**Hymenobacter kanuolensis sp. nov., a novel radiation-resistant bacterium**

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A Gram-reaction-negative, rod-shaped, non-motile, red-pigmented, radiation-resistant, aerobic bacterium designated T-3T was isolated from a soil sample from the Qinghai-Tibet Plateau in Tibet, China, after exposure to 10 kGy gamma radiation. Phylogenetic analysis based on 16S rRNA sequences indicated that this isolate represented a novel member of the genus *Hymenobacter*. Sequence identities of the 16S rRNA gene of strain T-3T with the type strains of species of the genus *Hymenobacter* with validly published names range from 89 % to 97 %, and the most closely related species is *Hymenobacter psychrotolerans* Tibet-IIU11T (97 %). The DNA–DNA relatedness between strain T-3T and *H. psychrotolerans* is 59.10 %. The major fatty acids of strain T-3T were iso-C_{15:0} (27.66 %), summed feature 4 (iso-C_{17:0} 3 \text{ 1} \text{ 1} and/or anteiso-C_{17:0} 3 \text{ 1} \text{ 1} 3 \text{ 0} \text{ 0}, 15.84 %), anteiso-C_{15:0} (14.08 %) and summed feature 3 (C_{16:1} \text{ 3} \text{ 0} \text{ 7} \text{ 1} \text{ 0} \text{ 7} 3 \text{ 1} \text{ 1} and/or C_{16:1} \text{ 3} \text{ 0} \text{ 6} \text{ 0} \text{ 6} \text{ 0} \text{ 6}, 12.38 %). The major menaquinone of strain T-3T was MK-7. Phosphatidylethanolamine (PE) was predominant in the polar lipid profile. The G+C content of the DNA of strain T-3T was 69.17 mol%. On the basis of the results of the polyphasic characterization presented in this study, it is concluded that strain T-3T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter kanuolensis* is proposed. The type strain is T-3T (=ACCC 05760\textsuperscript{T} =KCTC 32407\textsuperscript{T}).

The genus *Hymenobacter*, first proposed by Hirsch et al. (1998) and later amended by Buczolits et al. (2006), is part of the phylum *Bacteroidetes*, order *Sphingobacteriales*, family *Cytophagaceae*. Species of the genus *Hymenobacter* are Gram-reaction-negative, rod-shaped, non-motile, red-pigmented and non-spore-forming bacteria. The major respiratory quinone of this genus is menaquinone 7 (MK-7) and the main phospholipid is phosphatidylethanolamine (PE). Compared with other taxa of the phylum *Bacteroidetes*, the genomic DNA of species of the genus *Hymenobacter* shows a relatively high G+C content (55–65 mol%). At the time of writing, this genus comprises 25 species with validly published names: *Hymenobacter rososalivarius* (Hirsch et al., 1998), *H. actinosclerus* (Collins et al., 2000), *H. aerophilus* (Buczolits et al., 2002), *H. norwichensis*, *H. ocellatus*, *H. gelipurpurascens*, *H. chitinivorans* (Buczolits et al., 2006), *H. rigui* (Baik et al., 2006), *H. xinjiangensis* (Zhang et al., 2007), *H. psychrotolerans* (Zhang et al., 2008), *H. soli* (Kim et al., 2008), *H. daecheongensis* (Xu et al., 2009), *H. deserti* (Zhang et al., 2009), *H. perfusus*, *H. flocculans*, *H. metalli* (Chung et al., 2010), *H. algoricola*, *H. antarcticus*, *H. elongatus*, *H. fastidiosus*, *H. glaciei* (Klassen & Foght, 2011), *H. psychrophilus* (Zhang et al., 2011), *H. yonginensis* (Joung et al., 2011), *H. ginsengisoli* (Hoang et al., 2013) and *H. arizonensis* (Reddy & Garcia-Pichel, 2013). In addition *H. tibetensis* (Dai et al., 2009) has been described but the name is not validly published.

The Qinghai–Tibet plateau has been described as ‘The third pole of the planet’. It is a unique environment characterized by a high altitude, severe cold, oxygen deficiency and high UV radiation. These environmental characteristics make it a ‘natural laboratory’ for isolating radiation-tolerant micro-organisms. Amongst species of the genus *Hymenobacter*, only *H. actinosclerus* and *H. xinjiangensis* have been reported to be resistant to radiation, while *H. tibetensis* has been described as tolerant of high doses of UV irradiation. In our screening program for radiation-tolerant bacteria from the Qinghai-Tibet plateau, a strain belonging to the genus *Hymenobacter*, designated T-3T, was isolated from a soil sample collected from the mountain Kanuola, Tibet (28.89370 °N, 90.17190 °E, altitude 5019 m).

The soil sample (1.0 g) was exposed to 10 kGy gamma radiation from a {\textsuperscript{60}}Co source (CAIC), inoculated into 50 ml TGY liquid media (1.0 % peptone, 0.5 % yeast

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain T-3T (=ACCC 05760\textsuperscript{T} =KCTC 32407\textsuperscript{T}) is KC192770.

Three supplementary figures and a supplementary table are available with the online version of this paper.
extract, 0.1 % glucose) and cultivated at 30 °C, with shaking at 200 r.p.m. for 5 days. The resulting culture was used to inoculate a TGY agar plate (TGY medium with 1.5 % agar). Single colonies with red pigmentation were isolated and stored as glycerol suspensions (20 %, w/v) at −80 °C.

The genomic DNA of strain T-3T was extracted and purified using a TIANamp bacteria DNA Kit (Tiangen) according to the manufacturer’s instructions. DNA–DNA hybridization studies with H. psychrotolerans Tibet-IIU11T were carried out by the fluorometric microdilution plate method (Yuan et al., 2009). The G+C content was measured as described by Mesbah et al., (1989) using reversed-phase HPLC. The 16S rRNA gene was amplified by PCR with the universal primers 27F and 1492R and sequenced by TSINGKE (Beijing, China). The nearly full-length 16S rRNA gene (1380 bp) was assembled using VectorNTI 10 (Invitrogen). Related sequences were obtained from GenBank and multiple alignments were created in CLUSTAL_X (Larkin et al., 2007). Phylogenetic trees were reconstructed with the neighbour-joining method (Saitou & Nei, 1987) using MEGA version 4 (Tamura et al., 2007), and bootstrap values were calculated from 1000 replications (Fig. 1).

The Gram reaction was performed by the non-staining method. Colony morphologies were determined on R2A agar at 30 °C after 5 days of growth. Cell morphology was observed by light microscopy (Leica DM RAR) and scanning electron microscopy (Hitachi). Motility was observed by light microscopy (Leica DM RAR). The Gram reaction was performed by the non-staining method (Saitou & Nei, 1987) using MEGA version 4 (Tamura et al., 2007), and bootstrap values were calculated from 1000 replications (Fig. 1).

The fatty acid profile of strain T-3T includes iso-C_{15:0} (27.66 %), summed feature 4 (iso-C_{16:1}v_{7}\text{-}i/anteiso-C_{17:1}v_{10}), anteiso-C_{15:0} (14.08 %), summed feature 3 (C_{16:1}ω7c/C_{16:1}ω6c, 12.38 %), C_{16:1}ω9c (6.59 %), iso-C_{15:0}3-OH (5.77 %) and iso-C_{15:0}3-OH (3.77 %) (Table S1). The major menaquinone of strain T-3T is MK7, the same as for the other species of the genus Hymenobacter. Phosphatidylethanolamine (PE) was predominant in the polar lipid profile of strain T-3T and an unknown aminophospholipid was the second most prominent component, which was the same as for other species of the genus Hymenobacter. Some other unknown aminophospholipid and unknown aminolipid were also detected, which was quite different from H. psychrotolerans CGMCC 1.6365T (Fig. S2). Under the same incubation condition, strain T-3T displayed more polar lipid dots than H. psychrotolerans CGMCC 1.6365T, especially unknown polar lipids and unknown aminophospholipids. Strain T-3T was resistant to UV radiation at a dose of 2000 Jm⁻², compared with Escherichia coli K-12 CGMCC 1.3065 and different from H. psychrotolerans CGMCC 1.6365T (Fig. S3).

The G+C content of the DNA of strain T-3T was 69.17 mol%. This is significantly higher than the G+C content for most other species of the genus Hymenobacter (54–65 mol%) and only a little lower than that of H. arizonensis (70 %). The length of the 16S rRNA gene sequence of strain T-3T determined in this study was 1361 bp. Sequence database comparisons showed that the 16S rRNA gene of strain T-3T had the highest similarity to the rRNA sequences of the following strains: H. psychrotolerans Tibet-IIU11T (98 %), H. rigui WPBC131T (97 %), H. xinjiangensis X2-1gT (96 %), H. actinosclerus (MIDI) (Kämpfer & Kroppenstedt, 1996) and analysed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification Standard (MIS) Database TSBA6. Techniques for the extraction and analysis of quinones and polar lipids were reported previously (Yuan et al., 2009; Zhou et al., 2012). The conditions for assessing UV resistance were reported previously (Zhang et al., 2010).

Strain T-3T is a rod-shaped, Gram-reaction-negative, aerobic, non-motile, red-pigmented, radiation-resistant bacterium. After 3 days of growth on R2A agar at 30 °C, the colonies were red, circular, smooth, moist, translucent and convex with regular edges. After cultivation on R2A at 30 °C for 48 h, the cells were approximately 0.4–0.6 × 1.0–1.8 μm in size (Fig. S1, available in the online Supplementary Material). Growth occurred over a wide temperature range (4–37 °C) and at pH 6.0–8.0, but not in media containing 1–3 % NaCl. The morphological, cultural, physiological and biochemical characteristics of strain T-3T are listed in Table 1 and the results of enzyme activity tests are given in the species description. Strain T-3T was sensitive to ampicillin (50 μg ml⁻¹), chloramphenicol (35 μg ml⁻¹), rifampicin (80 μg ml⁻¹) and tetracycline (50 μg ml⁻¹), but resistant to hygromycin (50 μg ml⁻¹) and kanamycin (50 μg ml⁻¹).

Conventional biochemical tests were performed as described by Smibert & Krieg (1981) and Sneath (1986). These tests assessed nitrate reduction, indole production, urease activity, lysine decarboxylase activity, ornithine decarboxylase activity, arginine dihydrolase activity and hydrolysis of starch and casein. The API ZYM kit (bioMérieux) was used to test enzyme activities according to the manufacturer’s instructions. Antibiotic resistance was measured by spreading the culture on R2A agar plates containing the appropriate antibiotic and incubating the culture at 30 °C for 5 days.

Cellular fatty acids were determined from colonies grown on R2A agar medium at 30 °C for 3 days. Fatty acid methyl esters were prepared using the classical method of the Sherlock Microbial Identification System version 6.1.
Collins 1187T (95 %) and H. gelipurpurascens Txc1T (95 %). However, strain T-3T has low DNA–DNA relatedness with H. psychrotolerans Tibet-IIU11T (59.10 %). In the neighbour-joining phylogenetic tree (Fig. 1), strain T-3T was clustered within the genus Hymenobacter. Taken together, the phenotypic, chemotaxonomic and phylogenetic data indicates that strain T-3T represents a novel species of the genus Hymenobacter, for which the name Hymenobacter kanuolensis sp. nov. is proposed.

**Description of Hymenobacter kanuolensis sp. nov.**

*Hymenobacter kanuolensis* (ka.nu.ol.en’sis. N.L. masc. adj. kanuolensis pertaining to Mount Kanuola, Tibet, China, the source of the type strain).

Cells are rod-shaped, Gram-staining-negative, aerobic, non-motile, red-pigmented and radiation-resistant. Cells are approximately 0.4–0.6 μm wide and 1.0–1.8 μm long.

Growth occurs on TGY agar and R2A agar. Colonies are red, circular, smooth, moist, translucent and convex with regular edges on R2A agar at 30°C for 3 days. Growth temperature ranges between 4°C and 37°C, and the optimal growth temperature is 30°C. Growth occurs at pH 6.0–8.0 (optimum pH 7.0). The presence of more than 1 % NaCl inhibits growth on R2A agar. Catalase-positive but negative for lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase. Also negative for nitrate reduction, indole production and hydrolysis of starch and urease, but positive for hydrolysis of casein. It is positive according to the API ZYM test system for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase, but negative for lipase (C14), trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and β-fucosidase. Positive for utilization of maltose, trehalose, stachyose,
D-mannitol, D-arabitol, glycerol, D-glucose 6-phosphate, D-a
NR, not reported/not detected. Urease activity and indole production were negative for all strains, catalase activity was positive for all strains.

Strains: 1, H. kanuolensis

Table 1. Phenotypic characteristics that differentiate strain T-3T from other related members of the genus Hymenobacter

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<th>4</th>
<th>5</th>
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<td>Pigmentation</td>
<td>Red</td>
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<td>Brick red</td>
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<td>4 °C</td>
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<td>37 °C</td>
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<td>Nitrate reduction</td>
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<td>Starch hydrolysis</td>
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<td>+</td>
<td>+</td>
<td>(+)</td>
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<td>Casein hydrolysis</td>
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<td>NR</td>
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<td>(+)</td>
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<td>Arginine dihydrolase</td>
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<td>NR</td>
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<td>Assimilation of:</td>
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<td>–</td>
<td>NR</td>
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<td>–</td>
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<tr>
<td>Source of isolation</td>
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<td>Permafrost sediment</td>
<td>Water</td>
<td>Soil</td>
<td>Soil</td>
<td>Air</td>
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<td>DNA G+C content (mol%)</td>
<td>69</td>
<td>60</td>
<td>65</td>
<td>61</td>
<td>57-58</td>
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</table>

Acknowledgements

The authors wish to thank Professor István Molnár and Dr Yuquan Xu (Natural Products Center, School of Natural Resources and the Environment, The University of Arizona) for reading the manuscript. This work was supported by the Ministry of Science and Technology of China (National Basic Research Program 2013CB733900, 2010CB126504 and National High-Tech Program 2012AA063503), the National Natural Science Foundation of China (grant No. 31170105) and the Important National Science & Technology Specific Projects (2011ZX08012-001, 2011ZX08009-003). We would like to thank Dr Aihua Li (Institute of Microbiology, Chinese Academy of Sciences), Professor Honghui Zhu (Guangdong Institute of Microbiology) and Dr Xiaoxia Zhang (Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences) for helpful discussions. We also would like to thank Professor Zhimei Yang (College of Life Science, Capital Normal University, Beijing, China) for providing the soil samples.

References


