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, Cr7-05^T and Cr4-44^T, 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 C_{18:1}ω7c and summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) 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 Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T exhibited 16S rRNA 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 Cr7-05^T and Cr4-44^T represent two novel species within the genus *Devosia*, for which the names *Devosia psychrophila* sp. nov. (type strain Cr7-05^T = DSM 22950^T = CGMCC 1.10210^T = CIP 110130^T) and *Devosia glacialis* sp. nov. (type strain Cr4-44^T = CGMCC 1.10691^T = LMG 26051^T) 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 C_{18:1}ω7c 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 (Vanparrys *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 Cr7-05^T and Cr4-44^T, isolated from alpine glacier cryoconite that was sampled at different geographical locations.

Strain Cr7-05^T was isolated from alpine glacier cryoconite collected from the Pitztaler Jöchl 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 Cr4-44^T was isolated from cryoconite collected from the Pasterze glacier/Großglockner in the Hohe Tauern, Austria, at an altitude of 2200 m above

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Cr7-05^T and Cr4-44^T are GU441678 and HM474794, respectively.

Three supplementary figures and a supplementary table are available with the online version of this paper.

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, 0.03 % sodium pyruvate, 0.03 % K₂HPO₄, 0.005 % MgSO₄, 1.5 % agar, pH 7) that were incubated at 10 °C. *Devosia limi* DSM 17137^T, *Devosia chinhatensis* CCM 7426^T and *Devosia subaequoris* KCTC 12772^T 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 Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T 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 Cr7-05^T (96.6 %) and strain Cr4-44^T (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 17137^T and '*Candidatus D. euplotis*' in the neighbour-joining tree (Fig. 1). The relative position of strains Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T. Nucleotide signatures specific to the 16S rRNA gene sequence of strains Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T 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 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 Cr7-05^T and Cr4-44^T and all three reference strains (*D. limi* DSM 17137^T, *D. chinhatensis* CCM 7426^T and *D. subaequoris* KCTC 12772^T). 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 Cr7-05^T and Cr4-44^T, recognized members of the genus *Devosia* and representatives of some related taxa. The sequence of *Escherichia coli* ATCC 11775^T 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 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 Cr7-05^T and Cr4-44^T from the phylogenetically most closely related members of the genus *Devosia* are given in Table 1. Morphological, physiological and biochemical characteristics of strains Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T 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).

Strains Cr7-05^T and Cr4-44^T 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 Cr7-05^T could be distinguished from that of strain Cr4-44^T 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 Cr7-05^T and GL1–3 in extracts from strain Cr4-44^T enabled the two strains to be differentiated from *D. geojensis* and *D. yakushimensis*.

For fatty acid methyl ester analysis, strains Cr7-05^T and Cr4-44^T and the reference strains *D. limi* DSM 17137^T, *D. chinhatensis* CCM 7426^T and *D. subaequoris* KCTC 12772^T 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 Cr7-05^T were

Table 1. Phenotypic characteristics that differentiate strains Cr7-05^T and Cr4-44^T from phylogenetically closely related *Devosia* species and the type species of the genus *Devosia*

Strains: 1, *Devosia psychrophila* sp. nov. Cr7-05^T; 2, *Devosia glacialis* sp. nov. Cr4-44^T; 3, *D. limi* DSM 17137^T; 4, *D. chinhatensis* CCM 7426^T; 5, *D. subaequoris* KCTC 12772^T; 6, *D. riboflavina* IFO 13584^T (data from Nakagawa *et al.*, 1996; Yoon *et al.*, 2007). Data were obtained in this study unless indicated. +, Positive; –, negative; w, weak; ND, no data available. All strains are positive for motility and for activities of acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, *N*-acetyl- β -glucosaminidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase and β -glucosidase. All strains are negative for indole production, arginine dihydrolase, gelatin hydrolysis and β -glucuronidase. All strains are negative for assimilation of citrate, malic acid, capric acid, adipic acid and phenylacetic acid and for fermentation of D-glucose, D-mannitol, sucrose and inositol.

Characteristic	1	2	3	4	5	6
Isolation source	Glacier cryoconite	Glacier cryoconite	Nitrifying inoculum	Soil	Beach sediment	Soil
Colony colour	White	Light pink	White	White	White	Cream
Growth at/in the presence of/on:						
1 °C	+	+	w	–	–	ND
5 °C	+	+	+	–	–	ND
30 °C	–	–	+	+	+	+
3% (w/v) NaCl	–	–	+	+	+	ND
pH 6	–	w	+	w	–	ND
Nutrient agar	w	–	+	w	–	+
Nitrate reduction	–	+	–	+	+	+
Catalase activity	–	+	+	+	–	+
Urease activity	–	+	+	+	+	+
Assimilation of (API 20 NE):						
D-Glucose	+	–	–	+	+	+
L-Arabinose	+	–	–	+	+	+
D-Mannose	+	–	–	+	+	+
D-Mannitol	+	–	–	+	+	+
N-Acetylglucosamine	+	–	–	+	+	+
Maltose	+	–	–	+	+	+

C_{18:1}ω7c (43.9 %), summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) (21.7 %), C_{16:0} (7.5 %), 11-methyl C_{18:1}ω7c (6.3 %) and C_{18:0} (5.2 %), and those of strain Cr4-44^T were C_{18:1}ω7c (47.2 %), summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) (20.3 %), C_{16:0} (14.8 %) and C_{14:0} (7.2 %). Thus, the fatty acid profiles of strains Cr7-05^T and Cr4-44^T 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 (C_{16:1}ω7c and/or C_{16:1}ω6c) compared with other *Devosia* species. Details of the fatty acid profiles of strains Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T were 61.4 and 63.6 mol%, respectively. The DNA–DNA relatedness between strain Cr7-05^T and *D. limi* DSM 17137^T was 37.3 %, and that between isolates Cr7-05^T and Cr4-44^T 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 Cr7-05^T and Cr4-44^T are genotypically distinct from all other species of the genus *Devosia*.

The data presented in this study demonstrate that strains Cr7-05^T and Cr4-44^T 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 17137^T, *D. chinhatensis* CCM 7426^T and *D. subaequoris* KCTC 12772^T) 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 7426^T and *D. subaequoris* KCTC 12772^T, and faster growth at 1 °C compared with *D. limi* DSM 17137^T), 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 Cr7-05^T and Cr4-44^T 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 diphosphatidylglycerol, 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. *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, 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), α-fucosidase, β-glucuronidase and gelatinase. 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 C_{18:1}ω7c, summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c), C_{16:0}, 11-methyl C_{18:1}ω7c and C_{18:0}. Q-10 is the major ubiquinone. The polar lipid pattern contains diphosphatidylglycerol, 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 Cr7-05^T (=DSM 22950^T =CGMCC 1.10210^T =CIP 110130^T), isolated from glacier cryoconite collected from the Pitztaler Jöchler 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 ($0.6\text{--}0.8 \times 1.5\text{--}2.0\text{ }\mu\text{m}$ after 2 days and $0.8\text{--}1.0 \times 1.5\text{--}2.0\text{ }\mu\text{m}$ after 7 days at $20\text{ }^{\circ}\text{C}$ 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 $20\text{ }^{\circ}\text{C}$. Good growth occurs in liquid R2A medium and on agar plates at $1\text{--}20\text{ }^{\circ}\text{C}$; growth is absent at $30\text{ }^{\circ}\text{C}$. 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 H_2S production, utilization of citrate and activities of lysine dihydrolase, ornithine dihydrolase, arginine dihydrolase, tryptophan deaminase, α -fucosidase, 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, amygdalin and L-arabinose. The predominant cellular fatty acids are $\text{C}_{18:1\omega7c}$, summed feature 3 ($\text{C}_{16:1\omega7c}$ and/or $\text{C}_{16:1\omega6c}$), $\text{C}_{16:0}$ and $\text{C}_{14:0}$. Q-10 is the major ubiquinone. The polar lipids contain diphosphatidylglycerol, 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 Cr4-44^T (=CGMCC 1.10691^T =LMG 26051^T), isolated from glacier cryoconite collected from the Pasterze glacier/Großglockner in the Hohe Tauern, Austria.

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