Arthrobacter antarcticus sp. nov., isolated from an Antarctic marine sediment

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A bacterial strain, SPC26^T, was isolated from a sediment sample of the Southern Ocean off Antarctica. The strain was Gram-staining- and catalase-positive and contained lysine and alanine in the cell-wall peptidoglycan. The major cellular fatty acids were anteiso-C_{15:0} (54.92 %), iso-C_{15:0} (11.47 %), anteiso-C_{17:0} (6.48 %) and anteiso-C_{15:1} (6.38 %) and the major menaquinones were MK-8, MK-9 and MK-10. The major polar lipids were phosphatidylethanolamine and diphosphatidylglycerol. The G + C content was 68 ± 0.5 mol%. Based on 16S rRNA gene sequence similarities, the nearest phylogenetic neighbours of strain SPC26^T were identified as *Arthrobacter gangotriensis* Lz1y^T (98.8 %), *A. sulfureus* DSM 20167^T (98.6 %), *A. psychrophenicus* DSM 15454^T (97.9 %) and *A. kerguelensis* KGN15^T (97.5). With these strains, strain SPC26^T exhibited DNA–DNA relatedness values of 36, 21, 12 and 10 %, respectively. Therefore, on the basis of 16S rRNA gene sequence comparisons, phylogenetic analysis, phenotypic characteristics and DNA–DNA relatedness, it is proposed that strain SPC26^T represents a novel species of *Arthrobacter*, for which the name *Arthrobacter antarcticus* sp. nov. is proposed, with strain SPC26^T (=LMG 24542^T =NCCB 100228^T) as the type strain.

The genus *Arthrobacter* was first proposed by Conn & Dimmick (1947) with *Arthrobacter globiformis* as the type species. All *Arthrobacter* species are strictly aerobic, catalase-positive and sporogenous rod-shaped bacteria that display coryneform morphology and contain A-type (A3α or A4α) peptidoglycan with L-lysine as the dibasic amino acid (Schleifer & Kandler, 1972). The genus *Arthrobacter* is phenotypically heterogeneous, and over 52 species have so far been isolated from various sources such as soil (Reddy *et al.*, 2002; Lee *et al.*, 2003; Gupta *et al.*, 2004; Chen *et al.*, 2005), cheese (Irlinger *et al.*, 2005), clinical specimens (Funke *et al.*, 1996; Hou *et al.*, 1998; Wauters *et al.*, 2000; Huang *et al.*, 2005), paintings (Heyrman *et al.*, 2005), seals (Collins *et al.*, 2002), an alpine ice cave (Margesin *et al.*, 2004), fish (Osorio *et al.*, 1999), wastewater reservoir sediment (Roh *et al.*, 2008) and air (Li *et al.*, 2004). In this paper, we report the characteristics of a novel *Arthrobacter* strain isolated from a sediment sample of the Southern Ocean off Antarctica.

Sediment samples were collected from a depth of 400 m near the Larsemann Hills area (69° 22' S 76° 06' E) using a spade box corer and brought to the laboratory. The sample, after suspending 0.1 g in 1 ml sterile water by vortexing, plating on nutrient agar (NA) (1⁻¹: 10 g peptone, 10 g beef extract, 5 g NaCl and 20 g agar) and incubation at 22 °C for 7 days, yielded 7.9–15.2 × 10³ c.f.u. g⁻¹. Strain SPC26^T

was isolated by repeated subcultivation on NA plates and subjected to a detailed polyphasic taxonomic analysis. *Arthrobacter gangotriensis* DSM 15796^T, *A. sulfureus* DSM 20167^T, *A. psychrophenicus* DSM 15454^T and *A. kerguelensis* DSM 15797^T were used as reference strains.

Tryptone soy broth (TSB) (M290; HiMedia) containing (1⁻¹) 15 g pancreatic digest of casein and 5 g pancreatic digest of soybean meal or NA was used for growth and maintenance of the strain and determination of phenotypic and chemotaxonomic characteristics. A Leitz Diaplan phase-contrast microscope was used to ascertain the morphology and motility of cells. pH tolerance was determined using TSB adjusted to pH 4–10, at intervals of 1 pH unit, by the addition of HCl or NaOH. Tolerance of 0–8 % NaCl, at intervals of 1 %, was determined on NA with NaCl omitted. Results were scored after 72 h of incubation at 22 °C. Other physiological and biochemical characteristics were determined at 22 °C following Holding & Collee (1971) and Smibert & Krieg (1994). Acid production from carbohydrates was tested using a HiCarbohydrate kit (KB009; HiMedia). Utilization of various carbon compounds as the sole carbon source was tested at 22 °C for 15 days in minimal medium [containing 1⁻¹: 1.2 g (NH₄)₂SO₄, 0.5 g MgSO₄ · 7H₂O, 0.5 g KH₂PO₄, 0.1 g KCl; pH 6.5 ± 0.2] supplemented with 0.5 % (w/v) carbon source. The phenotypic characteristics of strain SPC26^T are given in Table 1 and the species description.

Menaquinones and polar lipids were determined in freeze-dried cells. Menaquinones were extracted as described

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SPC26^T is AM931709.

An extended neighbour-joining phylogenetic tree based on 16S rRNA gene sequences is available with the online version of this paper.

Table 1. Characteristics of strain SPC26^T and type strains of closely related *Arthrobacter* species

Strains: 1, *Arthrobacter antarcticus* sp. nov. SPC26^T; 2, *A. gangotriensis* DSM 15796^T; 3, *A. sulfureus* DSM 20167^T; 4, *A. psychrophenicus* DSM 15454^T; 5, *A. kerguelensis* DSM 15797^T. All strains were positive for urease and negative for β -galactosidase and glycerol utilization.

Characteristic	1	2	3	4	5
Aesculin hydrolysis	—	—	—	+	+
Casein hydrolysis	—	+	—	—	+
Nitrate reduction	+	—	—	+	—
Gelatinase	—	+	+	—	+
Phosphatase	+	+	—	—	+
Utilization of:					
L-Arabinose	+	+	—	—	—
D-Glucose	+	+	—	—	+
D-Galactose	+	+	—	—	+
Lactose	—	+	—	—	+
D-Ribose	—	+	—	—	+
L-Rhamnose	+	—	—	+	+
D-Xylose	+	+	—	—	+
Malonate	+	+	+	—	+
D-Xylitol	—	+	+	+	+
L-Histidine	—	—	+	—	+

by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Polar lipids were analysed by two-dimensional TLC using pure lipids as standards following the procedure of Minnikin *et al.* (1975). The presence of mycolic acids was examined using TLC (Minnikin *et al.*, 1980). DNA was isolated according to the procedure of Marmur (1961) and G+C content (mol%) was determined from the melting point (T_m) curves (Sly *et al.*, 1986) obtained using a Lambda 2 UV-Vis spectrophotometer equipped with Templab version 2.0 software (Perkin Elmer). Peptidoglycan and cell-wall sugars were prepared and analysed according to the method of Komagata & Suzuki (1987). The chemotaxonomic characteristics of strain SPC26^T are given in the species description.

For cellular fatty acid analysis, cells of strain SPC26^T were grown on TSA at 25 °C for 72 h. The fatty acid methyl esters were extracted from a loopful of culture according to the instructions of the Sherlock Microbial Identification

System (MIDI) and analysed using gas chromatography (MIDI 6890N GC; Agilent Technologies) equipped with an FID detector (Agilent Technologies). The cellular fatty acids of strain SPC26^T were anteiso-C_{15:0} (54.92 %), iso-C_{15:0} (11.47 %), anteiso-C_{17:0} (6.48 %), anteiso-C_{15:1} (6.38 %), C_{18:0} (2.42 %), C_{16:0} (2.14 %), iso-C_{16:0} (1.79 %), anteiso-C_{16:0} (1.76 %), C_{14:0} (1.30 %), iso-C_{15:1} (1.25 %) and iso-C_{19:0} (1.07 %).

The 16S rRNA gene of strain SPC26^T was amplified from the genomic DNA and sequenced according to Reddy *et al.* (2000). In the pairwise 16S rRNA gene sequence alignment performed using the ExPASy bioinformatics tool (<http://www.expasy.org>), strain SPC26^T showed 98.8 % similarity to *A. gangotriensis* Lz1y^T, 98.6 % to *A. sulfureus* DSM 20167^T, 97.9 % to *A. psychrophenicus* DSM 15454^T, 97.5 % to *A. kerguelensis* KGN15^T and less than 97.5 % similarity to strains of other species of *Arthrobacter* with validly published names. To determine the phylogenetic affiliation of strain SPC26^T, the 16S rRNA gene sequence of the strain was aligned with sequences from the type strains of other *Arthrobacter* species using CLUSTAL W (Thompson *et al.*, 1994) and phylogenetic analysis was carried out as described by Bhadra *et al.* (2008). In neighbour-joining phylogenetic trees constructed according to Kimura's two-parameter model (Kimura, 1980) using MEGA version 3.1 (Kumar *et al.*, 2004), strain SPC26^T formed a robust clade with *A. gangotriensis* Lz1y^T with bootstrap support of 97 % in a tree of the most closely related strains (Fig. 1) and 96 % in an extended tree (Supplementary Fig. S1, available in IJSEM Online).

There were various characteristics that indicated that strain SPC26^T is a member of the genus *Arthrobacter* and is most closely related to *A. gangotriensis* and *A. sulfureus*: anteiso-C_{15:0} as the major fatty acid, the presence of lysine and alanine as the diamino acids in the peptidoglycan, glutamic acid as the acyl type (A4 α variation), presence of MK-9, aerobic growth, positive catalase reaction, 16S rRNA gene sequence similarity and the results of phylogenetic analysis. The strain also differed from its nearest phylogenetic relatives in a number of phenotypic characteristics such as gelatinase production, nitrate reduction and utilization of L-rhamnose and D-xylitol as sole carbon sources (Table 1). In a DNA–DNA relatedness study, performed according to Reddy *et al.* (2000), strain

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain SPC26^T and the type strains of closely related *Arthrobacter* species. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. The sequence of *Brevibacterium oceanii* BBH7^T was used as an outgroup. Bar, 1 substitution per 100 nucleotide positions.

SPC26^T showed 35.6, 20.7, 12 and 10% DNA–DNA relatedness with *A. gangotriensis* DSM 15796^T, *A. sulfureus* DSM 20167^T, *A. psychrophenicus* DSM 15454^T and *A. kerguelensis* DSM 15797^T, respectively. Reverse DNA–DNA hybridizations yielded similar results. Considering 70% DNA–DNA relatedness as the cut-off point for species delineation (Wayne *et al.*, 1987) and the phenotypic and chemotaxonomic differences, strain SPC26^T is regarded as a representative of a novel species of *Arthrobacter*, for which the name *Arthrobacter antarcticus* sp. nov. is proposed.

Description of *Arthrobacter antarcticus* sp. nov.

Arthrobacter antarcticus (an.tarc'ti.cus. L. masc. adj. *antarcticus* southern, used to refer to the Antarctic, referring to the isolation of the type strain from Antarctic marine sediment).

Cells are Gram-staining-positive, motile, aerobic and have a rod–coccus cycle. Rod-shaped cells grown in TSB at 22 °C for 72 h are 1.5–2.2 µm long and 0.2–0.3 µm wide. Forms yellow colonies on TSA. Grows at 4–25 °C (at pH 7, optimum 22–25 °C), but not at 30 °C. On TSA, up to 6% NaCl is tolerated. Positive for catalase, phosphatase, citrate (Simmons'), lysine and ornithine decarboxylases and nitrate reduction, but negative for indole production, methyl red and Voges–Proskauer tests, H₂S production, gelatinase, lipase, β-galactosidase and DNase. Hydrolyses urea and starch but not aesculin or casein. Produces acid from D-arabinose, D-fructose, D-galactose, D-glucose, rhamnose, sucrose, trehalose, D-xylose and inositol, but not from D-mannose or erythritol. As sole carbon sources, utilizes L-arabinose, D-galactose, D-glucose, melezitose, raffinose, L-rhamnose, salicin, sucrose, trehalose, adonitol, inulin, sodium acetate, D-alanine, L-arginine, L-glutamic acid, glycine, L-lysine, L-proline and L-serine, but not cellobiose, lactose, maltose, D-mannose, melibiose, L-sorbose, dulcitol, erythritol, glycerol, mannitol, D-sorbitol, xylitol, L-asparagine, L-aspartic acid, L-glutamate, L-histidine, L-isoleucine, methionine, L-phenylalanine, L-threonine, tryptophan or L-tyrosine. Resistant to (µg ml⁻¹) norfloxacin (25), colistin (25) and nitrofurantoin (30), but sensitive to kanamycin (15), ampicillin (25), tetracycline (10), streptomycin (20) and rifampicin (15). The peptidoglycan diamino acids are lysine and alanine and the acyl type is glutamic acid (A4α variation). The major menaquinones are MK-8, MK-9 and MK-10. The major polar lipids are phosphatidylethanolamine and diphosphatidylglycerol. The cell-wall sugars are glucose, galactose and rhamnose. Mycolic acid is not present. The major cellular fatty acids (>5%) are anteiso-C_{15:0}, iso-C_{15:0}, anteiso-C_{17:0} and anteiso-C_{15:1}. The DNA G+C content of the type strain is 68 ± 0.5 mol%.

The type strain is SPC26^T (=LMG 24542^T =NCCB 100228^T), isolated from a Southern Ocean sediment from near the Larsemann Hills area of Antarctica.

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