Mucilaginibacter psychrotolerans sp. nov., isolated from peatlands

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Abstract

A Gram-stain-negative, rod-shaped, non-flagellated, pink, cold-tolerant bacterial strain, NH7-4T, was isolated from the Riganqiao peatlands on the Tibetan Plateau. The 16S rRNA gene sequence of the novel isolate shared a pairwise similarity ranging from 96.84 to 93.02 % with type strains of the genus Mucilaginibacter. Growth of strain NH7-4T occurred between 0 and 30 °C and at pH 5.0–9.0, with an optimum growth temperature at 20 °C and an optimum pH for growth of approximately 7.0. The major isoprenoid quinone was MK-7. The major cellular fatty acids were summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), iso-C15:0 3-OH, C16:0, iso-C15:0 3-OH and C16:1ω5c. The major polar lipid of strain NH7-4T was phosphatidylethanolamine. Strain NH7-4T did not assimilate any substrates in API 20NE strips without low concentrations of yeast extract being present and had a lower optimal growth temperature, which distinguished it from other type strains of species of the genus Mucilaginibacter. The DNA G+C content of strain NH7-4T was 48.6 mol%. Based on phylogenetic, phenotypic and chemotaxonomic data, strain NH7-4T (=JCM 30607T=CGMCC1.14937T) represents a novel species of the genus Mucilaginibacter for which the name Mucilaginibacter psychrotolerans sp. nov. is proposed.

The genus Mucilaginibacter, a member of the family Sphingobacteriaceae, phylum Bacteroidetes, was first proposed by Pankratov et al. [1] with the type strains of the species Mucilaginibacter paladis and Mucilaginibacter gracilis isolated from an acidic Sphagnum peat bog. Species of the genus were characterised as being Gram-stain-negative, non-spore-forming and non-motile rods, producing large amounts of extracellular polymeric substances. Most species were able to degrade pectin-, xylan- and laminarin. Typical fatty acids of the genus were Summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), iso-C15:0 3-OH, C16:0, iso-C15:0 3-OH and C16:1ω5c. Menaquinone-7 (MK-7) was the predominant isoprenoid quinone, phosphatidylethanolamine (PE) was the major polar lipid and the DNA G+C content ranged from 39.1 to 49.8 mol%. At the time of writing, the genus comprises at least 33 species with validly published names, isolated from a wide range of terrestrial and aquatic sites, including acidic peat, Arctic tundra soil, freshwater, forest, rice paddy fields and marine sand [1–26]. In a study of aerobic methanotroph diversity in the Riganqiao peatlands on the Tibetan Plateau, a cluster of red and mucus-producing bacterial strains were isolated. The area of wetlands on the Qinghai-Tibetan Plateau is estimated to be 1.33×104 km². These sites have distinguishing features and the Riganqiao peatlands (33.06° N, 102.38° E) belong to the Zoige wetlands. In phylogenetic analysis, the genomic DNA of these isolates was extracted with the method previously described [27] from cells grown on R2A for 72 h at 20 °C. The purity was assessed by NanoDrop (2000c, Thermo). The 16S rRNA gene was amplified with the universal primers, 27F (5¢-AGAGTTTGATCC TGGCTCAG-3¢) and 1492R (5¢-CGGTTACCTTGTTAC GACTT-3¢) [28]. Monoclonal sequencing was performed using vector M13 sequence-based primers flanking insert. When compared with the available sequences at GenBank using the blast program (NCBI) and the EzBioCloud [29], a strain designated as NH7-4T could be affiliated to the genus Mucilaginibacter and showed a similarity of less than 97 % with all the type strains of species of this genus. The sequence was then aligned with those of related species by using CLUSTAL W and phylogenetic trees were reconstructed by using the neighbour-joining, maximum-parsimony and maximum-likelihood methods with MEGA 5.05 software [30]. Bootstrap analysis (1000 replications) was used to evaluate the
topology of the trees (Fig. 1). Strain NH7-4\textsuperscript{T} was shown to be phylogenetically related to members of the genus Mucilaginibacter (96.84–93.02\%, similarities), with the most closely related type strain being Mucilaginibacter polysacchareus DRP28\textsuperscript{T}. It has been suggested that a 98.65\% or lower 16S rRNA gene sequence similarity can be used as the threshold for differentiating between two species, indicating that strain NH7-4\textsuperscript{T} may represent a novel species [31].

To determine further the taxonomic position of the novel isolate, a series of phenotypic and genotypic studies were conducted. Morphology was examined by transmission electron microscopy (JEM-1230; JEOL). The pH range for growth was determined in R2A broth at 10°C. pH values of <6, 6–9 and >9 for growth were tested in filter-sterilized broth adjusted with sodium acetate/acetic acid, Tris/HCl and Na\textsubscript{2}CO\textsubscript{3} buffers, respectively, with a modified method [32]. Growth in the presence of 0–8\% NaCl (w/v) with an

![Fig. 1. Neighbour-joining phylogenetic tree for strain NH7-4\textsuperscript{T} based on 16S rRNA gene sequence analysis. Numbers at nodes indicate bootstrap percentages (based on 1000 replications); only values >50% are shown. Bar, 0.01 accumulated changes per nucleotide.](image-url)
interval of 1% was also investigated in the same medium. Growth at various temperatures (0–40 °C, with an interval of 5 °C) was measured at 0 °C was maintained with an ice-water mixture, others were sustained using a constant-temperature incubator). OD_{600} was measured with a micro plate reader (MD Spectra Max M5) to assess the growth in experiments. Physiological and biochemical properties were determined with API 20NE and API ZYM strips according to the manufacturer’s instructions. The differences between the physiological characterization of strain NH7-4^T and other type strains of *Mucilaginibacter* with validly published names are given in Table 1 and in the species description of *Mucilaginibacter psychrotolerans* sp. nov. The bacterium grew at 0–30 °C (optimally at 20 °C) on R2A, at pH 5–9 (optimally at pH 7) and in 0–1% (w/v) NaCl. The relatively low optimal growth temperature and the inability to assimilate D-glucose, mannose (without the addition of 50 mg l^{-1} yeast extract), N-β-glucosamine and maltose distinguish strain NH7-4^T from other type strains of species of the genus *Mucilaginibacter* (Table 1). Strain NH7-4^T can be defined as a psychrotolerant microorganism [33].

Fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI, Version 6.0) using stationary phase cells harvested after 10 days of growth at 10 °C. No major differences were observed between the fatty acid profiles of the novel isolate and type strains of the genus *Mucilaginibacter*, but small quantitative differences were observed (Table 2). For example, the novel strain, NH7-4^T, had a relatively high proportion of C_{16:0}, C_{17:0}ω8c and anteiso-C_{15:0} when compared to other members of the genus *Mucilaginibacter*.

Isoprenoid quinones and polar lipids were analyzed using cells harvested after 72 h of growth at 20 °C in R2A broth. Isoprenoid quinones were analyzed with the method described by Hiraishi *et al.* [34], with Waters Acquity Ultra Performance liquid chromatography time of flight mass spectroscopy (UPLC)-Q-TOF-MS (Waters, Milford) in electrospray ionisation [35]. The predominant isoprenoid quinine of strain NH7-4^T was MK-7, which is one of the biochemical characteristics of the genus *Mucilaginibacter*. Polar lipids were extracted and analyzed by two-dimensional TLC [36, 37]. The major polar lipid of strain NH7-4^T was PE, there was also an unknown amino lipid present and two phospholipids. This is similar to what has been reported for other species of the genus *Mucilaginibacter* (Fig. S1, available in the online Supplementary Material). The genomic DNA G+C content of the strain was

### Table 1. Differential characteristics of strain NH7-4^T and type strains of related species of the genus *Mucilaginibacter*

<table>
<thead>
<tr>
<th>Strains: 1, NH7-4^T; 2, <em>M. polysaccharae</em> DRP28^T; 3, <em>M. litorea</em> BR-18^T; 4, <em>M. umbrosus</em> BR-3^T; 5, <em>M. rigui</em> WPCB133^T.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optimal growth temperature (°C)</strong></td>
<td>20</td>
<td>25†</td>
<td>25‡</td>
<td>25§</td>
<td>25</td>
</tr>
<tr>
<td><strong>Assimilation of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-β-Glucosamine</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Mannose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
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<td><strong>Enzyme activities</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>α-Fucosidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>48.6</td>
<td>42.7</td>
<td>42.4</td>
<td>49.85</td>
<td>47.0</td>
</tr>
</tbody>
</table>

*Data from: Han *et al.* [5].
†Data from: Kim *et al.* [26].
‡Data from: Yoon *et al.* [19].
§Data from: Baik *et al.* [39].

### Table 2. Fatty acid compositions of strain NH7-4^T and type strains of related species of the genus *Mucilaginibacter*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{14:0}</td>
<td>−</td>
<td>−</td>
<td>1.0</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>C_{16:0}</td>
<td>7.8</td>
<td>6.6</td>
<td>5.0</td>
<td>3.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C_{16:1}ω5c</td>
<td>8.5</td>
<td>9.3</td>
<td>9.0</td>
<td>10.0</td>
<td>7.1</td>
</tr>
<tr>
<td>C_{17:0}ω8c</td>
<td>1.4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>anteiso-C_{15:0}</td>
<td>1.2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>iso-C_{15:0}</td>
<td>14.0</td>
<td>16.6</td>
<td>24.1</td>
<td>21.6</td>
<td>20.4</td>
</tr>
<tr>
<td>iso-C_{15:1}ω3-OH</td>
<td>1.4</td>
<td>1.0</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>iso-C_{16:0}</td>
<td>−</td>
<td>−</td>
<td>1.7</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>iso-C_{16:1}ω3-OH</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C_{17:0}ω3-OH</td>
<td>6.3</td>
<td>7.5</td>
<td>3.7</td>
<td>6.7</td>
<td>7.0</td>
</tr>
<tr>
<td>iso-C_{17:0}ω9c</td>
<td>2.9</td>
<td>2.2</td>
<td>2.5</td>
<td>3.7</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Summed feature 3</strong></td>
<td>49.8</td>
<td>49.5</td>
<td>51.4</td>
<td>44.6</td>
<td>46.7</td>
</tr>
</tbody>
</table>

*Summed feature 3 included C_{16:1}ω7c and/or iso-C_{15:0} 2-0H.
estimated from the midpoint value (Tm) of the thermal denaturation profile [38], and was 48.6 mol%. The predominant isoprenoid quinones, major fatty acids and the major polar lipids are consistent with the general characteristics of the genus *Mucilaginibacter*. Strain NH7-4T contains a relatively high content of DNA G+C (48.6 mol%), compared with other species of the genus (range of 39.1–49.8 mol%).

Based on the genotypic and phenotypic data presented in this study, the psychrotrophic bacterium, NH7-4, represents a novel species of the genus *Mucilaginibacter* for which the name *Mucilaginibacter psychrotolerans* sp. nov. is proposed.

**DESCRIPTION OF MUCILAGINIBACTER PSYCHROTOLERANS SP. NOV.**

*Mucilaginibacter psychrotolerans* (psy.chro.to.le.rans. Gr. adj. psychros cold; L. pres. part. tolerans tolerating; N.L. part. adj. psychrotolerans cold tolerating).

Gram-stain negative, rod-shaped (approximately 0.6 µm wide and 2 µm long) without flagella. Colonies grown on R2A agar are pink, circular, convex and smooth. Growth occurs between 0 and 30 °C and at pH 5.0–9.0, with optimums at 20 °C and pH 7.0. Growth is inhibited at NaCl concentrations >1 %. Positive for catalase and oxidase, but negative for nitrate reductase. Hydrolyses esculin, but not gelatin. The major isoprenoid quinone is MK-7. The major cellular fatty acids are summed feature 3 (C₁₅:0 3-0H, C₁₆:0 3-0H, and C₁₆:1ω5c). The major polar lipid is phosphatidylethanolamine. Does not assimilate D-glucose, arabinose, mannosse (assimilates mannosse with the addition of 50 mg l⁻¹ yeast extract to the AUX medium), mannitol, N-β-glucosamine, maltose, glucosamine, capric acid, adipic acid, malic acid, citric acid and phenylacetic acid (API 20NE). In API ZYM tests, positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, saur phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, but negative for lipase (C14), α-chymotrypsin and β-glucuronidase.

The type strain, NH7-4T (=JCM 30607T=CGMCC 1.14937T), was isolated from the Riganqiao peatlands on the Tibetan Plateau. The DNA G+C content of the type strain is 48.6 mol%.

Conflicts of interest
The authors declare that there are no conflicts of interest.

**References**


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