**Arthrobacter cryoconiti** sp. nov., a psychrophilic bacterium isolated from alpine glacier cryoconite

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A Gram-stain-positive, aerobic, non-motile, psychrophilic bacterium, designated strain Cr6-08T, was isolated from alpine glacier cryoconite. Growth of strain Cr6-08T occurred at 1–25 °C. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain Cr6-08T is most closely related to members of the genus *Arthrobacter*. Strain Cr6-08T possessed chemotaxonomic properties consistent with those of the genus *Arthrobacter*, such as peptidoglycan type A3α (L-Lys–L-Ala4), MK-9(H2) as major menaquinone and anteiso- and iso-branched compounds (anteiso-C15:0 and iso-C15:0) as major cellular fatty acids. The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown glycolipid and three unknown polar lipids. The genomic DNA G+C content of strain Cr6-08T was 57.3 mol%. On the basis of phenotypic and chemotaxonomic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain Cr6-08T is considered to represent a novel species of the genus *Arthrobacter*, for which the name *Arthrobacter cryoconiti* sp. nov. is proposed. The type strain is Cr6-08T (=DSM 23324T =LMG 26052T =CGMCC 1.10698T).

The genus *Arthrobacter* was proposed by Conn & Dimmick (1947) and the description was later emended by Koch et al. (1995). Bacteria belonging to this genus show a rod–coccus cell cycle, aerobic metabolism and little or no acid production from glucose and have lysine in the peptidoglycan and a DNA G+C content of 59–66 mol% (Keddie et al., 1986). Members of the genus *Arthrobacter* fall into at least two groups (Stackebrandt & Schumann, 2006) as revealed by chemotaxonomic studies (Minnikin et al., 1978; Collins & Jones, 1981; Collins & Kroppenstedt, 1983; Stackebrandt et al., 1983). The type species, *Arthrobacter globiformis* (Conn & Dimmick, 1947), represents the ‘globiformis’ group, while the ‘nicotianae’ group is centred around *Arthrobacter nicotianae*. The ‘globiformis’ group is characterized by cell-wall peptidoglycan type A3α and the presence of menaquinone MK-9(H2), while members of the ‘nicotianae’ group contain peptidoglycan type A4α and unsaturated menaquinones with 8–10 isoprenoid units. *Arthrobacter* strains have been isolated from various environmental sources. A number of psychrophilic representatives have been described, such as *Arthrobacter psychrolactophilus* (Loveland-Curtze et al., 1999), *A. flavus* and *A. roseus* (Reddy et al., 2000, 2002), *A. chlorophenolicus* (Westerberg et al., 2000), *A. psychrophilenicus* (Margaris et al., 2004), *A. stackebrandtii* (Tvrzova et al., 2005), *A. alpinus* (Zhang et al., 2010) and *A. livingstonensis* (Ganzert et al., 2011). The present study deals with the characterization of a novel psychrophilic bacterium of the genus *Arthrobacter* isolated from alpine glacier cryoconite.

Strain Cr6-08T was isolated from cryoconite collected from the Banker glacier in the Ötzaler Alps in Tyrol, Austria, at an altitude of 2820 m above sea level, as described by Zhang et al. (2011). The sample was collected under sterile conditions in spring 2006. Subsequently, 1 g sample (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 (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; Reasoner & Geldreich, 1985) and incubated at 10 °C. One of the pure cultures was yellow-pigmented and was designated strain Cr6-08T. *A. stackebrandtii* DSM 16005T, *A. alpinus* DSM 22274T and *A. psychrolactophilus* DSM 15612T were used as reference strains; they were routinely grown on trypticase soy agar (TSA; 1.5% casein

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Cr6-08T is GU784867.

A supplementary figure is available with the online version of this paper.

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**A Supplementary Figure is Available with the Online Version of this Paper.**
peptone, 0.5 % soy peptone, 0.5 % NaCl, 1.5 % agar, pH 7) medium at 20 °C.

DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene was amplified by PCR with a pair of universal primers, 27F (5′-AGAGTTTGTATCTTGGCTCAG-3′) and 1541R (5′-AAGGAGGTGATCCAGCCGCA-3′). PCR products were purified by using the Gene JET PCR purification kit (Fermentas) and cloned in pGEM-T vectors (Promega) according to the manufacturer’s instructions. Searches for similar sequences in the GenBank and EMBL databases were performed with the program FASTA. Strain Cr6-08T showed highest 16S rRNA gene sequence similarities with A. stackebrandii CCM 2783T (98.3 %), A. alpinus S6-3T (98.0 %) and A. psychrolactophilus B7T (97.7 %). 16S rRNA gene sequence similarity between strain Cr6-08T and the type strain of the type species of the genus Arthrobacter (A. globiformis DSM 20124T) was 95.7 %.

Phylogenetic analysis was performed by using the MEGA 4.0 software package (Tamura et al., 2007) as well as the PHYLIP 3.69 package (Felsenstein, 2009) after multiple alignment of the data with CLUSTAL X 1.8 (Thompson et al., 1997). Analysis based on the neighbour-joining method showed that strain Cr6-08T grouped with members of the genus Arthrobacter and formed a cluster with A. stackebrandii CCM 2783T, A. alpinus S6-3T and A. psychrolactophilus B7T (Fig. 1). The relative position of strain Cr6-08T was also confirmed in the tree generated with the maximum-likelihood algorithm.

Strain Cr6-08T was routinely cultured in low-strength medium R2A and was maintained as a suspension in skimmed milk (10 %, w/v) at −80 °C. Cell morphology was examined by phase-contrast microscopy (×1000) of cells grown on R2A agar plates at 20 °C. Colony morphology was also observed on R2A agar plates. The following tests were all performed with strain Cr6-08T and reference strains A. stackebrandii DSM 16005T, A. alpinus DSM 22274T and A. psychrolactophilus DSM 15612T. The API M system (bioMérieux) and microscopic examination were used to evaluate cell motility. The Gram reaction was tested by Gram staining and was confirmed by the KOH lysis test. Catalase activity was determined by

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Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain Cr6-08T and related Arthrobacter species. Nocardioides simplex DSM 20130T was used as an outgroup. Asterisks indicate branches also found in the maximum-likelihood tree. Bootstrap values (%) are based on 1000 replicates. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 0.01 expected changes per site.
bubble production in 3 % (v/v) H2O2; oxidase activity was determined by using 1 % (w/v) N,N,N',N'-tetramethyl-p-phenylenediamine. API strips (API 20 E, API 20 NE, API ZYM; bioMérieux) incubated at 15–20 °C were used to determine physiological and biochemical characteristics as well as enzyme activities. In addition, the assimilation of lactose and Tweens 40 and 80 was tested in liquid culture by supplementing 10 ml of a pH-neutral phosphate-buffered mineral medium with a trace element solution (Margesin et al., 2002) and the carbohydrates (final concentration 0.2 %, w/v) as the sole carbon source. Activities of amylase and protease were additionally tested by using R2A agar plates supplemented with appropriate substrates (Margesin et al., 2003). Growth under anaerobic conditions was assessed after 5 days of incubation at 20 °C in an anaerobic jar [containing Anaerocult A (Merck)] to produce anaerobic conditions on R2A agar plates supplemented with 10 mM KNO3. Growth at 1, 5, 10, 15, 20, 25, 30 and 37 °C was assessed on R2A agar plates and in R2A liquid medium. Growth at pH 5, 6, 7, 8 and 9 and in the presence of 0, 1, 2, 3, 5, 7.5 and 10 % (w/v) NaCl was determined on R2A agar plates.

Morphological, physiological and biochemical characteristics of strain Cr6-08T are given in the species description and in Table 1. The characteristics that serve to differentiate strain Cr6-08T from the most closely related members of the genus Arthrobacter are given in Table 1.

For analysis of the peptidoglycan structure and cell-wall sugars, cells of strain Cr6-08T were cultured in shaken flasks of DSMZ medium 92 (http://www.dsmz.de/) at 20 °C for 3 days. The peptidoglycan structure was determined by using hydrolysates of purified cell walls according to Schleifer (1985). The amino acids and peptides were separated by two-dimensional ascending TLC on cellulose plates with the solvent systems described by Schleifer &

### Table 1. Phenotypic characteristics that differentiate strain Cr6-08T from the type strains of phylogenetically related *Arthrobacter* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Isolation source</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>Alpine glacier cryoconite</td>
<td>57.3</td>
<td>NA</td>
<td>61.9</td>
<td>60.6</td>
</tr>
<tr>
<td><strong>Cell-wall peptidoglycan type</strong></td>
<td>A3x (L-Lys–L-Ala4)</td>
<td>A3x (L-Lys–L-Ala2)a</td>
<td>A3x (L-Lys–L-Ala3)b</td>
<td>A3x (L-Lys–L-Thr–L-Ala3)c</td>
<td></td>
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<tr>
<td><strong>Major fatty acids†</strong></td>
<td>ai-C15:0, ai-C17:0</td>
<td>ai-C15:0, ai-C17:0</td>
<td>ai-C15:0, ai-C17:0</td>
<td>ai-C15:0, ai-C17:0</td>
<td></td>
</tr>
<tr>
<td><strong>Growth temperature range (°C)</strong></td>
<td>1–25</td>
<td>1–30</td>
<td>1–25</td>
<td>1–25</td>
<td></td>
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<tr>
<td><strong>Growth with 5% (w/v) NaCl</strong></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Nitratre reduction</strong></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td><strong>Enzyme activities</strong></td>
<td></td>
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<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Amylase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Gelatinase</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Alkaline phosphatase</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>α-Fucosidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td><strong>Assimilation of</strong></td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Potassium gluconate</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Phenylacetic acid</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Tween 40</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

*Data obtained from: a, Tvrzová et al. (2005); b, Zhang et al. (2010); c, Loveland-Curtze et al. (1999).†ai, Anteiso-branched; i, iso-branched.
The lipid pattern of strain Cr6-08\(^T\) was similar to those of phylogenetically related Arthrobacter species and is also the diagnostic diamino acid in the peptidoglycan of strain Cr6-08\(^T\). However, the peptidoglycan structure of strain Cr6-08\(^T\) (type A11.7) differed from those of its phylogenetic neighbours A. stackebrandtii DSM 16005\(^T\) (A11.5), A. alpinus DSM 22274\(^T\) and A. psychrolactophilus DSM 15612\(^T\) (both A11.28).

For analysis of sugars, cell walls were hydrolysed in 0.5 M \(\text{H}_2\text{SO}_4\) at 100 °C for 2 h. \(\text{H}_2\text{SO}_4\) was removed by shaking with 20 % \(\text{N}_2\text{-dioctylmethylamine in chloroform accord-}

For fatty acid methyl ester analysis, strain Cr6-08\(^T\) and the three reference strains were grown on TSA plates at 20 °C for 3 days. All the strains included in the fatty acid analyses showed similar growth behaviour and sufficient cells of comparable physiological age could be harvested from the third streak quadrant of the TSA plates after cultivation under the conditions applied. 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 by using the database TSBA (version 6.0). The predominant cellular fatty acids of strain Cr6-08\(^T\) were anteiso-C\(_{15}:0\) (41.3 %), iso-C\(_{17}:0\) (16.1 %), C\(_{18:1}\) \((8.9 %)\), C\(_{18:1}\) \((6.3 %)\) and C\(_{20:0}\) (5.3 %) (Table 2).

Cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986). The polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown glycolipid and three unknown polar lipids (see Supplementary Fig. S1 available in IJSEM Online). Although the polar lipids of Arthrobacter agilis, A. flavus, A. roseus, A. psychrophilicus, A. castelli, A. monumentii, A. parietis, A. pigments, A. tecti, A. tumbae, A. phenanthrenivorans and A. antarcticus have been analysed (Koch et al., 1995; Reddy et al., 2000, 2002; Margesin et al., 2004; Heyrman et al., 2005; Kallimanis et al., 2009; Pindi et al., 2010), no polar lipid profile images are available for visual comparison. The lipid pattern of strain Cr6-08\(^T\) was similar to those of A. agilis, A. psychrophilicus, A. castelli, A. monumentii, A. parietis, A. pigments, A. tecti and A. tumbae (with phosphatidylinositol as one of the major polar lipids), but differed from those of A. flavus, A. roseus, A. phenanthrenivorans and A. antarcticus (phosphatidylethanolamine as one of the major polar lipids).

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989). Strain Cr6-08\(^T\) contained MK-9(H\(_2\)) (87 %) as the major menaquinone, with minor amounts of MK-8(H\(_2\)) (9 %) and MK-10(H\(_2\)) (4 %).

The DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962) with Escherichia coli K-12 as the reference, and DNA–DNA hybridization was performed by the liquid renaturation method (De Ley et al., 1970) as modified by Huß et al. (1983). The two experiments were carried out by using a model Lambda 35 UV/Vis spectrometer equipped with a temperature programme controller (Perkin-Elmer). The DNA G+C content of strain Cr6-08\(^T\) was 57.3 mol%, Levels of DNA–DNA relatedness between strain Cr6-08\(^T\) and A. stackebrandtii DSM 16005\(^T\) and A. psychrolactophilus DSM 15612\(^T\) were 41.5, 26.6 and 33.9 %, respectively. These values were clearly below the value of 70 % DNA–DNA relatedness considered to be the threshold for the delineation of genomic species (Wayne et al., 1987).
Schumann, 2006). The strain is a psychrophilic representative of this group. In adaptation to the cold environment from which it was isolated, the strain was able to grow well at low temperatures and highest cell yields were obtained at 1–5 °C. The term psychrophile is used as a general one that describes 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 (Cavicchioli, 2006; Margesin, 2009). Strain Morita (1975) has been shown to be ambiguous and to define the optimum growth temperature as described by its different peptidoglycan type (A3\(\alpha\), L-Lys–L-Ala\(\alpha\)), its cellular fatty acid profile (larger amounts of iso-C\(15:0\) and smaller amounts of anteiso-C\(17:0\)) and its inability to hydrolyse starch and gelatin, to produce alkaline phosphatase and to grow in the presence of 5 % (w/v) NaCl. Based on the phenotypic, phylogenetic and genomic evidence, strain Cr6-08\(^T\) was identified as a representative of a novel species of the genus Arthrobacter, for which we propose the name *Arthrobacter cryoconiti* sp. nov.

**Description of *Arthrobacter cryoconiti* sp. nov.**

*Arthrobacter cryoconiti* (cry.o.co.ni’ti. N.L. gen. n. cryoconiti from cryoconite, referring to glacier cryoconite, the isolation source of the type strain).

Cells are irregular rods showing a rod–coccus cycle (0.5–0.7×0.8–1.8 μm after 4 days at 20 °C; 0.5–0.7×0.8–2.0 μm after 7 days) on R2A agar plates. Cells often occur in pairs as typical V-forms. Cells are Gram-stain-positive, aerobic and non-motile. Colonies on R2A agar are white after 3 days and sulfur-yellow after 7 days, round, convex, smooth, glossy and with an entire margin; colony diameter is 0.2–0.6 mm (1–1.5 mm) after 4 days (7 days) at 20 °C on R2A agar. Unable to grow under anaerobic conditions. Growth occurs in liquid R2A medium and on agar plates at 1–25 °C but not at 30 °C. Growth in liquid medium is fastest at 20–25 °C, while cell yield is highest at 1–5 °C. On R2A agar plates, growth occurs at pH 6–8 and in the presence of 0–3 % (w/v) NaCl. Growth at pH 9 is weak. Grows on R2A, nutrient agar (0.5 % peptone, 0.3 % meat extract, 1.5 % agar; pH 7) and TSA. Produces catalase; cytochrome oxidase is not produced. Positive for hydrolysis of aesculin, nitrate reduction, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acyetyl-β-glucosaminidase and α-amannosidase. Negative for indole production from tryptophan, H\(2\)S production, citrate utilization and hydrolysis of urea, starch and gelatin. Negative for alkaline phosphatase, α-chymotrypsin, α-fucosidase, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase and tryptophan deaminase. Assimilates D-glucose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, lactose, potassium gluconate, malic acid, trisodium citrate and phenylacetic acid, but not Tween 40 or 80, L-arabinose, capric acid or adipic acid. Negative for fermentation of D-glucose, D-mannitol, sucrose, inositol, D-sorbitol, L-rhamnose, melibiose, amygdalin and L-arabinose. The predominant cellular fatty acids are anteiso-C\(15:0\) and iso-C\(15:0\) 3-OH. Contains MK-9(H\(2\)) as the major menaquinone, with minor amounts of MK-8(H\(2\)) and MK-10(H\(2\)). The cell-wall peptidoglycan is of type A3\(\alpha\) (L-Lys–L-Ala\(\alpha\)). The predominant cell-wall sugars are galactose and rhamnose. The polar lipid profile contains diphasphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown glycolipid and three unknown polar lipids. The DNA G+C content of the type strain is 57.3 mol%.

The type strain, Cr6-08\(^T\) (=DSM 23324\(^T\) =LMG 26052\(^T\) =CGMCC 1.10698\(^T\)), was isolated from cryoconite from the Banker glacier in the Ötztaler Alps in Austria at an altitude of 2820 m above sea level.

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**References**


