Chelatococcus reniformis sp. nov., isolated from a glacier

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A Gram-stain-negative, non-motile, reniform bacterial strain, B2974T, was isolated from an ice core of the Muztagh Glacier, on the Tibetan Plateau, China. Strain B2974T grew optimally at pH 7.0–7.5 and 25–30°C in the presence of 0–2.0% (w/v) NaCl. 16S rRNA gene sequence similarity analysis indicated that strain B2974T was closely related to Chelatococcus asaccharovorans LMG 25503T at a level of 97.1%. The major quinone of strain B2974T was ubiquinone Q10. The predominant fatty acids were summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C19:0 cyclo ω8c. sym-Homospermidine was the major polyamine. The genomic DNA G+C content of the strain was 64 mol%. In DNA–DNA hybridization tests, strain B2974T shared 49.32% DNA–DNA relatedness with the type strain of Chelatococcus asaccharovorans LMG 25503T. Based on the results of phenotypic and chemotaxonomic characteristics, strain B2974T was considered as a novel species of the genus Chelatococcus, for which the name Chelatococcus reniformis sp. nov. is proposed. The type strain is B2974T (=JCM 30308T=CGMCC 1.12919T).

The genus Chelatococcus was first described by Auling et al. (1993) as a novel genus of the class Alphaproteobacteria (formerly alpha-subclass of proteobacteria). At the time of writing, the genus includes four species (http://www.bacterio.net/chelatococcus.html), which were isolated from different sources such as wastewater (Auling et al., 1993), textile waste matter (Yoon et al., 2008), hot springs (Panday & Das, 2010) and a sludge sample of a biofilm reactor (Jin et al., 2015). The members of the genus Chelatococcus are characterized as Gram-stain-negative, non-spore-forming and rod- or diplococcus-shape. The major isoprenoid quinone is Q10. C18:1ω7c and C19:0 cyclo ω8c are main components of cellular fatty profiles. sym-Homospermidine is the predominant compound in the polyamine pattern. Sometimes also, relatively high amounts of putrescine and spermidine are detected. The DNA G+C content of the strains of the genus Chelatococcus is 63.3–68.3 mol% (Egli & Auling, 2005). In this study, a novel reniform strain B2974T was isolated from the Muztagh Glacier on the Tibetan Plateau, China. Based on taxonomic characterization, we
consider the strain to represent a novel species of the genus *Chelatococcus*, for which we propose the name *Chelatococcus reniformis* sp. nov.

Strain B2974\(^T\) was isolated from a 164-metre-long ice core at a depth of 160 m. The ice core was drilled from the Muztagh Glacier on the Tibetan Plateau, China (36° 20.94’ N 87° 10.38’ E, 5770 m above sea level). Thawed water from sections of the ice core was used for cultivation. After incubation on R2A agar (containing 0.05 % yeast extract, 0.05 % peptone, 0.05 % casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K\(_2\)HPO\(_4\), 0.005 % MgSO\(_4\), 1.5 % agar; pH 7.2) at 4 °C for 30 days (Reasoner & Geldreich, 1985) bacterial colonies were visible. The colonies were picked and subcultured several times to confirm that they were pure cultures. Isolates were routinely cultured on R2A agar at 25 °C and maintained as a glycerol suspension (15 %, w/v) at −80 °C.

Genomic DNA was extracted from cells which were incubated in R2A broth at 25 °C for 5 days according to the methods of Marmur (1961), and the purity was assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific). The 16S rRNA gene was amplified with primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-CGGTTACCTTGTTACGACTT-3’) as described by Emb-ley (1991). Subsequently, the nearly full-length 16S rRNA gene sequence (1449 bp) was compared with sequence entries in EzBioCloud (Kim et al., 2012) in order to determine the approximate phylogenetic affiliation. For phylogenetic calculations, the software package MEGA 5.05 (Tamura et al., 2011) was used. The neighbour-joining, maximum-likelihood and maximum-parsimony algorithms were used to reconstruct phylogenetic trees with bootstrap values based on 1000 replications (Fig. 1). The 16S rRNA gene sequence comparison indicated that strain B2974\(^T\) was most closely related to *Chelatococcus asaccharovorans* LMG 25503\(^T\) with 97.1 % similarity, followed by *Camelimonas lactis* CCUG 58638\(^T\) (96.5 % similarity). Phylogenetic analyses revealed that strain B2974\(^T\) and species of the genera *Chelatococcus* formed a monophyletic group in the phylogenetic tree (Fig. 1). Based on 16S rRNA gene sequence analysis we considered that strain B2974\(^T\) represented a novel species of the genus *Chelatococcus*.

To determine the taxonomic position of the novel isolate, further extensive taxonomic methods were also used. Gram staining and catalase activity tests were conducted by the methods of Smibert & Krieg (1994). Growth was measured at temperatures from 0 to 40 °C with an interval of 5 °C after the cells were incubated in R2A broth for 2 weeks. The pH range for growth (pH 4.0–11.0, at intervals of 1.0 pH unit) was assessed after incubation for 2 weeks in R2A broth at 25 °C (Breznak & Costlow, 1994). Tolerance to salt was tested in R2A broth with 0–8 % (w/v) NaCl at 25 °C. Colony characteristics were observed on R2A agar plates after 5 days of growth. Morphology was observed by transmission electron microscopy (JEM-1400; JEOL) (Fig. S1, available in the online Supplementary Material) after the cells grew for 3 days. Other physiological and biochemical properties were determined by API 20E, API 20NE and API ZYM strips (bioMérieux) at the temperature recommended by the manufacturer. Motility was assessed by the hanging-drop method (http://microbeonline.com/procedure-hanging-drop-method-test-bacterial-motility/). The comparison of physiological and biochemical characteristics among strain B2974\(^T\) and reference strains of the genera *Chelatococcus* and *Camelimonas* are summarized in Table 1. Strain B2974\(^T\) could be distinguished from the other strains of the genus *Chelatococcus* and the genus *Camelimonas* by assimilating gluconate and not assimilating D-glucose. It could also be distinguished from the other strains of species of the genus *Chelatococcus* by being positive for valine arylamidase and negative for assimilation of L-arabinose.

**Fig. 1.** Neighbour-joining phylogenetic tree for strain B2974\(^T\), based on 16S rRNA gene sequence analysis. Numbers at nodes indicate bootstrap percentages (based on 1000 replications). Bar, 0.01 accumulated changes per nucleotide. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms.
Table 1. Phenotypic characteristics and DNA G+C content of strain B2974T and members of related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4a</th>
<th>5a</th>
<th>6b</th>
<th>7b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Icecore</td>
<td>Wastewater soil¹</td>
<td>Hot spring² rods³</td>
<td>Biofilm reactor rods</td>
<td>Textile waste matter rods</td>
<td>Placental tissue of a cow rods</td>
<td>Milk of camels rods</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Carbon assimilation</td>
<td></td>
<td></td>
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<tr>
<td>D-Glucose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Mannose</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>Maltose</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Gluconate</td>
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<td>Adipic Acid</td>
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<td>+</td>
<td>+</td>
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<td>ND</td>
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<td>Malic acid</td>
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<td>ND</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Phenylacetic acid</td>
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<td>–</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>Acid production from glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<td>Enzymic activities</td>
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<td>Valine arylamidase</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Trypsin</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>ND</td>
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<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64</td>
<td>63</td>
<td>67</td>
<td>68.7</td>
<td>68.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Data taken from: a, Jin et al. (2015); b, Kämpfer et al. (2012); c, Yoon et al. (2008).

The cellular fatty acids were extracted after cells were grown in R2A agar (pH 7.2) at 25 °C for 5 days. Fatty acid methyl esters were extracted and prepared according to the microbial identification system (http://www.midi-inc.com/pages/microbial_id.html). Cell biomasses used for fatty acid analysis were harvested at the exponential growth phase. The fatty acid profile of strain B2974T included C18:1ω7c (51.81 %), C19:0 cyclo ω8c (33.86 %), summed feature 2 (C12:0 aldehyde/unknown 10.98, 6.1 %), C16:0 (2.4 %), C18:0 3-OH (2.4 %), C18:0 (1.8 %), and minor amounts of other fatty acids (<1 %) (Table 2). No pronounced differences in the fatty acid profiles of strain B2974T and strain Chelatococcus asacharovorans LMG 25503T were found; however, slight differences could be observed (Table 2). Strain B2974T does not have C17:0 cyclo, C20:1ω7c or C18:1ω7c 11-methyl, in contrast to strain Chelatococcus asacharovorans LMG 25503T.

For isoprenoid quinone analysis, cells were incubated for 5 days at 25 °C. Quinones were extracted and analysed using the method described by Hiraishi et al. (1998). The predominant isoprenoid quinone of strain B2974T was Q10. For the polyamines analysis, cell biomasses were harvested at the late exponential growth phase. Polyamines were extracted and analysed as described previously (Busse & Auling, 1988; Busse et al., 1997). The polyamine pattern of strain B2974T contained 12.5 µmol sym-homospermidine (g dry weight)⁻¹, 1.7 µmol putrescine (g dry weight)⁻¹ and 0.3 µmol spermidine (g dry weight)⁻¹, 0.1 µmol cadaverine (g dry weight)⁻¹ and 0.05 µmol spermine (g dry weight)⁻¹. This polyamine pattern is clearly distinct from that of Camelimonas lactis CCM 7697, which was reported to contain the major compound spermidine and moderate amounts of putrescine and no sym-homospermidine (Kämpfer et al., 2010). This polyamine pattern also distinguished strain B2974T from Chelatococcus asacharovorans, which was reported to contain in addition to the major polyamine sym-homospermidine also relatively high amounts of spermidine and putrescine (Auling et al., 1993).

The G+C content of the DNA of strain B2974T was determined based on thermal denaturation of genomic DNA (De...
Table 2. Cellular fatty acid contents (percentages) of strain B2974<sup>T</sup>, Chelatococcus asaccharovorans, Chelatococcus sambhunathii, Chelatococcus caeni, Chelatococcus daegeuensis, Camelimonas abortus and Camelimonas lactis

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4&lt;sup&gt;*&lt;/sup&gt;</th>
<th>5&lt;sup&gt;*&lt;/sup&gt;</th>
<th>6&lt;sup&gt;†&lt;/sup&gt;</th>
<th>7&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>2.4</td>
<td>10.5</td>
<td>1.4</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
<td>7.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt; Cyclo</td>
<td>–</td>
<td>4.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;</td>
<td>1.8</td>
<td>0.9</td>
<td>0.6</td>
<td>1.9</td>
<td>1.7</td>
<td>5.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt; 11-methyl</td>
<td>–</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
<td>6.9</td>
<td>2.6</td>
<td>1.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; 10-methyl</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>33.9</td>
<td>25.9</td>
<td>7.0</td>
<td>9.0</td>
<td>10.7</td>
<td>52.5</td>
<td>67.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt; 2-OH</td>
<td>0.8</td>
<td>0.9</td>
<td>6.9</td>
<td>8.1</td>
<td>6.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt; 3-OH</td>
<td>2.4</td>
<td>0.4</td>
<td>1.3</td>
<td>3.0</td>
<td>3.5</td>
<td>3.3</td>
<td>1.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:1&lt;/sub&gt; ω7c</td>
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<td>1.1</td>
<td>–</td>
<td>–</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:2&lt;/sub&gt; ω9c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Summed feature 2&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>6.1</td>
<td>4.3</td>
<td>3.6</td>
<td>3.5</td>
<td>3.3</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Summed feature 3&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.9</td>
<td>1.8</td>
<td>0.5</td>
<td>–</td>
<td>0.4</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Summed feature 8&lt;sup&gt;§&lt;/sup&gt;</td>
<td>51.8</td>
<td>48.7</td>
<td>77.7</td>
<td>72.1</td>
<td>64.7</td>
<td>27.2</td>
<td>11.5</td>
</tr>
</tbody>
</table>

*Data taken from Kämpfer et al. (2012).
†Data taken from Jin et al. (2015).
‡Summed features represent two or more fatty acids that cannot be separated. Summed feature 2 comprises C<sub>12:0</sub> aldehyde and/or unknown 10:08. Summed feature 3 comprises C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c. Summed feature 8 comprises C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c.

Ley et al., 1970). The genomic DNA G+C content of strain B2974<sup>T</sup> was 64 mol%.

Since strain B2974<sup>T</sup> was phylogenetically most closely related to Chelatococcus asaccharovorans DSM 25503<sup>T</sup>, indicated from 97.1% 16S rRNA gene sequence similarity, we further compared strain B2974<sup>T</sup> and Chelatococcus asaccharovorans DSM 25503<sup>T</sup> at the genomic level by a DNA–DNA hybridization experiment, which was carried out by applying the optical renaturation method (Huss et al., 1983; De Ley et al., 1970). The temperature used in the optical renaturation method was 80°C. Hybridizations were repeated three times, and means of the resulting values were determined. The level of DNA–DNA relatedness between strain B2974<sup>T</sup> and Chelatococcus asaccharovorans DSM 25503<sup>T</sup> was 49.32±1.89%.

Phylogenetic analysis and 16S rRNA gene sequence similarity together with the major fatty acids (summed feature 8 (C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c) and C<sub>19:0</sub> cyclo ω8c) and the polyamine (sym-homospermidine) data support the affiliation of strain B2974<sup>T</sup> to the genus Chelatococcus. Furthermore, strain B2974<sup>T</sup> could also be distinguished from its closest phylogenetic neighbour, Chelatococcus asaccharovorans DSM 25503<sup>T</sup>, by DNA–DNA hybridization, relatively low contents of putrescine and spermidine in the polyamine pattern, carbon assimilation, morphology and optimum growth temperature.

Therefore, on the basis of the characters described above, strain B2974<sup>T</sup> represents a novel species of the genus Chelatococcus, for which the name Chelatococcus reniformis sp. nov. is proposed.

**Description of Chelatococcus reniformis sp. nov.**

Chelatococcus. reniformis (re.ni.for’mis. L. n. ren kidney; L. suff. formis shaped like; N.L. masc. adj. reniformis shaped like a kidney).

Cells are aerobic, Gram-stain-negative, non-motile and reniform (0.3–0.8 µm wide and 1.3–1.9 µm long). White, round, smooth, convex and opaque colonies are produced on R2A agar after incubation at 25°C for 4–5 days. Can grow at 5–40°C (optimally at 25–30°C) on R2A agar, at pH 5–10 (optimally at pH 7) and with 0–2% NaCl (optimally with 1% NaCl) in R2A broth. Oxidase and catalase activities are positive. Negative for Voges–Proskauer test and H<sub>2</sub>S production (API 20E test strip). Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and gelatin hydrolysis. Positive for aesculin hydrolysis and urease. Assimilates gluconate, l-arabinose, mannose mannitol, N-acetyl-β-glucosamine, maltose, capric acid, adipic acid, malic acid, citrate or phenylacetic acid (API 20NE test strip). In enzyme activities tests, positive for alkaline phosphatase, esterase (C4), esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, N-acetyl-
β-glucosaminidase, α-mannosidase and α-fucosidase. Negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, ε-galactosidase, β-galactosidase and β-glucuronidase (API ZYM test strip). The major fatty acids are summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C19:0 cyclo ω8c. sym-Homospermidine is the major polyamine. The quinone system contains exclusively ubiquinone Q-10.

The type strain is B2974T (=JCM 30308T=CGMCC 1.12919T), which was isolated from Muztagh ice core, the Tibetan Plateau, China. The genomic DNA G+C content of the type strain is 64 mol% (Tm).

Acknowledgements

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References


