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ORIGINAL PAPER

# 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

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**Abstract** A polyphasic taxonomic study was carried out on strains PB105$^T$ and PB108 isolated from a grass soil in Korea. The cells of the strains were Gram-stain negative, non-spore-forming, non-motile, and rod-shaped. Comparative 16S rRNA gene sequence studies showed a clear affiliation of these strains with *Bacteroidetes*, which showed high pairwise sequence similarities with *Hymenobacter algoricola* VUG-A23a$^T$ (99.2%), *Hymenobacter fastidiosus* VUG-A124a$^T$ (97.4%), and *Hymenobacter daecheongensis* Dae14$^T$ (96.9%). The phylogenetic analysis based on 16S rRNA gene sequences showed that the strains formed a clear phylogenetic lineage with the genus *Hymenobacter*. The major fatty acids were identified as $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:1}$ $\omega5c$, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3-OH, summed feature 3 ($C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c/t$), and summed feature 4 ($C_{17:1}$ anteiso B and/or $C_{17:1}$ iso I). The major cellular polar lipids were identified as phosphatidylethanolamine, an unidentified aminolipid, and two unidentified lipids. The respiratory quinone was identified as MK-7 and the genomic DNA G+C content was determined to be 64.5 mol% for strain PB105$^T$ and 64.1 mol% for strain PB108. DNA–DNA hybridization value of type strain PB105$^T$ with *H. algoricola* VUG-A23a$^T$ was 32.3% (reciprocal 39.2). Based on the combined genotypic and phenotypic data, we propose that strains PB105$^T$ and PB108 represent a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter daejeonensis* sp. nov. is proposed. The type strain is PB105$^T$ (= KCTC 52579$^T$ = JCM 31885$^T$).

**Keywords** Grass soil · PB105 · PB108 · *Hymenobacter daejeonensis*

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## Introduction

Phylogenetically, the genus *Hymenobacter* is an evolutionary lineage within the family *Hymenobacteraceae* of the phylum *Bacteroidetes*. The genus currently contains 63 validly named species (http://www.bacterio.net/index.html), since Hirsch et al.

(1998) proposed *Hymenobacter roseosalivarius* as a new genus in the family *Cytophagaceae*. The members of the genus *Hymenobacter* are found in a wide range of natural environments, soil, water, ice, air etc., including extreme environments (Klassen and Foght 2011; Jin et al. 2014a; Sedláček et al. 2017), and geographically different sites in the world (Dai et al. 2009; Jin et al. 2014a; Klassen and Foght 2011; Kojima et al. 2016; Zhang et al. 2007; Subhash et al. 2014; Buczolits et al. 2006; Sheu et al. 2017). All members of the genus *Hymenobacter* are Gram-stain non-motile, pink- to red-pigmented, and rod-shaped, and contain phosphatidylethanolamine as the major polar lipid and MK-7 as the predominant menaquinone (Hirsch et al. 1998; Buczolits et al. 2006; Kim et al. 2008; Srinivasan et al. 2015; Ten et al. 2017; Han et al. 2018). Herein, we describe two red-pigmented aerobic bacterial strains, PB105$^T$ and PB108, that were recovered from a grass soil sample in South Korea. Phylogenetic analysis of 16S rRNA gene sequences showed that strains PB105$^T$ and PB108 are closely related to members of the genus *Hymenobacter*. Based on a polyphasic approach including the determination of their phenotypic, chemotaxonomic properties and a detailed phylogenetic investigation, we propose strains PB105$^T$ and PB108 as a new species *Hymenobacter daejeonensis* sp. nov.

## Materials and methods

### Isolation, morphological and physiological characterization

Soil samples taken from KAIST campus (36°22′20″N, 127°21′37″E) grass were initially diluted serially with a 0.85% saline solution. A 100 µL sub-sample of the suspended material was aseptically transferred and spread on modified 1/10 R2A agar ($L^{-1}$: 0.05 g peptone, 0.05 g yeast extract, 0.05 g casamino acid, 0.05 g dextrose, 0.05 g soluble starch, 0.03 g $K_2HPO_4$, 0.005 g $MgSO_4$, 0.03 g sodium pyruvate, and 15 g agar), and the plates were incubated at 25 °C for 14 days under fluorescent light of 2400 lx. Two red colonies that appeared on the modified R2A plates were selected for further study. For long-term storage, the two isolates, PB105$^T$ and PB108, were routinely cultured on R2A plates at 30 °C under an aerobic condition and stored frozen at − 80 °C in 15% (v/v) glycerol stock solution. For most of the experiments, all strains were cultivated on R2A agar (BD, USA) or broth (MB cell; MB-R2230) at 30 °C for 48 h. All reference strains, *Hymenobacter algoricola* JCM 27214$^T$, *Hymenobacter fastidiosus* JCM 27227$^T$, and *Hymenobacter daecheongensis* KCTC 22258$^T$, were obtained from the JCM (Japan Collection of Microorganisms) and KCTC (Korean Collection for Type Cultures).

Macromorphology for colony, cell morphology, motility, Gram-staining, and biochemical properties were determined with cells grown on R2A agar plates at 30 °C for 48 h. The Gram reaction test was carried out using a Gram stain kit (Becton–Dickinson) following the manufacturer's instructions. Cell morphology and motility were observed under a phase-contrast microscope (Nikon Optiphot, 1000 × magnification). Transmission electron micrographs (Philips CM-20) were taken after negative staining with 1% (w/v) phosphotungstic acid. An oxidase activity test was carried out using 1% tetramethyl-*p*-phenylenediamine (Tarrand and Groschel 1982) and a catalase activity test using 3% $H_2O_2$. Growth was investigated on R2A agar at different temperatures (4, 8, 10, 15, 20, 30, 37, and 42 °C). The pH range (pH 5–10 at intervals of 1 unit) for growth was investigated in R2A broth, and different buffer systems were applied, as previously described (Jin et al. 2014b). NaCl tolerance for growth was carried out in R2A agar using different NaCl concentrations from 1 to 5% (w/v). Carbon-source utilization, enzyme activity, and additional physiological tests were performed using API 20NE, ID 32 DN, and API ZYM kits (bioMérieux) and the Biolog GN2 MicroPlate according to the manufacturer's instructions (bioMérieux).

### Chemotaxonomic characterization

For quantitative analysis of whole-cell fatty acid profiling, strains PB105$^T$, PB108, *H. algoricola* JCM 27214$^T$, *H. fastidiosus* JCM 27227$^T$, and *H. daecheongensis* KCTC 22258$^T$ were cultured on R2A agar at 20 °C for 72 h. and the harvesting of bacterial cells was standardized as specified by MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). To extract the fatty acids, we harvested the cell mass when the cells reached the late exponential phase. Separation and identification of the fatty acids were done by GC (Hewlett Packard 6890), and the TSBA 6 database

provided by Sherlock software 6.1. Extraction of isoprenoid quinine was completed as described by Komagata and Suzuki (1988), and the analysis was done by HPLC (Shimadzu) with an YMC-Pack ODS-A column. The polar lipids were analyzed by the Identification Service of the DSMZ. The polar lipids were extracted, determined using two-dimensional TLC, and identified following the method described by Tindall (1990).

### Molecular characterization

Phylogenetic positions of strains PB105$^T$ and PB108 were determined with a 16S rRNA gene sequence analysis. Genomic DNA was extracted using the FastDNA$^{TM}$ SPIN kit for soil DNA Extraction MP. Extracted DNA was then examined for purity on a ND2000 spectrometer (Nanodrop Technologies, Inc.). The 16S rRNA gene was amplified with the universal bacterial primer sets 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′; *Escherichia coli* position 8-27) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′; *E. coli* position 1492–1510) (Lane 1991), and the conditions for the PCR cycling were as follows: 95 °C for 5 min and 30 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min followed by a final extension step for 7 min at 72 °C. Two more primers, 785F (5′-GGA TTA GAT ACC CTG GTA-3′) and 800R (5′-TAC CAG GGT ATC TAA TCC-3′), were used for the sequence analysis (Lane 1991) by BIOFACT Co. Ltd (http://bio-ft.com/). To construct phylogenetic trees, sequence alignment and edition was carried out using CLUSTAL X (Thompson et al. 1997) and BIOEDIT (Hall 1999) software, respectively. Neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971), and maximum-likelihood (Felsenstein 1981) algorithms were applied in the MEGA 7 software (Kumar et al. 2016). Bootstrap values were calculated on 1000 resamplings of the sequences (Felsenstein 1985). For more accurate classification, housekeeping genes were applied to delineate our strains from their close species. Partial sequences of protein-encoding genes are useful for species identification and as phylogenetic markers. For this purpose, the house keeping genes, *gyr*B gene encoding DNA gyrase β subunit and *tuf* gene encoding the elongation factor Tu, were sequenced for the type strain and reference strains. The amplifying and sequencing primers and the PCR conditions were described by Klassen and Foght (2011) and Martineau et al. (2001).

DNA G+C contents (mol%) of genomic DNA was determined using HPLC after hydrolysis, as described by Tamaoka and Komagata (1984). Non-methylated λ DNA (Sigma) was used as a standard. For a more accurate genotypic analysis, DNA–DNA hybridization experiment was carried out between strains PB105$^T$, PB108, and type strains of *H. algoricola* and *H. fastidiosus*, selected as close phylogenetic neighbours. The hybridizations were carried out as described by Ezaki et al. (1989), and salmon sperm DNA (Sigma; D7656) was used as a control.

## Results and discussion

Strains PB105$^T$ and PB108 were observed to form visible colonies within 48 h on R2A agar when incubated at 30 °C. Growth was found to occur at temperatures ranging from 8 to 30 °C, but no growth was observed at 4 °C and 37 °C. Growth was found to occur at pH 7–8, but no growth was observed at pH 6 or 9. The colonies were observed to be red, smooth, convex, and circular with entire edges. The cells were found to be Gram-stain negative, catalase positive and oxidase negative, non-motile, and short rod-shaped (Supplementary Fig. S1). The strains were found to be positive for L-alanylglycine, L-aspartic acid, dextrin, glycogen, γ-hydroxybutyric acid, *myo*-inositol, L-leucine, D-mannitol, L-ornithine, L-phenylalanine, phenylethylamine (weak), L-proline, D-psicose, D-raffinose, and D-sorbitol, but negative for acetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-alaninamide, D-alanine, L-alanine, L-asparagine, γ-aminobutyric acid bromosuccinic acid, 2,3-butanediol, DL-carnitine, D-cellobiose, citric acid, i-erythritol, D-fructose, L-fucose, D-galactonic acid lactone, D-galactose, D-galacturonic acid, D-gluconic acid, α-D-glucose, α-D-glucose-1-phosphate, D-glucose-6-phosphate, D-glucosaminic acid, α-D-glucose, glucuronamide, D-glucuronic acid, L-glutamic acid, glycerol, DL-α-glycerol phosphate, glycyl L-aspartic acid, glycyl L-glutamic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, α- D-lactose, lactulose, malonic acid, D-mannose, D-melibiose, methyl β-D-glucoside, propionic acid, putrescine, pyruvic acid methylester,

quinic acid, L-rhamnose, D-saccharic acid, D-serine, L-serine, succinic acid monomethyl ester, sucrose, L-threonine, thymidine, D-trehalose, turanose, uridine, urocanic acid, xylitol; variable for *cis*-aconitic acid (positive for type strain), 2-aminoethanol (positive for strain PB108), L-arabinose (weakly positive for type strain), L-arabitol (weakly positive for strain PB108), α-cyclodextrin (positive for strain PB108), formic acid (weakly positive for type strain), gentiobiose (positive for type strain), L-histidine (positive for type strain), hydroxy-L-proline (positive for strain PB108), itaconic acid (positive for type strain), DL-lactic acid (positive for strain PB108), maltose (positive for type strain), L-pyroglutamic acid (positive for type strain), sebacic acid (positive for strain PB108), succinamic acid (positive for type strain), succinic acid (positive for strain PB108), Tween 40 (positive for type strain), Tween 80 (weakly positive for type strain). Positive for the following enzyme activities: *N*-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase, naphtol-AS-BI-phosphohydrolase and valine arylamidase; but negative for the following enzyme activities: α-chymotrypsin, α-fucosidase, α-galactosidase, *β*-galactosidase, *β*-glucosidase, *β*-glucuronidase, lipase (C14), α-mannosidase, and trypsin (Table 1).

The almost-complete 16S rRNA gene sequences of strains PB105$^T$ (EMBL accession number KY412787) and PB108 (EMBL accession number KY412788) were compared with the 16S rRNA gene sequences of representative species within the genus *Hymenobacter* and related genera. We used the EzTaxon-e server (Yoon et al. 2017) to search their close relatives. The results showed that strains PB105$^T$ and PB108 shared 99.2% pairwise similarity with *Hymenobacter algoricola* VUG-A23a$^T$, 97.4% with *Hymenobacter fastidiosus* VUG-A124a$^T$, 96.9% with *Hymenobacter daecheongensis* Dae14$^T$, and less than 96.0% with other species of the genus *Hymenobacter*. Strains PB105$^T$ and PB108 shared 100% 16S rRNA gene sequence similarity. It has been suggested that less than 98.7% similarity of the 16S rRNA gene sequence can be applied as a new alternative threshold value to avoid DNA–DNA hybridization (DDH) in bacterial classification (Kim et al. 2014; Rosselló-Móra and Amann 2015; Chun et al. 2018). Strains PB105$^T$ and PB108 shared high similarities with *H. algoricola* (99.2%), and thus the genomic delineation between strains PB105$^T$ and PB108 and the type strain of *H. algoricola* was supported by the DNA–DNA relatedness (the mean of triplicate experiments) data, for which the new isolates showed DNA–DNA relatedness values of 32.3% (reciprocal 39.2), 43.8% (reciprocal 48.2) with *H. algoricola* VUG-A23a$^T$, respectively (Supplementary Table S1). For more accurate delineation of the two novel strains from their close neighbours, two housekeeping genes, *gyr*B and *tuf*, were applied as phylogenetic markers. The *gyr*B and *tuf* gene sequences of PB105$^T$ and PB108 had 99.9 and 100% similarities, respectively, and 78.6–90.1% and 93.0–94.6% similarities with the *gyr*B and *tuf* gene sequences of *H. algoricola* VUG-A23a$^T$, *H. fastidiosus* VUG-A124a$^T$ and *H. daecheongensis* Dae14$^T$, respectively (Supplementary Table S2). Overall, phylogenetic analyses based on 16S rRNA, *gyr*B and *tuf* gene sequences revealed stable groups that are in good agreement with the currently recognized genera (Figs. 1, 2). The low DNA–DNA hybridization values which were below the 70% cut-off point for the delineation of genomic species (Wayne et al. 1987), together with *gyr*B and *tuf* gene similarities indicate that strains PB105$^T$ and PB108 should be classified in a novel species.

The G+C content of the genomic DNA was determined to be 64.1–64.5 mol%. The major fatty acids were identified as $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:1}$ *ω5c*, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3-OH, summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c/t*), and summed feature 4 ($C_{17:1}$ anteiso B and/or $C_{17:1}$ iso I) (Table 2). The major fatty acids in strains PB105$^T$ and PB108 were consistent with the major fatty acid components in species from the genus *Hymenobacter*. However, some qualitative and quantitative differences in the fatty acid profiles were found. Some differences in the presence/absence of several components were also observed (Supplementary Table S1). The major respiratory quinone was menaquinone-7 (MK-7). The polar lipids were composed of phosphatidylethanolamine (PE), three unidentified aminolipids (AL1, AL2, and AL3), two unidentified phospholipids (PL1, PL2), an unidentified aminophosopholipid, and two unidentified lipids (L1, L2) for the type strain; phosphatidylethanolamine (PE), four unidentified aminolipids (AL1, AL2, AL3, and AL4), two unidentified phospholipids (PL1, PL2), an unidentified aminophosopholipid, and two

**Table 1** Phenotypic and chemotaxonomic characteristics distinguishing strains PB105$^T$ and PB108 from some close members of *Hymenobacter*

| Characteristics | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Morphology | Rods (1–1.1 × 1.8–2.3 μm) | Rods (0.5–0.8 × 1.4–2.4 μm) | Rods or slightly vibriod (0.5–0.8 × 1–2 μm)[a] | Rods or slightly vibriod (0.5–1 × 1–3 μm)[a] | Rods (0.6–1.0 × 2–5 μm)[b] |
| Isolation source | Grass soil | Grass soil | Basal ice[a] | Basal ice[a] | Sediment[b] |
| Growth range (°C) | 8–30 | 8–30 | 4–20[a] | 4–20[a] | 4–30[b] |
| pH range | 7–8 | 7–8 | 6–11[a] | 7–10[a] | 5–10[b] |
| Oxidase | − | − | + | + | + |
| Catalase | + | + | − | − | + |
| Gelatine hydrolysis | − | − | + | − | − |
| Enzyme activities | | | | | |
| α-glucosidase | + | + | − | − | − |
| Carbon utilization | | | | | |
| L-Alaninamide | − | − | + | − | − |
| 2-Aminoethanol | − | + | − | − | − |
| L-Alanylglycine | + | + | − | − | − |
| L-Arabinose | w | − | − | − | − |
| D-Arabitol | − | w | − | − | − |
| *cis*-Aconitic Acid | + | − | − | − | − |
| L-Aspartic Acid | + | + | − | − | + |
| D,L-Carnitine | − | − | − | − | + |
| D-Cellobiose | − | − | − | − | + |
| α-Cyclodextrin | − | + | w | − | + |
| Dextrin | + | + | w | − | + |
| Formic acid | w | − | − | − | + |
| D-Galactose | − | − | − | − | + |
| Gentiobiose | + | − | − | − | − |
| L-Glutamic acid | − | − | − | + | − |
| α-D-Glucose-1-phosphate | − | − | − | − | + |
| Glycogen | + | + | − | − | + |
| L-Histidine | + | − | − | − | − |
| γ-Hydroxybutyric acid | + | + | − | − | − |
| Hydroxy-L-proline | − | + | − | − | − |
| *m*-Inositol | + | + | − | − | − |
| Itaconic acid | + | − | − | − | − |
| α-keto Butyric acid | − | − | − | + | − |

**Table 1** continued

| Characteristics | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| α-keto Glutaric acid | – | – | – | + | – |
| α-keto Valeric acid | – | – | – | + | – |
| D,L-Lactic acid | – | + | – | – | – |
| L-Leucine | + | + | – | – | – |
| Maltose | + | – | – | w | – |
| D-Mannitol | + | + | – | – | – |
| L-Ornithine | + | + | – | – | – |
| L-Phenylalanine | + | + | – | – | + |
| Phenyethyl-amine | w | w | – | – | + |
| L-Proline | + | + | + | – | + |
| D-Psicose | + | + | – | – | + |
| L-Pyroglutamic acid | + | – | – | – | – |
| D-Raffinose | + | + | + | – | + |
| D-Sorbitol | + | + | – | – | – |
| Sebacic acid | – | + | + | – | – |
| Succinic acid | – | + | – | – | – |
| Succinamic acid | + | – | – | – | – |
| Tween 40 | + | – | – | – | + |
| Tween 80 | w | – | – | – | – |
| Thymidine | – | – | – | – | + |
| Urocanic acid | – | – | – | – | w |
| DNA G + C content (mol%) | 64.5 | 64.1 | 64.0 | 62.0 | 62.2[§] |

Strains: 1 PB105$^T$; 2 PB108; 3 *H. algoricola* JCM 27214$^T$; 4 *H. fastidiosus* JCM 27227$^T$; 5 *H. daecheongensis* KCTC 22258$^T$. All data were from this study, unless indicated. All strains were observed to be positive for activities of *N*-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphtol-AS-BI-phosphohydrolase and valine arylamidase. All strains were observed to be negative for activities of α-chymotrypsin, α-fucosidase, α-galactosidase, *β*-galactosidase, *β*-glucosidase, *β*-glucuronidase, lipase (C14), α-mannosidase and trypsin; carbon assimilation of acetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-alanine, L-alanine, γ-aminobutyric acid, L-asparagine, bromosuccinic acid, 2,3-butanediol, citric acid, i-erythritol, D-fructose, L-fucose, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-D-glucose, D-glucose-6-phosphate, glucuronamide, D-glucuronic acid, glycerol, DL-α-glycerol phosphate, glycyl-L-aspartic acid, glycyl-L-glutamic acid, α-hydroxybutyric acid, *β*-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, α-D-lactose, lactulose, malonic acid, D-mannose, D-melibiose, methyl-*β*-D-glucoside, propionic acid, putrescine, pyruvic acid methylester, quinic acid, L-rhamnose, D-saccharic acid, D-serine, L-serine, succinic acid monomethyl ester, sucrose, D-trehalose, L-threonine, turanose, uridine and xylitol

+ Positive, - negative, w weakly positive

[a]Data taken from Klassen and Foght (2011)

[b]Xu et al. (2009)

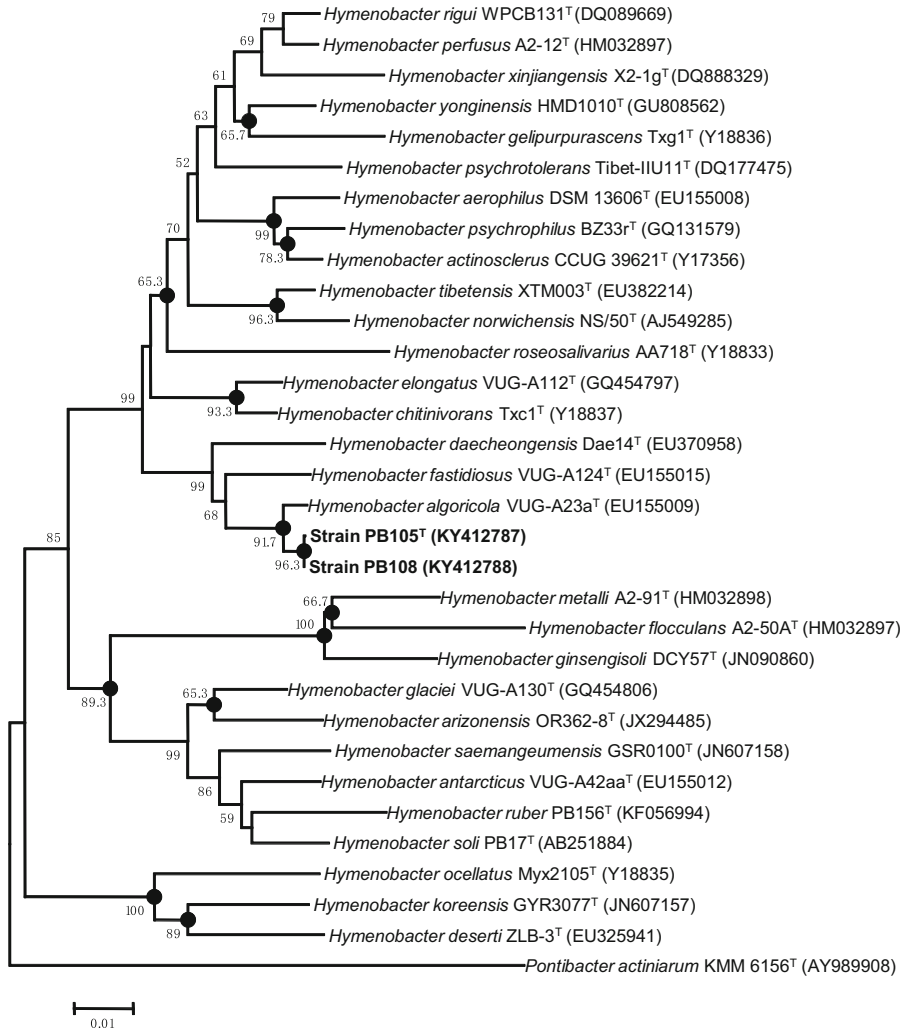
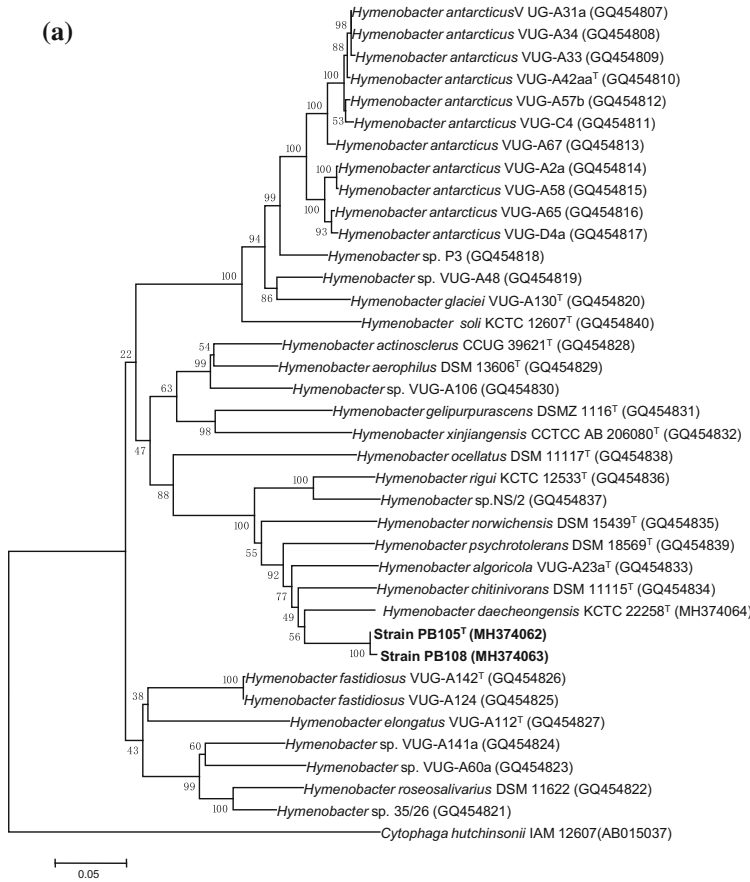


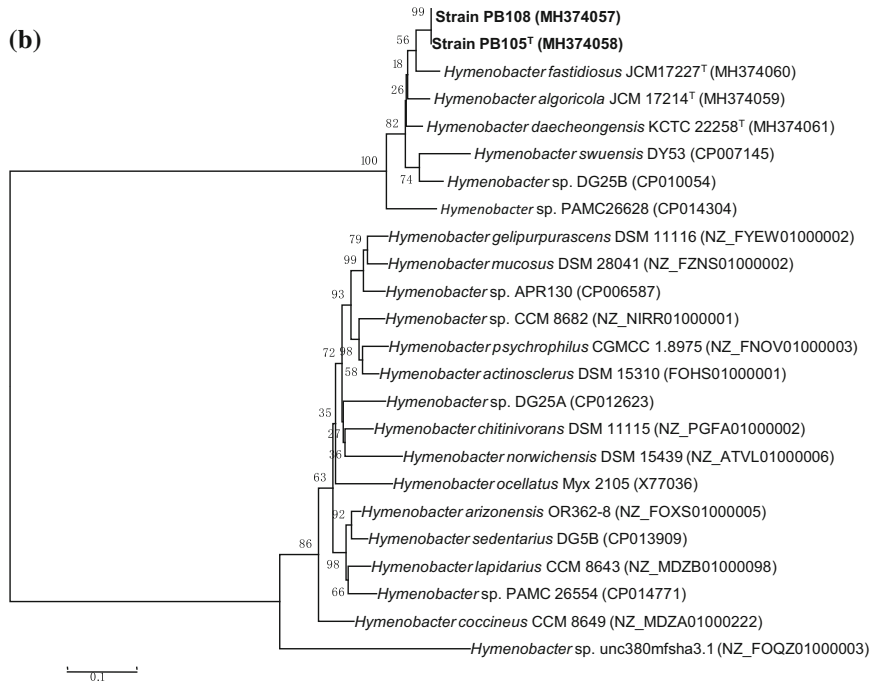

**Fig. 1** Phylogenetic tree based on 16S rRNA gene sequences using neighbour-joining method showing position of strains PB105$^T$ and PB108 among type strains within the genus *Hymenobacter*. Numbers at branching points refer to bootstrap values (1000 resamplings, only values above 50% shown). Filled circles indicate that the corresponding nodes were also calculated in trees generated with the algorithms of maximum-likelihood and maximum-parsimony. Bar, 1 substitution per 100 nt positions

unidentified lipids (L1, L2) for strain PB108 (Supplementary Fig. S2).

Based on phenotypic and phylogenetic characteristics, the new isolates are considered to be members of the genus *Hymenobacter*. Some physiological evidence, temperature growth range, carbon utilization, and enzyme activities, differentiated the two strains from their close formally described relatives. Strains PB105$^T$ and PB108 could be differentiated from the close species *H. algoricola* by assimilating L-alanylglycine, L-aspartic acid, glycogen, γ-hydroxybutyric acid, *myo*-inositol, L-leucine, D-mannitol, L-ornithine, L-phenylalanine, phenylethylamine, D-psicose, and D-sorbitol and by activities of oxidase, catalase, and α-glucosidase. Therefore, we conclude that strains PB105$^T$ and PB108 represent a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter daejeonensis* sp. nov. is proposed.

The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumbers for type strain PB105$^T$ is TA00522.


(a)
Hymenobacter antarcticusV UG-A31a (GQ454807)
Hymenobacter antarcticus VUG-A34 (GQ454808)
Hymenobacter antarcticus VUG-A33 (GQ454809)
Hymenobacter antarcticus VUG-A42aa$^T$ (GQ454810)
Hymenobacter antarcticus VUG-A57b (GQ454812)
Hymenobacter antarcticus VUG-C4 (GQ454811)
Hymenobacter antarcticus VUG-A67 (GQ454813)
Hymenobacter antarcticus VUG-A2a (GQ454814)
Hymenobacter antarcticus VUG-A58 (GQ454815)
Hymenobacter antarcticus VUG-A65 (GQ454816)
Hymenobacter antarcticus VUG-D4a (GQ454817)
Hymenobacter sp. P3 (GQ454818)
Hymenobacter sp. VUG-A48 (GQ454819)
Hymenobacter glaciei VUG-A130$^T$ (GQ454820)
Hymenobacter soli KCTC 12607$^T$ (GQ454840)
Hymenobacter actinosclerus CCUG 39621$^T$ (GQ454828)
Hymenobacter aerophilus DSM 13606$^T$ (GQ454829)
Hymenobacter sp. VUG-A106 (GQ454830)
Hymenobacter gelipurpurascens DSMZ 1116$^T$ (GQ454831)
Hymenobacter xinjiangensis CCTCC AB 206080$^T$ (GQ454832)
Hymenobacter ocellatus DSM 11117$^T$ (GQ454838)
Hymenobacter rigui KCTC 12533$^T$ (GQ454836)
Hymenobacter sp.NS/2 (GQ454837)
Hymenobacter norwichensis DSM 15439$^T$ (GQ454835)
Hymenobacter psychrotolerans DSM 18569$^T$ (GQ454839)
Hymenobacter algoricola VUG-A23a$^T$ (GQ454833)
Hymenobacter chitinivorans DSM 11115$^T$ (GQ454834)
Hymenobacter daecheongensis KCTC 22258$^T$ (MH374064)
Strain PB105$^T$ (MH374062)
Strain PB108 (MH374063)
Hymenobacter fastidiosus VUG-A142$^T$ (GQ454826)
Hymenobacter fastidiosus VUG-A124 (GQ454825)
Hymenobacter elongatus VUG-A112$^T$ (GQ454827)
Hymenobacter sp. VUG-A141a (GQ454824)
Hymenobacter sp. VUG-A60a (GQ454823)
Hymenobacter roseosalivarius DSM 11622 (GQ454822)
Hymenobacter sp. 35/26 (GQ454821)
Cytophaga hutchinsonii IAM 12607(AB015037)
0.05


(b)
Strain PB108 (MH374057)
Strain PB105$^T$ (MH374058)
Hymenobacter fastidiosus JCM17227$^T$ (MH374060)
Hymenobacter algoricola JCM 17214$^T$ (MH374059)
Hymenobacter daecheongensis KCTC 22258$^T$ (MH374061)
Hymenobacter swuensis DY53 (CP007145)
Hymenobacter sp. DG25B (CP010054)
Hymenobacter sp. PAMC26628 (CP014304)
Hymenobacter gelipurpurascens DSM 11116 (NZ_FYEW01000002)
Hymenobacter mucosus DSM 28041 (NZ_FZNS01000002)
Hymenobacter sp. APR130 (CP006587)
Hymenobacter sp. CCM 8682 (NZ_NIRR01000001)
Hymenobacter psychrophilus CGMCC 1.8975 (NZ_FNOV01000003)
Hymenobacter actinosclerus DSM 15310 (FOHS01000001)
Hymenobacter sp. DG25A (CP012623)
Hymenobacter chitinivorans DSM 11115 (NZ_PGFA01000002)
Hymenobacter norwichensis DSM 15439 (NZ_ATVL01000006)
Hymenobacter ocellatus Myx 2105 (X77036)
Hymenobacter arizonensis OR362-8 (NZ_FOXS01000005)
Hymenobacter sedentarius DG5B (CP013909)
Hymenobacter lapidarius CCM 8643 (NZ_MDZB01000098)
Hymenobacter sp. PAMC 26554 (CP014771)
Hymenobacter coccineus CCM 8649 (NZ_MDZA01000222)
Hymenobacter sp. unc380mfsha3.1 (NZ_FOQZ01000003)
0.1

◀**Fig. 2** Phylogenetic reconstructions based on individual analyses of the *gyr*B (**a**) and *tuf* (**b**) genes using the neighbour-joining method. Bars, 0.05 (**a**) and 0.1 (**b**) expected nucleotide substitutions per site. Only bootstrap values above 60% are shown (1000 resamplings) at branching points

**Table 2** Cellular fatty acid compositions (%) of strains PB105$^T$ and PB108 and related type strains

| Fatty acids | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $C_{15:0}$ iso | 20.5 | 18.9 | 18.9 | 13.3 | 18.6 |
| $C_{15:0}$ anteiso | 11.5 | 12.0 | 6.3 | 0.7 | 7.4 |
| $C_{16:1}$ iso H | 1.3 | 1.8 | 1.6 | tr | 5.5 |
| $C_{16:0}$ iso | 0.5 | 0.7 | 1.9 | 1.0 | 5.8 |
| $C_{16:1}$ $\omega5c$ | 6.2 | 5.8 | 19.5 | 21.4 | 15.1 |
| $C_{16:0}$ | 0.9 | 0.8 | 5.6 | 12.1 | 4.1 |
| $C_{15:0}$ iso 3-OH | 5.5 | 5.9 | 3.2 | 3.6 | 2.0 |
| $C_{17:0}$ iso | 1.8 | 1.9 | 1.9 | 3.0 | 5.1 |
| $C_{17:0}$ anteiso | tr | tr | tr | tr | 1.9 |
| $C_{16:0}$ iso 3-OH | tr | tr | 1.1 | tr | tr |
| $C_{16:0}$ 3-OH | tr | tr | tr | 1.5 | tr |
| $C_{17:0}$ iso 3-OH | 7.2 | 7.7 | 6.6 | 7.2 | 5.9 |
| $C_{17:0}$ 2-OH | 1.4 | 1.4 | tr | tr | tr |
| Summed Feature 1[a] | 3.0 | 3.0 | tr | tr | tr |
| Summed Feature 2[b] | tr | tr | tr | 1.1 | tr |
| Summed Feature 3[c] | 15.7 | 16.7 | 16.9 | 23.5 | 12.1 |
| Summed Feature 4[d] | 22.0 | 19.8 | 11.8 | 10.2 | 12.1 |

Strains: 1 PB105$^T$; 2 PB108; 3 *H. algoricola* JCM 27214$^T$; 4 *H. fastidiosus* JCM 27227$^T$; 5 *H. daecheongensis* KCTC 22258$^T$. All data were from present study. Cells of all strains were harvested after growth on R2A agar at 20 °C for 72 h. tr, less than 1% or not detected

[a]Summed Feature 1: $C_{13:0}$ 3-OH and/or C15:1 iso H

[b]Summed Feature 2: $C_{12:0}$ aldehyde and/or unknown 10.928

[c]Summed Feature 3: $C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c$

[d]Summed Feature 4: $C_{17:1}$ anteiso B and/or $C_{17:1}$ iso I

## Description of *Hymenobacter daejeonensis* sp. nov.

*Hymenobacter daejeonensis* (dae.je.on.en'sis. N.L. masc. adj. *daejeonensis* pertaining to Daejeon, a city in Korea, where the type strain was isolated).

Cells are Gram-stain negative, non-motile, rods after growth for 48 h at 30 °C on R2A agar. Colonies are smooth, circular, convex, and red-coloured on R2A agar. Growth occurs at 8–30 °C (optimum 30 °C), and at pH 7.0–8.0 (optimum pH 7.0). Cells do not tolerate NaCl. Catalase positive and oxidase negative. Negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase activities. The major fatty acids are $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:1}$ $\omega5c$, $C_{15:0}$ iso 3-OH, $C_{17:0}$ iso 3-OH, summed feature 3 ($C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c/t$), and summed feature 4 ($C_{17:1}$ anteiso B and/or $C_{17:1}$ iso I). The major polar lipids are phosphatidylethanolamine, an unidentified aminolipid, and two unidentified lipids. The DNA G+C content of the type strain is 64.5 mol% (determined using HPLC).

The type strain PB105$^T$ (= KCTC 52579$^T$ = JCM 31885$^T$) and strain PB108 (= KCTC 52580 = JCM 31886) were isolated from grass soil from Daejeon, Republic of Korea. The EMBL accession numbers for sequences generated in this study are as follows: KY412787–KY412788 (16S rRNA); MH374057–MH374061 (*gyr*B), MH374062–MH374064 (*tuf*).

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**Conflict of interest** The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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