

## ***Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica**

G. S. N. Reddy,<sup>1</sup> R. K. Aggarwal,<sup>1</sup> G. I. Matsumoto<sup>2</sup> and S. Shivaji<sup>1</sup>

Author for correspondence: S. Shivaji. Tel: +91 40 7172241. Fax: +91 40 7171195.  
e-mail: shivas@ccmb.ap.nic.in

<sup>1</sup> Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

<sup>2</sup> Department of Environmental Information Science, Otsuma Women's University, Tamashi, Tokyo 206, Japan

**CMS 19Y<sup>T</sup>, a psychrophilic bacterium, was isolated from a cyanobacterial mat sample from a pond in Antarctica and was characterized taxonomically. The bacterium was aerobic, Gram-positive, non-spore-forming, non-motile, exhibited a rod–coccus growth cycle and produced a yellow pigment that was insoluble in water but soluble in methanol. No growth factors were required and it was able to grow between 5 and 30 °C, between pH 6 and pH 9 and tolerated up to 11.5% NaCl. The cell wall peptidoglycan was Lys-Thr-Ala<sub>3</sub> (the A3 $\alpha$  variant) and the major menaquinone was MK-9(H<sub>2</sub>). The G+C content of the DNA was 64 $\pm$ 2 mol%. The 16S rDNA analysis indicated that CMS 19Y<sup>T</sup> is closely related to group I *Arthrobacter* species and showed highest sequence similarity (97.91%) with *Arthrobacter agilis*. Furthermore, DNA–DNA hybridization studies also indicated 77% homology between CMS 19Y<sup>T</sup> and *A. agilis*. It differed from *A. agilis*, however, in that it was psychrophilic, non-motile, yellow in colour, exhibited a rod–coccus growth cycle, had a higher degree of tolerance to NaCl and was oxidase- and urease-negative and lipase-positive. In addition, it had a distinct fatty acid composition compared to that of *A. agilis*: the predominant fatty acids were C<sub>15:0</sub><sup>0'</sup>, anteiso-C<sub>15:0</sub><sup>0'</sup>, iso-C<sub>16:0</sub><sup>0'</sup>, C<sub>17:0</sub><sup>0'</sup>, anteiso-C<sub>17:0</sub> and C<sub>18:0</sub><sup>0'</sup>. It is proposed, therefore, that CMS 19Y<sup>T</sup> should be placed in the genus *Arthrobacter* as a new species, i.e. *Arthrobacter flavus* sp. nov. The type strain of *A. flavus* is CMS 19Y<sup>T</sup> (= MTCC 3476<sup>T</sup>).**

**Keywords:** *Arthrobacter*, psychrophile, Antarctica, halotolerant, yellow pigment

### **INTRODUCTION**

Members of the genus *Arthrobacter* constitute a predominant group of micro-organisms in soils from various parts of the world. In addition to mesophilic arthrobacters (Schleifer & Kandler, 1972; Keddie & Jones, 1981; Keddie *et al.*, 1986), psychrophilic arthrobacters have been reported from samples of subterranean cave silts (Gounot, 1967), glacier silts (Moiroud & Gounot, 1969) and the soils of Antarctica (Johnson *et al.*, 1981; Johnson & Bellinoff, 1981; Madden *et al.*, 1979; Siebert & Hirsch, 1988; Shivaji *et al.*, 1989a). The psychrophilic isolates of *Arthrobacter* from Antarctica were identified on the basis of numerous phenotypic genus-specific charac-

teristics (Keddie *et al.*, 1986) such as morphology, physiological properties, cell wall composition, G+C content of the DNA (mol%), menaquinone type, nutritional requirements, antibiotic sensitivity and biochemical test results (Johnson *et al.*, 1981; Johnson & Bellinoff, 1981; Shivaji *et al.*, 1989a). Despite these similarities, the above Antarctic strains differed from the mesophilic species of *Arthrobacter* in that they were all psychrophilic, contained glucose as the cell wall sugar and did not hydrolyse starch. Pigmented *Arthrobacter* strains containing LL-diaminopimelic acid (LL-DAP) in the cell wall have also been reported from Antarctic soils (Siebert & Hirsch, 1988). Many of these *Arthrobacter* strains from Antarctica are unique in that they are psychrophilic and possess phenotypic traits by which they differ from the mesophilic *Arthrobacter* strains; thus they cannot be affiliated to any of the known species. These isolates were tentatively assigned to a group in the genus *Arthrobacter* known as the *Arthrobacter simplex/tumescens* group (Keddie

**Abbreviations:** DAP, diaminopimelic acid; NJ, neighbour-joining; UPGMA, unweighted pair group method with averages.

The EMBL accession number for the 16S rDNA sequence of CMS 19Y<sup>T</sup> is AJ242532.

& Jones, 1981; Johnson *et al.*, 1981; Johnson & Bellinoff, 1981; Siebert & Hirsch, 1988). *Arthrobacter simplex* and *Arthrobacter tumescens* have now been reclassified as *Pimelobacter simplex* and *Pimelobacter tumescens* (Suzuki & Komagata, 1983a, b).

All of the species of the genus *Arthrobacter* are Gram-positive, catalase-positive, aerobic and asporogenous bacilli with a coryneform morphology (Schleifer & Kandler, 1972) and have been separated into two groups on the basis of their peptidoglycan, which contains lysine as the diamino acid. Group I species of *Arthrobacter* contain the A3 $\alpha$  peptidoglycan variant in which cross-linkage of murein is brought about by interpeptide bridges involving monocarboxylic L-amino acids or glycine or both, as observed in most species of *Arthrobacter* including *Arthrobacter globiformis*, the type strain of the genus (Stackebrandt *et al.*, 1983). The group II species of *Arthrobacter* possess the A4 $\alpha$  peptidoglycan variant in which the peptidoglycan type is L-Lys-Ala-Glu, as in *Arthrobacter nicotianae*, *Arthrobacter uratoxydans*, *Arthrobacter protophormiae* (Schleifer & Kandler, 1972; Stackebrandt *et al.*, 1983), or L-Lys-L-Glu, as in *Arthrobacter sulfureus* (Stackebrandt *et al.*, 1995; Funke *et al.*, 1996). Recently, *Arthrobacter rhombi*, a new species (isolated from Greenland halibut) with A4 $\alpha$  peptidoglycan, was also included in *Arthrobacter* group II (Osorio *et al.*, 1999). Phylogenetic studies indicate that certain *Micrococcus* species such as *Micrococcus agilis*, *Micrococcus lylae* and *Micrococcus luteus* are intermixed with the species of *Arthrobacter* (Stackebrandt *et al.*, 1995) and detailed phylogenetic analysis along with phenotypic similarities have resulted in the reclassification of *M. agilis* as *Arthrobacter agilis* (Koch *et al.*, 1995). The genus also includes a number of other species for which the chemotaxonomic data are lacking, e.g. *Arthrobacter mysorens*, *Arthrobacter picolinophilus*, *Arthrobacter radiotolerans* and *Arthrobacter siderocapsulatus* (Keddie *et al.*, 1986). Detailed studies have now reclassified *A. picolinophilus* as *Rhodococcus erythropolis* (Koch *et al.*, 1995) and *A. radiotolerans* as *Rubrobacter radiotolerans* (Suzuki *et al.*, 1989). It has also been shown that *A. mysorens* is distinct from the *Arthrobacter* species recognized up to 1983 (Stackebrandt *et al.*, 1983). Furthermore, many other species that were included in the genus *Arthrobacter* (e.g. *Arthrobacter duodecadis*, *Arthrobacter viscosus*, *Arthrobacter variabilis*, *A. simplex* and *A. tumescens*) have now been excluded since they contain *meso*-DAP or *LL*-DAP in the peptidoglycan (Stackebrandt *et al.*, 1983).

In the present study, CMS 19Y<sup>T</sup>, a psychrophilic bacterium isolated from a cyanobacterial mat growing luxuriously in a pond in McMurdo Dry Valley, Antarctica, has been identified as belonging to the genus *Arthrobacter*. It possessed most of the phenotypic characteristics of *Arthrobacter*. Furthermore, the phylogenetic analysis based on the 16S rRNA gene of CMS 19Y<sup>T</sup> also indicated that it is closely related to group I *Arthrobacter* spp., which contain A3 $\alpha$ -variant

peptidoglycan. It differed, however, from all of the reported mesophilic and psychrophilic species with respect to many characteristics and was therefore designated as a new species, for which we propose the name *Arthrobacter flavus* sp. nov.

## METHODS

**Source of the organism, media and growth conditions.** Cyanobacterial mat samples were collected during the austral summer from the shores of an unnamed pond, designated E4 (77° 31' 7" S, 160° 45' 4" E), from the labyrinth of the Wright Valley, a constituent valley of McMurdo Dry Valley, Antarctica (Matsumoto, 1993; Matsumoto *et al.*, 1993). The sample was initially analysed microscopically ( $\times 1000$ ) and found to contain *Phormidium laminosum* as the most abundant cyanobacterium and also contained some green algae (Matsumoto *et al.*, 1993).

For the detection of bacteria, a piece of the above cyanobacterial mat sample (approx. 200 mg) was suspended in a tube containing 1 ml sterile saline (150 mM NaCl), teased with a glass rod and vortexed for approximately 2–5 min to obtain a suspension. An aliquot of the suspension (100  $\mu$ l) was plated on Antarctic Bacterial Medium (ABM) plates containing 0.5% (w/v) peptone, 0.1% (w/v) yeast extract and 1.5% (w/v) agar (pH 6.9) and incubated at 10 °C (Shivaji *et al.*, 1988, 1989a, b, 1991, 1992). The appearance of colonies was monitored on a regular basis and pure cultures of the bacteria were established. One of the pure cultures thus established was yellow in colour and was designated CMS 19Y<sup>T</sup>. The optimal temperature, pH and salt concentration for the growth of CMS 19Y<sup>T</sup> were determined by using ABM plates.

**Morphology and motility tests.** Cultures of CMS 19Y<sup>T</sup> in the lag-, log- and stationary phases of growth were observed under a phase-contrast microscope (1000 $\times$ ) to ascertain their shape and motility. Motility was also determined by the hanging drop method and staining of the flagellum was done by the method of silver impregnation (Blendin & Goldberg, 1965) using *Pseudomonas aeruginosa* as a positive control.

**Biochemical characteristics.** All of the tests were performed by growing the culture at 20 °C in the appropriate medium. The activities of catalase, oxidase, phosphatase, gelatinase, urease, arginine dihydrolase and  $\beta$ -galactosidase were determined according to standard methods (Holding & Collee, 1971). The production of indole, the utilization of citrate, the reduction of nitrate to nitrite and the hydrolysis of starch, aesculin and Tween 80 were measured according to the procedures described elsewhere (Stanier *et al.*, 1966; Holding & Collee, 1971; Stolp & Gadkari, 1981). The utilization of six different sugars, leading to the formation of acid with or without gas production, was monitored according to Hugh & Leifson (1953).

Fifty-four different carbon compounds were used to check the ability of the culture to utilize a carbon compound provided as the sole carbon source, using minimal medium without glucose but containing 0.2% (w/v) of the carbon source (Shivaji *et al.*, 1988, 1989a, b, 1992). The sensitivity of the culture to 24 different antibiotics was determined using antibiotic discs (HiMedia).

**Preparation of DNA and G + C content.** DNA was isolated according to the procedure of Marmur (1961) and the G + C content (mol%) was determined from the melting point ( $T_m$ ) curves obtained using a Hitachi Spectrophotometer as

described earlier (Shivaji *et al.*, 1989a, b, 1991, 1992). The equation of Schildkraut & Leifson (1965) was used to calculate the G + C content (mol%) of the DNA.

**DNA–DNA hybridization.** DNA–DNA hybridization between CMS 19Y<sup>T</sup> and *A. agilis* DNA was performed by using the membrane filter method (Tourova & Antonov, 1987) according to the protocol described earlier (Shivaji *et al.*, 1992). DNA was denatured by boiling in 0.2 M NaOH for 5 min and then quickly chilling on ice and neutralizing with HCl. Denatured DNA was dotted on to nitrocellulose and immobilized by baking at 80 °C for 2 h. The filters with the fixed DNA were prehybridized in a buffer containing 4 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5 × Denhardt's medium (Denhardt, 1966), calf-thymus DNA (100 µg ml<sup>-1</sup>) and 1% SDS for 2 h at 60 °C and then hybridized in the same buffer under similar conditions but in the presence of labelled DNA. DNA was labelled by nick translation (Rigby *et al.*, 1977).

**Analysis of cellular fatty acids.** Cellular fatty acid methyl esters were obtained using the method described by Stead *et al.* (1992) and were separated by GC on an Hewlett-Packard HP-5-type silicon capillary column (25 m × 0.25 mm). The fatty acids were identified by comparison with fatty acid standards run under similar gas chromatographic conditions and also by MS (VG AUTOSPEC) (Shivaji *et al.*, 1992).

**Analysis of isoprenoid quinones.** Menaquinones were extracted as described by Collins *et al.* (1977) and were separated by TLC using petroleum ether and diethyl ether (85:15, v/v). The menaquinone band corresponding to an *R<sub>F</sub>* value of 0.7 was separated, eluted with chloroform and further resolved into individual menaquinones by TLC, using silica-gel plates impregnated with silver nitrate with 15% methyl ethyl ketone in hexane as the solvent system (Dumphy *et al.*, 1971). The identities of the individual menaquinones were also confirmed by MS.

**Analysis of polar lipids.** Lyophilized cell pellets washed free of medium were used for the extraction of polar lipids by the method of Minnikin *et al.* (1975) and were identified by TLC.

**Peptidoglycan analysis.** Peptidoglycan was prepared according to the method described by Rosenthal & Dziarski (1994). The peptidoglycan thus obtained was hydrolysed with 4 M HCl at 120 °C for 60 min. The hydrolysate was vacuum-dried and the amino acids were extracted into acetate buffer (pH 4.6) and subjected to amino acid analysis on an automated amino acid analyser (model 80-2086-6314; Pharmacia). The composition of the peptidoglycan was determined according to the method of Schleifer & Kandler (1972).

**Cell wall sugars.** Cell wall sugars were prepared and analysed according to the method described by Komagata & Suzuki (1987).

**Bacterial pigment analysis.** Lyophilized cells of CMS 19Y<sup>T</sup> were suspended, extracted with methanol, centrifuged and the clear yellow supernatant recovered; the absorption spectrum was recorded in a Hitachi 330 spectrophotometer (Jagannadham *et al.*, 1991; Shivaji *et al.*, 1992; Chauhan & Shivaji, 1994).

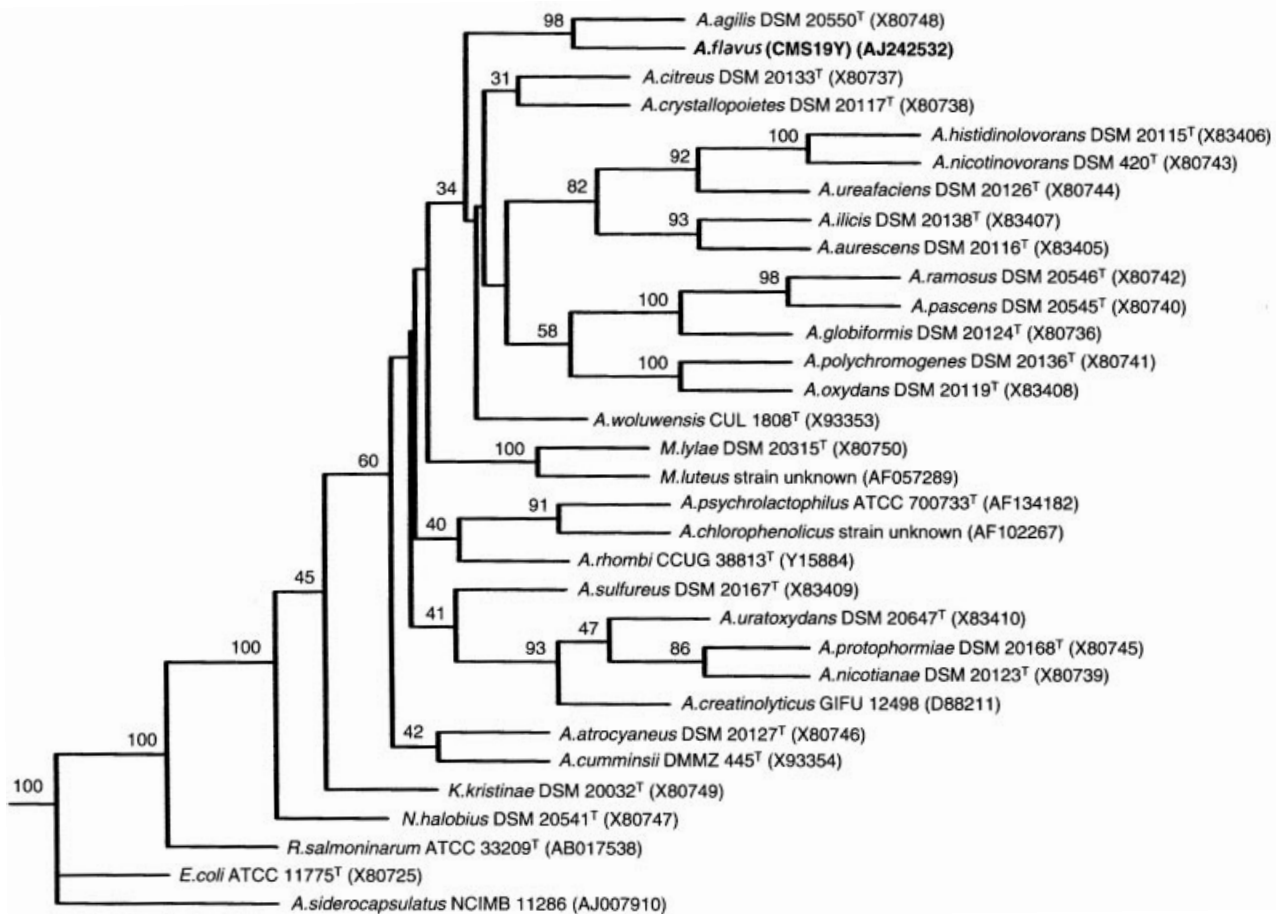
**PCR amplification of the 16S rRNA gene.** The small-subunit rRNA gene was amplified using the two primers 16S1 (5'-GAGTTTGATCCTGGCTCA-3') and 16S2 (5'-ACGGC-TACCTTGTACGACTT-3'), which are complementary to the conserved regions at the 5'- and 3' ends of the 16S

rRNA gene corresponding to positions 9–27 and 1477–1498 of the *Escherichia coli* 16S rRNA gene (Lane, 1991). The bacterial DNA (0.5 µg) was amplified by the PCR in a total volume of 50 µl containing 0.5 U *Taq* DNA polymerase, 10 mM TAPS, pH 8.8, 3 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% gelatin, 10 pmol of each of the two primers and 200 µM each of dATP, dCTP, dGTP and TTP. The amplification of DNA was carried out in a hot-air rapid thermocycler (model no. 1833; Idaho Technologies) programmed for 40 cycles of denaturation at 94 °C for 10 s, annealing at 48 °C for 20 s and extension at 72 °C for 30 s. A final extension of 5 min was carried out at 72 °C. In all of the amplification reactions, water was used in place of DNA for negative controls. The amplified DNA fragment (1.5 kb) was separated on 1% agarose gel, eluted from the gel and purified using the Clean Genei Kit (Bangalore Genei). The purified PCR product was used directly for DNA sequencing.

**16S rRNA gene sequencing.** The purified 1.5 kb DNA product was sequenced using the primers 16S1 and 16S2 and, in addition, a set of five forward primers [pB (TAACACATGCAAGTCGAACG), 50–70; pC (CTACGGAGGCAGCAGTGGG), 341–361; pD (CAGCAGCCGCGGTAATAC), 518–536; pE (AAACTCAAAGG-AATTGACGG), 908–928; and pF (CATGGCTGTCGT-CAGCTCGT), 1053–1073] and one reverse primer [pC°, (CCCACTGCTGCCTCCCGTAG), 301–341]. The nucleotide positions of the synthetic oligomers (as indicated in parentheses) are related to the 16S rDNA of *E. coli* (Woese *et al.*, 1983).

Sequencing of the purified PCR product (~200 ng/reaction) was carried out using 5 pmol of a given sequencing primer and 8 µl ready-reaction mix from either the Big Dye Terminator sequencing kit (Perkin Elmer) or the Thermo Sequenase Dye Terminator cycle sequencing kit (Amersham) in a total volume of 20 µl. Cycle sequencing was carried out in a GeneAmp PCR machine (9600; Perkin Elmer). The thermal sequence consisted of 30 cycles as follows: 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. After the PCR, the products were precipitated using 2 µl sodium acetate (3 M, pH 4.6) and 125 µl ethanol and stored on ice for 10 min. The pellet was recovered by centrifugation at 15000 r.p.m. for 20 min at 4 °C, washed with 70% ethanol, dried under vacuum and dissolved in 4 µl loading buffer [formamide:25 mM EDTA (4:1)]. The samples were denatured for 2 min at 90 °C before being loaded on to the sequencing gel (6% bis-acrylamide gel). Two-microlitre aliquots from each sample were loaded per gel lane and the gel was run for 10 h on a DNA sequencer (ABI Prism model 377 version 2.1.1).

**Phylogenetic analysis.** The 16S rDNA sequence of CMS 19Y<sup>T</sup> was aligned with 31 reference sequences (Fig. 1) from the EMBL database, using the multiple sequence alignment program CLUSTAL v (Higgins *et al.*, 1992). The aligned sequences were then manually checked for gaps. The pairwise evolutionary distances for the above aligned sequences were computed using the DNADIST program with the Kimura two-parameter model (Kimura, 1980). To obtain the confidence values for the rDNA sequence-based genetic affiliations, the original sequence data set was resampled 100 times using SEQBOOT and subjected to bootstrap analysis. The multiple distance matrices thus obtained were used to construct phylogenetic trees showing the relationships between CMS 19Y<sup>T</sup> and other reference micro-organisms, using various distance matrix-based clustering algorithms [FITCH, the unweighted pair group method with averages (UPGMA), KITCH and the neighbour-joining (NJ) method]



**Fig. 1.** UPGMA phenogram showing the phylogenetic relationship between CMS 19Y<sup>T</sup>, *Arthrobacter* species and other related reference micro-organisms, based on the 16S rDNA sequence analysis. The evolutionary distances were computed using the Kimura two-parameter model in DNADIST. The bootstrap analysis was done to check the reliability of the tree. The bootstrap values (%) are given at the nodes to which they apply; values below 30% are omitted. The branch lengths indicated in the phenogram are not to scale.

included in the Phylogeny Inference Package (Felsenstein, 1993). Parsimony analysis was also performed for the aligned sequence data set, using DNAPARS and DNAML. In all cases, the input order of species added to the topology being constructed was randomized via the jumble option with random seed of 7 and 10 replications. Majority-rule (50%) consensus trees were constructed for the topologies found by each method by using CONSENSE. All of these analyses were performed using the PHYLIP package, version 3.5C (Felsenstein, 1993).

**Reference strains.** *M. luteus* (ATCC 4698), *Micrococcus roseus* (reclassified as *Kocuria rosea*) (ATCC 412), *A. globiformis* (ATCC 8010), *A. protophormiae* (MTCC 693), *P. aeruginosa* (NCTC 675) and *Sphingobacterium antarcticus* (ATCC 51970) were used as controls in the studies relating to morphology, motility, biochemical tests, identification of fatty acids, etc.

## RESULTS AND DISCUSSION

A colony of CMS 19Y<sup>T</sup> was isolated and purified from a cyanobacterial mat sample from the Wright Valley located in the McMurdo Dry Valley region of

Antarctica. The cyanobacterial mat sample, following suspension in sterile water, plating on ABM and incubation at 10 °C, yielded approximately  $1 \times 10^4$  colonies per g mat sample after 15 d. All of the colonies were either white or orange in colour, except CMS 19Y<sup>T</sup> (which was yellow in colour). The yellow colonies of CMS 19Y<sup>T</sup> were 0.5–2 mm in diameter, round, smooth and convex. Table 1 summarizes some of the phenotypic characteristics of CMS 19Y<sup>T</sup>; details relating to pigment production, the UV-visible spectrum of the pigment, optimal growth conditions, the utilization of carbon compounds as sole carbon sources, the ability to oxidize or ferment sugars, sensitivity to antibiotics and other phenotypic characteristics are included below in the description of the species.

The cellular fatty acids were identified as C<sub>14:0</sub> (1.6%), C<sub>15:0</sub> (2.8%), anteiso-C<sub>15:0</sub> (52%), C<sub>16:0</sub> (2.2%), iso-C<sub>16:0</sub> (17%), C<sub>16:1</sub> (0.6%), C<sub>17:0</sub> (12.8%), anteiso-C<sub>17:0</sub> (7.2%), iso-C<sub>18:0</sub> (0.21%), C<sub>18:0</sub> (2.6%) and C<sub>20</sub> (1%). Glucose, ribose and galactose were the cell wall

**Table 1.** Phenotypic characteristics of CMS 19Y<sup>T</sup> and *Arthrobacter agilis*

+, Positive; —, negative.

Characteristic	CMS 19Y <sup>T</sup>	<i>A. agilis</i>
Colony diameter (mm)	0.5–2.0	—
Colony colour	Yellow	Red
Form	Rod–coccus	Coccoid
Motility	—	+ / —
Flagella	—	1–3
Spore formation	No	No
Gram reaction	Positive	Positive
Catalase	+	+
Oxidase	—	+
Urease	—	—
Lipase	+	—
Gelatinase	+	+
Phosphatase	—	—
β-Galactosidase	+	+
Aesculin hydrolysis	+	+
Starch hydrolysis	—	+
Nitrate to nitrite reduction	—	—
Indole test	—	—
Methyl red test	—	—
Voges–Proskauer test	—	—
Levan formation	—	—
Arginine dihydrolase		
Aerobic	+	—
Anaerobic	—	—
Peptidoglycan variant	A3α	A3α
Peptidoglycan type	Lys-Thr-Ala <sub>3</sub>	Lys-Thr-Ala <sub>3</sub>
Major menaquinone	MK-9(H <sub>2</sub> )	MK-9(H <sub>2</sub> )
Cellular fatty acids	C <sub>14:0</sub> , C <sub>15:0</sub> , anteiso-C <sub>15:0</sub> , C <sub>16:0</sub> , iso-C <sub>16:0</sub> , C <sub>16:1</sub> , C <sub>17:0</sub> , anteiso-C <sub>17:0</sub> , iso-C <sub>18:0</sub> , C <sub>18:0</sub> , C <sub>20:0</sub>	anteiso-C <sub>15:0</sub> , iso-C <sub>15:0</sub> , iso-C <sub>16:1</sub>
Cell wall sugars	Galactose, glucose, ribose	Glucosamine
DNA G+C content (mol%)	64.4 ± 2.44	67–70.0

sugars and the peptidoglycan type was L-Lys-Thr-L-Ala<sub>3</sub> of the A3α peptidoglycan variant. The major menaquinone was MK-9(H<sub>2</sub>) and the lipids were identified as phosphatidylglycerol, cardiolipin and phosphatidylethanolamine. The G+C content (mol%) of the DNA of CMS 19Y<sup>T</sup> was 64 ± 2 (*T*<sub>m</sub> 79.5 °C ± 1).

The characteristics of CMS 19Y<sup>T</sup> (see the description of the species, below) were observed to be consistent with the description of the genus *Arthrobacter* with respect to various phenotypic characteristics including cell wall composition, menaquinone type and the G+C content (mol%) of the DNA (Keddie & Jones, 1981; Keddie *et al.*, 1986). There are several other genera, which, like *Arthrobacter*, have a rod–coccus growth cycle, e.g. *Brevibacterium*, *Caseobacter*, *Rhodococcus* and *Microbacterium* (Keddie *et al.*, 1986; Takeuchi & Hatano, 1998). However, species in these genera have ornithine or DAP as the diamino

acid in the cell wall unlike species of *Arthrobacter*, which have lysine as the diamino acid in the cell wall (Keddie *et al.*, 1986).

CMS 19Y<sup>T</sup> differs from all of the reported psychrophilic and mesophilic species of *Arthrobacter* in a number of phenotypic characteristics. The genus *Arthrobacter* includes a few species that are unpigmented (*A. globiformis*, *Arthrobacter crystallopoietes*, *Arthrobacter pascens* and *Arthrobacter histidinovorans*) but a good majority of them produce a range of pigments, e.g. yellow (*Arthrobacter aurescens*, *Arthrobacter ilicis*, *Arthrobacter citreus*, *A. nicotianae*, *A. protophormiae*, *Arthrobacter uratoxydans*, *A. sulfureus* and *A. mysorens*), grey to yellow (*Arthrobacter urea-faciens*, *Arthrobacter oxydans* and *A. siderocapsulatus*), blue to black (*Arthrobacter atrocyaneus*) and red pigment (*A. agilis*) (Keddie *et al.*, 1986; Schleifer, 1986; Koch *et al.*, 1995). Thus CMS 19Y<sup>T</sup>, which produces a yellow pigment, could be any one of the eight above

**Table 2.** Some characteristics by which CMS 19Y<sup>T</sup> differs from the yellow-pigmented *Arthrobacter* spp. with A3 $\alpha$ -variant peptidoglycan and MK-9(H<sub>2</sub>) as the major menaquinone

Characteristic	<i>A. aurescens</i>	<i>A. ilicis</i>	<i>A. citreus</i>	CMS 19Y <sup>T</sup>
Motility	–	+	+	–
Peptidoglycan type	Lys-Ala-Thr-Ala	Lys-Ala-Thr-Ala	Lys-Thr-Ala <sub>2</sub>	Lys-Thr-Ala <sub>3</sub>
Cell wall sugars	Galactose (mannose)	Galactose, rhamnose, mannose	Galactose	Galactose, glucose, ribose
Starch hydrolysis	+	–	–	–
DNA G+C content (mol %)	61.5	61.5	62.9–65.1	64.4 ± 2.4

species of *Arthrobacter* that produce a yellow pigment. However, CMS 19Y<sup>T</sup> differs from the above yellow-pigmented species in that it has a peptidoglycan of the Lys-Thr-Ala<sub>3</sub> type of the A3 $\alpha$  variant. *A. aurescens*, *A. ilicis* and *A. citreus* also have A3 $\alpha$ -variant peptidoglycans but differ from CMS 19Y<sup>T</sup> in that their peptidoglycan type is Lys-Ala-Thr-Ala, as in *A. aurescens* and *A. ilicis*, and Lys-Thr-Ala<sub>2</sub>, as in *A. citreus* (Keddie *et al.*, 1986). The remaining five yellow *Arthrobacter* species have A4 $\alpha$ -variant peptidoglycan. Table 2 shows the characteristics by which CMS 19Y<sup>T</sup> differs from the yellow-pigmented *Arthrobacter* spp. having A3 $\alpha$ -variant peptidoglycan and MK-9(H<sub>2</sub>) as the major menaquinone. CMS 19Y<sup>T</sup> also differs from *A. duodecadis*, *A. variabilis* and *A. viscosus*, which have meso-DAP in the peptidoglycan (Keddie *et al.*, 1986), and have thus been excluded from the genus *Arthrobacter*.

In the mid-1990s, *M. agilis* was reclassified as *A. agilis* comb. nov. (Koch *et al.*, 1995). CMS 19Y<sup>T</sup> was observed to be similar to *A. agilis* with respect to many phenotypic characteristics, both have A3 $\alpha$ -variant peptidoglycan and the peptidoglycan in both was Lys-Ala-Thr<sub>3</sub> (Table 1). DNA–DNA hybridization studies indicated 77% homology between CMS 19Y<sup>T</sup> and *A. agilis*. Furthermore, the major menaquinone in both was MK-9(H<sub>2</sub>). Nonetheless, CMS 19Y<sup>T</sup> and *A. agilis* exhibit a number of distinct differences. The reported isolate (CMS 19Y<sup>T</sup>) is, unlike *A. agilis*, psychrophilic (it grows at 5–30 °C), halotolerant (it is able to grow in the presence of 11.5% NaCl), produces a yellow pigment, is non-motile, has a different fatty acid composition and has galactose, glucose and ribose as the cell wall sugars (Table 1).

In the present study, an attempt was also made to establish the identity and phylogenetic position of CMS 19Y<sup>T</sup> on the basis of its 16S rDNA sequence. Accordingly, the 16S rDNA sequence of CMS 19Y<sup>T</sup>, consisting of 1446 nucleotide base pairs, was compared with 31 corresponding sequences of closely related *Arthrobacter* species and other reference microorganisms retrieved from the EMBL database. The topology of the consensus phylogenetic trees shown in Fig. 1 is broadly in agreement with those reported

recently by Koch *et al.* (1995) and Hou *et al.* (1998). The genetic affiliations seen between different species were generally consistent across the different ‘distance as well as parsimony’-based clustering methods, viz. FITCH, KITSCH, UPGMA, NJ, DNAPARS and DNAML. Moreover, it was seen that while most of the *Arthrobacter* species were grouped into a few highly stable coherent clades (having high bootstrap values of > 50%), the inter-clade resolution was not very robust (Fig. 1), an observation also made by Hou *et al.* (1998). Interestingly, in most of these phylogenetic methods, the group I and group II species of *Arthrobacter* were clearly separated from each other, supporting the observations made with phenotypic data.

The 16S rDNA-based phylogenetic analysis clearly established CMS 19Y<sup>T</sup> as a member of the genus *Arthrobacter* (Fig. 1) and, more specifically, as a member of the group I *Arthrobacter* species, which include the type strain *A. globiformis* and all other species defined on the basis of the A3 $\alpha$  peptidoglycan variant (Keddie *et al.*, 1986). Within the group I *Arthrobacter* species, those containing the peptidoglycan Lys-Ser-Thr-Ala (*A. oxydans* and *Arthrobacter polychromogenes*) and those with Lys-Ala-Thr-Ala (*A. aurescens*, *A. ilicis*, *Arthrobacter ureafaciens*, *A. histidinolorans* and *Arthrobacter nicotinovorans*) appeared in two robust clades that were (relatively) closer to *A. pascens*, *Arthrobacter ramosus* and *A. globiformis*, which formed another robust clade according to most of the clustering methods (Fig. 1). Within group II *Arthrobacter* species (*A. nicotianae*, *A. protophormiae*, *Arthrobacter uratoxydans*, *Arthrobacter creatinolyticus*, *A. sulfureus*, *A. rhombi*, *Arthrobacter psychrolactophilus* and *Arthrobacter chlorophenolicus*) characterized as having the peptidoglycan variant A4 $\alpha$ , the first three and the last three were more closely related to each other, forming coherent clusters (Fig. 1). Overall, group II *Arthrobacter* species appeared to be closer to *M. lylae* and *M. luteus* than to group I species. In comparison, the genetic affiliations of the remaining known species of *Arthrobacter*, i.e. *A. atrocyaneus*, *Arthrobacter woluwensis*, *Arthrobacter cumminsii*, *A. citreus*, *A. crystallopoietes* and *A. siderocapsulatus* were well resolved, as none of these species exhibit close

affinity for any of the above species in particular. Against this background, it was most interesting to note that the new strain (CMS 19Y<sup>T</sup>) always formed a robust cluster with *A. agilis* in both phenetic and parsimony-based phylogenetic analysis, thus strongly defining its genetic affiliation as a member of the group I *Arthrobacter* spp. (the minimum evolutionary distance from *A. agilis*, according to the Kimura two-parameter model, being 2.09%).

Thus, the phylogenetic database of 16S rDNA sequences strongly suggests that CMS 19Y<sup>T</sup> is an *Arthrobacter* species (there being high levels of genetic similarity in the range 97.91–94.25%). The observed evolutionary distance of 2.09% between CMS 19Y<sup>T</sup> and its nearest *Arthrobacter* species (*A. agilis*) is significantly more than that seen between many of the other previously described known species of *Arthrobacter*. For instance, the distances between *A. pascens* and *A. globiformis*, *A. histidinolorans* and *A. nicotovorans*, *A. oxydans* and *A. polychromogenes* and *A. ramosus* and *A. pascens* were 0.34, 0.27, 0.07 and 0% respectively. These observations suggest that CMS 19Y<sup>T</sup>, which is phylogenetically most close to *A. agilis*, is differentiated enough not to be a strain/isolate or subspecies of the latter or of any other known species of the genus *Arthrobacter* and is thus defined as a new species. Recently, Stackebrandt & Goebel (1994) emphasized that DNA–DNA hybridization remains the optimal method for measuring degrees of relatedness and observed that when 16S rRNA sequence homology is below 97.5% it is unlikely that the two organisms have more than 60% DNA similarity. In the present study, it was observed that the sequence homology between CMS 19Y<sup>T</sup> and *A. agilis* was 97.91% and that the DNA–DNA similarity was 77%, thus indicating that the two are phylogenetically close. The genetic distinction of CMS 19Y<sup>T</sup> from *A. agilis* and from other species is even clearer in terms of absolute nucleotide changes along the 16S rDNA (data not shown). In *Arthrobacter* species, most of the nucleotide changes fall mainly into five broad hyper-variable regions, i.e. between nucleotide positions 59 and 81 (23 bp), 173 and 192 (20 bp), 603 and 664 (62 bp), 1006 and 1052 (47 bp) and 1143 and 1163 (21 bp). It was interesting to note that CMS 19Y<sup>T</sup> confirms the general pattern of nucleotide variation seen among *Arthrobacter* species and differed by only ~ 23 nucleotides from *A. agilis* over the whole length of the four hypervariable regions (~ 173 bp in length). In addition, it did not have an 11 bp sequence (CTGTCTTTTGG, between nucleotide positions 453 and 463) that is characteristic of *A. agilis* (data not shown).

Despite high 16S rRNA sequence homology (>97%) and DNA similarity between micro-organisms (>70%) phenotypic coherence among strains should be the deciding factor in the identification of species since it is still not established as to whether species should be delineated at the 60 or 80% DNA–DNA similarity level (Stackebrandt & Goebel, 1994). In the

present study, the genetic status of CMS 19Y<sup>T</sup> as a new *Arthrobacter* species is also supported by the phenotypic data, which established it as a member of the genus *Arthrobacter* but with a number of phenotypic characteristics different from those of *A. agilis* (Table 1) and other described species (Table 2). CMS 19Y<sup>T</sup> is also distinctly different from *A. psychrolactophilus* sp. nov., a psychrophilic *Arthrobacter* species (Loveland-Curtze *et al.*, 1999) that is more related to *A. polychromogenes*, *A. oxydans* and *A. globiformis*. Furthermore, in *A. psychrolactophilus* (unlike CMS 19Y<sup>T</sup>) pigment production is dependent on media and growth conditions; the two species also differ with respect to their fatty acid composition and other phenotypic traits (Loveland-Curtze *et al.*, 1999). LV7, another psychrophilic member of the genus *Arthrobacter*, is, like CMS 19Y<sup>T</sup>, an isolate from a cyanobacterial mat sample but differs from CMS 19Y<sup>T</sup> in that it does not grow above 24 °C, does not exhibit a rod–coccus cycle, tolerates only 3.5% NaCl in the medium and produces a cream or yellow pigment depending on media and growth conditions (Loveland-Curtze *et al.*, 1999). Therefore, we conclude that CMS 19Y<sup>T</sup> defines a new *Arthrobacter* species; it has been named *A. flavus* (wherein ‘flavus’ means ‘yellow’).

#### Description of *Arthrobacter flavus* sp. nov.

*Arthrobacter flavus* (fla'vus. L. adj. *flavus* yellow, the colour of a pigment that the bacterium produces).

Cells are aerobic, Gram-positive, non-spore-forming, non-motile, non-fermentative and exhibit a rod–coccus growth cycle. Colonies on peptone-yeast extract medium are yellow, round, smooth, convex and 0.5–2 mm in diameter. The pigment is insoluble in water but soluble in methanol and exhibits fine structure in its absorption spectrum with absorption maxima at 410, 440 and 470 nm. Pigment production is not dependent on growth conditions or media composition. No growth factors are required. Grows between 5 and 30 °C, at pH 6–9 and tolerates up to 11.5% (w/v) NaCl. Optimal growth is observed at 25 °C and pH 7. Positive for catalase, lipase, gelatinase and  $\beta$ -galactosidase but negative for oxidase, urease, phosphatase, indole production, nitrate reduction and levan formation. Unable to utilize a number of carbon compounds, such as sucrose, rhamnose, cellulose, arabinose, mellibiose, cellobiose, galactose, sucrose, fructose, mannose, trehalose, xylose, mannitol, raffinose, glycerol, ribose, lactose, lactic acid, adonitol, maltose, glucose, glucosamine, sorbitol, melezitol,  $\beta$ -hydroxy butyric acid, dulcitol, dextrose, polyethylene glycol, glycine, lysine, Na-citrate, Na-acetate, Na-succinate, cellulose, inulin, *meso*-inositol, glutamic acid, L-alanine, phenylalanine, methionine, glutamine, arginine, serine, potassium hydrogen phosphate, myristic acid, ammonium formate, creatine, methanol, tyrosine, sodium pyruvate, glycogen, erythritol, tryptophan, ethanol and sodium thioglycolate. Able to utilize sorbitol as the only source of carbon. Unable to oxidize or ferment glucose, galactose, sucrose, thio-

glycolate or mannose but able to acidify sucrose and hydrolyse aesculin but not starch or cellulose. The cell wall peptidoglycan type is Lys-Thr-Ala<sub>3</sub> (the A<sub>3</sub> variant) and the major menaquinone is MK-9(H<sub>2</sub>). The cell wall sugars are galactose, glucose and ribose. The cellular fatty acids are C<sub>14:0</sub>, C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, C<sub>16:0</sub>, iso-C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>17:0</sub>, anteiso-C<sub>17:0</sub>, iso-C<sub>18:0</sub>, C<sub>18:0</sub> and C<sub>20:0</sub>. The G+C content of the DNA is 64±2 mol%. Closely related phylogenetically to group I *Arthrobacter* species and exhibits maximum similarity to *A. agilis* (97.31%), as determined by 16S rDNA analysis. Sensitive to all antibiotics tested: carbenicillin (50 µg), tobramycin (15 µg), chlortetracycline (30 µg), polymyxin B (300 µg), oxytetracycline (30 µg), rifampicin (5 µg), nitrofurantoin (300 µg), penicillin (10 µg), bacitracin (10 µg), nitrofurazone (10 µg), gentamicin (10 µg), lincomycin (2 µg), furazolidone (50 µg), colistin (10 µg), furoxone (100 µg), kanamycin (30 µg), nystatin (100 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), ampicillin (10 µg), tetracycline (30 µg), amoxycillin (100 µg), trimethoprim (5 µg) and erythromycin (15 µg). Isolated from a cyanobacterial mat sample from McMurdo Dry Valley, Antarctica. The type strain is CMS 19Y<sup>T</sup> (= MTCC 3476<sup>T</sup>).

## REFERENCES

- Blenden, D. C. & Goldberg, M. S. (1965). Silver impregnation stain for *Leptospira* and flagella. *J Bacteriol* **89**, 899–900.
- Chauhan, S. & Shivaji, S. (1994). Growth and pigmentation in *Sphingobacterium antarcticus*, a psychrotrophic bacterium from Antarctica. *Polar Biol* **14**, 31–36.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Denhardt, D. T. (1966). A membrane filter technique for the detection of complementary DNA. *Biochem Biophys Res Commun* **23**, 641–646.
- Dumphy, P. J., Phillips, P. G. & Brodie, A. F. (1971). Separation and identification of menaquinones from microorganisms. *J Lipid Res* **12**, 442–449.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, USA.
- Funke, G., Hutson, R. A., Bernard, K. A., Pfyffer, G. E., Wauters, G. & Collins, M. D. (1996). Isolation of *Arthrobacter* spp. from clinical specimens and description of *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwendensis* sp. nov. *J Clin Microbiol* **34**, 2356–2363.
- Gounot, A. M. (1967). Role biologique des *Arthrobacter* dans les limons souterrains. *Ann Inst Pasteur Paris* **113**, 923–945.
- Higgins, D. G., Bleasby, A. T. & Fuchs, R. (1992). CLUSTAL V: improved software for multiple sequence alignment. *CABIOS* **8**, 189–191.
- Holding, A. J. & Collee, J. G. (1971). Routine biochemical tests. *Methods Microbiol* **6A**, 2–32.
- Hou, X. G., Kawakura, Y., Sultana, F., Shu, S., Hirose, K., Goto, K. & Ezaki, T. (1998). Description of *Arthrobacter creatinolyticus* sp. nov. isolated from human urine. *Int J Syst Bacteriol* **48**, 423–429.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J Bacteriol* **66**, 24–26.
- Jagannadham, M. V., Rao, V. J. & Shivaji, S. (1991). The major carotenoid pigment of a psychrotrophic *Micrococcus roseus*: purification, structure, and interaction of the pigment with synthetic membranes. *J Bacteriol* **173**, 7911–7917.
- Johnson, R. M. & Bellinoff, R. D. (1981). A taxonomic study of a dominant coryneform bacterial type found in Antarctic soils. *Antarct Res Ser* **30**, 169–184.
- Johnson, R. M., Inai, M. & McCarthy, S. (1981). Characteristics of cold desert Antarctic coryneform bacteria. *J Ariz-Nev Acad Sci* **16**, 51–60.
- Keddie, R. M. & Jones, D. (1981). Aerobic saprophytic coryneform bacteria. In *The Prokaryotes*, vol. 2, pp. 1838–1878. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. New York: Springer.
- Keddie, R. M., Collins, M. D. & Jones, D. (1986). Genus *Arthrobacter* Conn and Dimmick 1947. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1288–1301. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Koch, C., Schumann, P. & Stackebrandt, E. (1995). Reclassification of *Micrococcus agilis* (Ali-Cohen 1889) to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov., and emendation of the genus *Arthrobacter*. *Int J Syst Bacteriol* **45**, 837–839.
- Komagata, K. & Suzuki, K. I. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–206.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–147. Edited by E. Stackebrandt & M. Goodfellow. New York: Wiley.
- Loveland-Curtze, J., Sheridan, P. P., Gutshall, K. R. & Brenchley, J. E. (1999). Biochemical and phylogenetic analyses of psychrophilic isolates belonging to the *Arthrobacter* subgroup and description of *Arthrobacter psychrolactophilus* sp. nov. *Arch Microbiol* **171**, 355–363.
- Madden, J. M., Siegel, S. K. & Johnson, R. M. (1979). Taxonomy of some Antarctic *Bacillus* and *Corynebacterium* species. *Antarct Res Ser* **30**, 77–103.
- Marmur, J. (1961). Procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Matsumoto, G. I. (1993). Geochemical features of the McMurdo Dry Valley lakes, Antarctica. Physical and biogeochemical processes in Antarctic lakes. *Antarct Res Ser* **49**, 95–118.
- Matsumoto, G. I., Ohtani, S. & Hiroto, K. (1993). Biogeochemical features of hydrocarbons in cyanobacterial mats from the McMurdo Dry Valley, Antarctica. *Proc NIPR Symp Polar Biol* **6**, 98–105.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *J Gen Microbiol* **88**, 200–204.
- Moiroud, A. & Gounot, A. M. (1969). Sur une bacterie psychrophile obligatoire isolee de limons glaciaires. *Hebd Seances Acad Sci Ser D Sci Nat* **269**, 2150–2152.
- Osorio, C. R., Barja, J. L., Hutson, R. A. & Collins, M. D. (1999). *Arthrobacter rhombi* sp. nov. isolated from Greenland halibut (*Reinhardtius hippoglossoides*). *Int J Syst Bacteriol* **49**, 1217–1220.

- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977).** Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J Mol Biol* **113**, 237–245.
- Rosenthal, R. S. & Dziarski, R. (1994).** Isolation of peptidoglycan and soluble peptidoglycan fragment. *Methods Enzymol* **235**, 253–285.
- Schildkraut, C. & Leifson, S. (1965).** Dependence of the melting temperature of DNA on salt concentration. *Biopolymers* **3**, 195–208.
- Schleifer, K. H. (1986).** Family I. Micrococcaceae, Prevot 31<sup>AL</sup>. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1003–1008. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Shivaji, S., Rao, N. S., Saisree, L., Sheth, V., Reddy, G. S. N. & Bhargava, P. M. (1988).** Isolation and identification of *Micrococcus roseus* and *Planococcus* sp. from Schirmacher Oasis, Antarctica. *J Biosci* **113**, 409–414.
- Shivaji, S., Rao, N. S., Saisree, L., Reddy, G. S. N., Seshu Kumar, G. & Bhargava, P. M. (1989a).** Isolates of *Arthrobacter* from the soils of Schirmacher Oasis, Antarctica. *Polar Biol* **10**, 225–229.
- Shivaji, S., Rao, N. S., Saisree, L., Sheth, V., Reddy, G. S. N. & Bhargava, P. M. (1989b).** Isolation and identification of *Pseudomonas* sp. from Schirmacher Oasis, Antarctica. *Appl Environ Microbiol* **55**, 767–770.
- Shivaji, S., Ray, M. K., Seshu Kumar, G., Reddy, G. S. N., Saisree, L. & Wynn-Williams, D. D. (1991).** Identification of *Janthinobacterium lividum* from the soils of the islands of Scotia Ridge and from Antarctic peninsula. *Polar Biol* **11**, 267–272.
- Shivaji, S., Ray, M. K., Saisree, L., Jagannadham, M. V., Seshu Kumar, G., Reddy, G. S. N. & Bhargava, P. M. (1992).** *Sphingobacterium antarcticus* sp. nov. a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* **42**, 102–116.
- Siebert, J. & Hirsch, P. (1988).** Characterization of 15 selected coccid bacteria isolated from Antarctic rock and soil samples from the McMurdo valley (South Victoria Land). *Polar Biol* **9**, 37–44.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Fowler, J. J., Fiedler, F. & Seiler, H. (1983).** Taxonomic studies on *Arthrobacter nicotianae* and related taxa: description of *Arthrobacter uratoxydans* sp. nov. and *Arthrobacter sulfureus* sp. nov. and reclassification of *Brevibacterium protophormiae* as *Arthrobacter protophormiae* comb. nov. *Syst Appl Microbiol* **4**, 470–486.
- Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995).** Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Derma-coccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.
- Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966).** The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.
- Stead, D. F., Sellwood, J. G., Wilson, J. & Viney, I. (1992).** Evaluation of commercial microbial identification system based on fatty acid profiles and rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.
- Stolp, H. & Gadkari, D. (1981).** Nonpathogenic members of the genus *Pseudomonas*. In *The Prokaryotes*, vol. 1, pp. 719–741. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Suzuki, K. & Komagata, K. (1983a).** Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. *Int J Syst Bacteriol* **33**, 188–200.
- Suzuki, K. & Komagata, K. (1983b).** *Pimelobacter* gen. nov., a new genus of corenyform bacteria with LL-diaminopimelic acid in the cell wall. *J Gen Appl Microbiol* **29**, 59–71.
- Suzuki, K., Collins, M. D., Iijima, E. & Komagata, K. (1989).** Chemotaxonomic characterization of a radiotolerant bacterium, *Arthrobacter radiotolerans*: description of *Rubrobacter radiotolerans* gen. nov. comb. nov. *FEMS Microbiol* **52**, 33–40.
- Takeuchi, M. & Hatano, K. (1998).** Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* **48**, 739–747.
- Tourova, T. P. & Antonov, A. S. (1987).** Identification of microorganisms by rapid DNA–DNA hybridisation. *Methods Microbiol* **19**, 333–355.
- Woese, C. R., Gutell, R., Gupta, R. & Noller, H. F. (1983).** Detailed analysis of the higher order structure of 16S-like ribosomal ribonucleic acids. *Microbiol Rev* **47**, 621–669.