Antonie van Leeuwenhoek (2019) 112:1019–1028
https://doi.org/10.1007/s10482-019-01236-0

ORIGINAL PAPER

Hymenobacter persicinus sp. nov., a novel member of the family *Hymenobacteraceae*

Leonid N. Ten · Na Yeong Jeon · Weilan Li · Myung Kyum Kim · Young-Je Cho · Hee-Young Jung

Received: 10 December 2018 / Accepted: 18 January 2019 / Published online: 28 January 2019
© Springer Nature Switzerland AG 2019

Abstract A bacterial strain, 1-3-3-3^{T}, was isolated from a soil sample collected in Jeollabuk-do province, South Korea. Cells were observed to be Gram-stain negative, short rod-shaped and colonies to be red-pink in colour. Analysis of 16S rRNA gene sequences identified this strain as a member of the genus *Hymenobacter* in the family *Hymenobacteraceae*, with high levels of 16S rRNA sequence similarity with *Hymenobacter algoricola* VUG-A23a^{T} (98.0%), *Hymenobacter knuensis* 16F7C-2 (97.9%), *Hymenobacter fastidiosus* VUG-A124^{T} (97.1%), *Hymenobacter elongatus* VUG-A112^{T} (97.0%), *Hymenobacter chitinivorans* Txc1^{T} (97.0%) and *Hymenobacter aquaticus* 16F3P^{T} (96.7%). Growth of strain 1-3-3-3^{T} was observed at 10–30 °C, pH 6–8 and in the presence of 0–1.0% NaCl. The genomic G + C content was determined to be 61.6 mol %. The predominant respiratory quinone of the isolate was found to be MK-7; the major fatty acids were identified as iso-$C_{15:0}$ (19.9%), summed feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$, 19.7%), summed feature 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B, 17.8%), $C_{16:1}$ $\omega5c$ (12.5%) and anteiso-$C_{15:0}$ (11.2%), and the major polar lipid was found to be phosphatidylethanolamine. The phenotypic and chemotaxonomic data support the affiliation of strain 1-3-3-3^{T} with the genus *Hymenobacter*. However, the DNA-DNA relatedness between the isolate and its closest phylogenetic neighbours was lower than 34%. The DNA–DNA hybridization result and the differentiating phenotypic properties clearly indicate that strain 1-3-3-3^{T} represents a novel species in the genus *Hymenobacter*, for which the name *Hymenobacter persicinus* sp. nov. is proposed. The type strain is 1-3-3-3^{T} (= KCTC 52742^{T} = JCM 32191^{T}).

Keywords *Hymenobacter* · *Hymenobacteraceae* · Polyphasic taxonomy · Soil bacteria

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-019-01236-0) contains supplementary material, which is available to authorized users.

L. N. Ten · N. Y. Jeon · W. Li · H.-Y. Jung (✉)
School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea
e-mail: heeyoung@knu.ac.kr

M. K. Kim
Department of Bio and Environmental Technology, Seoul Women's University, Seoul 01797, Republic of Korea

Y.-J. Cho
School of Food Science and Biotechnology/Food and Bio-Industry Research Institute, Kyungpook National University, Daegu 41566, Republic of Korea

H.-Y. Jung
Institute of Plant Medicine, Kyungpook National University, Daegu 41566, Republic of Korea

Introduction

The genus *Hymenobacter* of the family *Cytophagaceae* was first proposed by Hirsch et al. (1998) and its description was later emended by Buczolits et al. (2006) and Han et al. (2014). Recently, the genus was placed together with *Adhaeribacter*, *Nibribacter*, *Pontibacter*, and *Rufibacter* in the new family *Hymenobacteraceae* (Munoz et al. 2016). Currently, the genus *Hymenobacter* comprises 65 species with validly published names (http://www.bacterio.net/hymenobacter.html). Members of this genus are Gram-stain negative, rod-shaped, red-pigmented, non-motile, aerobic bacteria that contain phosphatidylethanolamine as the major polar lipid, MK-7 as the predominant menaquinone, and iso-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$) and summed feature 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B) as the major fatty acids. *Hymenobacter* species occupy a wide range of ecological niches, such as air (Buczolits et al. 2002), fresh water (Ten et al. 2017), soil (Buczolits et al. 2006), and extreme environments like arid land (Reddy and Garcia-Pichel 2013), glacier (Klassen and Foght 2011), and continental Antarctic soils (Hirsch et al. 1998).

During a screening for novel bacteria, strain 1-3-3-3^T was isolated from a soil sample collected in Jeollabuk-do province, South Korea. Based on 16S rRNA gene sequence analysis, this isolate was assigned to the genus *Hymenobacter*. The strain was subjected to detailed investigation using a polyphasic taxonomic analysis that included genotypic, chemotaxonomic and phenotypic analyses. The results indicate that strain 1-3-3-3^T should be placed in the genus *Hymenobacter* as the type strain of a novel species, here named *Hymenobacter persicinus* sp. nov.

Materials and methods

Isolation of bacterial strain and culture conditions

Strain 1-3-3-3^T was isolated from a soil sample collected in Jeollabuk-do province, (35°41′04.50″N, 127°25′53.43″E), South Korea. The sampling location is a public area; therefore, no specific permissions were required to collect the material that was necessary for the present study, and the activities did not involve endangered or protected species. The soil texture was sandy clay with a pH of 7.2. The isolation was performed at 25 °C using the dilution plating technique with Reasoner's 2A (R2A) medium (Difco, Sparks, MD, USA), as described previously (Han et al. 2018). The novel strain was routinely cultured on R2A agar at 25 °C, maintained at 4 °C and stored in a 20% (w/v) glycerol suspension at − 70 °C. Strain 1-3-3-3^T has been deposited in the Korean Collection for Type Cultures (KCTC 52742^T) and the Japan Collection of Microorganisms (JCM 32191^T). *Hymenobacter algoricola* JCM 17214^T, *Hymenobacter fastidious* JCM17224^T, and *Hymenobacter elongatus* JCM 17223^T were obtained from the Japan Collection of Microorganisms. *Hymenobacter knuensis* KCTC 52538 and *Hymenobacter aquaticus* KCTC 32554^T were purchased from the Korean Collection for Type Cultures and *Hymenobacter chitinivorans* KACC 12068^T was obtained from the Korean Agricultural Culture Collection.

Sequencing of the 16S rRNA gene and phylogenetic analysis

Genomic DNA was extracted from strain 1-3-3-3^T using a commercial genomic DNA extraction kit (SolGent, Daejeon, South Korea). The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primers 9F and 1512R, as described previously (Weisburg et al. 1991), and purified PCR products were sequenced by Genotech (Daejeon, South Korea). A nearly complete 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR, Madison, WI, USA) and compared with sequences of closely related species using the EzBioCloud server (Yoon et al. 2017). The 16S rRNA gene sequences of related taxa were obtained from GenBank and assembled in BioEdit (Hall 1999). Multiple sequence alignments were performed with Clustal X 2.0 (Larkin et al. 2007). Phylogenetic trees were constructed using the neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981), and maximum-parsimony (MP) (Fitch 1971) methods in the program MEGA7 (Kumar et al. 2016). The NJ and ML phylogenetic trees were constructed using Kimura's two-parameter model (Kimura 1980) and the ML tree was constructed using the nearest neighbor interchange heuristic search method. The MP tree was constructed using subtree pruning and regrafting. The option of complete

deletion of gaps was applied for NJ, ML and MP tree construction. A bootstrap analysis with 1000 replicate datasets was performed to assess support for groups.

Phenotypic and biochemical characteristics

The Gram staining of strain 1-3-3-3^T was examined using a previously described method (Smibert and Krieg 1994). The morphology of the isolate, grown for 3 days at 25 °C on R2A agar, was observed under a light microscope at 1000× magnification (Olympus, Tokyo, Japan) and a HT7700 transmission electron microscope (Hitachi, Tokyo, Japan). Motility was tested by stab inoculation of semi-solid R2A medium containing 0.6% (w/v) agar (Tittsler and Sandholzer 1936) and gliding motility was investigated by phase-contrast microscopy of cells incubated for 24 h on microscope slides coated with R2A medium (0.7% agar) (Bowman 2000). The anaerobic growth test was performed as described previously (Ten et al. 2006). Catalase activity was determined by bubble production in 3% (v/v) H_2O_2 and oxidase activity was examined using 1% (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino and Sherman 2010). Aesculin hydrolysis was tested on R2A agar, as reported previously (Aslam et al. 2006). The ability to grow on different bacteriological media was assessed using R2A agar, Luria–Bertani (LB) agar, nutrient agar (NA), and trypticase soy agar (TSA) (all purchased from Difco). Growth at 4, 10, 15, 20, 25, 30, 37, 42 and 45 °C was assessed on R2A agar. Growth at different pH values (pH 4–10, in increments of 1 unit) and NaCl concentrations (0.5, 1, 1.5, 2, 3, 4, 5 and 10% [w/v]) was evaluated in R2A broth for 7 days as described previously (Lee et al. 2017a). Tests for nitrate and nitrite reduction were performed as described by La et al. (2005). Enzyme activities, assimilation of carbon sources, acid production from substrates and other physiological characteristics of the novel isolate and the reference strains were determined using the API ZYM, API 20 NE, API ID 32 GN, and API 50 CH kits according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). The API 50 CH strips were inoculated with cells suspended in API 50 CHB/E medium (bioMérieux). The API 20 NE, API ID 32 GN, and API 50CH strips were inspected after 5 days of incubation at 18 °C for *H. algoricola* JCM 17214^T, *H. fastidious* JCM17224^T, and *H. elongatus* JCM 17223^T or at 25 °C for other strains. The same temperature conditions and incubation time of 18 h were used to performed API ZYM tests with the above-mentioned strains.

Chemotaxonomic analyses

Following the growth conditions that were used for fatty acid analysis of the six references strains (Lee et al. 2017b; Ten et al. 2017), strain 1-3-3-3^T was grown on R2A agar at 18 °C for 7 days. Cellular fatty acid methyl esters were prepared according to a Sherlock Microbial Identification System (MIDI) protocol. Fatty acid methyl esters were then analysed by gas chromatography and Microbial Identification Software (Sherlock TSBA, version 6.0) (Sasser 1990). For the analysis of polar lipids and respiratory isoprenoid quinones, cells of strain 1-3-3-3^T were cultured, collected, and freeze-dried. Polar lipids were extracted using the procedure described by Minnikin et al. (1984) and identified by two-dimensional thin-layer chromatography, followed by spraying with appropriate detection reagents (Komagata and Suzuki 1987). Isoprenoid quinones were extracted and purified as described previously (Ten et al. 2008) and analysed according to the method of Hiraishi et al. (1996) with the following modifications. High-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with G1315A photodiode array detector and ChemStation software. The chromatographic separation was achieved using Atlantis T3 C_{18} column (4.6 mm × 250 mm, 5.0 μm; Waters, Milford, USA) as the stationary phase and methanol/isopropanol in the proportion of 7:5 (v/v) as the mobile phase at a flow rate of 1.2 ml/min.

DNA-DNA hybridization and determination of DNA G + C content

The genomic DNA of strain 1-3-3-3^T and the six closely related strains were extracted according to the standard cetyltrimethylammonium bromide/NaCl protocol (Wilson 1997). DNA-DNA hybridization was performed fluorometrically using the method described by Ezaki et al. (1989) with photobiotin-labelled DNA probes and microdilution wells. The DNA–DNA relatedness values presented are the mean ± standard deviation of five replicates.

The genomic DNA G + C content of strain 1-3-3-3^T was analysed by reverse-phase HPLC of the individual nucleosides that resulted from DNA hydrolysis and dephosphorylation using nuclease P1 and alkaline phosphatase (Mesbah et al. 1989). HPLC analysis was carried out on the above-mentioned Agilent 1100 HPLC system. Nucleosides were separated using a SunFire C18 column (4.6 mm x 250 mm, 5 μm; Waters, Milford, USA) and a mobile phase of 0.2 M ammonium phosphate (pH 4.0)/acetonitrile (40:1, v/v) at flow rate of 0.7 ml/min. Single-stranded DNA from salmon testes (D7656; Sigma; DNA G + C content, 41.2 mol %) was used as a standard.

Results and discussion

Phylogenetic analysis

The nearly complete 16S rRNA gene sequence determined for strain 1-3-3-3^T contains 1430 bp (NCBI GenBank/EMBL/DDBJ accession number LC315608). Based on 16S rRNA gene sequence similarity, the close relatives of the isolate were identified to be *H. algoricola* VUG-A23a^T (98.0%), *H. knuensis* 16F7C-2 (97.9%), *H. fastidiosus* VUG-A124^T (97.1%), *H. elongatus* VUG-A112^T (97.0%), *H. chitinivorans* Txc1^T (97.0%) and *H. aquaticus* 16F3P^T (96.7%). The sequence similarities between strain 1-3-3-3^T and other *Hymenobacter* species were less than 96.3%. The phylogenetic position of strain 1-3-3-3^T, determined using three tree-making algorithms (ML, NJ, and MP), revealed the novel strain was positioned within the radiation comprising members of the genus *Hymenobacter*, joining *H. algoricola* VUG-A23a^T in the ML and MP phylogenetic trees with moderate bootstrap values of 71 and 72%, respectively (Fig. 1).

A threshold of 97% similarity in 16S rRNA gene sequence was proposed for bacterial species delineation (Stackebrandt and Goebel, 1994), then later re-evaluated as 98.7% by Stackebrandt and Ebers (2006) and Chun et al. (2018). On the basis of the re-evaluated threshold value, the above data indicate that strain 1-3-3-3^T represents a novel species of the genus *Hymenobacter*. To confirm the taxonomic status of the novel isolate, DNA-DNA hybridization was performed with its five above-mentioned close phylogenetic neighbours. The DNA–DNA relatedness values of strain 1-3-3-3^T with *H. algoricola* JCM 17214^T (33.8 ± 6.0%), *H. knuensis* KCTC 52538 (26.9 ± 5.6%), *H. fastidious* JCM17224^T (24.8 ± 5.5%), *H. elongatus* JCM 17223^T (20.4 ± 3.9%), and *H. chitinivorans* KACC 12068^T (18.4 ± 3.8%) were lower than the threshold value of 70% suggested for the delineation of novel prokaryotic species (Wayne et al. 1987).

Phenotypic characteristics

Cells of strain 1-3-3-3^T were observed to be Gram-stain negative, aerobic, non-motile, red pink pigment-producing rods (0.9–1.2 μm wide and 1.8–3.8 μm long) (Supplementary Fig. S1). The isolate was found to be able to grow at 10–30 °C, but not at 4 or 37 °C, with optimum growth at 25 °C. Strain 1-3-3-3^T can grow at a pH range between 6.0 and 8.0, with the optimum being pH 7.0. The strain was found to tolerate 1% (w/v) NaCl, but not 1.5%. Growth was observed on NA, R2A and TSA agar but not on LB agar. Other phenotypic features of the isolate are summarised in the species description. In API ZYM tests, the strain is positive for acid phosphatase, alkaline phosphatase, cystine arylamidase (weak, w), esterase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase (w) and valine arylamidase activities, but negative for *N*-acetyl-*β*-glucosaminidase, *α*-chymotrypsin, esterase (C4), *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase and trypsin activities. In API ID 32 GN and API 20 NE tests, citrate, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate and D-melibiose are utilised, but acetate, *N*-acetyl-D-glucosamine, adipate, L-alanine, L-arabinose, caprate, L-fucose, gluconate, D-glucose, glycogen, DL-3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, malonate, D-maltose, D-mannitol, D-mannose, phenyl acetate, L-proline, propionate, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol, suberate, D-sucrose and n-valerate are not utilised. In API 50 CH tests, acid is produced from arbutin, D-cellobiose (w), D-fructose, D-galactose, D-melibiose, D-melezitose, methyl-*β*-D-xylopyranoside (w), L-sorbose, D-raffinose (w) and D-trehalose, but not from *N*-acetylglucosamine, D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, gentiobiose, gluconate, D-glucose, glycerol,

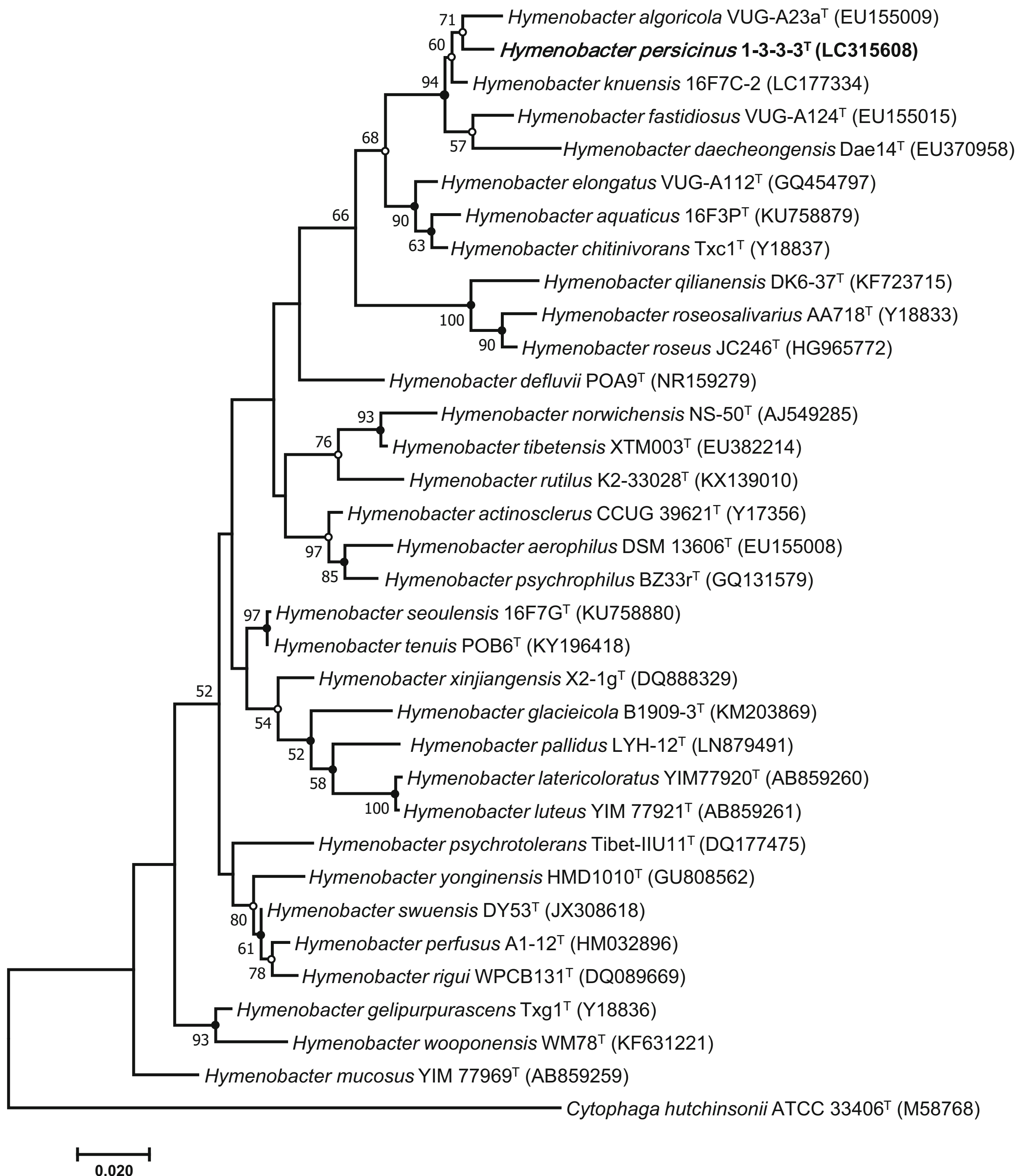

Fig. 1 Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain 1-3-3-3^{T} among related species of the genus *Hymenobacter*. Bootstrap values greater than 50% (percentages of 1000 replications) are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the neighbor-joining and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the neighbor-joining or maximum-parsimony algorithms. *Cytophaga hutchinsonii* ATCC 33406^{T} (M58768) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position

Table 1 Differential phenotypic characteristics of strain 1-3-3-3^T and phylogenetically related species of the genus *Hymenobacter*

Characteristic	1	2	3	4	5	6	7
Growth at/on/in							
4 °C	–	+	–	+	w	+	–
30 °C	+	–	+	–	–	+	+
TSA agar	+	–	–	–	–	–	–
NA agar	+	–	+	–	–	–	+
pH 5	–	–[a]	+	–[a]	–[a]	+	+
pH 10	–	+[a]	–	+[a]	+[a]	+	–
1% NaCl	+	–	+	–	–	+[b]	+
Catalase	+	–[a]	+	–[a]	–[a]	+[b]	+
Aesculin hydrolysis	+	–	+	–	–	+	w
Gelatin hydrolysis	+	–	+	–	–	+	+
Enzyme activity (API ZYM)							
N–Acetyl-*β*-glucosaminidase	–	–	+	–	–	+	–
Acid phosphatase	+	+	+	+	+	–	w
Cystine arylamidase	w	+	+	+	–	–	w
Esterase (C4)	–	+	w	+	–	+	w
β-Galactosidase	–	–	+	–	–	–	–
α-Glucosidase	–	–	+	–	w	w (–)[b]	–
Lipase (C14)	–	+	+	+	–	–	–
Naphthol-AS-BI-phosphohydrolase	w	+	+	+	+	–	w
Trypsin	–	+	–	w	–	–	–
Valine arylamidase	+	+	+	+	+	–	+
Assimilation of (API 20NE, API ID 32 GN)							
Citrate	+	–	–	–	–	–	–
L-Fucose	–	–	w	–	–	–	–
D-Glucose	–	+	–	+	+	–	–
Glycogen	–	–	w	–	–	–	+
L-Histidine	+	–	–	–	+	+	–
3-Hydroxybenzoate	+	–	–	–	+	+	–
4-Hydroxybenzoate	+	–	w	–	+	+	+
DL-Lactate	–	– (+)[c]	–	–	–	–	+
D-Maltose	–	–	–	–	+	–	+
D-Melibiose	+	–	–	–	–	–	–
D-Sorbitol	–	–	+	–	–	–	–
Suberate	–	w	+	w	–	–	–
D-Sucrose	–	–	+	–	–	–	–
Acid production from (API 50CH)							
Arbutin	+	–	+	–	–	–	–
D-Cellobiose	w	–	–	–	–	+	–
D-Fructose	+	–	+	+	–	+	+
D-Galactose	+	–	+	–	–	w	–
Gentiobiose	–	–	+	–	–	+	+
D-Glucose	–	+	–	–	–	–	–
Glycogen	–	–	+	–	–	–	+
Inulin	–	–	w	–	–	w	+

Table 1 continued

Characteristic	1	2	3	4	5	6	7
D-Lactose	–	–	+	–	–	w	–
D-Maltose	–	–	+	–	–	–	+
D-Melibiose	+	–	–	–	–	–	–
D-Melezitose	+	–	–	–	–	–	–
Methyl-β-D-xylopyranoside	w	–	–	–	–	–	w
D-Raffinose	w	–	+	–	–	w	+
D-Trehalose	+	–	w	–	–	–	w
L-Sorbose	+	–	–	–	–	–	–
D-Sucrose	–	–	+	–	–	–	–
D-Xylose	–	–	–	–	–	+	+
G + C content	61.6	64.1[c]	63.4[d]	64.0[c]	NR	61.0[b]	61.9[e]

Strains: 1, 1-3-3-3^T; 2, *H. algoricola* JCM 17214^T; 3, *H. knuensis* KCTC 52538; 4, *H. fastidious* JCM 17224^T; 5, *H. elongatus* JCM 17223^T; 6, *H. chitinivorans* KACC 12068^T; 7, *H. aquaticus* KCTC 32554^{T}All data were obtained in this study, unless otherwise indicated. Results deviating results from published data are shown in brackets. All strains are Gram-stain-negative, grow at pH 6-8 and on R2A agar but not on LB agar. All strains are positive for alkaline phosphatase, esterase (C8), leucine arylamidase and oxidase, but negative for reduction of nitrate to nitrite, indole production, arginine dihydrolase, α-chymotrypsin, α-fucosidase, α-galactosidase, *β*-glucosidase*,* *β*-glucuronidase, α-mannosidase, urease, and assimilation of acetate, *N*-acetyl-D-glucosamine, adipate, L-alanine, L-arabinose, caprate, gluconate, DL-3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, L-malate, malonate, D-mannitol, D-mannose, phenyl acetate, L-proline, propionate, L-rhamnose, D-ribose, salicin, L-serine, and valerate. All strains are negative for acid production from *N*-acetylglucosamine, D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, gluconate, glycerol, inositol, 2-ketogluconate, 5-ketogluconate, D-lyxose, D-mannitol, D-mannose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, L-rhamnose, D-ribose, salicin, sorbitol, starch, D-tagatose, D-turanose, xylitol, and L-xylose

+ Positive reaction, – negative reaction, *w* weakly positive reaction, *NR* not reported

[a]Data from Klassen and Foght (2011), [b]Data from Buczolits et al. (2006), [c]Data from Jin et al. (2018), [d]Data from Ten et al. (2017), [e]Data from Lee et al. (2017b)

glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, D-lactose, D-lyxose, D-maltose, D-mannitol, D-mannose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, L-rhamnose, D-ribose, salicin, D-sorbitol, starch, D-sucrose, D-tagatose, D-turanose, xylitol, D-xylose or L-xylose.

The morphological, physiological and biochemical characteristics differentiating the novel strain from *H. knuensis* KCTC 52538, *H. algoricola* JCM 17214^T, *H. fastidious* JCM17224^T, *H. elongatus* JCM 17223^T, *H. chitinivorans* KACC 12068^T and *H. aquaticus* KCTC 32554^T are listed in Table 1. In particular, strain 1-3-3-3^T can be readily differentiated from the reference strains by its ability to grow on TSA agar, to utilise citrate and D-melibiose, and to produce acid from D-melibiose, D-melezitose and L-sorbose.

Chemotaxonomic characteristics

The major fatty acids of strain 1-3-3-3^T were identified as iso-$C_{15:0}$ (19.9%), summed feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) (19.7%), summed feature 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B) (17.8%), $C_{16:1}$ *ω*5c (12.5%), and anteiso-$C_{15:0}$ (11.2%), together representing 81.1% of the total fatty acids present. As shown in Table 2, this fatty acid profile is similar to those of this strain's phylogenetically close relatives. However, under the same growth conditions, the isolate differed from the six reference strains in terms of the proportions of the major and some minor fatty acids. The major polar lipid in strain 1-3-3-3^T was observed to be phosphatidylethanolamine (Supplementary Fig. S2), which has also been identified as the main polar lipid in other *Hymenobacter* species (Buczolits et al. 2006; Lee et al. 2017b; Han et al. 2018; Ohn et al. 2018). In addition, the polar lipid profile of the isolate included three unidentified aminophospholipids, an unidentified

aminoglycolipid, two unidentified lipids and an unidentified phospholipid. The predominant isoprenoid quinone of strain 1-3-3-3[T] was found to be a menaquinone with seven isoprene units (MK-7), which is the major respiratory quinone found in other members of the genus *Hymenobacter* (Hirsch et al. 1998; Buczolits et al. 2006; Jin et al. 2018; Ten et al. 2018).

DNA G + C content

The genomic DNA G + C content of strain 1-3-3-3[T] was determined to be 61.6 mol %, which lies within the range of values recorded for recognised members of the genus *Hymenobacter* (55.0–70.0 mol%) (Buczolits et al. 2006; Han et al. 2014).

Taxonomic conclusion

The DNA G + C content, respiratory quinone composition, polar lipid profile and major cellular fatty acids of strain 1-3-3-3[T] are generally in agreement with those of the members of the genus *Hymenobacter*, consistent with the assignment of the novel isolate into the genus. However, based on its phylogenetic distance from established species within the genus *Hymenobacter*, the low level of DNA relatedness with closely related species and the specific combination of phenotypic characteristics (Table 1), it is clear that strain 1-3-3-3[T] is distinct from any recognised *Hymenobacter* species. These results support the conclusion that strain 1-3-3-3[T] represents a novel species of the genus *Hymenobacter*, for which the

Table 2 Fatty acid profiles of strain 1-3-3-3[T] and phylogenetically related species of the genus *Hymenobacter*

Fatty acids	1	2	3	4	5	6	7
Saturated							
Iso-$C_{15:0}$	19.9	8.7	12.6	22.2	12.4	22.4	13.0
Anteiso-$C_{15:0}$	11.2	7.5	10.4	tr	–	8.2	6.3
$C_{16:0}$	tr	10.6	1.4	2.1	5.1	1.7	19.8
Iso-$C_{16:0}$	tr	1.9	1.5	2.2	–	1.2	–
Iso-$C_{17:0}$	1.1	tr	2.7	3.3	–	3.9	–
Hydroxy							
Iso-$C_{15:0}$ 3-OH	4.0	2.9	4.0	3.2	4.0	2.3	1.9
Iso-$C_{17:0}$ 3-OH	5.0	3.9	7.1	5.1	6.6	5.8	3.0
Unsaturated							
Iso-$C_{15:1}$ G	–	tr	–	2.3	1.9	1.4	2.5
Iso-$C_{16:1}$ H	1.9	2.9	2.5	3.1	–	1.4	–
$C_{16:1}$ $\omega 5c$	12.5	12.5	10.5	12.3	10.9	10.6	10.7
Summed feature[a]							
1 (iso-$C_{15:1}$ H/$C_{13:0}$ 3-OH)	1.6	1.3	1.0	1.8	1.1	1.0	–
3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$)	19.7	15.7	19.5	12.4	13.9	11.1	15.4
4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B)	17.8	13.6	15.9	19.5	18.9	14.4	6.9

Strains: 1, 1-3-3-3[T]; 2, *H. algoricola* JCM 17214[T]; 3, *H. knuensis* KCTC 52538; 4, *H. fastidious* JCM 17224[T]; 5, *H. elongatus* JCM 17223[T]; 6, *H. chitinivorans* KACC 12068[T]; 7, *H. aquaticus* KCTC 32554[T]. Data for reference taxa 2 - 7 are from our previous studies (Lee et al. 2017b; Ten et al. 2017)

All strains were grown on R2A agar at 18 °C for 7 days prior to fatty acid analysis. Values are percentages of the total fatty acid content, and only fatty acids detected in at least four strains and accounting for at least 1% of the total are presented. *H. elongatus* JCM 17223[T] contained $C_{18:1}$ $\omega 9c$ (3.9%) and unidentified fatty acids with ECL 16.35 (9.6%) and ECL 15.76 (9.6%)[b]. $C_{18:0}$ was detected in *H. algoricola* JCM 17214[T] (6.8%) and *H. aquaticus* KCTC 32554[T] (12.5%). *H. chitinivorans* KACC 12068[T] contained $C_{16:0}$ N alcohol (9.7%).–, not detected; tr, trace (< 1.0%)

[a]Summed features contained two or three fatty acids that could not be separated by gas–liquid chromatography with the Sherlock Microbial Identification (MIDI) System

[b]Unidentified fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; ECL, equivalent chain-length

name *Hymenobacter persicinus* sp. nov. is proposed. The Digital Protologue Database (Rosselló-Móra et al. 2017) TaxoNumber for strain 1-3-3-3^T is TA00817.

Description of *Hymenobacter persicinus* sp. nov.

Hymenobacter persicinus (per.si.ci'nus. N.L. masc. adj. *persicinus* peach-coloured because of its red-pink colour).

Cells are Gram-stain negative, non-motile, aerobic rods without flagella that are 0.9–1.2 µm wide and 1.8–3.8 µm long. After 3 days of incubation at 25 °C on R2A agar, colonies are convex, smooth, circular, red-pink, and approximately 0.9-1.1 mm in diameter. Growth occurs at 10–30 °C and pH 6–8. Grows on NA, R2A, and TSA agar but not on LB agar; tolerates 1% but not 1.5% NaCl. No growth is observed under anaerobic conditions. Positive for catalase, oxidase, aesculin and gelatin hydrolysis but negative for nitrate reduction, urea hydrolysis and indole production. The major cellular fatty acids are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$), summed feature 4 (iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B), $C_{16:1}$ ω5c, and anteiso-$C_{15:0}$. The predominant respiratory quinone is MK-7. The major polar lipid is phosphatidylethanolamine. The genomic DNA G + C content of the type strain is 61.6 mol %.

The type strain 1-3-3-3^T (= KCTC 52742^T = JCM 32191^T) was isolated from a soil sample collected in Jeollabuk-do province, (35°41′04.50″N, 127°25′53.43″E), South Korea. The 16S rRNA gene sequence of strain 1-3-3-3^T has been deposited in NCBI GenBank/EMBL/DDBJ under the accession number LC315608.

Acknowledgements This work was supported by Brain Pool Program (Grant No. 2018H1D3A2065415) through the National Research Foundation (NRF) funded by the Ministry of Science and ICT, Republic of Korea.

Author's contribution Leonid N. Ten analysed the data and wrote the manuscript, Na Yeong Jeon made the original isolation and performed the phenotypic characterization, Weilan Li performed TEM imaging and conducted the fatty acid analysis, Myung Kyum Kim performed 16S rRNA phylogeny, Young-Je Cho performed the phylogenetic analysis and taxonomic analysis, Hee-Young Jung designed, planned the study and wrote the manuscript. All authors approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aslam Z, Im WT, Ten LN, Lee MJ, Kim KH, Lee ST (2006) *Lactobacillus siliginis* sp. nov., isolated from wheat sourdough in South Korea. Int J Syst Evol Microbiol 56:2209–2213

Bowman JP (2000) Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. Int J Syst Evol Microbiol 50:1861–1868

Buczolits S, Denner EBM, Vybiral D, Wieser M, Kämpfer P, Busse HJ (2002) Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. Int J Syst Evol Microbiol 52:445–456

Buczolits S, Denner EB, Kämpfer P, Busse HJ (2006) 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 chitinivorans* sp. nov., respectively, and emended description of the genus *Hymenobacter* Hirsch et al. 1999. Int J Syst Evol Microbiol 56:2189–2192

Cappuccino JG, Sherman N (2010) Microbiology: a Laboratory Manual, 9th edn. Benjamin Cummings, San Francisco

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Hana Y, 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

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric DNA–DNA hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Han L, Wu SJ, Qin CY, Zhu YH, Lu ZQ, Xie B, Lv J (2014) *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 105:971–978

Han J, Ten LN, Lee DH, Kang IK, Jung HY (2018) *Hymenobacter agri* sp. nov., a novel bacterium isolated from soil. Antonie Van Leeuwenhoek 111:1815–1823

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

Jin L, Wu X, Ko SR, Jin FJ, Li T, Ahn CY, Oh HM, Lee HG (2018) Description of *Hymenobacter daejeonensis* sp. nov., isolated from grass soil, based on multilocus sequence analysis of the 16S rRNA gene, *gyr*B and *tuf* genes. Antonie Van Leeuwenhoek 111:2283–2292

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

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 KI (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–205

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

La HJ, Im WT, Ten LN, Kang MS, Shin DY, Lee ST (2005) *Paracoccus koreensis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket (UASB) reactor. Int J Syst Evol Microbiol 55:1657–1660

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Lee JJ, Lee YH, Park SJ, Lee SY, Kim BO, Ten LN, Kim MK, Jung HY (2017a) *Spirosoma knui* sp. nov., a radiation-resistant bacterium isolated from the Han River. Int J Syst Evol Microbiol 67:1359–1365

Lee JJ, Park SJ, Lee YH, Ten LN, Jung HY (2017b) *Hymenobacter aquaticus* sp. nov., a radiation-resistant bacterium isolated from a river. Int J Syst Evol Microbiol 67:1206–1211

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnella AG, Goodfellowb M, Aldersonb G, Athalyeb M, Schaala A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Munoz R, Rosselló-Móra R, Amann R (2016) Revised phylogeny of *Bacteroidetes* and proposal of sixteen new taxa and two new combinations including *Rhodothermaeota* phyl. nov. Syst Appl Microbiol 39:281–296

Ohn JE, Ten LN, Kim BO, Cho YJ, Jung HY (2018) *Hymenobacter rufus* sp. nov., a bacterium isolated from soil. Int J Syst Evol Microbiol 68:2983–2989

Reddy GS, Garcia-Pichel F (2013) Description of *Hymenobacter arizonensis* sp. nov. from the southwestern arid lands of the United States of America. Antonie Leeuwenhoek 103:321–330

Rosselló-Móra R, Trujillo ME, Sutcliffe IC (2017) Introducing a digital protologue: a timely move towards a database-driven systematic of archaea and bacteria. Antonie Van Leeuwenhoek 110:455–456

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

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc, Newark

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Ten LN, Baek SH, Im WT, Lee M, Oh HW, Lee ST (2006) *Paenibacillus panacisoli* sp. nov., a xylanolytic bacterium isolated from soil in a ginseng field in South Korea. Int J Syst Evol Microbiol 56:2677–2681

Ten LN, Jung HM, Yoo SA, Im WT, Lee ST (2008) *Lysobacter daecheongensis* sp. nov., isolated from sediment of stream near the Daechung dam in South Korea. J Microbiol 46:519–524

Ten LN, Lee JJ, Lee YH, Park SJ, Lee SY, Park S, Lee DS, Kang IK, Kim MK, Jung HY (2017) *Hymenobacter knuensis* sp. nov., isolated from river water. Curr Microbiol 74:515–521

Ten LN, Lim SJ, Kim BO, Kang IK, Jung HY (2018) *Hymenobacter segetis* sp. nov., isolated from soil. Arch Microbiol 200:1167–1175

Tittsler RP, Sandholzer LA (1936) The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 31:575–580

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O et al (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Wilson K (1997) Preparation of genomic DNA from bacteria. In: Ausubel FM (ed). Current protocols in molecular biology, Jonh, New York, pp 2.4.1–2.4.5

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.