Two psychrophilic strains, Cr7-05T and Cr4-44T, isolated from alpine glacier cryoconite, were characterized by using a polyphasic approach. Both strains were psychrophilic, showing good growth over a temperature range of 1–20 °C. The chemotaxonomic characteristics of these isolates included the presence of C₁₈:₁ω7c and summed feature 3 (C₁₈:₁ω7c and/or C₁₆:₁ω6c) as the major cellular fatty acids, Q-10 as the predominant ubiquinone and diphasphatidylglycerol, phosphatidylglycerol and unknown glycolipids as major polar lipids. The DNA G+C contents of strains Cr7-05T and Cr4-44T were 61.4 and 63.6 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two isolates belong to the genus *Devosia*. The 16S rRNA gene sequence similarity between the two strains was 98.6 %, but DNA–DNA hybridization indicated 54 % relatedness. Strains Cr7-05T and Cr4-44T exhibited 16S rDNA gene sequence similarity of 94.7–97.2 and 94.9–96.9 %, respectively, to the type strains of recognized *Devosia* species. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strains Cr7-05T and Cr4-44T represent two novel species within the genus *Devosia*, for which the names *Devosia psychrophila* sp. nov. (type strain Cr7-05T = DSM 22950T = CGMCC 1.10210T) and *Devosia glacialis* sp. nov. (type strain Cr4-44T = CGMCC 1.10691T = LMG 26051T) are proposed. An emended description of the genus *Devosia* is also provided.

The genus *Devosia* falls within the class Alphaproteobacteria (Nakagawa et al., 1996) and was created by the reclassification of *Pseudomonas riboflavina* (Foster, 1944) as *Devo*sis *riboflavina* (Nakagawa et al., 1996). The genus accommodates Gram-negative, rod-shaped, obligately aerobic, oxidase-positive bacteria and contains Q-10 or Q-11 as the predominant respiratory quinone. The fatty acid profile consists of unsaturated and straight-chain fatty acids, 11-methyl C₁₈:₁ω7c and hydroxy or branched fatty acids. The DNA G+C content ranges from 59.5 to 66.2 mol% (Nakagawa et al., 1996; Yoon et al., 2007). At the time of writing, 11 species and three uncultured *Devosia* strains with the provisional name *Candidatus Devosia euplotis* (Vannini et al., 2004) have been described within the genus. The culturable species of the genus have been isolated from a variety of sources including nodules of legume plants (Rivas et al., 2003; Bautista et al., 2010), a nitrifying inoculum (Vanparys et al., 2005), soil (Yoo et al., 2006; Yoon et al., 2007; Kumar et al., 2008; Ryu et al., 2008; Verma et al., 2009) and beach sediment (Lee, 2007). The uncultured *Candidatus D. euplotis* strains were endosymbionts of a marine ciliate (Vannini et al., 2004). Some representatives of the genus *Devosia* are able to grow at 5 °C (Kumar et al., 2008; and results from this study); however, members of the genus have not yet been described from cold regions. In this study, we report the characterization of two psychrophilic bacterial strains, designated Cr7-05T and Cr4-44T, isolated from alpine glacier cryoconite that was sampled at different geographical locations.

Strain Cr7-05T was isolated from alpine glacier cryoconite collected from the Pitztaler Jochl glacier in the Oetztaler Alps in Tyrol, Austria, at an altitude of 2875 m above sea level (46°56'31.22"N 10°55'20.15"E), as described by Zhang et al. (2011). Strain Cr4-44T was isolated from cryoconite collected from the Pasterze glacier/Großglockner in the Hohe Tauern, Austria, at an altitude of 2200 m above

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*Devosia psychrophila* sp. nov. and *Devosia glacialis* sp. nov., from alpine glacier cryoconite, and an emended description of the genus *Devosia*

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sea level (47° 04' 28.12" N 12° 44' 38.96" E). Cryoconite samples were collected under sterile conditions in spring 2006. Part of the sample (1 g dry mass) was shaken with 9 ml sterile 1% sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile saline solution (0.9% NaCl), were plated on R2A agar plates (Reasoner & Geldreich, 1985) (0.05% yeast extract, 0.05% peptone, 0.05% Casamino acids, 0.05% glucose, 0.05% starch, 0.05% sodium pyruvate, 0.03% K2HPO4, 0.005% MgSO4, 1.5% agar, pH 7) that were incubated at 10°C. *Devosia limi* DSM 17137T, *Devosia chinhatensis* CCM 7426T and *Devosia subaequoris* KCTC 12772T were used as reference strains and were routinely grown on R2A agar plates at 20°C.

DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene was amplified by PCR with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCCGCA-3'). PCR products were cloned by using pGEM-T vectors (Promega) according to the manufacturer's instructions. On the basis of pairwise comparisons of 16S rRNA gene sequences using the EzTaxon program (Chun et al., 2007), strains Cr7-05T and Cr4-44T exhibited 16S rRNA gene sequence similarity of 94.7–97.2 and 94.9–96.9%, respectively, with respect to the type strains of recognized *Devosia* species. The 16S rRNA gene sequence similarity between strains Cr7-05T and Cr4-44T was 98.6%. The type strain of *Devosia riboflavina*, the type species of the genus, shared relatively low 16S rRNA gene sequence similarity with strain Cr7-05T (96.6%) and strain Cr4-44T (96.5%). Phylogenetic analysis was performed using the MEGA 4.0 software (Tamura et al., 2007) and the PHYLIP 3.69 package (Felsenstein, 2009) after multiple alignment of data using CLUSTAL X version 1.8 (Thompson et al., 1997). For neighbour-joining analysis (Saitou & Nei, 1987), genetic distances were calculated using Kimura's two-parameter model (Kimura, 1980). Phylogenetic analysis based on the neighbour-joining method showed that the two strains formed a coherent cluster at a bootstrap resampling value of 90% and also formed a distinct phyletic line in the clade of *D. limi* DSM 17137T and 'Candidatus D. euplotis' in the neighbour-joining tree (Fig. 1). The relative position of strains Cr7-05T and Cr4-44T was also confirmed in the maximum-likelihood tree.

To find the 16S rRNA sequence signatures that distinguish the genus *Devosia* from other taxa in the phylum *Proteobacteria* (Nakagawa et al., 1996), we aligned the sequences of strains Cr7-05T and Cr4-44T. Nucleotide signatures specific to the 16S rRNA gene sequence of strains Cr7-05T and Cr4-44T were the same as those reported by Nakagawa et al. (1996) for the genus *Devosia*, except that the signature sequence at positions 445:489 and 1419:1481 was G–C instead of A–U (Escherichia coli numbering; Brosius et al., 1978; Kumar et al., 2008).

Strains Cr7-05T and Cr4-44T were routinely cultured on R2A agar plates at 20°C and maintained as a suspension in skimmed milk (10%, v/v) at −80°C. Cell morphology was studied by phase-contrast microscopy (×1000) and by transmission electron microscopy (Zeiss Libra 120 EFTEM) of cells grown on R2A agar plates at 20°C. Motility was examined by microscopy (×1000), by the API M system (bioMérieux) and by the DSMZ on soft agar. Colony morphology was observed on R2A agar plates that were incubated at 20°C.

The tests described in this paragraph were done with strains Cr7-05T and Cr4-44T and all three reference strains (*D. limi* DSM 17137T, *D. chinhatensis* CCM 7426T and *D. subaequoris* KCTC 12772T). Gram staining was performed by using the bioMérieux Gram stain kit. Catalase activity was determined by bubble production in 3% (v/v) H2O2.

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic positions of strains Cr7-05T and Cr4-44T, recognized members of the genus *Devosia* and representatives of some related taxa. The sequence of *Escherichia coli* ATCC 11775T was used as an outgroup. Asterisks indicate nodes that were also found in the maximum-likelihood tree. Bootstrap values (%) are based on 1000 replicates and are given for branches with more than 50% support. GenBank accession numbers are given in parentheses. Bar, 2% sequence divergence.
and oxidase activity was determined using 1 % (w/v) N,N,N′N′-tetramethyl-p-phenylenediamine. API 20 E, API 20 NE and API ZYM strips (bioMérieux) incubated at 20 °C for 20 h (API ZYM) or 7 days (API 20 E, API 20 NE) were used to determine physiological and biochemical characteristics as well as enzyme activities. Activities of β-galactosidase, amylase, lipase and protease were additionally tested by using R2A agar plates supplemented with appropriate substrates (Margesin et al., 2003). Growth at 1–37 °C was assessed on R2A agar plates and in R2A liquid medium at 150 r.p.m. Growth at pH 5–9 and tolerance of 0–10 % (w/v) NaCl were determined on R2A agar plates.

Growth at/in the presence of/on:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Glacier cryoconite</td>
<td>Glacier cryoconite</td>
<td>Nitrifying inoculum</td>
<td>Soil</td>
<td>Beach sediment</td>
<td>Soil</td>
</tr>
<tr>
<td>Growth at/in the presence of/on:</td>
<td>White</td>
<td>Light pink</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Cream</td>
</tr>
<tr>
<td>1 °C</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>5 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>30 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3 % (w/v) NaCl</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>pH 6</td>
<td>–</td>
<td>W</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>W</td>
<td>–</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Urease activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Assimilation of (API 20 NE):</td>
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<td></td>
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</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
The data presented in this study demonstrate that strains Cr7-05T and Cr4-44T resemble those of other Devosia species (Rivas et al., 2003; Yoon et al., 2007; Lee, 2007), with the exception that the two strains contained larger amounts of the unsaturated fatty acid profiles of strains Cr7-05T and Cr4-44T resembled those of other Devosia species. Details of the fatty acid profiles of strains Cr7-05T and Cr4-44T and the reference strains are available in Supplementary Table S1.

The DNA G+C content was determined by the thermal denaturation method with *Escherichia coli* K-12 as the reference, and DNA–DNA hybridization was done by the liquid renaturation method (De Ley et al., 1970) as modified by Huß et al. (1983). Both experiments were carried out using a model Lambda 35 UV/Vis spectrometer equipped with a temperature program controller (Perkin-Elmer). The DNA G+C contents of strains Cr7-05T and Cr4-44T were 61.4 and 63.6 mol%, respectively. The DNA–DNA relatedness between strain Cr7-05T and *D. limi* DSM 17137T was 37.3%, and that between isolates Cr7-05T and Cr4-44T was 54.1%. Thus, both DNA–DNA relatedness values are lower than the hybridization threshold (70%) recommended for species delineation (Wayne et al., 1987). 16S rDNA gene sequence similarities <97.0% were found with other recognized species of the genus *Devosia*. According to Stackebrandt & Goebel (1994), bacterial strains with less than 97% 16S rDNA gene sequence similarity exhibit levels of DNA–DNA hybridization that are less than 70%. Therefore, we can conclude that strains Cr7-05T and Cr4-44T are genotypically distinct from all other species of the genus *Devosia*.

The data presented in this study demonstrate that strains Cr7-05T and Cr4-44T are psychrophilic members of the genus *Devosia*. We use the term ‘psychrophilic’ as a general term to describe a micro-organism that grows in a cold environment (Margesin et al., 2008); the use of growth rates to define the optimum growth temperature as described by Morita (1975) has been shown to be ambiguous and inappropriate (Feller & Gerday 2003; Cavicchioli, 2006; Margesin, 2009).

The two isolates described in this study showed differences in terms of polar lipid patterns, nitrate reduction, urease activity and assimilation of a range of carbon sources, and their colonies differed in colour (Table 1). Both isolates can be easily differentiated from their closest phylogenetic neighbours (*D. limi* DSM 17137T, *D. chinhatensis* CCM 7426T and *D. subaequoris* KCTC 12772T) by their psychrophilic growth characteristics (weak growth at 25 °C and absence of growth at 30 °C; ability to grow at 1 °C, in contrast to *D. chinhatensis* CCM 7426T and *D. subaequoris* KCTC 12772T, and faster growth at 1 °C compared with *D. limi* DSM 17137T), their inability to grow in the presence of 3% (w/v) NaCl and the relative amount of certain cellular fatty acids (summed feature 3). Based on phenotypic, phylogenetic and genomic data, it is evident that strains Cr7-05T and Cr4-44T represent separate novel species of the genus *Devosia*, for which the names *Devosia psychrophila* sp. nov. and *Devosia glacialis* sp. nov., respectively, are proposed.

**Emended description of the genus Devosia Nakagawa et al. 1996**

The description is as given by Nakagawa et al. (1996) and emended by Rivas et al. (2003), Yoo et al. (2006) and Yoon et al. (2007), with the following further amendments. Cells are positive or negative for catalase and urease. Pigmentation of colonies on R2A agar plates differs between species. Major polar lipids are diphostatidylglycerol, phosphatidylglycerol and unknown glycolipids.

**Description of Devosia psychrophila sp. nov.**

*Devosia psychrophila* (psy.chro’phi.la. Gr. adj. psychros cold; Gr. adj. philos liking, loving; N.L. fem. adj. psychrophi.la cold-loving).

Cells are aerobic, Gram-staining-negative, motile (polar flagellation; Supplementary Fig. S2) and rod-shaped (0.4–0.6 × 1.0–1.5 μm after 2 days and 0.4–0.6 × 4–5 μm after 7 days at 20 °C on R2A agar plates). Colonies on R2A agar are white, convex, smooth and round with entire margins and produce slime. Colony diameter is <1 mm after 2 days and 1.0–1.5 mm after 7 days on R2A agar at 20 °C. Good growth occurs in liquid R2A medium and on agar plates at 1–20 °C; growth is weak at 25 °C and absent at 30 °C. Growth on trypticase soy agar (TSA) and nutrient agar is very weak. On R2A agar plates, grows at pH 7–8 and in the presence of 0–1% (w/v) NaCl; no growth in the presence of 3% (w/v) NaCl. Positive for activities of cytochrome c oxidase, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, leucine arylamidase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and β-glucosidase. Negative for indole and H2S production, nitrate reduction and utilization of citrate and for activities of catalase, urease, protease, amylase, lysine dihydrolase, ornithine dihydrolase, arginine dihydrolase, tryptophan deaminase, lipase (C14), dihydrolase, ornithine dihydrolase, β-galactosidase and β-glucosidase. Negative for indole and H2S production, nitrate reduction and utilization of citrate and for activities of catalase, urease, protease, amylase, lysine dihydrolase, ornithine dihydrolase, arginine dihydrolase, tryptophan deaminase, lipase (C14), α-fucosidase, β-glucuronidase and galactosidase. Assimilates D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, D-mannitol and maltose but not gluconate, citrate, malic acid, capric acid, adipic acid or phenylacetic acid. Negative for fermentation of D-glucose, D-mannitol, sucrose, inositol, sorbitol, L-rhamnose, melibiose, amygdalin and L-arabinose. The predominant cellular fatty acids are C18:1ω7c, summed feature 3 (C16:1ω7c and/or C16:1ω6c), C16:0, 11-methyl C18:1ω7c and C18:0. Q-10 is the major ubiquinone. The polar lipid pattern contains diphostatidylglycerol, phosphatidylglycerol, five unknown glycolipids and six unknown polar lipids. The G+C content of DNA of the type strain is 61.4 mol%.

The type strain is Cr7-05T (=DSM 22950T =CGMCC 1.10210T =CIP 110130T), isolated from glacier cryoconite collected from the Pitztaler Jochler glacier in the Oetztaler Alps, Austria.
**Description of Devosia glacialis** sp. nov.

*Devosia glacialis* (gl.a.ci.a’lis. L. fem. adj. *glacialis* icy, full of ice, referring to the frozen, icy environment from which the type strain was isolated).

Cells are aerobic, Gram-staining-negative, motile (polar flagellation; Supplementary Fig. S3) and rod-shaped (0.6–0.8 × 1.5–2.0 μm after 2 days and 0.8–1.0 × 1.5–2.0 μm after 7 days at 20 °C on R2A agar plates). Colonies on R2A agar are light pink, convex, smooth and round with entire margins and produce slime. Colony diameter is about 1 mm after 7 days on R2A agar at 20 °C. Good growth occurs in liquid R2A medium and on agar plates at 1–20 °C; growth is absent at 30 °C. Growth on TSA and nutrient agar is absent. On R2A agar plates, grows well at pH 7–8 and in the presence of 0–2% (w/v) NaCl; no growth occurs in the presence of 3% (w/v) NaCl. Positive for nitrate reduction and for activities of cytochrome c oxidase, catalase, urease, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, leucine arylamidase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase. Negative for indole and H₂S production, utilization of citrate and activities of lysine dihydrolase, ornithine dihydrolase, arginine dihydrolase, tryptophan deaminase, and activities of arginine dihydrolase, ornithine dihydrolase, and ornithine dihydrolase. 

**References**


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