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Hymenobacter amundsenii sp. nov. resistant to ultraviolet radiation, isolated from regoliths in Antarctica☆,☆☆,★

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ABSTRACT

A group of thirteen bacterial strains was isolated from rock samples collected in a deglaciated northern part of James Ross Island, Antarctica. The cells were rod-shaped, Gram-stain-negative, non-motile, catalase positive, and produced moderately slimy, ultraviolet light (UVC)-irradiation-resistant and red–pink pigmented colonies on R2A agar. A polyphasic taxonomic approach based on 16S rRNA gene sequencing, extensive biotyping, fatty acid profile, chemotaxonomy analyses, and whole genome sequencing were applied in order to clarify the taxonomic position of these isolates. Phylogenetic analysis based on the 16S rRNA gene indicated that all isolates constituted a coherent group belonging to the genus *Hymenobacter*. The closest relatives to the representative isolate P5136^T were *Hymenobacter psychrophilus* BZ33r^T and *Hymenobacter rubripertinctus* CCM 8852^T, exhibiting 97.53% and 97.47% 16S rRNA pairwise similarity, respectively. Average nucleotide identity calculated from the whole-genome sequencing data supported the finding that P5136^T represents a distinct *Hymenobacter* species. The major components in fatty acid profiles were Summed Feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$), $C_{16:1}$ $\omega5c$, $C_{15:0}$ iso and $C_{15:0}$ anteiso. The cellular quinone content contained unanimously menaquinone MK-6 and MK-7 (ratio 1:5.1). The predominant polar lipid was phosphatidylethanolamine, and moderate to minor amounts of two unknown polar lipids, two unknown aminolipids, one unknown glycolipid and two unknown glycophospholipids were present. The G + C content of genomic DNAs is 60.31 mol%. Based on all the obtained results, we propose a novel species for which the name *Hymenobacter amundsenii* sp. nov. is suggested, with the type strain P5136^T (= CCM 8682^T = LMG 29687^T).

Introduction

Investigations of the diversity and taxonomy of psychrotrophs have increased over the last two decades, and revealed a high complexity within Antarctic microbiota. Numerous novel species, mainly from the *Cytophaga–Flavobacterium–Bacteroides* group, have been discovered by cultivation as well as by molecular methods [1–4], which has led to rapid changes in the taxonomy of the phylum *Bacteroidetes* [5,6]. The genus *Hymenobacter* was described twenty years ago [7] and subsequently the genus description was amended [8,9]. At present, the genus *Hymenobacter* represents a member of the family *Hymenobacteraceae*, phylum *Bacteroidetes*, including 65 species with validly published names (http://www.bacterio.net/hymenobacter.html; accessed September, 2018) [10]. Based on the phylogenetic clustering of *Hymenobacter* spp., two

Abbreviations: ANI, average nucleotide identity; FAME, fatty acid methyl esters; WGS, whole-genome sequencing; MK, menaquinone.

☆ Subject category: New taxa (*Bacteroidetes*).

☆☆ The GenBank/EMBL/DDBJ accession number for the complete 16S rRNA gene sequence of *Hymenobacter amundsenii* P5136^T = CCM 8682^T is MF782439. The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA genes of other *H. amundsenii* isolates are MF782440-MF782451.

★ The Whole-Genome Shotgun project of *H. amundsenii* P5136^T have been deposited at GenBank/EMBL/DDBJ under the accession NZ_NIRR00000000.

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new genera *Siccationidurans* and *Parahymenobacter* were proposed [11] but these names had not yet been validated.

The majority of *Hymenobacter* species are pigmented brick-red or pink with many shades of colour [12], which facilitates their isolation from primocultures. *Hymenobacter* spp. have been retrieved from various polar environments in recent years, e.g. from sediment in the permafrost [9,13], Antarctic soils [7,14], fragmentary rocks [15], glacial till [16] or glacier ice [17]. Some hymenobacters are unique in being radiation-resistant [18–20], UV-resistant [21] or desiccation-resistant [8,22].

Here we describe the novel UV-resistant species of the genus *Hymenobacter*, isolated in 2013 from regoliths in Antarctica. A total of several hundred strains were isolated in the frame of the project focused on psychrophilic soil bacteria from Antarctica and analysed by 16S rRNA sequencing. A small group of 13 red–pink pigmented isolates was nearly identical in 16S rRNA sequence analysis and in this study these strains were characterized further.

Material and methods

Isolation, cultivation and reference strains

Sampling sites were situated in a deglaciated northern part of James Ross Island, Antarctica (Table 1). Sampling was carried out by dispersing 1 g of regolith in 5 ml of sterile saline solution, and 100 μl of this suspension was spread by L-loop on the surface of an R2A (Oxoid) agar plate and cultivated at 15 °C for up to 5 days. A total of several hundred strains were isolated in the frame of the project focused on psychrophilic soil bacteria from Antarctica. Individual red–pink pigmented colonies were continuously picked out, purified by repeated streaking on R2A medium at 15 °C, and the obtained pure cultures were maintained at −70 °C until analysed. A small group of 13 red–pink pigmented isolates was nearly identical in 16S rRNA gene sequence analysis and these strains were characterized further in this study. Reference strains of the phylogenetic relatives *Hymenobacter psychrophilus* CCM 8561^T, *Hymenobacter actinosclerus* CCM 8740^T, *Hymenobacter aerophilus* CCM 8584^T and *Hymenobacter rubripertinctus* CCM 8852^T were retrieved from the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm/).

Construction of phylogenetic trees based on 16S rRNA genes

DNA for molecular analyses was extracted with FastPrep™ Lysing Matrix type B and a FastPrep Homogenizer (MP Biomedicals) and purified with a High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's recommendations. The 16S rRNA genes were sequenced as described previously [15] and compared with those extracted from whole-genome shotgun (WGS) data using RNAmmer version 1.2 [23]. Partial 16S rRNA gene of type strain *H. rubripertinctus* CCM 8852^T was resequenced in the frame of this study (GenBank accession no. MH763858) since the 5′ end of sequence MF770258 was not properly corrected. Pairwise sequence alignment and calculation of similarity values was carried out by the algorithm used in the EzBioCloud (formerly EzTaxon) service [24]. 16S rRNA gene sequences of other species of the genus *Hymenobacter* retrieved from the Gen-Bank/EMBL/DDJB database were used for phylogenetic analysis performed using the software MEGA version 7 [25]. The evolutionary history was inferred using the neighbour-joining (NJ) and maximum-likelihood (ML) methods, using a bootstrap test based on 500 replications.

Genome analysis and calculating average nucleotide identity (ANI) values

WGS sequencing was performed using an Ion Torrent™ Personal Genome Machine (Ion PGM™). The purified genomic DNA of strain P5136^T was used for preparing a 400-bp sequencing library with an Ion Plus Fragment Library Kit (Thermo Fisher Scientific) as described previously [26]. The sample was loaded onto a 318v2 chip and sequenced using an Ion PGM Hi-Q View sequencing kit (Thermo Fisher Scientific). Quality trimming of the reads were performed with the Ion Torrent Suite of software (version 5.0.4) with default settings. The assembly computation and error correction was performed using the Assembler SPAdes 3.9 with default parameters for Ion Torrent data. The total length of the assembly contained 4,582,236 bp. Assembled contigs larger than 200 bp were used for subsequent analysis. For primal analysis, the genome was annotated using RAST [27]. To calculate the ANI value, the OrthoANI algorithm implemented on the EzBioCloud server (http://www.ezbiocloud.net/tools/ani) was used [28]. Whole-genome sequences of the related *H. psychrophilus* CGMCC 1.8975^T (GenBank accession NZ_FNOV00000000) and *H. actinosclerus* DSM 15310^T (GenBank accession NZ_FOHS01000001) were obtained from the NCBI database. Whole-genome sequence of *H. rubripertinctus* CCM 8852^T was determined in this study (GenBank accession NZ_QYCN00000000).

Further genotypic differentiation of isolates

The genomic relatedness among isolated strains was studied by automated ribotyping with the *Eco*RI restriction enzyme using a RiboPrinter Microbial Characterization System (DuPont Qualicon) in accordance with the protocol provided by the manufacturer. Numerical analysis and dendrogram construction was done using the software BioNumerics 7.1 (Applied Maths). The ribotype patterns were exported into the BioNumerics database using the load samples import script provided by the manufacturer.

Table 1
Sources of isolation of *H. amundsenii* sp. nov. strains.

Strain no.	CCM no.	Source; locality	GPS coordinates
P5053		Hyaloclastite brecchia; Lachman Crags	57°51′30″W; 63°49′45″S
P5104		Stone with lichen; hillside behind Bibby Point	57°57′05″W; 63°48′25″S
P5124		Stone fragment; south-east hillside of Berry Hill	57°49′15″W; 63°48′30″S
P5132		Stone fragment; south-east part of Panorama Pass	57°50′50″W; 63°49′05″S
P5135		Sandy soil, Crame Col	57°55′40″W; 63°49′30″S
P5136^T	CCM 8682^T	Stone fragment; north part of Panorama Pass	57°50′55″W; 63°48′50″S
P5148		Stone fragment; south part of Panorama Pass	57°50′21″W; 63°49′05″S
P5171	CCM 8683	Soil; mound nearby Big Lachman Lake	57°48′05″W; 63°47′45″S
P5308	CCM 8684	Stony soil; valley nearby Mendel Base	57°54′15″W; 63°48′50″S
P5309		Stone with lichen; hillock nearby Mendel Base	57°52′50″W; 63°48′40″S
P5317		Stone fragment; hillside behind Bibby Point	57°57′30″W; 63°48′30″S
P5328		Soil; hillside behind Bibby Point	57°57′48″W; 63°48′35″S
P5343		Sandstone; Hidden Lake Formation	57°53′45″W; 63°48′15″S

Phenotypic and physiological characterization

The Gram-staining results of the analysed strains were confirmed by the KOH lysis test method [29]. The presence of flexirubin-type pigments was investigated using a 20% (w/v) KOH solution [30]. The UV-resistance test was performed as described by Hirsch et al. [31] with minor modifications [15]. Growth on several media such as Plate Count Agar (PCA), (Oxoid), Tryptone Soya Agar (TSA), (Oxoid), Nutrient Agar CM03 (Oxoid), Mac Conkey Agar (Becton Dickinson) and Brain Heart Infusion Agar (BHI), (Oxoid) at 20 °C was evaluated. Anaerobic growth on R2A agar (Oxoid) was tested at 20 °C for 72 h and compared with those cultivated in the ambient atmosphere. Growth at different temperatures (1, 5, 10, 15, 20, 25, 30 and 35 °C) and tolerance to various NaCl concentrations (0.5, 1, 2, 3, 4 and 5% w/v) were determined based on cultivation on R2A agar plates for up to 4 days [15]. The pH range for growth was tested on R2A agar plates adjusted to pH 5.0–10.0 using the buffer system (pH 5.0–8.0, 0.1M KH_2PO_4/0.1M NaOH; pH 9.0–10.0, 0.1M $NaHCO_3$/0.1M Na_2CO_3; at interval of 1 pH unit) for one week at 20 °C [32]. Liquid cultures were not used because of the poor growth of hymenobacter isolates in broth media [17]. The basic phenotyping was performed using conventional tube and plate tests relevant for Gram-negative rods as described previously [30,33–35]. The activities of amylase and protease were tested by using R2A agar plates supplemented with appropriate substrates [36]. These key tests were inoculated with cells grown at 20 °C for 48 h on R2A agar (Oxoid). Additional biotyping using identification test kits GN2 MicroPlate™ (Biolog) and API ZYM (bioMérieux) according to the manufacturer's instructions enabled a comprehensive characterization of isolates. Inoculated kits were incubated at 20 °C, and the results were read after 18 h (API ZYM) or 24–48 h (GN2 MicroPlate). Differences in the antibiotic resistance patterns were tested by the disc diffusion method on R2A agar (Oxoid) for 2 days at 20 °C. Sixteen antibiotic discs generally used for Gram-negative rods were chosen [37–39]. EUCAST/CLSI standards were strictly followed for cultivation and inhibition zone diameter reading [37,38].

Chemotaxonomic characterization

Fatty acid methyl ester analysis (FAME) was performed with cells growing on R2A agar (Difco) incubated at 20 °C ± 2 °C for 72 h, as described by Švec et al. [39]. Quinones and polar lipids were extracted from freeze-dried biomass grown on R2A medium (Oxoid) and analysed as described previously [40–43]. Polyamines were extracted from biomass that was harvested at the late exponential growth phase accoring to Busse and Auling [44]. Analysis by HPLC was carried out as described by Busse et al. [45] and the HPLC equipment used was described by Stolz et al. [41].

GenBank accession numbers

The accession numbers of 16S rRNA gene sequences of isolates P5136^T, P5309, P5317, P5308, P5132, P5343, P5053, P5104, P5135, P5124, P5148, P5171, and P5328 are MF782439-MF782451, respectively. The accession number of the WGS assembly of strain P5136^T is NZ_NIRR00000000.

Results and discussion

Phenotypic characteristics of Hymenobacter *isolates*

All 13 isolated strains were aerobic, Gram-stain negative rods and produced moderately slimy colonies with a pink–red colour. The set of 13 strains represented psychrophilic, non-fermenting rods, and cells occurred singly or in irregular clusters (Supplementary Fig. S1, available in SYAPM online). The complete morphological and biochemical/physiological characterization of 13 isolated strains is summarized in the species description below. The tests distinguishing the proposed novel species from the phylogenetically closest recognized *Hymenobacter* spp. are shown in Table 2.

Table 2
Phenotypic characteristics that differentiate *H. amundsenii* sp. nov. from closely related *Hymenobacter* spp.

Test	1[a]	2	3	4	5
Growth at 5 °C	+	+	–	–	–
Growth at 30 °C	–	–	+	+	–
Growth at pH 6	–	–	+	+	+
Growth on Plate Count Agar	–	+	+	+	w
Acid from xylose	–	–	w	–	+
Hydrolysis of: esculin	+	–	–	–	+
Starch	+	+	–	w	+
Tween 80	–	+	+	+	–
Casein	+	+	+	–	+
API ZYM: α-glucosidase	+	–	–	w	w
Cystin arylamidase	w	–	–	–	–
N-Acetyl-β glucosaminidase	+	–	–	–	+

Strains: 1. *H. amundsenii* sp. nov. P5136^T; 2. *H. psychrophilus* CCM 8561^T; 3. *H. actinosclerus* CCM 8740^T; 4. *H. aerophilus* CCM 8584^T; 5. *H. rubripertinctus* CCM 8852^T. +, Positive; w, weak positive; –, negative; all data were taken from this study.
[a] Data are uniform for all isolates of *H. amundsenii*.

Phylogenetic relationship based on 16S rRNA gene sequence identity

Identification based on the 16S rRNA gene sequence placed all 13 strains within the genus *Hymenobacter* and comparison with EZ taxon database showed *H. psychrophilus* BZ33r^T, *H. rubripertinctus* CCM 8852^T and *H. actinosclerus* CCUG 39621^T to be the closest relative species, with 97.53%, 97.47% and 97.14% 16S rRNA gene sequence similarity with P5136^T. Other *Hymenobacter* spp. did not exhibit higher sequence similarities than 97%. The phylogenetic analysis (Fig. 1) showed that all analysed strains belonged to the *Hymenobacter roseosalivarius* phylogenetic clade and formed a common branch with *H. rubripertinctus*, *H. psychrophilus*, *H. actinosclerus*, and *H. aerophilus* with bootstrap values 94% in NJ and 76% in ML tree. The majority of the internal branches of the reconstructed ML tree were identical to those in the NJ tree (Fig. 1).

Basic genome characterization with focus on UV-resistance

The size of the draft P5136^T genome was 4.60 Mb, comprised of 131 contigs > 200 bp (N50 = 103,435 bp, mean coverage 292×) with an average G + C content of 60.3 mol%. A total of 3973 coding genes were predicted by RAST, where 42% were hypothetical. Since the isolate was UV-resistant, we focused on the analysis of the genes contributing to the high tolerance to UV-light irradiation and survival in adverse climatic conditions. A total of 50 genes associated with DNA repair were identified. These genes were divided into several groups based on their function: Uracil-DNA glycosylase (2), the bacterial MutL–MutS system (6), UvrABC system (5), bacterial photolyase (1), 2-phosphoglycolate salvage (1), the DNA repair system including RecA and MutS (2), the bacterial RecFOR pathway (9), DNA repair by base excision (10), bacterial UvrD-related helicases (1), and other bacterial DNA repair (13). In addition, the strain P5136^T possesses a large number of genes for tolerance to stress conditions (82) and for resistance to heavy metals, antibiotics and toxic compounds (58).

UV-light irradiation has serious consequences for cell survival. Pyrimidine-pyrimidone (6–4) photoproducts and cyclobutane pyrimidine dimers are induced by UV-light in genomic DNA. Mutagenic UV-light-induced DNA damage can be repaired by the nucleotide excision repair (NER) process or by photolyases repair-

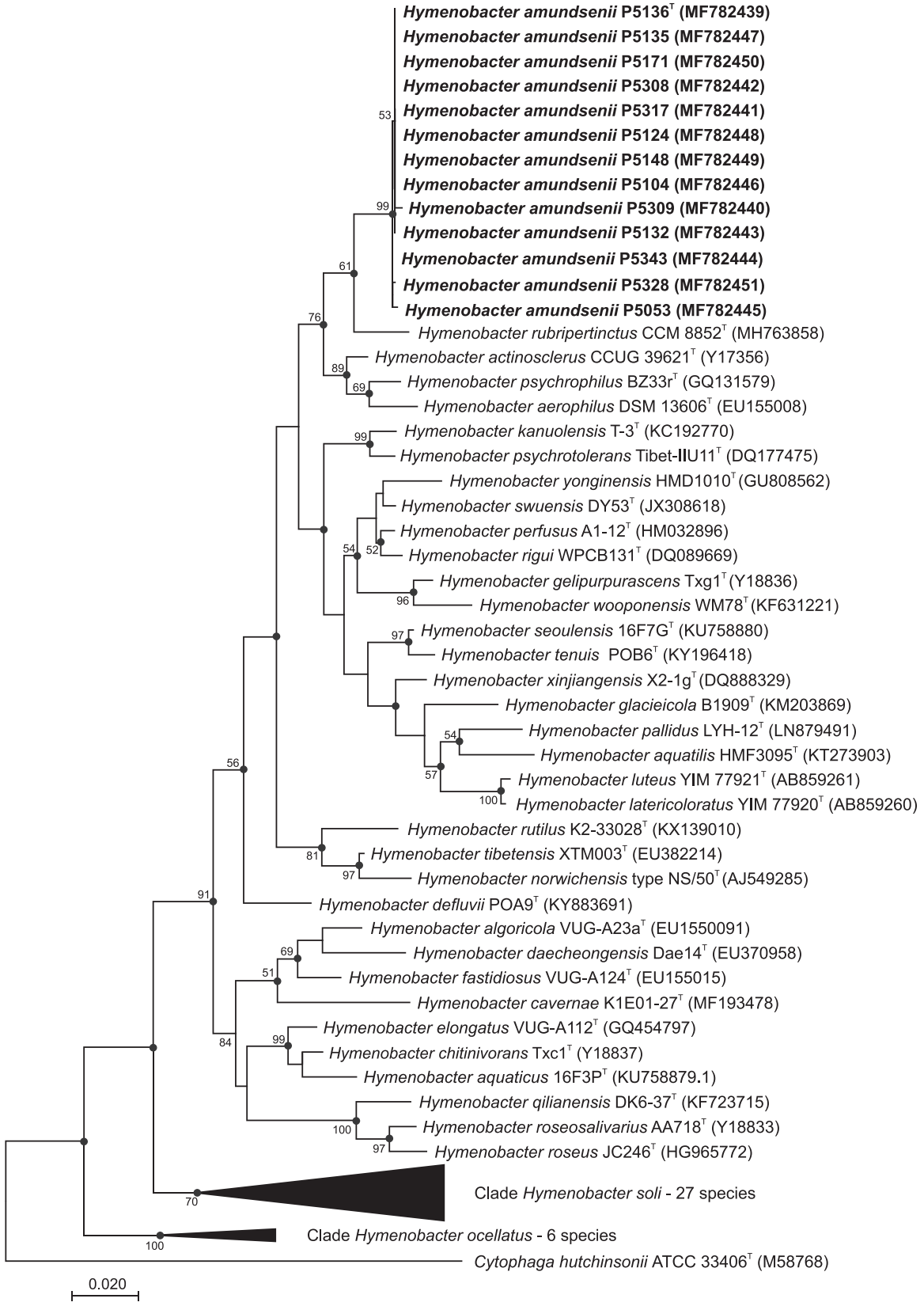

Fig. 1. Unrooted phylogenetic tree based on 16S rRNA gene sequence comparison showing phylogenetic position of *H. amundsenii* sp. nov. within the *H. roseosalivarius* phylogenetic clade. The evolutionary history was inferred by using the maximum-likelihood method based on the Kimura 2-parameter model. The percentage of 500 tree replications above 50% in which the associated taxa clustered together is shown next to the branches. Filled circles indicate that the corresponding nodes are also obtained in the neighbour-joining tree. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. *Cytophaga hutchinsonii* was used as an outgroup. The analysis involved 70 nucleotide sequences. There were a total of 1547 positions in the final alignment.

ing cyclobutane pyrimidine dimer lesion. From the comparison of UV-resistance features it is obvious that the strain P5136^T has the same UV defence mechanisms as some representatives of the genus *Hymenobacter* [21]. The complete UvrABC system involved in NER was present [46]. The *uvrA*, *uvrB* and *uvrC* genes encoding three excinuclease subunits A–C that shared 95–99% amino acid identity with UvrABC in related *H. psychrophilus*, *H. actinosclerus* and *H. aerophilus*. In addition to NER, a gene encoding deoxyribodipyrimidine photolyase (GenBank accession no. OWP62326) that could have potential biotechnological applications [47] was present. Similar deoxyribodipyrimidine photolyases sharing 89–90% amino acid identity were encoded by *H. psychrophilus*, *H. aerophilus* and *H. actinosclerus.*

Average nucleotide identity with related Hymenobacter *species*

To evaluate the intergenomic distances between the genome sequences of strain P5136^T and reference type strains belonging to the phylogenetically closest *Hymenobacter* spp., average nucleotide identity (ANI) values were determined. The analyses were carried out with the type strain of the species, whose 16S rRNA gene sequence similarities lie above 97%. The calculated ANI values for *H. psychrophilus* (81.89%), *H. rubripertinctus* (83.18%) and *H. actinosclerus* (82.09%) were below the threshold value of 95–96% suggested for species delineation [48]. These results confirmed that the strain P5136^T represents a distinct *Hymenobacter* species.

Genotyping by RiboPrinter

Numerical analysis of the obtained ribotypes clustered ten isolates into a homogenous cluster with similarities above 90%, the remaining three isolates P5053, P5343 and P5309 exhibited more distant fingerprints (Supplementary Fig. S2). As clearly shown in the dendrogram, ribotype patterns separated the investigated pigmented isolates from each other and demonstrated their non-clonal relationship. The most distinct ribotype profile showed isolate P5309, obtained from a stone fragment with lichen (*Usnea* sp.). On the other hand, the closest ribotype patterns were revealed by pairs of isolates P5132 with P5317 (98.6% similarity) and by P5136^T with P5328 (99% similarity), but both pairs were isolated in different locations, which supports the proposal that they are not identical.

Chemotaxonomic characterization

The predominant fatty acids were Summed Feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c)*, $C_{16:1}$ *ω5c*, $C_{15:0}$ iso and $C_{15:0}$ anteiso. The complete cellular fatty acids composition of the analysed 13 strains is given in Supplementary Table S1 (available in SYAPM online). For comparison purposes, the cellular fatty acids content of the closest neighbours of *Hymenobacter* spp. was tested as well and included in Table S1 in Supplementary material. The obtained FAME profiles were in agreement with those typically found in other *Hymenobacter* species [8,13,49]. In the polyamine pattern only *sym*-homospermidine [4.2 μmol (g dry weight)$^{-1}$] was detectable. The major respiratory quinones of the strain P5136^T were unanimously menaquinone MK-6 (16.4%) and MK-7 (83.4%). The predominant polar lipid was phosphatidylethanolamine. The strain P5136^T also contained moderate to minor amounts of two unidentified polar lipids lacking a functional group, two unidentified aminolipids, one unidentified glycolipid and two unidentified glycophospholipids (Supplementary Fig. S3). All these data corresponded to *Hymenobacter* classification as mentioned by Buczolits et al. [8] and Buczolits and Busse [49].

Conclusions

The polyphasic classification of 13 strains using 16S rRNA gene sequencing, whole-genome sequencing, ribotyping, chemotaxonomic analyses (menaquinone, polar lipids and FAME), and extended phenotyping differentiated the set of strains from their closest relatives. Our results demonstrated that the aforementioned group of strains isolated from regoliths in Antarctica represents a novel *Hymenobacter* species for which the name *Hymenobacter amundsenii* sp. nov. is proposed (Digital Protologue Taxonumber TA00419). The numbers of isolated strains obtained from different localities on James Ross Island indicate the presence of *H. amundsenii* as an autochthonous and stable part of the soil microbiome in the Antarctic ecosystem.

Description of H. amundsenii *sp. nov.*

H. amundsenii sp. nov. (a.mund.se′ni.i. N.L. gen. n. amundsenii, of R. E. G. Amundsen, a Norwegian explorer of Polar Regions). The description of the species is based on 13 strains.

Its cells are Gram-stain-negative non-spore-forming rods, occurring predominantly separately or in irregular clusters and non-motile. Cells wide is about 900 nm and length around 2000 nm in size (Fig. S1 in Supplementary material). Colonies on R2A agar are circular, whole margin, convex, smooth, glistening, moderately slimy, reddish pigmented and 1–2 mm in diameter after 3 days cultivation at 20 °C. Non-flexirubin type of pigment. Resistant to UVC irradiation. Growth occurs on R2A agar only. No growth was observed on PCA, TSA, BHI, Mac Conkey agar or Nutrient agar at 20 °C. Aerobic, there was no growth on R2A agar under anaerobic conditions. Growth is observed between 5 °C and 25 °C, but not at 30 °C. Cells only grow in pH range 7.0–8.0. Good growth on R2A medium in presence of 0.5% NaCl (w/v) and weak in presence of 1% NaCl; the presence of 2% NaCl inhibits growth. Glucose is not fermented to acid in OF test medium. Catalase, alkaline phosphatase, acid phosphatase, esterase lipase (C8) (weak), leucine arylamidase, valine arylamidase, cystine arylamidase (weak), α-glucosidase and *N*-acetyl-β-glucosaminidase positive by API ZYM. Acid production from glucose and maltose positive. Gelatine, casein, esculin and starch hydrolysis positive. Lipase (C14), trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase negative by API ZYM. Nitrate and nitrite reduction, fluorescein (King B medium), acid from xylose and mannitol, urease, oxidase, lysine and ornithine decarboxylase, arginine dihydrolase, Simmons citrate, acetamide and malonate utilization negative. Hydrolysis of Tween 80, ONPG, tyrosine and lecithin negative. Variable phenotypic reactions of *H. amundsenii* strains are listed in Table S2 in Supplementary material. Resistant to aztreonam, cefixim and ceftazidime, but sensitive to carbenicillin, cephalothin, ciprofloxacin, chloramphenicol, imipenem, kotrimoxazol, piperacillin, polymyxin B, streptomycin and tetracycline. Sensitivity or resistance to ampicillin, gentamicin and kanamycin were strain-dependent (Table S2 in Supplementary material).

All strains were positive for the utilization (Biolog) of α-cyclodextrin and dextrin as carbon sources and negative for utilization of Tween 40, *N*-acetyl-D-galactosamine, D-arabitol, D-cellobiose, erythritol, D-fructose, myo-inositol, lactulose, D-mannitol, β-methyl-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, turanose, succinic acid monomethyl ester, formic acid, D-glucuronic acid, α-hydroxybutyric acid, *p*-hydroxy phenyl acetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, D,L-lactic acid, propionic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, glucuronamide, L-alanine, L-histidine, hydroxyl-L-proline, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-threonine, urocanic acid, inosine, thymidine, phenethylamine, 2,3-butanediol, D,L-α-glycerol phosphate and *α*-

D-glucose-1-phosphate. Variable results of *H. amundsenii* strains obtained in the Biolog GN2 Micro Plate are shown in Table S3 (available in SYAPM online).

Type strain is P5136^T (= CCM 8682^T = LMG 29687^T). The DNA G + C content of strain P5136^T is 60.31 mol%. Almost all characteristics of the type strain P5136^T are in agreement with the species description. The strain-dependent test results of P5136^T are as follows: positive growth at 1 °C (weak), hydrolysis of DNA, naphthol-AS-Bi-phosphohydrolase and β-glucosidase activity (weak). Negative for esterase (C4) and acid production from fructose. Resistant to ampicillin and gentamicin, but sensitive to kanamycin. On the Biolog GN2 Micro Plate, the type strain P5136^T is able to utilise α-D-glucose, β-hydroxybutyric acid, α-keto valeric acid, malonic acid, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid and L-serine, but unable to utilise glycogen, Tween 80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, L-fucose, D-galactose, gentiobiose, α-D-lactose, maltose, D-mannose, D-melibiose, D-psicose, sucrose, D-trehalose, xylitol, pyruvic acid methyl ester, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, γ-hydroxybutyric acid, quinic acid, succinamic acid, L-alaninamide, D-alanine, L-alanyl-glycine, glycyl-L-aspartic acid, L-leucine, D-serine, D,L-carnitine, γ-aminobutyric acid, uridine, putrescine, 2-aminoethanol, glycerol and D-glucose-6-phosphate. The utilization of acetic acid was borderline.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.12.004.

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