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TAXONOMIC DESCRIPTION
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# *Flavitalea antarctica* sp. nov., isolated from Fildes Peninsula, Antarctica

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**Abstract**

A bright-yellow, Gram-stain-negative, rod-shaped, gliding and aerobic bacterium, designated strain AQ6-291$^T$, was isolated from the Fildes Peninsula, Antarctica, and its taxonomic position was investigated by genotypic, phenotypic and chemotaxonomic analyses. Growth occurred at 4–28 °C (optimum 20 °C) and at pH 5.0–8.0 (optimum pH 7.0). Strain AQ6-291$^T$ contained iso-$C_{15:1}$ G, iso-$C_{15:0}$, $C_{16:1}\omega5c$, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) as the major cellular fatty acids. The main polar lipids were phosphatidylethanolamine, unknown aminophospholipids, unknown phospholipids, five unknown aminolipids and two unknown polar lipids. MK-7 was the major respiratory quinone. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain AQ6-291$^T$ belonged to the genus *Flavitalea*. The DNA G+C content was 48.1 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain AQ6-291$^T$ is considered to represent a novel species of the genus *Flavitalea*, for which the name *Flavitalea antarctica* sp. nov. is proposed. The type strain is AQ6-291$^T$ (=CCTCC AB 2016109$^T$=KCTC 52491$^T$).

The genus *Flavitalea* was first described by Wang *et al.* [1] and this description was later emended by Zhang *et al.* [2]. At the time of writing, the genus *Flavitalea* comprised two recognized species, isolated from soil. Both are Gram-stain-negative, strictly aerobic, rod-shaped and non-motile, and have MK-7 as the major menaquinone, DNA G+C contents of 46.8–47.1 mol% and homospermidine as the major polyamine. In this study, an Antarctic bacterial isolate was subjected to a polyphasic analysis and was identified as a novel member of the genus *Flavitalea*.

A bacterial strain, designated AQ6-291$^T$, was isolated from a soil sample collected from Great Wall Station (62° 12.986′ S 058° 57.861′ W) in the Fildes Peninsula, Antarctica. The soil sample was diluted serially with a sterile 0.9 % (w/v) NaCl solution, and isolation was carried out using the standard dilution plating method on 0.5× R2A agar (Becton, Dickinson and Company; BD) at 4 °C. Single colonies on these plates were purified by transferring them onto new plates and subjecting them to additional incubation for 14 days at 4 °C. The isolate was routinely cultivated on R2A agar at 20 °C and stored following lyophilization at 4 °C.

Genomic DNA was prepared from a fresh culture of strain AQ6-291$^T$ following the methods of Sambrook *et al.* [3]. The 16S rRNA gene of strain AQ6-291$^T$ was amplified by PCR with universal bacterial primer pair 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [4] and the PCR products were sequenced by Invitrogen Biotechnology. Sequence similarity was investigated using NCBI BLAST and pairwise alignment was calculated using the EzTaxon database [5]. Further phylogenetic analysis was performed by using the software package MEGA version 6.0 [6] after multiple alignment of the data via CLUSTAL X [7]. Phylogenetic trees were reconstructed by using the neighbour-joining [8], maximum-likelihood [9] and minimum-evolution [10] methods. Evolutionary distances for the neighbour-joining algorithm were calculated with Kimura's two-parameter method [11] and the topologies of the phylogenetic trees were evaluated by the bootstrap resampling method of Felsenstein [12] with 1000 replicates. The root position was estimated by using the sequence of *Flavobacterium aquatile* ATCC 11947$^T$ (GenBank accession number M62797) as an outgroup.

The 16S rRNA gene sequence of strain AQ6-291$^T$ was a continuous stretch of 1420 bp. According to the EzTaxon-e server, the closest relatives of strain AQ6-291$^T$ were *Flavitalea populi* HY-50R$^T$ (95.1 % 16S rRNA gene sequence similarity) and *Flavitalea gansuensis* JCN-23$^T$ (94.6 %). The

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AQ6-291$^T$ is KX146487.
One supplementary figure is available with the online Supplementary Material.

neighbour-joining tree (Fig. 1) showed that strain AQ6-291$^T$ was grouped with members of the genus *Flavitalea* and formed a distinct cluster distinguishable from other genera. The topology of the maximum-likelihood and minimum-evolution trees was the same as that of the neighbour-joining tree. On the basis of the phylogenetic data, it is clear that strain AQ6-291$^T$ should be classified in the genus *Flavitalea*. To determine the exact taxonomic position of the new isolate, *F. populi* HY-50R$^T$, *F. gansuensis* JCN-23$^T$ and *Pseudoflavitalea soli* KIS20-3$^T$ were selected as reference strains. Prior to testing, all strains were incubated aerobically for 1 or 3 days on R2A agar (BD) at the optimum temperature for their growth.

Growth of strain AQ6-291$^T$ was evaluated on several standard bacteriological media: R2A agar, 0.5× R2A agar, tryptic soy broth (TSB) agar, 0.1× TSB agar, nutrient agar (NA), marine broth 2216 agar (MA) and MacConkey agar (all from BD). Growth at different temperatures (4, 10, 15, 20, 28, 30, 37 and 42 °C) was investigated on R2A agar for up to 1 week. Tolerance of NaCl for growth was determined at 0, 0.5, 1, 2, 3, 4 and 5 % (w/v) on R2A agar. The pH range for growth was investigated by using filter-sterilized R2A broth adjusted to pH 4.0–11.0 by using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M $KH_2PO_4$/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M $Na_2CO_3$; pH 11.0, 0.05 M $Na_2HPO_4$/

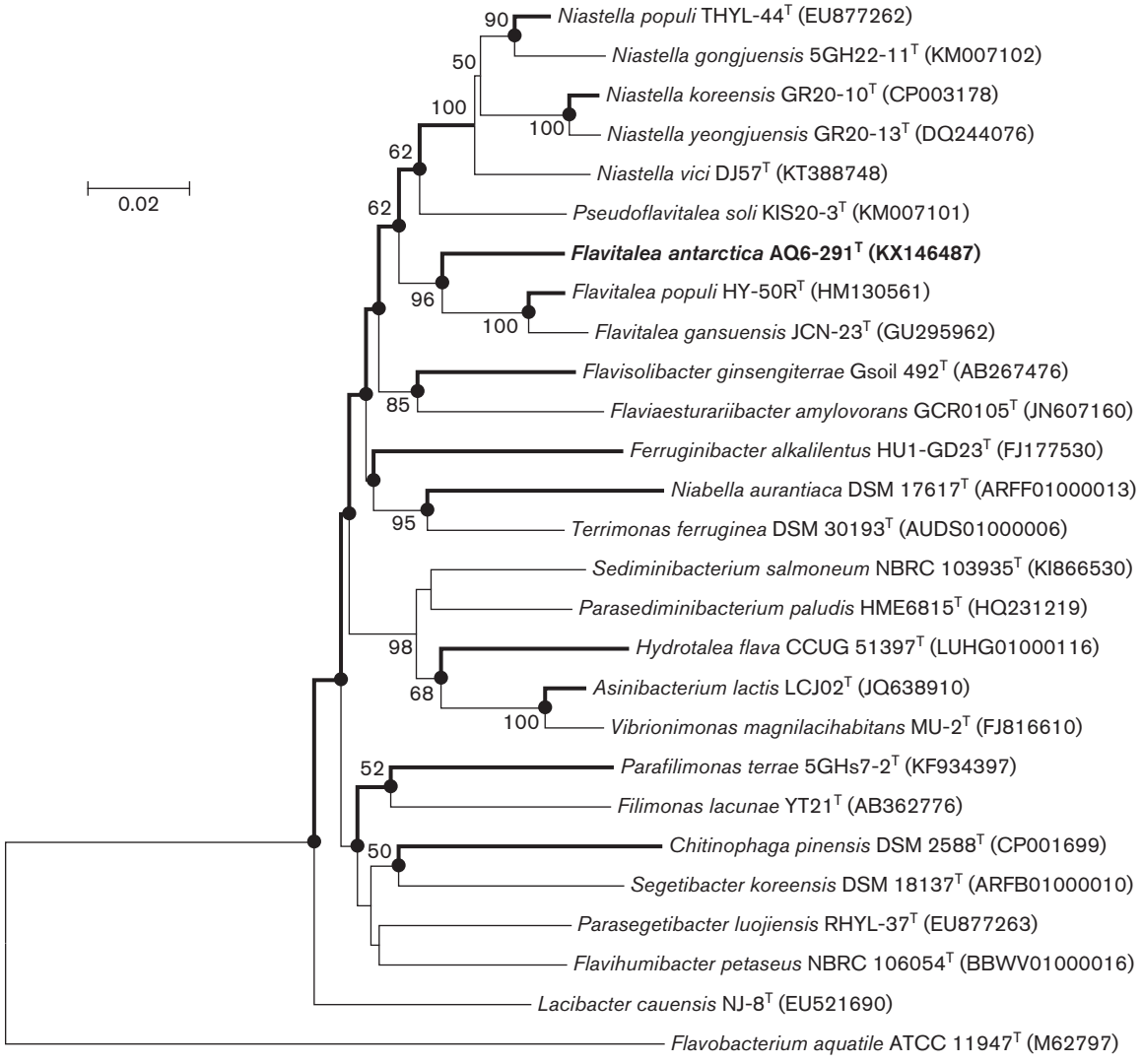


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain AQ6-291$^T$ in relation to *F. populi* HY-50R$^T$ and *F. gansuensis* JCN-23$^T$. Bootstrap values (expressed as percentages of 1000 replications) of 50 % or above are shown at branch points. The 16S rRNA gene sequence of *Flavobacterium aquatile* ATCC 11947$^T$ was used as an outgroup. Bar, 0.02 substitutions per nucleotide position. Filled circles indicate nodes of the tree supported by both the maximum-likelihood and the minimum-evolution algorithms.

0.1 M NaOH. Cell morphology was examined by phase-contrast (Olympus BX51) and transmission electron (Hitachi 8100) microscopy using cells grown on R2A agar at 20 °C for 3 days. Gliding motility was tested according to the procedure described by Bowman [13]. Gram staining was performed according to the classical Gram procedure described by Doetsch [14]. Anaerobic growth was assessed in an anaerobic chamber (Oxoid) on R2A agar supplemented with 0.1 % $KNO_3$ for 1 month. Oxidase activity was evaluated via the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine [15]. Catalase activity was determined by measurement of bubble production after the application of 3 % (v/v) hydrogen peroxide solution. Hydrolysis of casein, starch, gelatin, CM-cellulose, chitin or tyrosine was determined according to the methods of Smibert and Krieg [16], using R2A agar as the basal medium. Determination of flexirubin-type pigments was investigated using the bathochromatic shift test with a 20 % (w/v) KOH solution [17, 18]. Cellular pigments were extracted according to Weeks [19] and a spectrum was obtained using an enzyme-labelled instrument (BioTek). Other physiological properties and enzyme activities were determined with API 20NE, API 20E, ID 32GN and API ZYM galleries (bioMérieux), according to the manufacturer's instructions.

Cells of strain AQ6-291$^T$ were Gram-stain-negative, non-endospore-forming, motile by gliding, aerobic, rods (0.2–0.4×1.5–2.5 μm). Strain AQ6-291$^T$ grew well on R2A agar, 0.5× R2A agar, 0.1× TSB agar and NA, but not on TSB agar, MA or MacConkey agar. Colonies on R2A agar were circular, convex, smooth, shiny, bright yellow and 1.0–2.0 mm in diameter within 3 days at 20 °C. The temperature range for growth of strain AQ6-291$^T$ was 4–28 °C (optimum, 20 °C). Growth occurred at pH 5.0–8.0 (optimum pH 6.0–7.0) and the range of NaCl for growth was 0–0.5 % (w/v); optimum growth occurred without NaCl added to R2A agar. Weakly positive for oxidase and positive for catalase. Other physiological characteristics of strain AQ6-291$^T$ are summarized in the species description. Selected characteristics that differentiate strain AQ6-291$^T$ from related species of the genus *Flavitalea* and *P. soli* are shown in Table 1.

To measure the G+C content of the chromosomal DNA, genomic DNA from the novel strain was extracted and purified as described by Moore and Dowhan [20] and degraded enzymically into nucleosides and the G+C content was then investigated by reversed-phase HPLC (UltiMate 3000; Dionex) according to the method of Mesbah and Whitman [21]. Respiratory quinones were extracted and identified by HPLC as described by Xie and Yokota [22]. For analysis of cellular fatty acids, strain AQ6-291$^T$ and the two most closely related type strains, *F. populi* HY-50R$^T$ and *F. gansuensis* JCN-23$^T$, were grown on R2A agar plates at 20 °C, and cells from the late exponential phase of growth were used in this study. Harvesting, saponification, methylation and extraction of cellular fatty acids were carried out according to the protocol of the Sherlock Microbial Identification System (MIDI) version 6.0. Separation and identification of fatty acid methyl esters was performed using a Hewlett Packard 6890 N gas chromatograph, with MIDI Sherlock TSBA 6 (version of the database) [23]. For polar lipid analysis, polar lipids were extracted and detected using two-dimensional TLC (silica gel plates, layer thickness 0.2 mm; Merck) as described by Tindall [24]. The polyamines of strain AQ6-291$^T$ were extracted and analysed as described by Busse and Auling [25] and Busse *et al.* [26].

**Table 1.** Differential characteristics between strain AQ6-291$^T$ and related species

Strains: 1, AQ6-291$^T$; 2, *F. populi* HY-50R$^T$; 3, *F. gansuensis* JCN-23$^T$; 4, *P. soli*KIS20-3$^T$. +, Positive reaction; −, negative reaction; w, weakly positive reaction. Data were taken from this study unless otherwise indicated.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Motility | + | − | + | +[c] |
| Growth at (in): | | | | |
| 4 °C | + | −[a] | −[b] | −[c] |
| 37 °C | − | +[a] | +[b] | +[c] |
| 1 % (w/v) NaCl | − | +[a] | +[b] | +[c] |
| Oxidase | + | − | w | +[c] |
| Catalase | w | + | + | +[c] |
| Hydrolysis of: | | | | |
| Casein | − | − | − | +[c] |
| Gelatin | − | − | − | +[c] |
| Tyrosine | − | − | − | +[c] |
| Assimilation of: | | | | |
| Sucrose | w | + | − | +[c] |
| Maltose | − | − | + | +[c] |
| D-Glucose | − | − | + | +[c] |
| Salicin | − | − | + | +[c] |
| Melibiose | − | − | + | +[c] |
| Capric acid | + | − | − | −[c] |
| Enzyme activity: | | | | |
| Valine arylamidase | w | w | + | +[c] |
| α-Mannosidase | − | w | w | −[c] |
| Arginine dihydrolase | + | − | − | −[c] |
| DNA G+C content (mol %) | 48.1 | 46.8[a] | 47.1[b] | 55.7[c] |

Reference data were taken from: *a*, Wang *et al.* [1]; *b*, Zhang *et al.* [2]; *c*, Kim *et al.* [27].

The DNA G+C content of strain AQ6-291$^T$ was 48.1 mol%, which is relatively higher than that previously reported for the genus *Flavitalea* (46.8–47.1 mol%). MK-7 was the only respiratory quinone of strain AQ6-291$^T$. Strain AQ6-291$^T$ contained iso-$C_{15:1}$ G (23.65 %), iso-$C_{15:0}$ (13.03 %), $C_{16:1}$ ω5*c* (16.32 %), iso-$C_{17:0}$ 3-OH (13.55 %) and summed feature 3 (comprising $C_{16:1}$ω7*c* and/or $C_{16:1}$ω6*c*, 16.03 %) as the major cellular fatty acids. The fatty acid profile of the novel isolate was similar to that of other recognized species of the genus *Flavitalea*, but differed in terms of proportions. A comparison of the fatty acid profiles of strain AQ6-291$^T$ and the reference strains is shown in Table 2. The main

**Table 2.** Comparation of the cellular fatty acid contents of strain AQ6-291$^T$ and the type strains of phylogenetically related species

Strains: 1, AQ6-291$^T$; 2, *F. populi* HY-50R$^T$; 3, *F. gansuensis* JCN-23$^T$ (data for 1–3 are all from this study); 4, *P. soli* KIS20-3$^T$ (data from Kim *et al.* [27]). Values are percentages of the total fatty acids; fatty acids amounting to <1 % of the total fatty acids in all strains listed are omitted. –, Not detected; TR, trace amount (<1 %).

| Fatty acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| iso-$C_{13:0}$ | TR | 5.54 | 3.67 | 1.0 |
| iso-$C_{13:0}$ 3-OH | – | 3.99 | 3.4 | – |
| iso-$C_{15:1}$ G | 23.65 | 14.04 | 1.98 | 19.3 |
| anteiso-$C_{15:1}$ A | – | 4.91 | TR | 1.0 |
| iso-$C_{15:0}$ | 13.03 | 15.25 | 28.83 | 30.9 |
| anteiso-$C_{15:0}$ | TR | 4.97 | 4.33 | 4.0 |
| $C_{16:1}\omega5c$ | 16.32 | 3.14 | – | – |
| $C_{16:0}$ | 3.46 | 2.21 | 4.85 | 2.1 |
| $C_{16:0}$ 3-OH | 2.87 | – | – | 2.4 |
| iso-$C_{17:0}$ | TR | 7.63 | 1.72 | – |
| anteiso-$C_{17:0}$ | TR | 1.55 | TR | – |
| $C_{18:1}\omega9c$ | TR | | TR | – |
| iso-$C_{17:0}$ 3-OH | 13.55 | 16.84 | 16.33 | 22.9 |
| Summed features* | | | | |
| 1 | – | 4.13 | – | – |
| 3 | 16.03 | 4.82 | 7.8 | 5.6 |
| 4 | – | 4.72 | – | – |
| 5 | TR | 1.33 | TR | – |
| 8 | TR | 1.01 | – | – |
| 9 | – | – | 19.4 | – |

*Summed features are groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ h; summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 4 contained anteiso-$C_{17:1}$ B and iso-$C_{17:1}$ I; summed feature 5 contained anteiso-$C_{18:0}$ and/or $C_{18:2}\omega6$, 9*c*; summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9 contained $C_{17:1}\omega9c$ and/or $C_{16:0}$10-methyl.

polar lipids of strain AQ6-291$^T$ were phosphatidylethanolamine, unknown aminolipids and unknown polar lipids. In addition, unknown aminophospholipids, an unknown phospholipid and two unknown lipids distinguished strain AQ6-291$^T$ from the two reference strains (Fig. S1, available in the online Supplementary Material). The polyamine pattern of strain AQ6-291$^T$ was homospermidine and was the same as that of *F. gansuensis* JCN-23$^T$, as previously reported [2].

On the basis of 16S rRNA gene sequence analysis, and physiological and biochemical characteristics, strain AQ6-291$^T$ should be classified as a member of a novel species of the genus *Flavitalea*, for which the name *Flavitalea antarctica* sp. nov. is proposed.

## DESCRIPTION OF *FLAVITALEA ANTARCTICA* SP. NOV.

*Flavitalea antarctica* (ant.arc'ti.ca. L. fem. adj. *antarctica* southern, and by extension pertaining to Antarctica).

Cells are Gram-stain-negative, non-endospore-forming, motile by gliding, strictly aerobic rods (approximately 0.2–0.4×1.5–2.5 µm). Colonies are bright yellow, circular, convex and smooth after growth for 3 days at 20 °C on R2A agar. Growth occurs at 4–28 (optimum 20 °C), at pH 5.0–8.0 (optimum pH 7.0) and in the presence of 0–0.5 % (w/v) NaCl (optimal growth without NaCl). Grows well on R2A agar, 0.5× R2A agar, 0.1× TSB agar and NA, but not on TSB agar, MA or MacConkey agar. Flexirubin-type pigments are not formed and carotenoid pigments with absorbance peaks at 450 and 470–480 nm are not produced. Weakly positive for oxidase and positive for catalase. Hydrolyses aesculin but not gelatin, starch, casein, tyrosine, chitin or CM-cellulose. Nitrate is not reduced to nitrite and hydrogen sulfide is not produced. Indole production, glucose acidification, arginine dihydrolase and urease are negative but citrate utilization is positive. Positive for β-glucosidase, L-arabinose, alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and β-fucosidase, weakly positive for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase and α-mannosidase, but negative for trypsin, α-chymotrypsin and β-glucuronidase. Does not utilize the following compounds as sole carbon sources: L-rhamnose, *N*-acetylglucosamine, L-rhamnose, inositol, sucrose, maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, melibiose, L-fucose, D-sorbitol, L-arabinose, propionic acid, caprate, valeric acid, citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline. The only respiratory quinone is MK-7, the major polyamine is homospermidine and the predominant cellular fatty acids are iso-$C_{15:1}$ G, iso-$C_{15:0}$, $C_{16:1}\omega5c$, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The major polar lipid is phosphatidylethanolamine; unknown aminophospholipids, unknown phospholipids, five unknown aminolipids and two unknown polar lipids are also detected.

The type strain, AQ6-291$^T$ (=CCTCC AB 2016109$^T$=KCTC 52491$^T$), was isolated from a soil sample collected from Great Wall Station in the Fildes Peninsula, Antarctica. The DNA G+C content of the type strain is 48.1 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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