Antonie van Leeuwenhoek (2021) 114:1025–1031
https://doi.org/10.1007/s10482-021-01573-z

ORIGINAL PAPER

Hymenobacter negativus sp. nov., bacteria isolated from mountain soil collected in South Korea

Soohyun Maeng · Myung Kyum Kim · Yoonjee Chang

Received: 29 January 2021 / Accepted: 1 April 2021 / Published online: 4 May 2021

Abstract Two novel Gram-negative bacterial strains BT442^T and BT584 were isolated from dry soil collected in mountains Busan and Guri, Korea during wintertime. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains BT442^T and BT584 both belong to a distinct lineage within the genus *Hymenobacter* (family Hymenobacteraceae, order Cytophagales, class Cytophagia). Strain BT442^T was closely related to *Hymenobacter soli* PB17^T (98.0% 16S rRNA gene similarity) and *Hymenobacter terrae* POA9^T (97.6%). No other recognized bacterial species showed more than 97% 16S rRNA gene sequence similarity to strains BT442^T. The genome size of strain BT442^T was 5,143,362 bp. Bacterial growth was observed at 10–30 °C (optimum 25 °C), pH 6.0–8.0 (optimum pH 6.0) in R2A agar and in the presence up to 1% NaCl. The major cellular fatty acids of strains BT442^T and BT584 were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ $\omega6c$ / $C_{16:1}$ $\omega7c$). In addition, their predominant respiratory quinone was MK-7. The major polar lipids of strains BT442^T and BT584 were identified to be phosphatidylethanolamine, aminophospholipid, and aminolipid. Based on the biochemical, chemotaxonomic, and phylogenetic analyses, strains BT442^T and BT584 are novel bacterial species within the genus *Hymenobacter,* and the proposed name is *Hymenobacter negativus*. The strain type of *Hymenobacter negativus* is BT442^T (= KCTC 72902^T = NBRC XXXX^T).

Keywords *Hymenobacteraceae* · *Hymenobacter* · Novel species · Taxonomy

Introduction

The genus *Hymenobacter* was first described by Hirsch et al. (1998) and allocated to the family *Hymenobacteraceae* (Munoz et al. 2017). At the time of writing (November 2020), the genus comprised 87 validated species (http://www.bacterio.net/hymenobacter.html). Members of the genus *Hymenobacter* were found in various environments, including freshwater wetlands (Baik et al. 2006), grassy soils (Kim et al. 2008), sediments (Xu et al. 2009), an artificial lake (Joung et al. 2011) and soil (Li et al. 2019). Some species of the genus *Hymenobacter* were isolated from extreme environments, including Antarctica, glacial ice,

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-021-01573-z.

S. Maeng · M. K. Kim (✉)
Department of Bio and Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea
e-mail: biotech@swu.ac.kr

Y. Chang (✉)
Department of Food and Nutrition, Kookmin University, Seoul 02707, Republic of Korea
e-mail: ychang@kookmin.ac.kr

permafrost, Antarctic lakes, sanitized clean-room facilities, leaves, snow, irradiated meat, and temperate desert (Klassen & Foght 2011).

Mostly, the cells of the genus *Hymenobacter* are rod shaped, red to pink in color, Gram-stain negative, and aerobic. The major cellular fatty acids of the genus were iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ $\omega5c$, summed feature 3 ($C_{16:1}$ $\omega7c$/ $C_{16:1}$ $\omega6c$), and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B). This genus has MK-7 as a major respiratory quinone and phosphatidylethanolamine as a main polar lipid (Zhu et al. 2017).

In this study, two newly isolated strains, BT442^T and BT584, were characterized. According to the 16S rRNA sequence analysis, BT442^T and BT584 showed less than 98.7% 16S rRNA gene sequence similarity to other strains in the genus *Hymenobacter*. Phenotypic, chemotaxonomic, and phylogenetic analyses showed that strains BT442^T and BT584 were distinct from other previously reported *Hymenobacter* species.

Materials and methods

Strain isolation

Strain BT442^T was isolated from a soil sample collected from mountain in Busan, Korea. And strain BT584 was isolated a soil sample collected from mountain in Guri, Korea during Winter. Isolation of strains from soil samples was performed using the standard dilution plating technique on an R2A medium (Difco) agar (Staley 1968) at 25 °C for 7 days. The 16S rRNA gene sequences of the purified colonies were identified using the EzBioCloud server (https://www.ezbiocloud.net/).

Morphology, physiology, and biochemical analysis

Cell morphology was tested using transmission electron microscopy (JEOL, JEM1010, Tokyo, Japan) with negative staining. The Gram stain was performed using a commercial kit (bioMérieux, Marcy-l'Étoile, France), following the manufacturer's instructions. Catalase and oxidase activities were determined using 1% (w/v) tetramethyl-p-phenylenediamine and 3% (w/v) H_2O_2 solution, respectively (Cappuccino and Sherman 2002). Bacterial growth was tested at 25 °C under various pH conditions (4 to 10, 0.5 pH intervals, pH 4.0 ~ 5.5: acetic acid buffer, pH 6.0 ~ 8.0: potassium phosphate buffer, pH 8.5 ~ 10.0: Glycine—NaOH buffer) and different NaCl concentrations (0 to 5% [w/v%], 1% intervals). Bacterial growth on R2A agar plate was observed for 3 days at different temperatures (4, 10, 15, 18, 25 and 30 °C). The bacteria were grown on R2A agar plates, Luria–Bertani (LB) agar, nutrient agar (NA), MacConkey (MAC) agar, and trypticase soy agar (TSA) (all purchased from Difco, New Jersey, United States) and were observed for three days at different temperatures (4 °C, 10 °C, 25 °C, 30 °C). Biochemical and physiological tests were performed using API 20NE kits, and enzymic activities were tested using an API ZYM kit (bioMérieux) following the manufacturer's instructions. Two reference strains, *H. soli* PB17^T and *H. terrae* DG7A^T were obtained from the Korean Collection for Type Cultures and were cultured under the same conditions for comparative analysis.

Genome sequencing

Genomic DNA was extracted using a genomic DNA extraction kit (Solgent, Daejeon, Korea).

The DNA sequencing library was prepared by the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, United States), and whole-genome sequencing was accomplished using iSeq 100 (150 bp paired-end). The genome sequence was assembled using SPAdes 3.10.1 (Algorithmic Biology Lab, St. Petersburg Academic University of the Russian Academy of Sciences). The whole-genome sequences of strains BT442^T and BT584 were deposited in the GenBank (www.ncbi.nlm.nih.gov/) database. The genome sequences of strains BT442^T and BT584 were annotated using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016).

Phylogenetic analysis

The 16S rRNA genes of strains BT442^T and BT584 were amplified by 27F and 1492R universal bacterial primers (Macrogen) using BT442^T and BT584 genomic DNA as a template. Then, sequencing was performed using the 337F, 518R, 785F and 926R universal primers (Macrogen). To determine the taxonomic positions of strains BT442^T and BT584,

16S rRNA sequences of related taxa were obtained from EzBioCloud and compared with those of BT442^T and BT584 using the EzEditor2 program (Jeon et al. MEGA). A phylogenetic tree was constructed using the MEGAX program (Kumar et al. 2018) with the neighbor-joining (Saitou and Nei 1987), maximum-likelihood and maximum-parsimony algorithms (Zi-heng 1995). Evolutionary distances were calculated according to the Kimura two-parameter model (Kimura 1983). A bootstrap analysis was conducted with 1,000 replicates (Felsenstein 1985). DNA sequence similarities among the strains BT442^T, BT584, and the closest type strain were evaluated using average nucleotide identity (ANI) analysis and in silico DNA-DNA hybridization. ANI values were calculated using the Orthologous Average Nucleotide Identity Tool version 0.98 (Lee et al. 2016) and digital DNA-DNA hybridization was performed using Genome-to Genome Distance Calculator (GGDC) with the recommended formula 2 (Meier-Kolthoff et al. 2013).

Chemotaxonomic characteristics

Polar lipids were extracted from the cells according to a previously described method (Minnikin et al. 1984) and separated by two-dimensional thin-layer chromatography (TLC). Spots were detected with detection reagents sprayed onto the lipids (Komagata and Suzuki 1987). Respiratory quinones were extracted using Sep-Pak Vac cartridges (Waters, Massachusetts, USA) and were analyzed by high-performance lipid chromatography (HPLC) based on the method of Hiraishi et al. (1996). For cellular fatty acid analysis, strains BT442^T and BT584 were grown on R2A agar plates for 3 days at 25 °C, and 300 mg of cells were harvested and freeze-dried. Cellular fatty acids were purified by saponification, methylation, and extraction procedures (Sasser 1990), then identified using the Sherlock Microbial Identification System V6.01 (MIS, database TSBA6, MIDI Inc., Newark, USA).

Results and discussion

Morphology, physiology, and biochemical analysis

Strains BT442^T and BT584 were isolated from dry soil collected in Busan and Guri, Korea. Strains BT442^T and BT584 were Gram negative and rod shaped (Fig. S1). Colonies of strain BT442^T and BT584 were convex, smooth, circular, and pink in color after incubation for three days at 25 °C. Cells could grow at 10–30 °C (optimum 25 °C) and pH 6.0–8.0 (optimum 6.0) in the R2A medium. Different features between two novel strains and reference strains are provided in Table 1. Features from API 20NE and API ZYM test were listed in Table S1.

Genome sequence analysis and phylogenetic analysis

The draft genome of strain BT442^T consisted of 5,143,362 bp with a coverage of 20-fold. A total of 4,202 protein-coding genes and 44 RNA genes (3 rRNA genes, 41 tRNA genes) were predicted by NCBI Prokaryotic Annotation Pipeline (PGAP) analysis. The genome sequence of the strain BT442^T was deposited in GenBank under accession number NZ_JAEDAE010000000. The draft genome of strain BT584 consisted of 5,156,552 bp with a coverage of 55-fold. A total of 4209 protein-coding genes and 43 RNA genes (3 rRNA genes, 40 tRNA genes) were predicted by NCBI Prokaryotic Annotation Pipeline (PGAP) analysis. The genome sequence of the strain BT584 has been deposited in GenBank under accession number NZ_JAEDAD000000000.

Based on its sequence similarity to the 16S rRNA gene, strain BT442^T was affiliated with the family *Hymenobacteraceae*, and it showed high sequence similarities with the genus *Hymenobacter*. 16S rRNA sequence from PCR results was identical with that of whole genome. The similarity of the 16S rRNA gene sequence between the BT442^T and BT584 strains was 99.23%, which indicated that they represent nearly identical species. The novel isolate BT442^T was related to *Hymenobacter soli* PB17^T (98.0% 16S rRNA gene similarity) and *Hymenobacter terrae* DG7A^T (97.6%). According to Chun et al (2018), BT442^T could be regarded a new species as its 16S rRNA similarity was 98.7% or less. The OrthoANI value between strains BT422^T and the closest type strain *Hymenobacter terrae* DG7A^T was 81.74%, and the OrthoANI values between strain BT584 and *H. terrae* DG7A^T was 81.73%. The ANI and dDDH values between strain BT422^T and *H. terrae* DG7A^T was 80.40% and 17.0%, respectively. The ANI and dDDH values between strain BT584 and *H. terrae*

Table 1 Different characteristics of *Hymenobacter negativus* sp. nov., and closely related species

Characteristic	1	2	3	4
Size (μm long)			2.0–4.0	2.1–3.0
Size (μm wide)			0.6–1.0	0.6–1.1
Colony color (Yellow, White)	Red	Red	Pink red	Yellow
Oxidase			−	+
Catalase			+	−
Temperature range (°C)	30	25–30	30	30
NaCl % range	0	1	1	3
Production of acid from glucose	−	−	−	−
Enzyme activity				
N–Acetyl-*β*-glucosaminidase	−	−	w	+
Acid phosphatase	w	w	+	+
α-Chymotrypsin	−	−	−	−
Cystine arylamidase	−	−	+	+
Esterase (C4)	−	−	+	+
Esterase (C8)	−	−	+	+
α-Galactosidase	−	−	−	+
β-Galactosidase (ONPG)	w	−	+	+
β-Galactosidase (PNPG)	−	w	+	+
α-Glucosidase (starch hydrolysis)	w	−	−	−
β-Glucosidase (Esculin hydrolysis)	+	+	+	w
β-Glucosidase	w	w	−	w
β-Glucuronidase	−	−	−	−
Leucine arylamidase	w	+	+	+
Lipase (C14)	−	−	−	−
Naphtol-AS-BI-phosphohydrolase	w	w	+	+
Protease (gelatin hydrolysis)	+	+	−	+
Trypsin	−	−	−	−
Valine arylamidase	w	w	+	+
G + C content	61.2	61.2	58.8	63.5

Taxa: 1, strain BT442^T (data from this study); 2, strain BT584 (data from this study); 3, *H. soli* PB17^T (data from Ten et al. 2017); 4, *H. terrae* DG7A^T (data from Srinivasan et al. 2014)

All strains were positive for alkaline phosphatase, and all strains were negative for nitrate reduction to NO_2, nitrate reduction to N_2, production of indole, arginine dihydrolase, α-fucosidase, α-mannosidase, urease, adipate, gluconate, L-malate, phenyl acetate, and D-mannose

+ , positive; − , negative; w, weak positive

DG7A^T was 80.38% and 17.0%, respectively. In the neighbor-joining, maximum-likelihood and maximum parsimony tree, strain BT442^T formed an independent cluster (Fig. 1). The phylogenetic analysis result clearly showed that strain BT442^T is a novel species within the genus *Hymenobacter*.

Chemotaxonomic characterization

The fatty acid profiles of strains BT442^{T}and BT584 were compared with those of the closely related three species of the genus *Hymenobacter* (Table 2). The major fatty acids of strains BT442^T and BT584 were $C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ *ω5c*; summed feature 3 ($C_{16:1}$ *ω7c*/ $C_{16:1}$ *ω6c*); and summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B). The fatty acid profiles of strains BT442^T and BT584 were similar to those of the two most closely related *Hymenobacter* species.

The polar lipids of strain BT442^T consisted of phosphatidylethanolamine, phospholipid, glycolipid, two aminolipids, four aminophospholipids, and two unknown polar lipids (Fig. S2). Polar lipids of strain BT584 consisted of one phosphatidylethanolamine, one phospholipid, one glycolipid, two aminolipids, two aminophospholipids, and two unknown lipids (Fig. S3). The dominant respiratory quinone of strain BT442^T was MK-7.

The morphological, biochemical, and chemotaxonomic characters of strains BT442^T and BT584 were consistent with descriptions of the genus

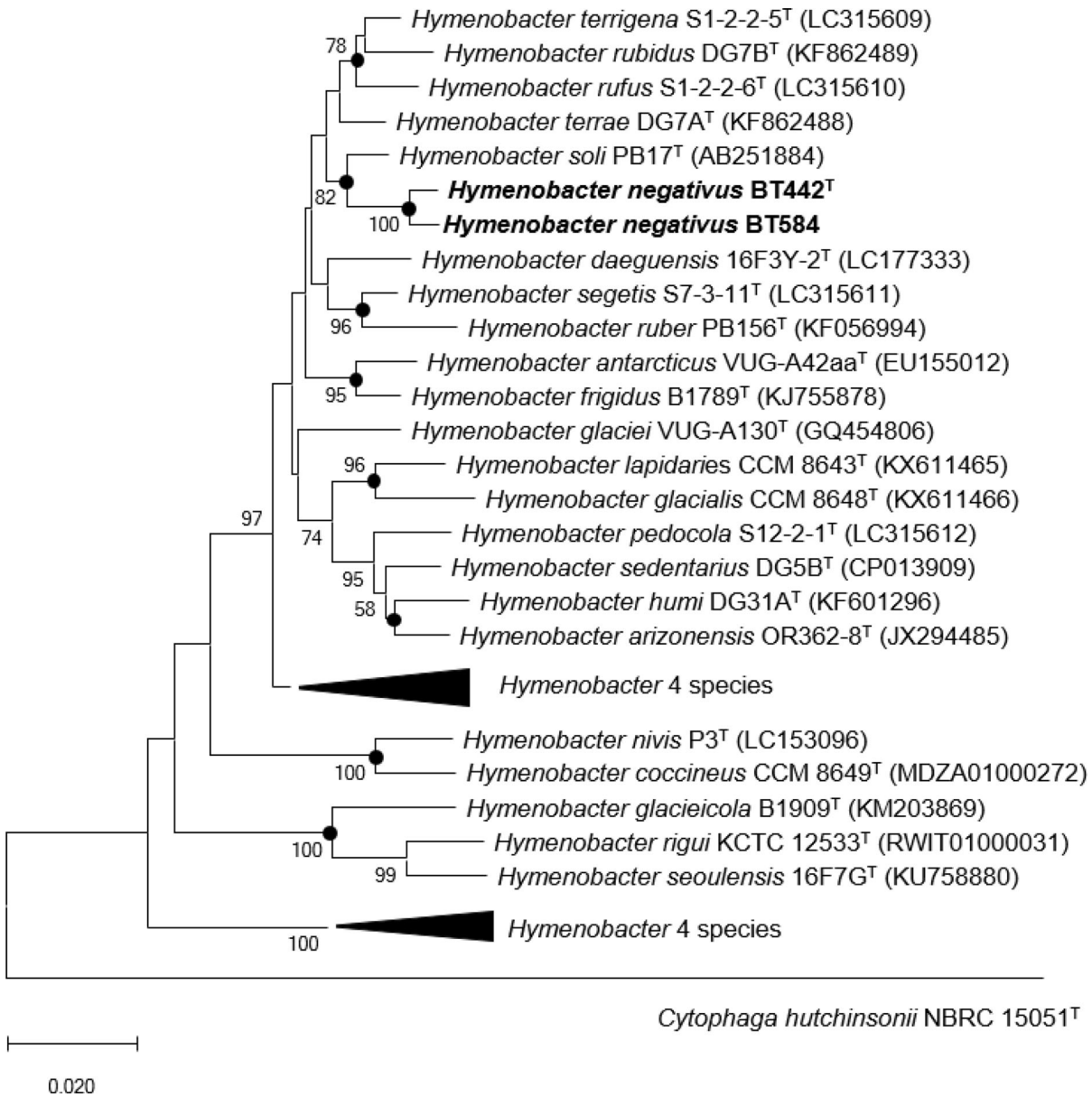

Fig. 1 Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strains BT442^T and BT584 with closely related validly published species. Bootstrap values (> 70%) based on the neighbor-joining method are shown at the branch nodes. Circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 0.020 substitutions per nucleotide position. *Chitinophaga hutchinsonii* NBRC 15051 ^{T} was used as the outgroup. The compact triangles represent other species of genus *Hymenobacter*

Hymenobacter. On the basis of the phylogenetic differences between strain BT442^T and species of the genus *Hymenobacter*, a novel species, *Hymenobacter negativus* sp. nov., has been proposed, with BT442^T as the strain type.

Description of *Hymenobacter negativus* sp. nov

Hymenobacter negativus (ne.g.,a.ti'vus. L. masc. adj. *negativus*, negative, because of the Gram-negative staining reaction).

Table 2 Cellular fatty acid profiles of *Hymenobacter negativus* sp. nov., and closely related species

Fatty acids	1	2	3	4
Saturated				
14:0	1.2	1.3	1.2	–
14:0 iso	1.6	2.0	–	2.0
15:0 iso	25.8	26.1	51.8	24.9
15:0 anteiso	15.3	13.4	6.2	21.4
15:0 iso 3OH	2.5	2.6	2.1	2.0
16:0	1.8	1.6	4.2	5.3
16:0 iso	tr	tr	–	1.7
16:0 3OH	1.1	1.1	1.0	tr
17:0 iso	tr	tr	1.1	2.6
17:0 anteiso	–	–	–	1.4
17:0 2OH	tr	tr	–	1.7
17:0 iso 3OH	1.0	1.1	1.2	1.1
Unsaturated				
15:1 iso G	1.7	1.8	–	1.6
16:1 iso H	tr	tr	1.0	1.9
16:1 ω 5*c*	7.0	9.4	11.0	6.7
16:0 iso 3OH	tr	tr	–	1.0
Summed Feature 3 (16:1 ω 6*c* / 16:1 ω 7c)	28.7	29.2	14.4	15.0
Summed Feature 4 (17:1 iso I / 17:1 anteiso B)	4.8	3.9	3.5	3.2

Taxa: 1, strain BT442^T (data from this study); 2, strain BT584 (data from this study); 3, *H. soli* PB17^T (data from Kim et al. 2008); 4, *H. terrae* DG7A^T (data from Srinivasan et al. 2014). All strains were grown on R2A agar at 25 °C for three days

For unsaturated fatty acids, the location of the double bond was presented by counting the number from the methyl (ω) end of the carbon chain

tr, trace (< 1%); -, not detected

Cells are Gram negative and rod shaped. Colonies on R2A agar are irregular, convex, and pink in color after the three-day incubation at 25 °C. The cell size of strain BT442^T is approximately 1.2–1.5 μm in diameter and approximately 5.3–5.8 μm in length. Growth occurs at 10–30 °C (optimum 25 °C) and pH 6.0–9.0 (optimum 6.0). Cells grow on R2A agar, TSA, and NA but not on LB agar and Macconkey agar. Cells are oxidase activity negative and catalase activity positive and do not assimilate D-mannitol. The major respiratory quinone is MK-7. The dominant cellular fatty acids are $C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}$ ω5*c* and summed feature 3 ($C_{16:1}$ ω7*c*/ $C_{16:1}$ ω6*c*), summed feature 4 (iso-$C_{17:1}$ I / anteiso-$C_{17:1}$ B). The major polar lipids are phosphatidylethanolamine (PE), aminophospholipid (APL), and aminolipid (AL). The whole genome sequences of strains BT442^T and BT584 have been deposited in GenBank under accession numbers NZ_JAEDAE000000000 and NZ_JAEDAD000000000, respectively. The GenBank accession number for the 16S rRNA gene sequence of strains BT442^T and BT584 are MT815535 and MT893355, respectively. The strain type BT442^T (= KCTC 72902 ^{T} = NBRC XXXX^T) was isolated from a soil sample collected in Korea.

Acknowledgements This work was supported by a research grant from Seoul Women's University (2021) and a grant from the National Institute of Biological Resources (NIBR), which was funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202002108). We are grateful to Dr. Aharon Oren (The Hebrew University of Jerusalem, Israel) for helping with the etymology.

Authors contributions Conceptualization, funding acquisition, and supervision: MKK; Laboratory work, data analysis and writing-original draft: SM; Writing-review and editing: YC.

Funding This work was supported by a research grant from Seoul Women's University (2021) and by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202002203).

Availability of data and materials The GenBank accession numbers for the 16S rRNA gene sequences of strain BT442^T and strain BT584 are MT815535 and MT893355, respectively. The whole genome sequences of strain BT442^T and strain BT584 have been deposited in GenBank under accession numbers NZ_JAEDAE000000000 and NZ_JAEDAD000000000, respectively.

Code availability Not applicable.

Declarations

Conflict of interest All authors certify that there is no conflict of interest.

References

Baik KS, Seong CN, Moon EY, Park YD, Yi H, Chun J (2006) *Hymenobacter rigui* sp. nov., isolated from wetland freshwater. Int J Syst Evol Microbiol 56:2189–2192

Cappuccino JG, Sherman N (2002) Microbiology: a laboratory manual, 6th edn. Pearson Education, Inc., Benjamin

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466

Felsenstein J (1985) Confidence limit on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high performance liquid chromatography and photodiode array detection. J Gen Appl Microbiol 42:457–469

Hirsch P, Ludwig W, Hethke C, Sittig M, Hoffmann B, Gallikowski CA (1998) *Hymenobacter roseosalivarius* gen. nov., sp. nov. from Continental Antarctic Soils and Sandstone: Bacteria of the *Cytophaga/Flavobacterium/Bacteroides* Line of Phylogenetic Descent. Syst Appl Microbiol 21:374–383

Joung Y, Cho SH, Kim H, Kim SB, Joh K (2011) *Hymenobacter yonginensis* sp. nov., isolated from a mesotrophic artificial lake. Int J Syst Evol Microbiol 61:1511–1514

Kim KH, Im WT, Lee ST (2008) *Hymenobacter soli* sp. nov., isolated from grass soil. Int J Syst Evol Microbiol 58:941–945

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge

Klassen JL, Foght JM (2011) Characterization of *Hymenobacter* isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. Extremophiles 15:45–57

Komagata K, Suzuki K (1987) 4 Lipid and cell-wall analysis in bacterial systematics. Method Microbiol 19:161–207

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096

Lee I, Kim YO, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103

Li YD, Zhou XK, Mo MH, Jiao JY, Yang DQ, Li WJ, Duan YQ (2019) *Hymenobacter terrae* sp. nov., a Bacterium Isolated from Soil. Int J Syst Evol Microbiol 69:2082–2088. https://doi.org/10.1099/ijsem.0.003440

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Meth 2:233–241

Munoz R, Rossello MR, Amann R (2017) Revised phylogeny of *Bacteroidetes* and proposal of sixteen new taxa and two new combinations including *Rhodothermaeota* phyl. nov. Syst Appl Microbiol 39:281–296. https://doi.org/10.1016/j.syapm.2016.04.004

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Bio Evol 4:406–425

Sasser M (1990) Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. MIDI Technical Note 101. Newark, DE: MIDI Inc

Srinivasan S, Lee JJ, Park KR et al (2014) *Hymenobacter terrae* sp. nov., a bacterium isolated from soil. Curr Microbiol 70:643–650

Staley JT (1968) Prosthecomicrobium and Ancalomicrobium: new prosthecate freshwater bacteria. J Bacteriol 95:1921–1942

Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569

Xu JL, Liu QM, Yu HS, Jin FX, Lee ST, Im WT (2009) *Hymenobacter daecheongensis* sp. nov., isolated from stream sediment. Int J Syst Evol Microbiol 59:1183–1187

Zhu HZ, Yang L, Muhadesi JB et al (2017) *Hymenobacter cavernae* sp. nov., isolated from a karst cave. Int J Syst Evol Microbiol 67:4825–4829

Ziheng Y (1995) Phylogenetie analysis using parsimony and likelihood methods. J Mol Evol (1996) 42:294–307