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TAXONOMIC DESCRIPTION
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MICROBIOLOGY SOCIETY

Hymenobacter artigasi sp. nov., isolated from air sampling in maritime Antarctica

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Abstract

A rod-shaped and Gram-stain-negative bacterial strain, $1B^T$, was isolated from an air sample collected at King George Island, maritime Antarctica. Strain $1B^T$ is strictly aerobic, psychrophilic, catalase-positive, oxidase-positive and non-motile. Growth of strain $1B^T$ is observed at 0–20 °C (optimum, 10 °C), pH 6.0–8.0 (optimum, pH 8.0) and in the presence of 0–1.0% NaCl (optimum, 0.5% NaCl). Phylogenetic analysis based on 16S rRNA gene sequences places strain $1B^T$ within the genus *Hymenobacter* and shows the highest similarity to *Hymenobacter antarcticus* VUG-A42aa^T (97.5%). The predominant menaquinone of strain $1B^T$ is MK-7 and the major fatty acids (>10%) comprise summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 32.5%), iso-$C_{15:0}$ (17.6%) and anteiso $C_{15:0}$ (12.3%). The polar lipid profile consists of the major compounds phosphatidylethanolamine, phosphatidylserine, two unidentified aminolipids and one unidentified phospholipid. The DNA G+C content based on the draft genome sequence is 61.2 mol%. Based on the data from the current polyphasic study, $1B^T$ represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter artigasi* sp. nov. is suggested. The type strain is $1B^T$ (=CCM 8970^T=CGMCC 1.16843^T).

The genus *Hymenobacter* [1] belongs to the family *Hymenobacteraceae* [2], order *Cytophagales*, phylum *Bacteroidetes* and was first proposed by Hirsch *et al.* in 1998 [1] and emended by Buczolits *et al.* (2006) [3], Reddy (2013)] [4] and Han *et al.* (2014)] [5]. It is a genus of rod-shaped, red-pink or pink pigmented, aerobic and heterotrophic bacteria [6] with a preference for oligotrophic media. Species of this genus have recently been isolated from many cold environments, e.g. sediment in the permafrost [5, 7], Antarctic rocks [8], Antarctic soils [1, 9] and snow [10, 11]. At the time of writing, there were 82 species of the genus *Hymenobacter* recorded on the LPSN (https://lpsn.dsmz.de/genus/hymenobacter, accessed March 2020).

The colonization success of propagules undergoing airborne transport to Antarctica depends upon survival in harsh conditions and viability in the new niche. Here, classification of one airborne isolate and its description as a novel species of the genus *Hymenobacter* is reported. This study shows that Antarctic air is an interesting ecosystem for isolating novel species.

ISOLATION AND ECOLOGY

With the aim of studying the diversity of cultivable bacteria from air in the Fildes peninsula, King George Island (62° 08′–62° 14′ S and 59° 02′–58° 51′ W, maritime Antarctica), air samples were collected on Reasoner's 2A (R2A) agar (Oxoid) media and incubated at 5 °C for up to 30 days to obtain isolated colonies. The sample was collected at 1.5 m height in February 2018 from outdoor air at Antarctic Scientific Base Artigas (62° 11′ 04″ S 58° 51′ 07″ W) area. A Gram-stain-negative rod, designated strain $1B^T$, was purified and selected for further characterization from a collection of more than 100 strains collected in different days. This unique strain was routinely grown on R2A agar medium and maintained at 4 °C

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Keywords: *Hymenobacter*; psychrophiles; bacterial colonization; airborne bacteria; maritime Antarctica; oligotrophy.
Abbreviations: ANI, average nucleotide identity; BHI, brain heart infusion; FAME, fatty acid methyl ester; LB, Luria–Bertani; NA, nutrient agar; NB, nutrient broth; PCA, plate count agar; R2A, Reasoner's 2A; TSA, tryptic soy agar; TSB, tryptic soy broth; VRBGA, violet red bile glucose agar.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence for strain $1B^T$ is MN007169. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAAVTK000000000. The version described in this paper is version JAAVTK010000000.
Two supplementary tables and four supplementary figures are available with the online version of this article.

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for short-term preservation or lyophilized for long storage. Reference strains *Hymenobacter antarcticus* CCM 8582^T and *Hymenobacter glaciei* CCM 8583^T were obtained from the Czech Collection of Microorganisms (www.sci.muni.cz/ccm/) and were used for subsequent comparison. All strains were routinely cultivated on R2A agar for 48–72 h at 15 °C.

16S rRNA GENE PHYLOGENY

Extraction of genomic DNA was carried out with FastDNA (MP Biomedicals) Lysing Matrix type A and amplification of nearly full-length 16S rRNA gene fragments was performed as described previously [12]. The sequence was compared to other publicly available sequences in GenBank of type strains with validly published prokaryotic names. The pairwise sequence similarity was calculated using the global alignment algorithm, which was implemented at the EzBioCloud server [13]. Phylogenetic trees were inferred by three different methods: neighbour-joining [14], maximum-likelihood [15] and maximum-parsimony [16]. The phylogenetic analysis used 1347 positions. Trees were generated by using the MEGA version 7.0 software package [17]. Kimura's two-parameter model [18] was used to calculate evolutionary distance matrices of the neighbour-joining and maximum-likelihood phylogenetic trees. Bootstrap analyses (1000 replicates) were performed in order to assess the reliability of the inferred branches [19].

Analysis of a near-complete 16S rRNA gene sequence of strain 1B^T in the EzBioCloud server revealed that it had highest sequence similarities to *H. antarcticus* VUG-A42aa^T (97.5%) and below 97.0% with other *Hymenobacter* species type strains with validly published names. The phylogenetic tree reconstructed from evolutionary distances of Kimura's two-correction parameter by the neighbour-joining method (Fig. 1) revealed clear affiliations of the novel isolate to the genus *Hymenobacter* and clustered in a clade with *H. antarcticus* VUG-A42aa^T and *Hymenobacter frigidus* B1789^T with moderate bootstrap support (62%). The phylogenetic position was also confirmed by the trees generated using the maximum-likelihood and maximum-parsimony methods with similar topologies (Figs S1 and S2, available in the online version of this article).

GENOME FEATURES

The draft genome of strain 1B^T was generated at the DOE Joint Genome Institute (JGI) using Illumina technology [20]. Plate-based DNA library preparation for Illumina sequencing was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using the Kapa Biosystems library preparation kit. Sample DNA (200 ng) was sheared to 300 bp using a Covaris LE220 focused-ultrasonicator. An Illumina standard shotgun library was reconstructed and sequenced using the Illumina NovaSeq S4 platform which generated 12904748 reads totaling 1948616948 bp. Raw Illumina sequence was quality filtered using BBTools [21] per SOP 1061. The following steps were then performed for assembly:artifact-filtered and normalized Illumina reads were assembled with SPAdes (version 3.13.0; –phred-offset 33 –cov-cutoff auto -t 16 m 64–careful -k 25,55,95) [22] and contigs were discarded if the length was <1 kb (BBTools reformat.sh: minlength=1000ow=t). CheckM [23] was used to derive the level of completeness and contamination of the final assembly, which yielded estimates of 99.7 and 0.6%, respectively. Authenticity of the genome was verified via MegaBlast against 16S rRNA gene using the Silva SSU database [24].

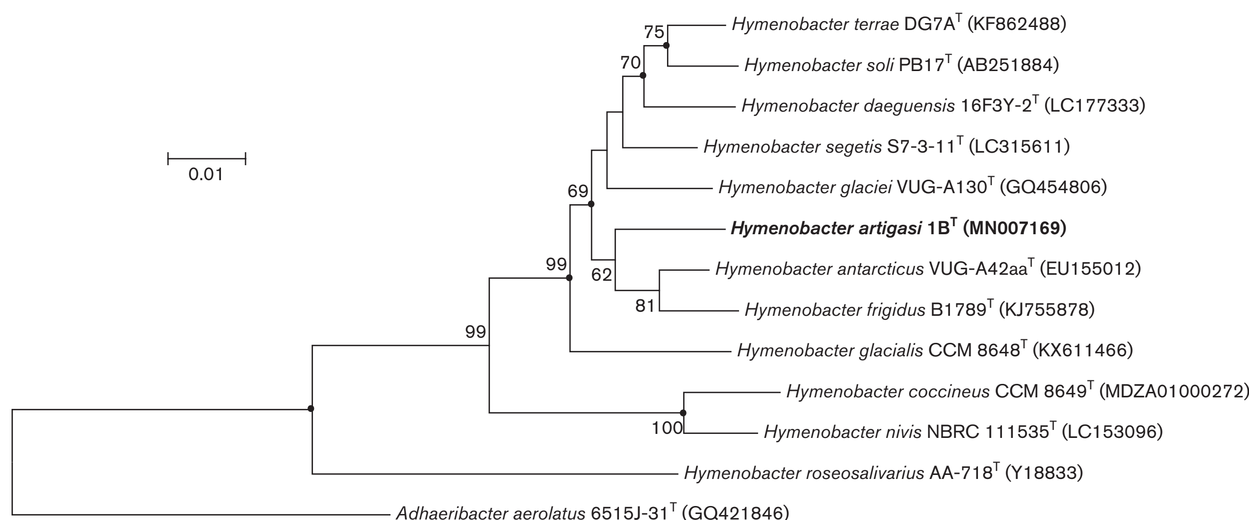

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relation of strain 1B^T and closely related species. Bootstrap values (above 50%) based on 1000 re-samplings are listed at the nodes. Filled circles indicate branches that were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. *Adhaeribacter aerolatus* 6515 J-31^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

16S rRNA gene sequence extracted from the genome shared 100% identity with the partial sequence obtained from PCR and sequenced by Sanger method (accession MN007169). The final draft assembly contained 45 contigs in 39 scaffolds, totalling 5509658 bp in size with an N50 contig size of 228689 (max length 867450) and had a G+C content of 61.2 mol%, which corresponded to the range of 55–70 mol% observed in *Hymenobacter* species [6]. The final assembly was based on 1499299891 bp of Illumina data with a mapped coverage of 273.0×.

Genome annotation was performed using the DOE-JGI annotation pipeline [25]. Additional analysis and functional annotation were performed within the Integrated Microbial Genomes (IMG) platform [26] developed by JGI. 4600 protein-coding genes were predicted in the genome of strain sp. $1B^T$ and 42 putative tRNAs genes.

Comparison analysis of the genome sequences of strain $1B^T$ and other strains of the genus *Hymenobacter* revealed clear differences, as detailed in Table S1. Some genes were common to all strains (data not shown) and some differed among them. Particularly, function genes related to NADPH:quinone oxidoreductase and formate hydrogenase only existed in the genome of strain $1B^T$. In contrast, a gene related to glutathionylspermidine and trypanothione whose function is defense against oxidative damage, was not present in strain $1B^T$ but was present in the other strains. Even though genes related to urea degradation were found in strain $1B^T$, the urea hydrolysis test was negative, suggesting that genes were not functional at all or the test should be modified. The same negative results were obtained for *Hymenobacter gelipurpurascens* $Txg1^T$ and *Hymenobacter aquaticus* $16F3P^T$, both having genes for urea hydrolysis (accession numbers FYEW00000000.1 and SRLC00000000.1, respectively) but negative results for urea hydrolysis tests. Regarding the carbohydrate related genes, the presence of genes associated with mannose, xylose, L-arabinose, D-galacturonate and D-glucuronate utilization support the results obtained for the phenotypic test. Apart from these, it is also interesting to note that strain $1B^T$ was also observed to possess stress-response genes, including five genes that are designated to sigmaB stress-response regulation and three genes coding periplasmic stress-response proteins and a gene coding a cold shock, cspA family of proteins, that are induced in response to temperature downshifts, enabling cells to adapt to cold temperatures.

Average nucleotide identity (ANI) values were calculated to estimate the relatedness between the genome sequence of strain $1B^T$ and other related species of *Hymenobacter* species with genome sequences available, using the OrthoANI algorithm [27] provided by the EzBioCloud e-server. As expected from the 16S rRNA gene similarity to type strains with validly published names, the ANI values (Table S2) were lower than the cut off value (95–96%) for species demarcation [28], even for strain IS2118 (also isolated from Antarctica) with a 16S rRNA gene sequence similarity of 99.28%. Finally, a species tree was reconstructed with the Insert Genome Into SpeciesTree (version 2.2.0) app [29] of KBase [30] using a set of 49 core, universal genes defined by Clusters of Orthologous Groups gene families of a set of closely related genomes selected from the public KBase genomes import of RefSeq. Bootstrap analyses (1000 replicates) were performed in order to assess the reliability of the inferred branches [19]. The resulting phylogenomic tree (Fig. S3) shows that strain $1B^T$ formed a clade together with *Hymenobacter* sp. IS2118, *Hymenobacter lapidarius* CCM 8643^T and *Hymenobacter glacialis* CCM 8648^T.

Table 1. Cellular fatty acid composition (%) of strain $1B^T$ and closest related species.

Strains: 1, $1B^T$; 2, *Hymenobacter antarcticus* CCM 8582^T. Major components (>10%) are shown in bold type. Fatty acids representing less than 0.5% are not shown. All data were taken from this study using cells grown in the late exponential phase (72 h) on R2A agar medium at 20 °C. TR, traces (<1.0%)

Fatty acid	1	2
$C_{14:0}$	TR	TR
iso-$C_{15:1}$ G	8.0	2.4
anteiso-$C_{15:1}$ A	1.3	TR
iso-$C_{15:0}$	**17.6**	**11.9**
anteiso-$C_{15:0}$	**12.3**	**11.4**
$C_{16:1}$ ω5*c*	9.1	**16.0**
$C_{16:0}$	1.0	1.3
iso-$C_{15:0}$ 3OH	1.8	2.2
iso-$C_{16:0}$ 3OH	1.0	1.4
$C_{16:0}$ 3OH	1.0	1.7
iso-$C_{17:0}$	1.0	TR
$C_{17:1}$ ω6*c*	TR	TR
Summed feature 1*	TR	TR
Summed feature 3*	**32.5**	**37.4**
Summed feature 4*	8.6	6.9

*Summed features comprise groups of two or more fatty acids that could not be separated under the given conditions Summed feature 1 comprises iso-$C_{15:0}$h and/or $C_{13:0}$ 3OH; summed feature 3 comprises: $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; summed feature 4 comprises anteiso-$C_{17:1}$ B/iso-$C_{17:1}$ I.

CHEMOTAXONOMY

Fatty acid methyl ester (FAME) analysis was performed by following the standard protocol [31]. Biomass of strain $1B^T$ and the reference strain were harvested from cultures grown under the same conditions on R2A agar at 20 °C for 72 h. Extraction and subsequent analysis of FAMEs were performed using an Agilent 7890B gas chromatograph according to the standard protocol of the Sherlock MIDI Identification System (version 6.3; MIDI database RTSBA 6.21). The predominant fatty acids (Table 1) were summed feature 3 ($C_{16:1}$ ω7*c* and/or

$C_{16:1}$ $\omega 6c$; 32.5%), iso-$C_{15:0}$ (17.6%) and anteiso $C_{15:0}$ (12.3%). The complete cellular fatty acid composition of the isolate was in agreement with those typically found in other *Hymenobacter* species [6]. However, there are some differences in abundance of iso-$C_{15:1}$ G and $C_{16:1}$ ω5*c* between strain 1B^T and *H. antarcticus* VUG-A42aa^T.

For polyamine, quinone and polar lipid analyses, cells were grown in R2A agar at 20 °C for 72 h. Polyamines were extracted following the protocol of Busse and Auling [32] and analysed by HPLC as described by Busse *et al.* [33]. Quinones and polar lipids were extracted by an integrated procedure and analysed as described by Tindall [34, 35] and Altenburger *et al.* [36]. HPLC equipment was described by Stolz *et al.* [37]. The polyamine pattern of strain 1B^T consisted of the major compound *sym*-homospermidine [12.3 µmol g (dry weight)$^{-1}$] and traces of putrescine [0.2 µmol g (dry weight)$^{-1}$] and spermidine [0.1 µmol g (dry weight)$^{-1}$]. The quinone system contained 99.6% menaquinone MK-7 and 0.5% MK-8. Both polyamine pattern and quinone system are well in agreement with the emended description of the genus *Hymenobacter* [3]. The polar lipid profile was very complex and consisted of 16 detectable lipids (Fig. S4). Major polar lipids were phosphatidylethanolamine, phosphatidylserine, one unidentified phospholipid (PL1), and two unidentified aminolipids (AL1, AL2). Additionally, moderate to minor amounts of three unidentified aminophospholipids (APL1–3), two unidentified aminophosphoglycolipids (APGL1, 2), one unidentified glycolipid (GL1) and six unidentified lipid (L1–6) lacking a functional group were detected. This polar lipid profile with several lipids was listed in the emended genus description [3], though differences were detected compared to other established species of the genus including the close phylogenetic neighbours *Hymenobacter segetis* [38] and *H. frigidus* [39] and other species such as *Hymenobacter ocellatus* [3] and *Hymenobacter aerophilus* [40], *Hymenobacter psychrophilus* [41], *Hymenobacter amundsenii* [42] and *Hymenobacter humicola* [9].

The presence of flexirubin-type pigments was investigated using a 20% (w/v) KOH solution [43].

PHYSIOLOGY

Growth on/in several media such as plate count agar (PCA; Difco), tryptic soy agar (TSA; Difco), tryptic soy broth (TSB; Difco), nutrient agar (NA; Oxoid), nutrient broth (NB; Oxoid), Luria–Bertani (LB) agar, MacConkey agar (Difco), violet red bile glucose agar (Difco) and brain heart infusion (BHI) broth (Oxoid) at 15 °C for 7 days was evaluated. Anaerobic growth on R2A agar was tested at 15 °C for 7 days using the Anaerocult A Mini (Merck) gas generator system. Growth at different temperatures (from −5–30 °C at intervals of around 5 °C) was performed in R2A broth (supplemented with 5% glycerol for −5 and 0 °C). The pH range for growth (pH 5.0–9.0, at intervals of 1.0 pH units) was determined in R2A broth supplemented with K_2HPO_4–KH_2PO_4 buffer (for pH 5.0–8.0) and $NaHCO_3$–NaOH buffer (for pH 9.0). Initial pH was carefully adjusted with HCl or NaOH as required, and media was sterilized by filtration. Growth at different NaCl concentrations was tested in R2A broth without NaCl and with 0.5, 1, 2, 3% (w/v) final NaCl concentration. Growth was monitored by measuring the OD_{660}. Slopes of OD_{660} versus time were plotted and averaged from triplicate runs at each pH, NaCl concentration and temperature for the determination of optimal growth rate at each condition. The basic phenotypic classification was performed using traditional key tests applicable for Gram-stain-negative rods. Catalase activity was performed in a slide with 3% hydrogen peroxide solution and oxidase activity with Bactident oxidase (Merck). Further tests were done as follows: Tween 20, Tween 60 and Tween 80 hydrolysis tests were performed according to Barrow and Felthman [44] using R2A agar as the basal media. The oxidation-fermentation test was carried out in Hugh and Leifson medium. Acetamide, aesculin, casein, DNA, gelatin (method 2), starch and urea hydrolysis, arginine dihydrolase activity, citrate utilization (method 1), indole production, lecithinase activity, nitrate reduction, nitrite reduction and denitrification assays were carried out according to Tindall *et al.* [45], except that R2A was used as the basal media when required. Motility (hanging drop technique) and shape were observed under phase contrast microscope (Zeiss Axioplan) in cultures at the exponential phase of growth, cultivated in R2A broth at 15 °C. Further extended phenotyping test performed using API ZYM, API 20NE and API 50CH kits (bioMérieux), and the GEN III MicroPlate with protocol C1 (Biolog) according to the manufacturer's instructions, enabled a comprehensive characterization of the isolate. Inoculated kits were incubated at 15 °C, and the results were read after 18 h (API ZYM), 3 days (GEN III MicroPlate) or 7 days (20NE and API 50CH).

Susceptibility to UV radiation exposure was tested as described by Sedláček *et al.* [9] with minor modifications. The results indicated that the strain was sensitive to UV radiation.

TAXONOMIC CONCLUSION

Growth characteristics (temperature, pH and salinity ranges) of strain 1B^T were consistent with environmental parameters of its habitat. Phenotypic and phylogenetic properties of this strain support its inclusion in the genus *Hymenobacter*. Differentiating characteristics of strain 1B^T and closely related type strains are shown in Table 2. Strain 1B^T differed from *H. antarcticus* VUG-A42aa^T in catalase activity, DNA and lecithin hydrolysis and differed from *H. segetis* S7-3-11^T in gelatin, DNA, Tween 20 and Tween 80 hydrolysis. Moreover strain 1B^T differed significantly in the pH range as well as growth with 1% NaCl.

DNA–DNA hybridization experiments were not performed between strain 1B^T and closely related strains, as they showed 16S rRNA gene sequence similarity values below the 98.7% cutoff for consideration of DNA–DNA hybridization or other genome sequence relatedness experiments [46]. Therefore, based on the distinctive phenotypic, chemotaxonomic and genotypic characteristics, strain 1B^T represents a novel species within the genus *Hymenobacter*, for which the name *Hymenobacter artigasi* sp. nov. is proposed.

Table 2. Differential characteristics of strain 1B^T and related species of the genus *Hymenobacter*

Strains: 1, 1B^T; 2, *Hymenobacter antarcticus* CCM 8582^T; 3, *Hymenobacter glaciei* CCM 8583^T; 4, *Hymenobacter segetis* S7-3-11^T; 5, *Hymenobacter frigidus* B1789^T; 6, *Hymenobacter lapidarius* CCM 8643^T; 7, *Hymenobacter glacialis* CCM 8648^T; 8, *Hymenobacter roseosalivarius* AA-718^T. All data, unless otherwise indicated, were from this study. Nr, not reported; +, positive; w, weakly positive; –, negative. All organisms are positive for oxidase and negative for citrate utilization

Characteristic	1	2	3	4*	5†	6‡	7‡	8§
Isolation source	Air	Ice	Ice	Soil	Ice	Stone	Volcanic tuff	Soil
Growth with 1% NaCl (w/v)	+	–‖	–‖	–	+	–	–	+
pH range for growth	6.0–8.0	6.0–11.0‖	5.0–11.0‖	4.0–10.0	6.0–7.0	7.0–9.0	7.0–8.0	5.5–10.5
Temperature range for growth (°C)	0–20	4–18‖	4–28‖	4–30	5–20	1–25	5–25	3–25
Catalase activity	+	–	+	+	+	+	+	+
Hydrolysis of:								
Aesculin	+	+	w	w	–	+	+	Nr
Gelatin	+	+	+	–	–	w	+	+
Lecithin	+	–	–	Nr	Nr	–	–	Nr
Starch	+	w	+	+	–	–	+	+
Tween 20	w	+	+	–	Nr	Nr	Nr	Nr
Tween 60	w	–	+	Nr	Nr	Nr	Nr	+
Tween 80	w	–	w	–	Nr	–	+	+
DNA	+	–	–	–	Nr	–	+	–

*Data from Ten *et al.* [38].
†Data from Gu *et al.* [39].
‡Data from Sedláček *et al.* [8]
§Data from Hirsch *et al.* [1].
‖Data from Klassen *et al.* [10].

DESCRIPTION OF *HYMENOBACTER ARTIGASI* SP. NOV.

Hymenobacter artigasi [ar.ti.ga'si. N.L. gen. n. *artigasi*, name of the Uruguayan Antarctic Scientific Base (BCAA, Base Científica Antártica Artigas), where the strain was isolated].

Cells of strain 1B^T are Gram-stain-negative rods, occurring singly or in pairs, non-motile and non-spore-forming, and measure 0.6–0.9 µm in diameter and 1.8–2.9 µm long. Catalase-positive and oxidase-positive. Strictly aerobic. Growth occurs on R2A agar and PCA, but not on/in BHI agar or broth, TSA, TSB, LB agar or broth, NA agar or broth, VRBGA and MacConkey agar at 15 °C. No growth on R2A agar under anaerobic conditions. Colonies on R2A agar are circular with an entire margin, flat, smooth, glistening, reddish-pigmented and 2–3 mm in diameter after 5 days of cultivation at 15 °C. Non-flexirubin type of pigment. Grows at pH 6.0–8.0 (optimum, pH 8.0) and in the presence of 0–1.0% NaCl (optimum, 0.5%) and at 0–20 °C (optimum, 10 °C). Does not grow in Hugh and Leifson OF media supplemented with glucose. Positive results are obtained for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, lipase (C14), α-mannosidase and trypsin in the API ZYM kit. Aesculin, casein, DNA, gelatin, lecithin and starch hydrolysis are positive, and Tween 20, 60 and 80 hydrolysis results are weakly positive. Acetamide, arginine dihydrolase and citrate utilization, denitrification, indole production, reduction of nitrates to nitrites and urease tests are negative. Cells assimilate (API 20NE) D-glucose, maltose and weakly L-arabinose, D-mannitol, D-mannose, but not *N*-acetyl-glucosamine, adipic acid, capric acid, phenylacetic acid, potassium gluconate, malic acid or tri-sodium citrate. In API 50CH assays, acid is produced from *N*-acetylglucosamine, D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, glycerol, lactose, D-lyxose, D-mannitol, D-mannose, methyl α-D-mannopyranoside,melibiose, L-rhamnose, D-ribose, D-sorbitol, turanose, trehalose, D-xylose, D-raffinose and xylitol. Acid is not produced from arbutin, cellobiose, dulcitol, gentiobiose, gluconate, D-glucose, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, maltose, melezitose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, salicin, L-sorbose, starch,sucrose, D-tagatose or L-xylose. Strain 1B^T was positive

for the utilization (Biolog) of L-aspartic acid, cellobiose, gelatin, gentiobiose, D-glucuronic acid, glycyl-L-proline, L-histidine, α-keto glutaric acid, maltose, myo-inositol, trehalose and Tween 40 as carbon sources, and negative for utilization of acetic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl neuraminic acid, L-alanine, γ-amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, bromo-succinic acid, citric acid, dextrin, formic acid, D-fucose, L-fucose, D-gluconic acid, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acid, p-hydroxy phenylacetic acid, inosine, α-keto butyric acid, L-lactic acid, D-malic acid, L-malic acid, D-mannitol, melibiose, 3-methyl glucose, mucic acid, propionic acid, L-pyroglutamic acid, quinic acid, raffinose, D-saccharic acid, D-salicin, L-serine, D-serine, D-sorbitol and D-turanose. Borderline results of $1B^T$ strain obtained in the Biolog GENIII MicroPlate were acetoacetic acid, *N*-acetyl-D-galactosamine, D-fructose, D-fructose-6-PO4, D-galactonic acid lactone, D-galacturonic acid, α-D-glucose, D-glucose-6-PO4, glucuronamide, L-glutamic acid, glycerol, D-lactic acid methyl ester, lactose, pectin, methyl pyruvate, D-mannose, methyl β-D-glucoside, L-rhamnose, stachyose and sucrose.

The predominant quinone is menaquinone MK-7. The polar lipid profile consists of the major polar lipids phosphatidylethanolamine, phosphatidylserine, two unidentified aminolipids (AL1, AL2) and one unidentified phospholipid (PL1). Moderate to minor amounts of three unidentified aminophospholipids (APL1–3), two unidentified aminophosphoglycolipids (APGL1, 2), one unidentified glycolipid (GL1), and six unidentified lipid (L1–6) lacking a functional group are also detectable. Major fatty acids are summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), iso-$C_{15:0}$ and anteiso $C_{15:0}$. The type strain, $1B^T$ (=CCM 8970^T=CGMCC 1.16843^T), was isolated from air sampled at Antarctic Scientific Base Artigas (62° 11′ 04″ S 58° 51′ 07″ W), peninsula Fildes, King George Island, maritime Antarctica. The DNA G+C content is 61.2mol%. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence for strain $1B^T$ is MN007169. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAAVTK000000000. The version described in this paper is version JAAVTK010000000.

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

References

1. Hirsch P, Ludwig W, Hethke C, Sittig M, Hoffmann B *et al*. *Hymenobacter roseosalivarius* gen. nov., sp. nov. from continental Antartica soils and sandstone: bacteria of the Cytophaga/Flavobacterium/Bacteroides line of phylogenetic descent. *Syst Appl Microbiol* 1998;21:374–383.
2. Munoz R, Rosselló-Móra R, Amann R. Revised phylogeny of *Bacteroidetes* and proposal of sixteen new taxa and two new combinations including *Rhodothermaeota* phyl. nov. *Syst Appl Microbiol* 2016;39:281–296.
3. Buczolits S, Denner EBM, Kämpfer P, Busse HJ. Proposal of *Hymenobacter norwichensis* sp. nov., classification of '*Taxeobacter ocellatus*', '*Taxeobacter gelupurpurascens*' and '*Taxeobacter chitinovorans*'as *Hymenobacter ocellatus* sp. nov., *Hymenobacter gelipurpurascens* sp. nov. and *Hymenobacter chitinivor*. *Int J Syst Evol Microbiol* 2006;56:2071–2078.
4. Reddy GS. Phylogenetic Analyses of the Genus *Hymenobacter* and Description of *Siccationidurans* gen. nov., and *Parahymenobacter* gen. nov. *J Phylogen Evol Biol* 2013;01:1–9.
5. Han L, Wu S-J, Qin C-Y, Zhu Y-H, Lu Z-Q *et al*. *Hymenobacter qilianensis* sp. nov., isolated from a subsurface sandstone sediment in the permafrost region of Qilian Mountains, China and emended description of the genus *Hymenobacter*. *Antonie Van Leeuwenhoek* 2014;105:971–978.
6. Buczolits S, Busse H-J. Hymenobacter. In: Whitman WB (editor). *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons, Inc., in association with Bergey's Manual Trust; 2015. pp. 1–11.
7. Zhang G, Niu F, Busse H-J, Ma X, Liu W *et al*. *Hymenobacter psychrotolerans* sp. nov., isolated from the Qinghai--Tibet Plateau permafrost region. *Int J Syst Evol Microbiol* 2008;58:1215–1220.
8. Sedláček I, Králová S, Kýrová K, Mašlaňová I, Busse H-J *et al*. Red-pink pigmented Hymenobacter coccineus sp. nov., Hymenobacter lapidarius sp. nov. and Hymenobacter glacialis sp. nov., isolated from ROCKs in Antarctica. *Int J Syst Evol Microbiol* 2017;67:1975–1983.
9. Sedláček I, Pantůček R, Holochová P, Králová S, Staňková E *et al*. *Hymenobacter humicola* sp. nov., isolated from soils in Antarctica. *Int J Syst Evol Microbiol* 2019;69:2755–2761.
10. Klassen JL, Foght JM. Characterization of *Hymenobacter* isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* 2011;15:45–57.
11. Kojima H, Watanabe M, Tokizawa R, Shinohara A, Fukui M. *Hymenobacter nivis* sp. nov., isolated from red snow in Antarctica. *Int J Syst Evol Microbiol* 2016;66:4821–4825.
12. Machin EV, Asem MD, Salam N, Iriarte A, Langleib M *et al*. *Nesterenkonia natronophila* sp. nov., an alkaliphilic actinobacterium isolated from a soda lake, and emended description of the genus *Nesterenkonia*. *Int J Syst Evol Microbiol* 2019;69:1960–1966.
13. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al*. Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
14. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
15. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
16. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406–416.
17. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
18. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
19. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
20. Bennett S. Solexa Ltd. pharmacogenomics 2004;5:433–438.

21. Bushnell B. BB tools software package. URL http://bbtools.jgi.doe.gov.
22. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
23. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590–D596.
25. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D *et al*. The standard operating procedure of the DOE-JGI microbial genome annotation pipeline (MGAP v.4). *Stand Genomic Sci* 2015;10:4–9.
26. Chen I-MA, Chu K, Palaniappan K, Pillay M, Ratner A *et al*. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res* 2019;47:D666–D677.
27. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
28. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al*. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
29. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One* 2010;5:e9490–10.
30. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL *et al*. KBase: the United States department of energy systems biology knowledgebase. *Nat Biotechnol* 2018;36:566–569.
31. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, 101, MIDI Tech Note; 1990. pp. 1–6.
32. Busse J, Auling G. Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. *Syst Appl Microbiol* 1988;11:1–8.
33. Busse H-J, Bunka S, Hensel A, Lubitz W. Discrimination of members of the family Pasteurellaceae based on polyamine patterns. *Int J Syst Evol Microbiol* 1997;47:698–708.
34. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
35. Tindall BJ. A Comparative Study of the Lipid Composition of *Halobacterium saccharovorum* from Various Sources. *Syst Appl Microbiol* 1990;13:128–130.
36. Altenburgera P, Kämpferb P, Makristathisc A, Lubitza W, Bussea H-J. Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* 1996;47:39–52.
37. Stolz A, Busse H-J, Kämpfer P. *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* 2007;57:572–576.
38. Ten LN, Lim SJ, Kim B-O, Kang I-K, Jung H-Y. *Hymenobacter segetis* sp. nov., isolated from soil. *Arch Microbiol* 2018;200:1167–1175.
39. Gu Z, Liu Y, Xu B, Wang N, Jiao N *et al*. *Hymenobacter frigidus* sp. nov., isolated from a glacier ice core. *Int J Syst Evol Microbiol* 2017;67:4121–4125.
40. Buczolits S, Denner EBM, Vybiral D, Wieser M, Kämpfer P *et al*. Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* 2002;52:445–456.
41. Zhang D-C, Busse H-J, Liu H-C, Zhou Y-G, Schinner F *et al*. *Hymenobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from soil. *Int J Syst Evol Microbiol* 2011;61:859–863.
42. Sedláček I, Pantůček R, Králová S, Mašlaňová I, Holochová P *et al*. *Hymenobacter amundsenii* sp. nov. resistant to ultraviolet radiation, isolated from regoliths in Antarctica. *Syst Appl Microbiol* 2019;42:284–290.
43. Bernardet J-F, Nakagawa Y, Holmes B. Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
44. Barrow GI, Feltham RKA. *Cowan and Steel's Manual for Identification of Medical Bacteria*. Cambridge: Cambridge University Press; 2003.
45. Tindall BJ, Sikorski J, Smibert RA, Krieg NR *et al*. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM *et al*. (editors). *Methods for General and Molecular Microbiology*, 3rd ed. Washington, DC: ASM; 2014. pp. 330–393.
46. Kim M, Oh H-S, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.

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