**Dyadobacter tibetensis** sp. nov., isolated from glacial ice core

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A Gram-stain-negative, rod-shaped, aerobic, non-motile bacterium, designated Y620-1T, was isolated from a glacier on the Tibetan Plateau, China. The 16S rRNA gene sequence of the novel isolate shared 93.6–95.1% similarity with type strains of species of the genus *Dyadobacter*. The major fatty acids of strain Y620-1T were summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH), iso-C_{15:0}, C_{16:1ω5c} and iso-C_{17:0} 3-OH. The predominant isoprenoid quinone and polar lipid were MK-7 and phosphatidylethanolamine (PE), respectively. The DNA G+C content was 44.4 ± 0.3 mol% (Tm). Flexirubin-type pigment was produced. The novel isolate was classified in the genus *Dyadobacter*, but a number of phenotypic characteristics distinguished the novel isolate from type strains of species of the genus *Dyadobacter*. From these genotypic and phenotypic data, it is evident that strain Y620-1T represents a novel species of the genus *Dyadobacter*, for which the name *Dyadobacter tibetensis* sp. nov. is proposed. The type strain is Y620-1T (=JCM 18589T=CGMCC 1.12215T).

The genus *Dyadobacter* was first proposed by Chelius & Triplett (2000) for strain NS114T isolated with a nitrogen-limited medium. Members of the genus *Dyadobacter* are characterized as aerobic, stain Gram-negative, contain several typical fatty acids, e.g. iso-C_{15:0}, C_{16:1ω9c} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH) and produce a flexirubin-like pigment (Reddy & Garcia-Pichel, 2005). MK-7 is the predominant isoprenoid quinone of this genus. The DNA G+C content ranges from 44.0 to 50.0 mol%. At the time of writing, a total of 10 species with validly published names have been described (Baik et al., 2007; Chaturvedi et al., 2005; Chelius & Triplett 2000; Dong et al., 2007; Liu et al., 2006; Reddy & Garcia-Pichel, 2005; Tang et al., 2009; Zhang et al., 2010; Lee et al., 2010; Chen et al., 2012).

Ice core samples were drilled from Yuzhufeng Glacier on the Tibetan Plateau, China (94°14.77′ E 35°39.64′ N). The ambient condition is extremely cold on the Yuzhufeng Glacier, therefore, bacteria recovered from this area are able to endure low temperatures. Thawed water from separate sections along the depth of the ice core was used for cultivation. After incubation at 4°C for 30 days with R2A medium (Reasoner & Geldreich, 1985) some bacterial colonies were recovered, bacterial colonies were restreaked several times for purification.

Genomic DNA of strains isolated from Yuzhufeng Glacier was extracted using the method previously described by Marmur (1961) from cells grown on R2A for 2 days at 28°C. The purity of genomic DNA was assessed by NanoDrop (2000c; Thermo). The 16S rRNA gene was amplified with universal primers 27F (5′-AGAGTTTGATCCTTGCTCAG-3′) and 1492R (5′-CGGTACCTTGGTACGACTT-3′) (Embley, 1991). Nearly full-length 16S rRNA gene sequences were aligned with sequences available in the GenBank database by employing the BLAST program.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y620-1T is JQ824420.

Two supplementary figures are available with the online version of this paper.

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(NCBI) to determine the approximate phylogenetic affiliation of these strains. The 16S rRNA gene sequences were analysed with the software package MEGA 5.05 (Tamura et al., 2011). Phylogenetic trees were reconstructed using the neighbour-joining and maximum-likelihood methods with bootstrap values based on 1000 replications (Fig. 1). One bacterial strain recovered from 59 m beneath the glacier, designated Y620-1T, was shown to be phylogenetically related to members of the genus Dyadobacter (93.6–95.1 % 16S rRNA gene sequence similarity).

To further determine the taxonomic position of the novel isolate, a series of phenotypic and genotypic approaches were employed. The morphology was examined by transmission electron microscopy (JEM-1230; JEOL) (Fig. 2). Phenotypic characteristics such as Gram staining, catalase activity, and hydrolysis of casein, hypoxanthine, Tween 80 and starch were performed using the methods of Smibert & Krieg (1994). The pH range for growth was determined in R2A broth at 30 °C. The pH4-10 and 0-4 of the medium was adjusted (in 1 unit increments) with citrate phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Growth in the presence of 0–5 % (w/v) NaCl (in 1 % increments) was investigated in the same medium. Growth at various temperatures (0–40 °C) was measured in R2A broth in 5 °C increments. Anaerobic growth was determined at 30 °C according to the method described by Liu et al. (2006). Other physiological and biochemical properties were further determined with API 20NE and API ZYM strips (bioMérieux) according to the manufacturer’s instructions. The differences in physiological characteristics between strain Y620-1T and type strains of other species of the genus Dyadobacter are given in Table 1.

Flexirubin-like pigments were tested by measuring the absorbance UV–visible spectrum of ethanol and alkaline-ethanol extracts of lysed cells (Weeks, 1981). The strain exhibited three peaks characteristic of flexirubin-type pigment at 428, 452 and 478 nm in ethanol (Fig. S1, available in IJSEM Online) (Chelius & Triplett, 2000; Reddy & Garcia-Pichel, 2005) and the addition of 1 % KOH broadened the peak. This supported the affiliation of strain Y620-1T to the genus Dyadobacter.

Whole-cell fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDi, version 6.0) with cells of

![Fig. 1](http://ijs.sgmjournals.org)

**Fig. 1.** Neighbour-joining phylogenetic tree for strain Y620-1T, based on 16S rRNA gene sequence analysis. Numbers at nodes indicate bootstrap percentages (based on 1000 replications); only values >50 % are shown. Bar, 0.02 accumulated changes per nucleotide.

![Fig. 2](http://ijs.sgmjournals.org)

**Fig. 2.** Transmission electron micrograph of cells of strain Y620-1T after 24 h of growth on R2A. Bar, 1 μm.
strains Y620-1<sup>T</sup> and D. ginsengisoli Gsoil 043<sup>T</sup> harvested from TSA after 48 h of incubation at 30 °C. No remarkable differences in fatty acid profiles were found between the novel isolate and type strains of species of the genus *Dyadobacter*, but small quantitative differences were observed, such as strain Y620-1<sup>T</sup> contained a relatively low proportion of summed feature 3 (C16:1<sub>ω7c</sub> and/or iso-C<sub>15:0 2-OH</sub>). (Table 2). Polar lipids were extracted and analysed by two-dimensional TLC (Altenburger et al., 1996; Tindall, 1990). The predominant polar lipid of strain Y620-1<sup>T</sup> was phosphatidylethanolamine (PE). (Fig. S2).

For the analysis of isoprenoid quinones cells were harvested after 48 h of incubation at 30 °C. Isoprenoid quinones were analysed as described by Hiraishi et al. (1998), using a Waters Acuity Ultra Performance LC (UPLC)-Q-TOF-MS spectrometer (Waters) with an electrospraying ionization method (Romano et al., 2006). The predominant isoprenoid quinone of strain Y620-1<sup>T</sup> was MK-7. The genomic DNA G+C content of the strain was estimated from the midpoint value (T<sub>m</sub>) of the thermal denaturation profile (Mandel et al., 1970). The genomic DNA G+C content of strain Y620-1<sup>T</sup> was 44.4 ± 0.3 mol%. The predominant isoprenoid quinones and the DNA G+C content of the bacterium Y620-1<sup>T</sup> are consistent with the general characteristics of the genus *Dyadobacter*.

Based on genotypic and phenotypic data presented in this study, the novel psychrotolerant bacterium, strain Y620-1<sup>T</sup>, represents a novel species of the genus *Dyadobacter*, for which the name *Dyadobacter tibetensis* sp. nov. is proposed.

**Description of Dyadobacter tibetensis sp. nov.**

*Dyadobacter tibetensis* (ti.bet.en’sis. N.L. masc. adj. tibetensis of or belonging to Tibet, referring to the isolation of the type strain from the Tibetan Plateau).

Cells are aerobic, Gram-stain-negative, non-motile and rod-shaped, approximately 0.5 μm wide and 1.5 μm long. Yellow, round, smooth, convex and opaque colonies are produced on R2A after incubation at 30 °C for 2–3 days. Grows at 4–35 °C (optimally at 30 °C) on R2A. Positive result in tests for catalase activity and hydrolysis of starch, but negative result for hydrolysis of Tween 80. Degrades glucose, mannitol, arabinose and mannose, but not maltose, gluconate, capric acid, adipic acid, malic acid, citric acid or phenylacetic acid (API 20NE test strips). In API ZYM tests, positive result for alkaline phosphatase, esterase lipase (C14), esterase (C4), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, a-galactosidase, b-galactosidase, a-glucosidase, b-glucosidase, N-acetyl-b-glucosaminidase and a-mannosidase activities, but negative result for lipase (C14), trypsin, x-chymotrypsin, b-glucuronidase and a-fucosidase activities. The major fatty acids are summed feature 3 (C<sub>16:1ω7c</sub> and/or iso-C<sub>15:0 2-OH</sub>), iso-C<sub>15:0</sub> C<sub>16:1ω7c</sub> and iso-C<sub>17:0 3-OH</sub>. The major respiratory quinone is MK-7, and the predominant polar lipid is phosphatidylethanolamine (PE).

The type strain, Y620-1<sup>T</sup> (=JCM 18589<sup>T</sup> =CGMCC 1.12215<sup>T</sup>), was isolated from a 59-metre-deep ice core
section drilled from Yuzhufeng Glacier, Tibetan Plateau, China. The DNA G+C content of the type strain is 44.4 ± 0.3 mol% ($T_m$).

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**References**


