen and gluconate. Acid was not produced from L-arabinose, methyl \(\beta\)-D-xylidoside, galactose, mannitol, methyl D-xylo-D-mannoside, methyl D-xylo-D-glucoside, arbutin, salicin, lactose, melibiose, sucrose, raffinose, gentiobiose, turanose or 5-ketoglucurate.

The novel strain also showed a fatty acid composition different from those of the reference strains of related species using a previously described method (Athalye \textit{et al.}, 1985; Reddy \textit{et al.}, 2008). In our study, the novel strain and three reference type strains were grown on TSA medium for 4 days at 20 °C, then the cells were harvested and the fatty acids were prepared and identified by the standard method of the MIDI Sherlock Microbial Identification System (Library RTS6 6.0, MIDI Sherlock software...
Cells are Gram-stain-positive, aerobic, motile with a polar flagellum, rod-shaped, 0.6 μm wide by 2.5 μm long. Terminal ellipsoidal spores are formed in swollen sporangia. Colonies are circular, slightly convex, shallow and yellow on TSA under optimal growth temperature (20 °C) and pH (7). Can grow at temperatures between 4 °C and 25 °C, between pH 6 and 7.5, and weakly with up to 4% (w/v) NaCl. Utilizes dextrin, N-acetyl-D-glucosamine, L-arabinose, D-fructose, α-D-glucose, maltose, maltotriose, D-mannose, methyl β-D-glucoside, D-ribose, trehalose, D-xylene, acetic acid, succinic acid monomethyl ester, glycerol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, 3-methyl-D-glucose (weakly), palatinose (weakly), salicin (weakly), pyruvic acid methyl ester (weakly) and pyruvic acid (weakly) as sole carbon sources. Positive for hydrolysis of arginine and starch. Acid is produced from ribose, D-xylene, fructose, mannose, glycogen and gluconate. The fatty acid is anteiso-C15:0. The diphosphoglycerol is meso-diaminopimelamic acid. MK-7 is the major isoprenoid quinone and phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol are the main polar lipids.

The type strain is SSB001T (=ACCC 19728T=DSM 29321T), isolated from alpine swamp meadow soil in Tibet, China. Its DNA G+C content is 40.18 mol% (HPLC).

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### References


### Description of Paenibacillus tibetensis sp. nov.

Paenibacillus tibetensis (ti.bet.en’sis. N.L. masc. adj. tibetensis of or pertaining to Tibet, where the organism was isolated).


