The genus *Polaromonas* was first proposed by Irgens et al. (1996) for a psychrophilic, marine bacterium isolated from Antarctica, *Polaromonas vacuolata*. At the time of writing, this genus comprised seven recognized species: *Polaromonas naphthalenivorans* (Jeon et al., 2004), *Polaromonas aquatica* (Kämpfer et al., 2006), *Polaromonas hydrogenivorans* (Sizova & Panikov, 2007), *Polaromonas jejuensis* (Weon et al., 2008), *Polaromonas glacialis* (Margesin et al., 2012), *Polaromonas cryoconitii* (Margesin et al., 2012) and the type species, *P. vacuolata* (Irgens et al., 1996).

Members of the genus *Polaromonas* have been isolated from various environmental sources, such as marine water (Irgens et al., 1996), tap water (Kämpfer et al., 2006), soil (Weon et al., 2008; Sizova & Panikov, 2007), sediment (Jeon et al., 2004) and glacier cryoconite (Margesin et al., 2012). A novel bacterial strain, *B717-2^T*, was isolated from an ice core at a depth of 38 m. The whole ice core was 120 m in length drilled from Muztagh Glacier (83.7° E, 26.4° N) on the Tibetan Plateau, China. Climate on the glacier is extremely cold. Therefore, bacteria recovered from this area may survive in an inactive state at low temperature. The taxonomic position of the novel strain was investigated by using a polyphasic approach.

The ice core was preserved at −20°C before analysis. The ice core was cut into 5 cm long sections using a band saw within walk-in freezers. All samples were decontaminated by cutting away an outer annulus of about 4 mm with a...
sterilized saw tooth knife, before rinsing the remaining inner cores with cold ethanol (95 %) and finally with cold autoclaved water. No particulate sterile suits were worn during the sampling process. All work was done in a sterile operating room. The samples were then melted in sterile Nalgene bottles at 4 °C. We subsequently incubated 200 μl of meltwater at 4 °C for 30 days on an R2A agar plate containing 0.05 % yeast extract, 0.05 % peptone, 0.05 % Casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K₂HPO₄, 0.005 % MgSO₄, 1.5 % agar; pH 7.2 (Reasoner & Geldreich, 1985), and several bacterial colonies were recovered.

DNA of the novel strain was extracted according to the method of Marmur (1961) from cells grown on R2A agar for 10 days at 15 °C. Purity was assessed using a NanoDrop spectrophotometer (2000c; Thermo). PCR amplification was via universal primers 27F (5'-AGAGTTTGATCCTGGC TGAG-3') and 1492R (5'-CGGGTACCTTGTAGATCT-3') (Embley, 1991), and the PCR products were sequenced at Sangon using an ABI PRISM 3730xl sequencer. The sequence was then analysed against the GenBank database with the BLAST program (NCBI) and EzBioCloud (http://www.ezbiocloud.net/eztaxon) (Kim et al., 2012) to reveal the most similar sequences deposited in GenBank. In comparisons with the type strains of Polaromonas species, strain B717-2 T appeared to be related most closely to P. vacuolata 34-P T, with a 16S rRNA gene sequence similarity of 97.7 %, followed by similarities of 97.4, 97.4, 97.1 and 97.1 % with P. jejeunis JS12-13 T, P. aquatica CCUG 39402 T, P. glacialis Cr4-12 T and P. cryoconitii Cr4-35 T, respectively. Based on the threshold value of 98.65 % (Kim et al., 2014), strain B717-2 T may thus represent a novel species. The phylogenetic position of the novel strain was reconstructed by treeing using the software package MEGA 5.0.5 (Tamura et al., 2011). A phylogenetic tree was reconstructed using the neighbour-joining and maximum-likelihood methods with bootstrap values based on 1000 replications (Fig. 1). In the neighbour-joining phylogenetic tree, strain B717-2 T formed a distinct cluster in a phyletic line with P. vacuolata 34-P T, and the maximum-likelihood tree displayed a similar topology (Fig. 1). DNA–DNA hybridization experiments were carried out applying the optical renaturation method (De Ley et al., 1970; Huš et al., 1983; Jahnke, 1992). The genomic DNA G+C content of strain B717-2 T, which was estimated from the midpoint value (\(T_m\)) of the thermal denaturation profile, was 63.4 mol% (Mandel et al., 1970). The temperature used in the optical renaturation method was 79 °C. Mean levels of DNA–DNA relatedness between strain B717-2 T and P. jejunis JS12-13 T, P. vacuolata 34-P T, P. aquatica CCUG 39402 T, P. glacialis Cr4-12 T and P. cryoconitii Cr4-35 T were 37.0±1.9, 30.0±1.7, 26.0±0.9, 23.4±0.5 and 18.4±1.9 %, respectively. These data again indicate that strain B717-2 T may represent a novel species of the genus Polaromonas.

To further determine the taxonomic position of the novel isolate, a series of phenotypic and genotypic approaches were used. Cell morphology was examined by transmission electron microscopy (JEM-1230; JEOL) (Fig. 2), using cells that had been grown on R2A agar at 15 °C. Gram staining and catalase activity tests were conducted according to the methods described by Smibert & Krieg (1994). Physiological and biochemical characteristics and other enzyme activities were determined by using API 20E, API 20NE and API ZYM systems (bioMérieux) at 15 °C. All tests were positive for catalase and oxidase. Gram staining, motility, and the results of API ZYM tests were consistent with those of Polaromonas species. The results of the phenotypic analysis are given in Table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Polaromonas species</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>Polaromonas</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Motility</td>
<td>Polaromonas</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Catalase</td>
<td>Polaromonas</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Oxidase</td>
<td>Polaromonas</td>
<td>Positive</td>
<td>Negative</td>
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</table>

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain B717-2 T.

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 obtained in the maximum-likelihood tree.
carried out simultaneously with strain B717-2T and the seven reference strains. *P. vacuolata* 34-P, *P. aquatica* CCUG 39402T, *P. hydrogenivorans* DSM 17735T and *P. jejuensis* JS12-13T were obtained from China General Microbiological Culture Collection Center, while *P. naphthalenivorans* Cj2T, *P. glacialis* Cr4-12T and *P. cryoconiti* Cr4-35T were obtained from Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures. Growth at various temperatures (0–35 °C) was tested in R2A broth at increments of 5 °C. The pH range (5.0–11.0) for growth was determined in R2A broth at 15 °C. The pH values of <6, 6–9 and >9 were obtained by using sodium acetate/acetic acid, Tris/HCl and Na2CO3 buffers, respectively. Growth in the absence of NaCl and in the presence of 1, 2, 3, 4, 5 and 6% (w/v) NaCl was also investigated in the same medium at 15 °C. Differences in the physiological characteristics between strain B717-2T and the seven reference strains (Irgens et al., 1996; Jeon et al., 2004; Kämpfer et al., 2006; Sizova & Panikov, 2007; Weon et al., 2008; Margesin et al., 2012) are given in Table 1.

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 strain B717-2T grown on R2A agar for 10 days at 15 °C. Biomasses used for fatty acid analyses were harvested from the logarithmic phase of growth. The fatty acid profile of strain B717-2T resembled those of members of the genus *Polaromonas*.

Table 1. Phenotypic characteristics that differentiate strain B717-2T from closely related members of the genus *Polaromonas*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Rods</td>
<td>Rods&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Rods&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cocci&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Rods&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Cocci&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Rods&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Rods&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>Temperature range (°C)</td>
<td>5–20</td>
<td>0–12&lt;sup&gt;g&lt;/sup&gt;</td>
<td>25–30&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0–25&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5–30&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4–25&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1–25&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1–25&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>Isolation source</td>
<td>Ice core</td>
<td>Soil&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tap water&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Soil&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Soil&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Sediment&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Glacier cryoconite&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Glacier cryoconite&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>pH range</td>
<td>7.0–9.0</td>
<td>6.0–9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>6.0–7.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.0–9.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0–9.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0–7.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Nitrate reduction</td>
<td>+</td>
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<td>–</td>
<td>+</td>
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<td>Arginine dihydrolase</td>
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<td>ND</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>–</td>
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<td>Citrate utilization</td>
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<td>–</td>
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<td>Urease production</td>
<td>–</td>
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<td>Acid production from:</td>
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<td>d-Glucose</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>d-Mannitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>w</td>
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<tr>
<td>Succrose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Melibiose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Amygdalin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>w</td>
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<td>Enzyme activities:</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Lipase</td>
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<td>+</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>63.4</td>
<td>52.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>62.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>61.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>60.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data taken from: a, Irgens et al. (1996); b, Kämpfer et al. (2006); c, Sizova & Panikov (2007); d, Weon et al. (2008); e, Jeon et al. (2004); f, Margesin et al. (2012).
Polaromonas (Irgens et al., 1996; Jeon et al., 2004; Kämpfer et al., 2006; Sizova & Panikov, 2007; Weon et al., 2008; Margesin et al., 2012). As shown in Table 2, the predominant cellular fatty acids of strain B717-2\textsuperscript{T} were summed feature 3 (C\textsubscript{16:1}\textomega 7c and/or iso-C\textsubscript{15:0} 2-OH; 52.7%), summed feature 8 (C\textsubscript{18:1}\textomega 7c, C\textsubscript{18:1}\textomega 6c; 23.1%) and C\textsubscript{16:0} (21.9%). There were no significant differences in the fatty acid components between strain B717-2\textsuperscript{T} and the reference strains, but some quantitative differences were observed (Table 2). Strain B717-2\textsuperscript{T} had a relatively higher proportion of summed feature 8 compared with recognized species of the genus Polaromonas.

For respiratory quinone analyses, cells were harvested after 10 days of growth at 15°C. Respiratory quinones were extracted and purified according to the method of Collins (1985). They were then analysed by HPLC according to Wu et al. (1989). The only respiratory quinone of strain B717-2\textsuperscript{T} was Q-8. The reference strains of the genus Polaromonas showed a quinone system with ubiquinone Q-8 predominant (Jeon et al., 2004; Kämpfer et al., 2006; Sizova & Panikov, 2007; Weon et al., 2008; Margesin et al., 2012). The profile of polar lipids mainly consisted of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. One unknown phospholipid and two unknown polar lipids were also found in strain B717-2\textsuperscript{T} (Fig. 3). The major quinone and polar lipids of strain B717-2\textsuperscript{T} were consistent with the genus Polaromonas.

Based on the phenotypic and genotypic data presented in this study, the eurypsychrotolerant bacterium strain B717-2\textsuperscript{T} represents a novel species of the genus Polaromonas, for which the name Polaromonas eurypsychrophila sp. nov. is proposed.

**Description of Polaromonas eurypsychrophila sp. nov.**

Polaromonas eurypsychrophila [eu.ry psy.chro’phi.la. Gr. adj. eurys wide, broad; Gr. adj. psychros cold; N.L. adj. philus -a -
um (from Gr. adj. philos -ē -on) friend, loving; N.L. fem. adj. eurypsychrophila loving a broad range of low temperatures).

Cells are aerobic, Gram-stain-negative and rod-shaped (1.8–2.0 μm long and 0.71–0.77 μm wide). Colonies on R2A agar after incubation at 15°C for 10 days are beige, round, smooth, convex and opaque. Growth occurs at 5–20°C (optimally at 15°C) on R2A agar, at an initial pH of 7–9 (optimally at pH 7) and with 0–2% (w/v) NaCl (optimally with 2% NaCl). The fatty acid profile is mostly composed of summed feature 3 (C₁₅:₀,ω7c and/or iso-C₁₅:₀ 2-OH), summed feature 8 (C₁₈:₁ω7c, C₁₈:₁ω6c and C₁₆:₀. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The sole respiratory quinone is Q-8. In API ZYM tests, cells are positive for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase and N-acetyl-β-glucosaminidase. Negative for esterase lipase (C8), lipase (C14), valine arylamidase, trypsin, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucosidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In API 20NE and API 20E tests, does not degrade gelatin, aesculin or starch. Assimilates glucose, N-acetylglucosamine, capric acid and trisodium citrate, but not l-arabinose, d-mannose, maltose, malic acid, adipic acid or phenylacetic acid.

The type strain, B717-2T (=CGMCC 1.15322T =JCM 31171T), was isolated from an ice core in Muztagh Glacier, Tibet, China. The DNA G+C content of the type strain is 63.4 mol% (Tm).

Acknowledgements

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References


