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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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 (C16 : 1ω7c and/or C16 : 1ω6c) as the major cellular fatty acids, Q-10 as the predominant ubiquinone and diphosphatidylglycerol, 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 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 = CIP 110130T) 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 Devosia 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 Joch 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 sea level (46° 33′ 25.01″N 12° 23′ 17.63″E), as described by Zhang et al. (2011).
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. Appropriately diluted samples, 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, 0.03% 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 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, 2004) for the genus *Devosia* and for other recognized members of the genus *Devosia* as well as 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 shown in parentheses. Bar, 2% sequence divergence.

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 sequences 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%, w/v) at −80 °C. Cell morphology was studied by phase-contrast microscopy (×1000) and by transmission electron microscopy (Zeiss Libra 120 ETEM) 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. The features that serve to differentiate strains Cr7-05T and Cr4-44T from phylogenetically closely related members of the genus Devosia are given in Table 1. Morphological, physiological and biochemical characteristics of strains Cr7-05T and Cr4-44T are given in the species descriptions or are shown in Table 1. Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989). Strains Cr7-05T and Cr4-44T contained Q-10 as the predominant ubiquinone.

Cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986). The predominant cellular fatty acids of strain Cr7-05T were identified using the database TSBA (version 6.0). Strains Cr7-05T and Cr4-44T showed the presence of some polar lipids in common, including diphosphatidylglycerol, phosphatidylglycerol, three unknown glycolipids (GL1–3) and two unknown polar lipids (PL1–2) (Supplementary Fig. S1, available in IJSEM Online). However, the polar lipid pattern of strain Cr7-05T could be distinguished from that of strain Cr4-44T by the presence of GL4–5 and more unknown polar lipids. Though the polar lipids of Devosia geojensis and D. yakushimensis have been analysed (Ryu et al., 2008; Bautista et al., 2010), no images of the polar lipid profiles were shown and the profiles are therefore not accessible for visual comparison. The presence of GL1–5 in extracts from strain Cr7-05T and GL1–3 in extracts from strain Cr4-44T enabled the two strains to be differentiated from D. geojensis and D. yakushimensis.

For fatty acid methyl ester analysis, strains Cr7-05T and Cr4-44T and the reference strains D. limi DSM 17137T, D. chinhatensis CCM 7426T and D. subaequoris KCTC 12772T were grown on R2A agar plates at 20 °C for 3 days. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI, version 6.0) (Sasser, 1990) and were identified using the database TSBA (version 6.0). The predominant cellular fatty acids of strain Cr7-05T were

![Table 1. Phenotypic characteristics that differentiate strains Cr7-05T and Cr4-44T from phylogenetically closely related Devosia species and the type species of the genus Devosia](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Colony source</td>
<td>Glacier cryoconite</td>
<td>Glacier cryoconite</td>
<td>Nitrifying inoculum</td>
<td>Soil</td>
<td>Beach sediment</td>
<td>Soil</td>
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<tr>
<td>Colony colour</td>
<td>White</td>
<td>Light pink</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Cream</td>
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<td>Growth at/in the presence of/on:</td>
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<td>1 °C</td>
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<td>W</td>
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<td>5 °C</td>
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<td>30 °C</td>
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<td>3 % (w/v) NaCl</td>
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<td>Nutrient agar</td>
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<td>Nitrate reduction</td>
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<td>Catalase activity</td>
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<td>Urease activity</td>
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<td>Assimilation of (API 20 NE):</td>
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<tr>
<td>D-Glucose</td>
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<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
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<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>–</td>
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<td>Maltose</td>
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</table>
C18:1ω7c (43.9 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c) (21.7 %), C16:0 (7.5 %), 11-methyl C18:1ω7c (6.3 %) and C18:0 (5.2 %), and those of strain Cr4-44T were C18:1ω7c (47.2 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c) (20.3 %), C16:0 (14.8 %) and C14:0 (7.2 %). Thus, the fatty acid profiles of strains Cr7-05T and Cr4-44T resembled 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 summed feature 3 (C16:1ω7c and/or C16:1ω6c) compared with 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 rRNA 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 rRNA 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 ‘psychrophile’ 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. subaer quis 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. subaequis 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 amounts 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 dihexadecylglycerol, phosphatidylglycerol and unknown glycolipids.

### Description of Devosia psychrophila sp. nov.

*Devosia psychrophila* (psy.chro’phi.1a. Gr. adj. psychros cold; Gr. adj. philos liking, loving; N.L. fem. adj. psychrophila 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, growths 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), α-fucosidase, β-glucuronidase and galatase. Assimilates D-glucose, L-arabinose, D-mannose, N-acetylgalactosamine, D-mannitol and maltose but not gluconate, citric acid, malic acid, capric acid, adipic acid or phytelactonic acid. Negative for fermentation of D-glucose, D-mannitol, sucrose, inositol, sorbitol, D-mannose, 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 C18:0-7c and Q-10 is the major ubiquinone. The polar lipid pattern contains diphasphatidylglycerol, 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 Jöchler glacier in the Öetztal Alps, Austria.
**Description of *Devosia glacialis* sp. nov.**

*Devosia glacialis* (gla.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, growth 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-phospho-hydrolase, β-galactosidase, α-glucosidase and β-glucosidase. Negative for indole and H2S production, utilization of citrate, malate, glutarate, propionate, succinate, adipate, 3-hydroxy fatty acids or phenylacetic acid. Negative for arginine dihydrolase, tryptophan deaminase, N-acetyl-L-β-glucosaminidase, β-glucosaminidase, β-glucuronidase, gelatinase, protease and amylase. Does not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, gluconate, N-acetylglucosamine, citrate, malic acid, capric acid, adipic acid or phenylacetic acid. Negative for fermentation of D-glucose, D-mannitol, sucrose, inositol, sorbitol, L-rhamnose, melibiose, myo-inositol and L-arabinose. The predominant cellular fatty acids are C18:1ω7c, summed feature 3 (C16:1ω7c and/or C16:1ω6c), C16:0 and C14:0. Q-10 is the major ubiquinone. The polar lipids contain diphasphatidylglycerol, phosphatidylglycerol, three unknown glycolipids and three unknown polar lipids. The G+C content of DNA of the type strain is 63.6 mol%.

The type strain is CGMCC 26051T (LB 7401T), isolated from glacier cryoconite collected from the Pasterze glacier/Großglockner in the Hohe Tauern, Austria.

**Acknowledgements**

R. M. thanks the ‘Aktion D. Swarovski & Co. 2009’ at the University of Innsbruck for financial support. We are grateful to W. Salvenmoser (Institute of Zoology, University of Innsbruck) for performing transmission electron microscopy and we thank P. Thurnbichler and J. Mair for technical assistance.

**References**


Devosia psychrophila and D. glacialis spp nov.