Flavobacterium antarcticum sp. nov., a novel psychrotolerant bacterium isolated from the Antarctic

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A yellow-pigmented, Gram-negative and aerobic bacterial strain, designated AT1026^T, was isolated from a terrestrial sample from the Antarctic. Results of 16S rRNA gene sequence analysis indicated that the Antarctic isolate belonged to the genus Flavobacterium, with the highest sequence similarity to Flavobacterium tegetincola (96.4%). Cells were non-motile, non-gliding and psychrotolerant, with optimum and maximum temperatures of about 20 and 25 °C. Flexirubins were absent. The major isoprenoid quinone (MK-6), predominant cellular fatty acids (iso-C₁₅:1 G, iso-C₁₅:0 and a mixture of C₁₆:1ω7c and/or iso-C₁₅:0 2-OH) and DNA G+C content (38 mol%) of the Antarctic isolate were consistent with those of the genus Flavobacterium. In contrast, several phenotypic characters can be used to differentiate this isolate from other flavobacteria. The polyphasic data presented in this study indicated that this isolate should be classified as a novel species in the genus Flavobacterium. The name Flavobacterium antarcticum sp. nov. is therefore proposed for the Antarctic isolate; the type strain is AT1026^T (=IMSNU 14042^T = KCTC 12222^T = JCM 12383^T).

A soil sample was collected from a penguin habitat near the King Sejong Station on King George Island, Antarctica (62° 14' 01.2" S 58° 46' 47.4" W). Isolation was carried out using marine agar 2216 (MA; Difco) at 10 °C following enrichment for 2 days in marine broth 2216 at 4 °C. The isolate was cultured routinely on R2A (Difco) at 15 °C and maintained as a glycerol suspension (20%, w/v) at −80 °C.

16S rRNA genes were enzymically amplified from a single colony. Primers, PCR conditions and sequencing methods were described elsewhere (Chun & Goodfellow, 1995). The sequence of strain AT1026^T was aligned manually with representative sequences of the family Flavobacteriaceae obtained from GenBank. Phylogenetic trees were inferred using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). Resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at http://chunlab.snu.ac.kr/jphydit) and PAUP 4.0 (Swofford, 1998) as described previously (Chun et al., 2000). An almost complete 16S rRNA gene sequence of strain AT1026^T (1406 bp) was obtained. Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was related closely to the genus Flavobacterium. The newly determined sequence was then aligned manually against representatives of Flavobacterium species using bacterial 16S rRNA secondary structure. The regions available for all sequences (positions 46–1477; Escherichia coli numbering system), excluding positions likely to show ambiguous alignment (positions 76–94), were used to...
construct the phylogenetic trees. Based on 16S rRNA gene sequence similarity, the Antarctic isolate shared 91.3–96.4% similarity with members of the genus *Flavobacterium* and the closest bacterial relatives with validly published names were *Flavobacterium tegetincola* (96.4%), *Flavobacterium flevense* (95.9%), *Flavobacterium micromati* (95.6%) and *Flavobacterium xinjiangense* (95.5%). Strain AT1026T and *F. tegetincola* formed a robust (96% bootstrap value) monophyletic clade (Fig. 1) in all trees inferred in this study, and formed a further monophyletic clade with *F. flevense* and *Flavobacterium johnsoniae* in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. On the basis of our phylogenetic analysis, it is evident that our isolate is a member of the genus *Flavobacterium* and represents a novel genomic species.

Growth on several bacteriological media was tested; Anacker and Ordal agar [AOA; Bacto agar (Difco), 10 g; Casitone (Difco), 0.5 g; yeast extract (Difco), 0.5 g; sodium acetate, 0.2 g; beef extract (Difco), 0.2 g; distilled water, 1 l], cetrimide agar (Difco), MacConkey agar (Difco), MA, nutrient agar (NA; Difco), R2A, tryptic soy agar (TSA; Difco) and sea-salt-free Zobell’s agar [Bacto agar, 15 g; Bacto peptone (Difco), 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water, 1 l]. Maximum growth was observed on R2A and abundant growth was observed on AOA, MA, NA, TSA and sea-salt-free Zobell’s agar. No growth was observed on cetrimide or MacConkey agar. The temperature range for growth was determined in a temperature-gradient incubator (TVS 126MA; Advantec) using R2A broth in the range 5–35°C (50, 9.7, 12.4, 15.1, 17.4, 19.6, 21.8, 24.1, 26.6, 28.9, 31.9 and 35.0°C). To determine cardinal temperatures, the resultant data were fitted to the Ratkowsky temperature growth model (Ratkowsky et al., 1983) by non-linear regression using the R 1.8.1 package (R Foundation for Statistical Computing, 2003). Square-root growth rate–temperature plots showed that the notional minimum, optimum and maximum growth temperatures were respectively −14.5, 21.2 and 25.1°C (Fig. 2). When tested on R2A (between 5 and 35°C at 5°C intervals), strain AT1026T grew at 25°C, but not at 30°C. From the definition of Isaksen & Jørgensen (1996), our Antarctic isolate can be defined as a psychrotolerant bacterium. The minimum doubling time of strain AT1026T was about 4.9 h. Growth at different pH (between pH 4 and 12 with an interval of 1) and NaCl concentrations [between 0 and 7% (w/v)] at 1% concentration.
intervals] was determined using sea-salt-free ZoBell’s
medium. KOH and HCl (both at 6 M) were used to
adjust the final pH. Anaerobic and microaerophilic growth
was checked under anaerobic (with 4–10 % CO₂) and
microaerobic (with 5–15 % O₂ and 5–12 % CO₂) condi-
tions, using GasPak Plus and CampyPak Plus systems (BBL)
at 15 °C for up to 1 month. The results of tests for growth
conditions are given in the species description.

Morphological and physiological tests were performed
using R2A as the basal medium at 15 °C. Cellular morpho-
logy and motility were examined by SEM and phase-
contrast microscopy using 3-, 5- and 10-day-old cells. Gliding
motility was observed by direct microscopic
examination of the edge of colonies in exponential phase
on AOA, R2A and CY agar [casitone (Difco), 3 g; yeast
extract, 1 g; CaCl₂2H₂O, 1 g; sea salts (Sigma), 40 g; Bacto
agar, 15 g; distilled water, 1 l] plates, and motility was
observed by the hanging drop technique for cells in
exponential phase in R2A and CY broth. The presence of
flexirubin-type pigments was determined by flooding the
agar plate or biomass with 20 % (w/v) KOH and confirmed
by measuring the absorbance spectrum of an ethanol
and alkaline-ethanol extract of lysed cells (Weeks, 1981). Congo
red adsorption was tested by directly flooding colonies on
agar plates with 0·01 % aqueous Congo red solution.

Standard physiological and biochemical tests were per-
formed at 15 °C as described previously (Smibert & Krieg,
1994). Hydrolysis of alginate (0·5 %, w/v), casein [50 %
skimmed milk (Difco), v/v], carboxymethyl cellulose
[0·5 % carboxymethyl cellulose (Sigma), w/v], chitin (0·5 %
colloidal chitin, w/v), egg yolk (5 %, w/v), elastin (0·5 %,
w/v), starch (0·2 %, w/v), Tween 80 (1 %, v/v) and
L-tyrosine (0·5 %, w/v) was tested using R2A as the basal
medium. PEK7 agar (Reichenbach, 1991) and DNase test
agar (Difco) were respectively used for pectinase and DNase
assays. Production of H₂S was investigated using triple-
sugar iron agar (Difco). Phenylalanine deaminase activity
was determined on phenylalanine agar (Smibert & Krieg,
1994; yeast extract, 3 g; l-phenylalanine, 1 g; Na₂HPO₄, 1 g;
NaCl, 5 g; Bacto agar, 12 g; distilled water, 1 l). Alkaline
reaction on Christensen’s citrate was tested on Christensen
citrate agar (Christensen, 1949). Arginine dihydrolase
and urease activities were determined using Thornley’s semi-
solid medium (Thornley, 1960) and Christensen urea agar
(Christensen, 1946), respectively. Acid production from
carbohydrates was examined for up to 1 month using
modified O/F agar plates (Leifson, 1963; casitone, 1·0 g;
yeast extract, 0·1 g; ammonium sulfate, 0·5 g; Tris base,
0·5 g; phenol red, 0·01 g; Bacto agar, 15 g; distilled water,
1 l; adjusted to pH 7·0). Nitrate and nitrite reduction,
indole production, aesculine, gelatinase, β-galactosidase
and assimilation of sole carbon sources (glucose, arabinose,
mannose, mannitol, N-acetylgalcosamine, maltose, glucos-
ate, caprate, adipate, malate, citrate and phenylacetate)
were tested using the API 20NE kit (bioMérieux), and other
enzymic activities were determined using the API ZYM kit
(bioMérieux). The results of morphological, biochemical
and physiological tests are given in Table 1 and the species
description.

Chemotaxonomic and genotypic characteristics were deter-
mined from cultures grown at 15 °C on R2A. Menaquinone
was isolated from 7-day-old cells according to Minnikin
et al. (1984) and analysed by HPLC (Waters) as described
by Collins (1985). DNA G+C content was determined by
HPLC analysis of deoxyribonucleosides as described by
Mesbah et al. (1989), using a reverse-phase column
(Supelco). Fatty acid methyl esters analysis was performed by
GLC according to the Microbial Identification (MIDI)
System using 5-day-old cells. The chemotaxonomic
properties of the test strain are consistent with those of
the genus Flavobacterium and are given in the species
description. The major fatty acid composition of our isolate
is similar to those of phylogenetically related species, but
differs somewhat from them in quantities (Bernardet et al.,
1996; McCammon & Bowman, 2000).

In the phylogenetic trees, our Antarctic isolate clearly
belongs to the genus Flavobacterium and forms a distinct
phyletic line with low 16S rRNA gene sequence similarity
(96·8 %), which indicates that it represents a novel genomic
species (Stackebrandt & Goebel, 1994). Moreover, a number
of phenotypic characteristics (Table 1) readily differentiate
the Antarctic strain from other related Flavobacterium
species. The polyphasic data obtained in this study clearly
show that the test strain merits novel species status within
the genus Flavobacterium. The name Flavobacterium anta-
rcticum sp. nov. is therefore proposed for strain AT1026ᵀ.

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<td>β-Galactosidase activity</td>
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*On API 20NE test strip.
Description of Flavobacterium antarcticum sp. nov.

Flavobacterium antarcticum (ant.arc’ti.cum. L. neut. adj. antarcticum southern, and, by extension, pertaining to Antarctica).

Gram-negative, oxidase- and catalase-positive and psychrotolerant. Cells are rod-shaped with rounded ends, approximately 0.5–1.3 x 0.3–0.4 μm and non-motile. Colonies are convex, translucent, glistening, butyrous, yellow, circular with entire margins and becoming mucoid after prolonged incubation on R2A and AOA. Does not glide or adhere to agar plates. Flexirubin-type pigment is absent. Congo red is not adsorbed. Spores are not formed. Growth occurs on R2A, AOA, MA, NA and TSA, but not on cetrimide or MacConkey agar. Growth is aerobic. Grows weakly under microaerobic conditions (with about 5–10 % O2 and 5–12 % CO2 created by CampyPak Plus system) and poorly under anaerobic conditions (with about 4–10 % CO2 created by GasPak Plus system). Growth occurs at pH 6–10 (optimum pH 7) and 0–4 % NaCl (optimum 0 %). Grows at 5–24 °C, with notional minimum, optimum and maximum growth temperatures of −14.5, 21.2 and 25.1 °C. Minimum doubling time is 4.9 h. Decomposes Tween 80, but not alginates, chitin, carboxymethyl cellulose, elastin, starch or tyrosine. Produces brown pigment weakly on tyrosine agar. Positive reaction for arginine dihydrolase. Negative reactions for nitrate reduction, urease, L-phenylalanine deaminase, H2S production, indole production and alkalization on Christensen’s citrate agar. Produces acid from D-glucose and maltose, but not from D-cellobiose, D-xylose, lactose, L-arabinose, L-rhamnose or sucrose. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, -mannitol, D-rafﬁnose, D-salicyl, D-trehalose, D-xylose, lactose, L-arabinose, L-rhamnose or sucrose. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive; trypsin, α-glucosidase and N-acetyl-β-glucosaminidase are weakly positive; esterase (C4), lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-1,3-mannosidase and α-fucosidase are negative in API ZYM kits. Cannot assimilate any of the compounds contained in API 20NE kits as sole carbon sources. Other physiological and biochemical characteristics are given in Table 1. Maximum absorption peak of pigment is at 452 nm and the next shoulder peak is at 479 nm. Major physiological and biochemical characteristics are given in Table 1. Maximum absorption peak of pigment is at 452 nm and the next shoulder peak is at 479 nm. Major physiological and biochemical characteristics are given in Table 1. Maximum absorption peak of pigment is at 452 nm and the next shoulder peak is at 479 nm. Major physiological and biochemical characteristics are given in Table 1.

The type strain, AT1026T (=IMSMNU 14042T = KCTC 12222T = JCM 12383T), was isolated from a soil sample of a penguin habitat near the King Sejong Station on King George Island, Antarctica.

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References


