Hymenobacter soli sp. nov., isolated from grass soil

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A Gram-negative, strictly aerobic, non-spore-forming, rod-shaped, red–pink bacterium, designated strain PB17^T, was isolated from grass soil in the Republic of Korea and its taxonomic position was investigated by means of a polyphasic approach. Phylogenetic analysis of 16S rRNA gene sequences showed that strain PB17^T belonged to the genus *Hymenobacter*. Sequence similarities between strain PB17^T and the type strains of *Hymenobacter* species with validly published names ranged from 90.1 to 91.8 %. The predominant cellular fatty acids in strain PB17^T were iso-C_{15:0}, summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), C_{16:1}ω5c and anteiso-C_{15:0}. The DNA G + C content was 58.8 mol%. The results of phylogenetic, chemotaxonomic and phenotypic characterization indicated that strain PB17^T can be distinguished from all known *Hymenobacter* species and represents a novel species, for which the name *Hymenobacter soli* sp. nov. is proposed. The type strain is PB17^T (=KCTC 12607^T =LMG 24240^T).

Hirsch *et al.* (1998) proposed a novel genus and species, *Hymenobacter roseosalivarius*, by describing five strains isolated from Antarctic soil. *Hymenobacter actinosclerus* (a radiation-resistant bacterium isolated from irradiated pork) and *Hymenobacter aerophilus* (isolated from air) were subsequently added to the genus (Collins *et al.*, 2000; Buczolits *et al.*, 2002). Recently, four more species were proposed: *Hymenobacter norwichensis*, *H. ocellatus*, *H. gelipurpurascens* and *H. chitinivorans* (Buczolits *et al.*, 2006). The members of the genus *Hymenobacter* have high G + C contents (55–65 mol%) compared with other members of the family 'Flexibacteraceae' in the class 'Sphingobacteria' (Garrity & Holt, 2001) (27–48 mol%) (Buczolits *et al.*, 2002).

During the course of a study on culturable, aerobic, pigmented bacterial strains from soil and a stream in Daejeon, Republic of Korea, a large number of novel bacterial strains were isolated. One of these isolates, strain PB17^T, was found to be a member of the genus *Hymenobacter* in the phylum *Bacteroidetes* and was subjected to further taxonomic investigation. In this study, we analysed its genotypic, chemotaxonomic and phenotypic characteristics in order to establish its phylogenetic affiliation.

Strain PB17^T was isolated from grass soil in April 2004. The soil sample was thoroughly suspended in 50 mM

phosphate buffer (pH 7.0) and the suspension was spread on R2A agar (Scharlau). After incubation at 25 °C for 1 or 2 weeks, the pigmented colonies that formed were picked and subcultured several times to confirm that they were pure cultures. Isolates were routinely cultured on R2A agar at 30 °C and maintained as glycerol suspensions (20 %, w/v) at –70 °C.

The Gram reaction was performed using the non-staining method, as described previously (Buck, 1982). Cell morphology was examined by using light microscopy (E600; Nikon). Catalase activity was determined from bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Flexirubin-type pigments were detected by suspending cells in 20 % KOH (Fautz & Reichenbach, 1980). Enzyme activities, substrates that could be utilized as sole carbon sources and some physiological characteristics were determined with API ZYM, API 20E, API 50 CH and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). The defined liquid mineral salts medium used for the API 50 CH and API 20NE tests contained (l⁻¹): 1.8 g K₂HPO₄, 1.08 g KH₂PO₄, 0.5 g NaNO₃, 0.5 g NH₄Cl, 0.1 g KCl, 0.1 g MgSO₄ and 0.05 g CaCl₂, supplemented with vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984). The pH of the medium was adjusted to 6.8. A test for anaerobic growth was performed in a serum bottle containing R2A broth supplemented with sodium thioglycolate (1 g l⁻¹) and the gas phase was replaced with nitrogen gas. A nitrate-reduction test was performed in a serum bottle containing R2A broth supplemented with KNO₃ (10 mM) and the reduction of nitrate was

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain PB17^T is AB251884.

Table 1. Differential characteristics of strain PB17^T and members of the genus *Hymenobacter*

Strains: 1, PB17^T; 2, *H. ocellatus* Myx2105^T; 3, *H. roseosalivarius* DSM 11622^T; 4, *H. aerophilus* I/26-Cor1^T; 5, *H. actinosclerus* CCUG 39621^T; 6, *H. chitinivorans* Txcl^T; 7, *H. gelipurpurascens* Txgl^T; 8, *H. norwichensis* DSM 15439^T; 9, *Hymenobacter* sp. DSM 15438. Data for reference strains are from Hirsch *et al.* (1998), Collins *et al.* (2000) and Buczolits *et al.* (2002, 2006). All of the strains are negative for assimilation of L-arabinose, D-adonitol, inositol, D-sorbitol, phenylacetate and L-rhamnose and for indole production. +, Positive; −, negative; (+), weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Growth at/in:									
1 % NaCl	−	+	+	+	ND	(+)	(+)	−	−
3 % NaCl	−	+	ND	(+)	ND	(+)	(+)	−	−
4 °C	+	−*	+	+	−	+	(+)	(+)	−
37 °C	−	+	−	−	+	−	−	−	−
Assimilation of:									
Arbutin	+	−	−	−	(+)	−	−	(+)	−
D-Galactose	+	−	−	−	−	−	−	(+)	−
D-Mannose	+	−	−	(+)	(+)	−	−	(+)	−
D-Ribose	(+)	−	−	−	(+)	−	−	(+)	−
D-Glucose	−	−	−	(+)	(+)	−	−	(+)	−
D-Fructose	−	−	−	(+)	−	−	−	(+)	−
Cellobiose	−	−	−	−	(+)	−	−	(+)	−
N-Acetyl-D-glucosamine, gluconate	−	−	−	−	(+)	−	−	−	−
D-Xylose, salicin, maltose, trehalose, D-mannitol	−	−	−	−	−	−	−	(+)	−
Sucrose	−	−	−	(+)	−	−	−	−	−
Adipate, citrate	−	−	−	−	(+)	−	−	−	−
Arginine dihydrolase	+	−	ND	−	ND	−	−	−	−
Urease	+	−	ND	−	−	−	−	−	−
Enzyme activities (API ZYM)									
Alkaline phosphatase	+	+	ND	+	+	+	+	+	+
Leucine arylamidase	+	(+)	ND	+	+	+	+	+	+
Cystine arylamidase	+	−	ND	−	+	−	−	−	+
Valine arylamidase	+	−	ND	+	+	−	(+)	(+)	+
Naphthol-AS-BI-phosphohydrolase	+	(+)	ND	+	ND	(+)	(+)	+	+
Esterase (C4)	+	−	ND	+	ND	(+)	(+)	(+)	+
Esterase lipase (C8)	+	(+)	ND	+	+	(+)	(+)	(+)	+
α-Galactosidase	(+)	−	+	−	−	−	−	−	+
β-Galactosidase	+	−	ND	−	−	−	−	−	−
β-Glucuronidase, lipase (C14), α-mannosidase, α-fucosidase, chymotrypsin, trypsin	−	−	ND	−	−	−	−	−	−
α-Glucosidase	+	−	+	−	ND	−	−	−	−
β-Glucosidase	(+)	−	+	−	−	−	−	(+)	−
N-Acetyl-β-glucosaminidase	(+)	−	ND	−	+	−	−	−	−
Acid phosphatase	+	−	ND	+	+	−	−	(+)	+
Media allowing growth†	R2A	PYES, R2A, C-D, TSA	PYGV, R2A	PYES, R2A, TSA	YB agar, TSA	PYES, R2A, C-D, TSA	PYES, R2A, C-D, TSA	PYES, R2A	PYES, R2A
Media not allowing growth†	TSA, MC	MC	ND	C-D, MC	ND	MC	MC	TSA,‡ C-D, MC	TSA,‡ C-D, MC
Source of isolation	Soil	Soil	Soil	Air	Pork	Soil	Soil	Air	Air
DNA G + C content (mol%)	58.8	~65	56	63.1	62	61	57–58	ND	ND

*No growth at 5 °C (Collins *et al.*, 2000).

†C-D, Czapek–Dox agar; MC, MacConkey agar; PYES, PYES agar (Buczolits *et al.*, 2002); PYGV, peptone-yeast extract-glucose-vitamins; TSA, trypticase soy agar; YB, yeast broth.

‡Poor growth on this medium.

monitored using an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Growth at different temperatures (4, 15, 25, 30, 37 and 42 °C) and various pHs (pH 5.0–10.0, in increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation.

To measure the G+C content of the chromosomal DNA, genomic DNA was extracted and purified as described by Ausubel *et al.* (1987) and was determined as described by Mesbah & Whitman (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and then re-extracted in n-hexane-water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for cells grown on R2A agar for 3 days at 28 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed with a gas chromatograph (model 6890; Hewlett Packard) and identified using the Microbial Identification software package (Sasser, 1990).

PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The complete 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR). Related sequences were obtained from the databases of Ribosomal Database Project II (Cole *et al.*, 2005) and GenBank (Benson *et al.*, 2005) and multiple alignment was done using the CLUSTAL_X program (Thompson *et al.*, 1997). Some ambiguous sequences, gaps and 5' and 3' ends of the alignment were edited manually using the program BioEdit (Hall, 1999). The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) within the program MEGA3 (Kumar

et al., 2004), with bootstrap analysis based on 1000 resamplings (Felsenstein, 1985). Distance matrices were calculated using Kimura's two-parameter model (Kimura, 1983).

Cells of strain PB17^T were Gram-negative, non-motile, non-spore-forming and rod-shaped. Colonies grown on R2A agar for 3 days were circular, entire, smooth, red-pink, transparent and 0.5–2.0 mm in diameter. After about 5–7 days, colonies became mucoid and slimy; an extracellular polymer was produced, forming a thin, transparent layer around the colonies. Capsules were observed with cells that had been negatively stained with nigrosin. Cells became shorter and almost coccoid in the stationary growth phase. An extracellular polymer, a thin layer, a capsule and changes in cell size were also reported for *H. roseosalivarius* (Hirsch *et al.*, 1998). Strain PB17^T could grow at 4–30 °C but did not grow at 37 or 42 °C on R2A agar. It could grow at pH 5.0–8.5 but did not grow at pH 9.0 or 10.0. The presence of 1 % NaCl or more inhibited growth. The strain could not grow under anaerobic or denitrifying conditions. Physiological characteristics of strain PB17^T are summarized in the species description. Selected characteristics that serve to differentiate strain PB17^T from related species of the genus *Hymenobacter* are shown in Table 1.

An almost-complete 16S rRNA gene sequence (1473 bp in length) was obtained for strain PB17^T. Comparisons with corresponding sequences showed that strain PB17^T had the highest levels of similarity with respect to the following type strains: *H. actinosclerus* CCUG 39621^T (91.8 %), *H. chitinivorans* Txc1^T (91.7 %), *H. aerophilus* I/26-Cor1^T (91.4 %), *H. roseosalivarius* DSM 11622^T (91.2 %), *H. gelipurpurascens* Txg1^T (90.8 %), *H. ocellatus* Myx 2105^T (90.3 %) and *H. norwichensis* DSM 15439^T (90.1 %).

In the neighbour-joining tree constructed using the 16S rRNA gene sequences of the novel strain and strains of the genus *Hymenobacter*, PB17^T clustered within the radiation of the genus *Hymenobacter* but was separate from recognized *Hymenobacter* species. Its distinct phylogenetic position was supported by high bootstrap percentages

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for strain PB17^T and related species. Bootstrap percentages (based on 1000 replications) >70 % are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.

(96–100 %) (Fig. 1). Low levels of similarity with respect to recognized *Hymenobacter* species (90.1–91.8 %) indicate that PB17^T represents a novel species.

The predominant isoprenoid quinone was determined as MK-7. The DNA G+C content of strain PB17^T was 58.8 mol%: such relatively high values are unique to the genus *Hymenobacter*, distinguishing it from other genera of the family 'Flexibacteraceae'.

The major cellular fatty acids of strain PB17^T were iso-C_{15:0}, summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7*clt*), C_{16:1}ω5*c* and anteiso-C_{15:0}. The profile of strain PB17^T was qualitatively similar to those of members of the genus *Hymenobacter*, but it showed quantitative differences, e.g. a large proportion of iso-C_{15:0} and a small proportion of summed feature 5 (iso-C_{17:1} I and/or anteiso-C_{17:1} B) (Table 2).

Morphological similarities, the relatively high DNA G+C content and the phylogenetic data indicated that PB17^T belongs to the genus *Hymenobacter*. However, the low levels of 16S rRNA gene sequence similarity (90.1–91.8 %) between PB17^T and members of the genus *Hymenobacter*, its quantitative fatty acid profile and its differential phenotypic characteristics supported the notion that strain PB17^T represents a novel species. On the basis of the data and observations described above, therefore, strain PB17^T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter soli* sp. nov. is proposed.

Description of *Hymenobacter soli* sp. nov.

Hymenobacter soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-negative, rod-shaped, non-spore-forming and non-motile. Obligately aerobic, chemo-organotrophic. Cells are 0.6–1.0 µm wide and 2.0–4.0 µm long. Cells grow on R2A agar, but not on trypticase soy agar or MacConkey agar. Colonies on R2A agar are circular, entire, smooth, red–pink, transparent and slimy. Cells produce extracellular polymer and form thin, spreading layers around colonies on agar surfaces. A capsule is observed in negatively stained cells. Cells become shorter and almost coccoid in the stationary growth phase on R2A agar. No flexirubin-type pigments are formed. Oxidase- and catalase-positive. Grows at 4–30 °C, but not at 37 °C. The presence of more than 1 % NaCl inhibits growth on R2A agar. No growth occurs under anaerobic or denitrifying conditions. The DNA G+C content of the type strain is 58.8 mol%. The major respiratory quinone is MK-7. The major cellular fatty acids are iso-C_{15:0}, summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7*clt*), C_{16:1}ω5*c* and anteiso-C_{15:0}. Does not hydrolyse agar, xylan, cellulose, skimmed milk, chitin or olive oil. Arbutin, aesculin, D-galactose, 5-ketogluconate and D-mannose are assimilated. Weakly positive for assimilation of glycerol and D-ribose. The following substrates are not assimilated: N-acetyl-D-glucosamine, D-adonitol, starch, amygdalin, D- and

Table 2. Cellular fatty acid profiles of strain PB17^T and its closest relatives

Strains: 1, PB17^T (data from this study); 2, *H. ocellatus* Myx2105^T (Buczolits *et al.*, 2006); 3, *H. roseosalivarius* DSM 11622^T; 4, *H. aerophilus* 1/26-Cor1^T; 5, *H. actinosclerulus* CCUG 39621^T (data in columns 3–5 from Buczolits *et al.*, 2002); 6, *H. chitinivorans* Txc1^T; 7, *H. gelipurpurascens* Txg1^T; 8, *H. norwichensis* DSM 15439^T; 9, *Hymenobacter* sp. DSM 15438 (data in columns 6–9 from Buczolits *et al.*, 2006). Culture conditions: strains 1 and 3 were grown on R2A for 72 h at 28 °C; strains 2, 6 and 7 were grown on TSA; strains 4 and 5 were grown on TSA for 72 h at 28 °C; strains 8 and 9 were grown on PYES agar (Buczolits *et al.*, 2002) at 25 °C. Values are percentages of total fatty acids; –, not detected; ECL, equivalent chain length. Discriminative components are highlighted in bold.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated									
C _{14:0}	1.2	–	–	–	–	–	–	1.3	0.3
C _{15:0}	–	–	–	–	–	–	–	0.5	–
C _{16:0}	4.2	0.6	1.1	1.7	–	–	–	2.2	1.0
Unsaturated									
C _{15:1} ω6 <i>c</i>	–	–	–	0.8	0.8	–	–	0.5	–
C _{16:1} ω5 <i>c</i>	11.0	2.0	23.3	7.9	3.7	8.9	11.3	13.6	7.4
C _{16:1} ω7 <i>c</i> alcohol	0.5	–	0.7	–	–	–	–	–	–
C _{17:1} ω6 <i>c</i>	–	1.2	1.1	–	0.7	–	–	–	0.5
Branched-chain									
iso-C _{13:0}	–	0.6	–	–	–	0.6	–	–	–
iso-C _{14:0}	–	1.5	–	–	–	0.5	–	0.8	0.6
iso-C _{15:0}	51.8	36.7	8.3	10.8	22.3	31.1	17.3	27.3	18.7
iso-C _{15:1} G	–	–	–	–	–	0.6	0.8	–	–
anteiso-C _{15:0}	6.2	3.9	–	22.3	25.8	3.7	23.1	10.6	16.2
anteiso-C _{15:1} A	–	–	–	–	–	–	1.1	–	1.0
iso-C _{16:0}	–	2.9	2.1	0.8	–	1.4	2.7	1.2	1.3
iso-C _{16:1} H	1.0	1.3	2.7	1.5	1.5	2.0	3.7	1.4	2.0
iso-C _{17:0}	1.1	2.8	1.7	4.5	1.8	2.6	1.7	1.6	2.0
iso-C _{17:1} ω9 <i>c</i>	–	0.6	–	–	–	0.7	–	–	0.5
anteiso-C _{17:0}	–	–	–	2.3	0.7	–	2.0	–	1.3
Hydroxy fatty acids									
C _{15:0} 2-OH	–	–	–	–	–	–	0.9	–	1.0
iso-C _{15:0} 2-OH	–	–	–	0.9	0.8	–	–	–	–
iso-C _{15:0} 3-OH	2.1	4.3	2.7	1.6	1.6	4.1	2.3	2.2	2.0
C _{16:0} 3-OH	1.0	–	1.2	–	–	0.5	–	0.6	0.4
iso-C _{16:0} 3-OH	–	–	1.0	–	–	–	–	–	0.3
C _{17:0} 2-OH	–	–	–	1.3	–	–	1.7	–	1.0
iso-C _{17:0} 2-OH	–	–	–	–	2.0	–	–	–	–
iso-C _{17:0} 3-OH	1.2	6.8	5.8	3.5	3.1	6.2	3.7	3.0	3.6
Summed features*									
2	0.9	2.7	–	0.8	2.3	2.1	–	0.8	2.8
4	14.4	5.4	29.8	21.4	13.1	13.9	17.6	23.6	18.5
5	3.5	26.9	18.5	17.7	19.9	20.8	9.4	8.3	17.2
Unknown fatty acids									
ECL 13.565	–	–	–	–	–	0.5	0.8	–	0.4
ECL 16.580	–	–	–	–	–	–	–	–	0.5

*Summed features represent groups of two or more fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 consists of one or more of iso-C_{15:1} I, iso-C_{15:1} H and C_{13:0} 3-OH, summed feature 4 consists of iso-C_{15:0} 2-OH and/or C_{16:1}ω7*clt* and summed feature 5 consists of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

L-arabinose, D- and L-arabitol, cellobiose, dulcitol, erythritol, D-fructose, D- and L-fucose, gentiobiose, gluconate, D-glucose, glycogen, inositol, inulin, 2-ketogluconate, D-lactose, D-lyxose, maltose, D-mannitol, methyl α -D-glucopyranoside, melezitose, melibiose, methyl α -D-mannopyranoside, methyl β -D-xylose, raffinose, L-rhamnose, sucrose, salicin, D-sorbitol, L-sorbose, D-tagatose, trehalose, turanose, xylitol and D- and L-xylose (API 50 CH). Negative for assimilation of caprate, adipate, malate, citrate and phenylacetate (API 20NE). Negative for lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities and for production of hydrogen sulfide and indole. Weakly positive for acetoin production (Voges-Proskauer reaction). Acid is not produced from D-glucose, melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose or sucrose (API 20E). Positive for arginine dihydrolase, urease and aesculin hydrolysis and negative for glucose acidification (API 20NE). In API ZYM tests, positive results are obtained for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase lipase (C8), esterase (C4), β -galactosidase, α -glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase, weakly positive results are obtained for N-acetyl- β -glucosaminidase, α -galactosidase and β -glucosidase and negative results are obtained for α -chymotrypsin, α -fucosidase, β -glucuronidase, lipase (C14), α -mannosidase and trypsin.

The type strain, PB17^T (=KCTC 12607^T =LMG 24240^T), was isolated from grass soil in Daejeon, Republic of Korea.

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