A novel Gram-negative, mesophilic, slightly halophilic, catalase- and oxidase-positive, obligately aerobic bacterium, designated strain YIM-C238\textsuperscript{T}, was isolated from a sediment sample from a salt lake in the Qaidam Basin, north-west China. Cells were non-sporulating, non-motile, straight to slightly curved rods. Coccolid bodies and filaments of varying length developed in older cultures. Growth occurred with 0.5–6 % (w/v) NaCl [optimum, 2–3 % (w/v) NaCl] at pH 6.0–10.0 (optimum, pH 7.0–8.0) and at 10–40 °C (optimum, 25–30 °C). The major cellular fatty acids were anteiso-C\textsubscript{15}:0, 3-OH iso-C\textsubscript{16}:0 and anteiso-C\textsubscript{15}:1\texttt{O}10c, and menaquinone 6 was the sole respiratory quinone. Non-diffusible carotenoid pigments were produced. Flexirubin pigments were absent. The genomic DNA G+C content was 35.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM-C238\textsuperscript{T} should be assigned to the genus Psychroflexus. The sequence similarities between the isolate and the type strains of members of the genus Psychroflexus were in the range 95.5–97.0 %. The combination of phylogenetic analysis, DNA–DNA hybridization data, phenotypic characteristics and chemotaxonomic differences supported the view that strain YIM-C238\textsuperscript{T} represents a novel species of the genus Psychroflexus, for which the name Psychroflexus sediminis sp. nov. is proposed. The type strain is YIM-C238\textsuperscript{T} (=CCTCC AA 207030\textsuperscript{T}=KCTC 22166\textsuperscript{T}).

The genus Psychroflexus, in the family Flavobacteriaceae, was first proposed by Bowman et al. (1998) with the description of Psychroflexus torquis as well as the reclassification of Flavobacterium gondwanense (Dobson et al., 1993) as Psychroflexus gondwanensis. The genus was defined as Gram-negative, catalase- and oxidase-positive, strictly aerobic, heterotrophic, non-motile or motile via gliding motility, slightly or moderately halophilic, psychrophilic or psychrotrophic rods, with menaquinone 6 (MK-6) as the predominant isoprenoid quinone and a DNA G+C content range of 32–36 mol% (Bowman et al., 1998). Psychroflexus tropicus (Donachie et al., 2004) was the first mesophilic member of the genus. In a recent study of the microbial diversity of the Qaidam Basin in Qinghai Province, north-west China, a novel strain, designated YIM-C238\textsuperscript{T}, was isolated from a sediment sample collected from the Dachaidamu salt lake. The lake is located at 37° 46’ N–37° 55’ N and 95° 22’ E–95° 33’ E. The water temperature was 18 °C, pH 6.4–7.8 and had a salinity of 27.4 % (w/v). Based on the results of a polyphasic taxonomic study, strain YIM-C238\textsuperscript{T} is proposed to represent a novel species, Psychroflexus sediminis sp. nov.

Strain YIM-C238\textsuperscript{T} was isolated from a sediment sample by plating 1 : 10 serial dilutions of the sample on Difco marine agar 2216 (MA; pH 7.5) followed by incubation at 28 °C for 14 days. After primary isolation and purification, the isolate was preserved both on MA slants at 4 °C. The water temperature was 18 °C, pH 6.4–7.8 and had a salinity of 27.4 % (w/v). Based on the results of a polyphasic taxonomic study, strain YIM-C238\textsuperscript{T} is proposed to represent a novel species, Psychroflexus sediminis sp. nov.
Table 1. Characteristics that differentiate strain YIM-C238T from other Psychroflexus species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility</td>
<td>Non-motile</td>
<td>Gliding</td>
<td>Gliding</td>
<td>Non-motile</td>
</tr>
<tr>
<td>NaCl range</td>
<td>0.5–6</td>
<td>1–20</td>
<td>1.5–4.6</td>
<td>0–17.5</td>
</tr>
<tr>
<td>NaCl optimum</td>
<td>2–3</td>
<td>7.5–10</td>
<td>2.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>10–40</td>
<td>4–43</td>
<td>−16 to 20</td>
<td>−5 to 30</td>
</tr>
<tr>
<td>β-Glucuronidase activity</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ararbitol</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Lactose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

(GeminiXPS; Molecular Devices) was employed for fluorescence measurements.

The DNA G+C content of strain YIM-C238T was 35.8 mol%. An almost-complete 16S rRNA gene sequence (1475 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM-C238T was closely related to the type strains of the three recognized species of the genus Psychroflexus (Bowman et al., 1998). The four strains formed a robust cluster in the phylogenetic tree (bootstrap value, 100%), in which strain YIM-C238T was phylogenetically most closely related to P. tropicus ATCC BAA-734T (Donachie et al., 2004), and the two strains formed a distinct subclade with significant bootstrap support (96%) (Fig. 1). The sequence similarities between the isolate and the type strains of members of the genus Psychroflexus were 97.0% (P. tropicus ATCC BAA-734T), 96.5% (P. gondwanensis ACAM 44T) and 95.5% (P. torquis ACAM 623T). To establish the precise taxonomic position of strain YIM-C238T, DNA–DNA hybridizations were performed between the novel isolate and the reference strain P. tropicus DSM 15496T; the level of DNA–DNA relatedness between the two strains was 8.4%, which was far below the threshold value of about 70% recommended by Wayne et al. (1987) for assigning strains to the same species. Therefore, based on phylogenetic analysis and the DNA–DNA hybridization data, it is

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evident that strain YIM-C238\textsuperscript{T} represents a novel species of the genus Psychroflexus (Wayne et al., 1987; Stackebrandt & Goebel, 1994).

Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). Fatty acids were determined as described by Sasser (1990) using the Microbial Identification System (MIDI; Microbial ID) with cells grown in MB in flasks on a rotary shaker at 200 r.p.m at 30 °C for 3 days. The bathochromic shift test with 10 % (w/v) KOH was used to detect flexirubin pigments. The test was performed on a small mass of cells collected with a loop and deposited on a glass slide placed on a white background; another similar mass of cells was deposited on the slide as a control (Bernardet et al., 2002). Carotenoid pigments were extracted using methanol according to Schmit et al. (1994). Absorption spectra were determined between 250 and 700 nm using a Shimadzu UV–visible spectrophotometer (UV1601). Chemotaxonomic data for strain YIM-C238\textsuperscript{T} were compatible with its assignment to the genus Psychroflexus (Bowman et al., 1998). MK-6 was the sole respiratory quinone detected. The fatty acid profile of strain YIM-C238\textsuperscript{T} was similar to those of the type strains of the genus Psychroflexus (see Supplementary Table S1, available in IJSEM Online). The major fatty acids of strain YIM-C238\textsuperscript{T} were anteiso-C\textsubscript{15:0} (29.4 %), 3-OH iso-C\textsubscript{16:0} (16.1 %) and anteiso-C\textsubscript{15:1\textsuperscript{\textcircled{c}}}\textsubscript{10} (10.7 %). The strain produced carotenoid pigments with absorbance peaks at 455 and 480 nm. Flexirubin pigments were absent.

The results of the phylogenetic analysis and chemotaxonomic studies supported the view that strain YIM-C238\textsuperscript{T} should be assigned to the genus Psychroflexus (Bowman et al., 1998). However, the strain could be distinguished by using a number of phenotypic properties (Table 1) and by its discriminative fatty acid profile (Supplementary Table S1), although the latter may partly result from different culture conditions. The presence of noticeable amounts of unbranched monounsaturated fatty acids (making up 5.0 % of the total), whereas the absence of saturated straight-chain fatty acids in the fatty acid pool of strain YIM-C238\textsuperscript{T} (Supplementary Table S1), together with its ability to produce acid from D-lactose, to hydrolyse gelatin and to exhibit \(\beta\)-glucuronidase activity, as well as the inability to grow at less than 10 °C (Table 1), differentiated strain YIM-C238\textsuperscript{T} markedly from the three recognized Psychroflexus species. In addition to all the differences presented here, strain YIM-C238\textsuperscript{T} could be clearly distinguished from its closest phylogenetic neighbour \(P.\) tropicus by some other discriminative taxonomic markers, such as its ability to hydrolyse aesculin and Tween 80 and to produce acids from maltose, as well as its ~3.5-fold
increment in the ratio of anteiso- to iso-branched fatty acids (Table 1 and Supplementary Table S1) and the low level of DNA–DNA relatedness between the species. Overall, the results of the polyphasic taxonomic study presented above allowed us to assign the novel isolate as representing a novel species, for which we propose the name *Psychroflexus sediminis* sp. nov.

**Description of Psychroflexus sediminis** sp. nov.

*Psychroflexus sediminis* (sed.i’m.is. L. gen. n. *sediminis* of sediment).

Cells are Gram-negative, straight to slightly curved rods, approximately 0.4–1.0 μm wide and 3.0–6.0 μm long, and occur singly. Coccolid bodies and filaments of varying length develop in older cultures. Endospores are not formed. Devoid of flagella and gliding motilities. Strictly aerobic, catalase- and oxidase-positive. Colonies are orange-pigmented, flat and non-translucent with glistening surfaces and circular/SLightly irregular margins, and 2–3 mm in diameter after incubation for 3–5 days at 28 °C on MA. Non-diffusible orange carotenoid pigments are produced. Flexirubin pigments are absent. Mesophilic and slightly halophilic, growth occurs at 10–40 °C (optimum, 25–30 °C) with 0.5–6% (w/v) NaCl (optimum, 2–3% (w/v) NaCl). Growth occurs at pH 6.0–10.0, with optimum growth at pH 7.0–8.0. Aesculin, gelatin and TWEENs 20 and 80 are hydrolysed, but casein, cellulose (carboxymethyl-cellulose and filter paper), chitin, DNA, starch, L-tyrosine, xanthine or TWEENs 40 and 60 are not hydrolysed. Nitrate is reduced to nitrite. Urease is not produced. Acids are produced from gentiobiose, glycogen, D-mannitol, methyl β-D-glucoside, D-psicose, L-serine, succinic acid, sucrose, trehalose and turanose (API 50CH). The following carbon sources are oxidized (Biolog GN2 MicroPlate): L-alanlye glycine, L-aspartic acid, dextrin, glycogen, L-fucose, gentiobiose, maltose, D-mannitol, methyl β-D-glucoside, D-psicose, L-serine, succinic acid, sucrose, trehalose and TWEEN 80. Constitutive enzymes expressed include acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), β-glucuronidase, leucine arylamidase, valine arylamidase and naphthol-AS-Bl-phosphohydrolase (API ZYM). Major fatty acids are anteiso-C15:0, 3-OH iso-C16:0 and anteiso-C15:1ω10c. MK-6 is the sole respiratory quinone. The DNA G+C content of the type strain is 35.8 mol%.

The type strain, YIM-C238T (=CCTCC AA 207030T=KCTC 22166T), was isolated from a sediment sample collected from the Dachaidamu salt lake in the Qaidam Basin in Qinghai Province, north-west China.

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**References**


