Hymenobacter rigui sp. nov., isolated from wetland freshwater

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A non-motile, rod-shaped and pinkish-red-pigmented bacterium, designated strain WPCB131^T, was isolated from freshwater of Woopo wetland, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that this isolate represents a novel member of the genus *Hymenobacter* with sequence similarities of 93.2–95.5 % to known *Hymenobacter* species. No other taxa in the phylum *Bacteroidetes* showed more than 90 % sequence similarity to the isolate. Cells were Gram-negative, oxidase-negative and catalase-positive. Optimum growth was observed at pH 6 and 30 °C. The major fatty acids were iso-C_{15:0} (34.8 %), C_{16:1ω5c} (15.0 %), anteiso-C_{17:1} B/iso-C_{17:1} I (14.4 %) and C_{16:1ω7c}/iso-C_{15:0} 2-OH (13.8 %). The DNA G + C content of strain WPCB131^T was 65 mol%. The isolate could be readily distinguished from other *Hymenobacter* species using a combination of phenotypic characters. On the basis of the polyphasic evidence presented in this study, it is proposed that strain WPCB131^T (=IMSNU 14116^T = KCTC 12533^T = NBRC 101118^T) represents a novel species, *Hymenobacter rigui* sp. nov.

The genus *Hymenobacter* Hirsch *et al.* 1998 represents a deep branch within the phylum *Bacteroidetes* and displays a distinct taxonomic status in view of the high G + C content of genomic DNA (55–65 mol%) of *Hymenobacter* species, which is nearly unique in this phylum. Currently, there are three species in the genus, namely *Hymenobacter roseosalivarius* (Hirsch *et al.*, 1998), *Hymenobacter actinosclerulus* (Collins *et al.*, 2000) and *Hymenobacter aerophilus* (Buczolits *et al.*, 2002). In the course of our study on wetland microbial diversity, a *Hymenobacter*-like bacterial strain, designated WPCB131^T, was isolated from a freshwater sample and was the subject of a taxonomic investigation.

Strain WPCB131^T was isolated from a freshwater sample collected from the wetland in Woopo (35° 33' N 128° 25' E), Republic of Korea, during July 2000 using the standard dilution plating technique. Isolation was achieved using PYGV (Staley, 1968) at 25 °C for 7 days. The isolate was routinely cultured on R2A (Oxoid) and maintained as a glycerol suspension (20 %, w/v) at –80 °C.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant

sequence of strain WPCB131^T was aligned manually against sequences obtained from GenBank. Phylogenetic trees were inferred from the regions available for all sequences (positions 43–1438, *Escherichia coli* numbering system) using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the program jPHYDIT (Jeon *et al.*, 2005) and PAUP 4.0 (Swofford, 1998) as described previously (Yi & Chun, 2006).

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was closely related to members of the genus *Hymenobacter*. The newly determined sequence was then aligned manually against representatives of the genus *Hymenobacter*. Strain WPCB131^T showed the highest 16S rRNA gene sequence similarity to *H. actinosclerulus* CCUG 39621^T (95.5 %); this value is well below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994). Similarities between the 16S rRNA gene sequence of strain WPCB131^T and those of *H. aerophilus* DSM 13611^T and *H. roseosalivarius* DSM 11622^T were 94.5 and 93.2 %, respectively. No other

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WPCB131^T is DQ089669.

described bacterial species showed more than 90 % 16S rRNA gene sequence similarity to our isolate. This relationship between our isolate and other members of the genus *Hymenobacter* is also evident in the phylogenetic tree. Strain WPCB131^T represented a distinct phyletic line within the genus *Hymenobacter* and formed a robust monophyletic clade together with other hymenobacters in all trees inferred in this study (Fig. 1). It is clear from 16S rRNA gene sequence similarity data and phylogenetic analysis that strain WPCB131^T represents a novel species in the genus *Hymenobacter*.

Growth on various standard bacteriological media was tested using nutrient agar (NA; Difco), tryptone soy agar (TSA; Oxoid) and R2A agar (Oxoid). Cells grown on TSA at 28 °C for 3–7 days were used for physiological and biochemical tests. Motility was examined by phase-contrast microscopy using wet mounts. Growth in various pH (pH 4–12), temperature (4–42 °C) and NaCl concentration (0, 1, 2, 3, 5 and 10 %, w/v) ranges was tested using TSA for up to 1 week. Anaerobic growth was checked in an anaerobic cabinet (CO₂/H₂/N₂, 10:10:80 by vol.; Sheldon Manufacturing) using TSA. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. Other physiological and biochemical tests were performed using API 20E, API 20NE and API 50CHB (bioMérieux) test panels. Enzyme activities were tested using the API ZYM kit (bioMérieux) following the manufacturer's instructions. The detailed results of physiological and biochemical analyses are given in Table 1 and the species description. It is evident from Table 1 that there are several phenotypic characters that readily differentiate strain WPCB131^T from other *Hymenobacter* species.

Cellular fatty acids of strain WPCB131^T were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI). The G + C content of the DNA was determined using the thermal denaturation

Table 1. Phenotypic characteristics that differentiate strain WPCB131^T from other *Hymenobacter* species

Species/strains: 1, WPCB131^T; 2, *H. actinoscleris*; 3, *H. aerophilus*; 4, *H. roseosalivarius*. Data from Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002) and this study. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4
Pigmentation	Pinkish-red	Red	Red	Reddish
Growth at (°C):				
4	+	–	+	+
28	+	+	+	–
37	+	+	–	–
40	–	+	–	–
42	–	+	–	–
Oxidase (Kovacs' reagent)	–	+	+	+
Assimilation of:				
N-Acetyl-D-glucosamine	–	+	–	–
Arbutin	–	+	–	–
D-Cellobiose	+	+	–	–
D-Fructose	+	–	+	–
Gluconate	–	+	–	–
D-Glucose	+	+	+	–
D-Mannose	–	+	+	–
D-Maltose	+	–	–	–
D-Ribose	–	+	–	–
Sucrose	+	–	+	–
Salicin	W	–	–	–
D-Trehalose	+	–	–	–
Enzyme activity (API ZYM):				
N-Acetyl-β-glucosaminidase	+	ND	–	ND
Cystine arylamidase	W	+	–	ND
DNA G + C content (mol%)	65	62	63	56

method of Marmur & Doty (1962). The cellular fatty acid composition of strain WPCB131^T was similar to those of other *Hymenobacter* species, but differed from them in

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain WPCB131^T and related taxa within the phylum *Bacteroidetes*. Numbers at the nodes are the percentages of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) are also recovered in maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T (U01330) was used as an outgroup. Bar, 0.1 nucleotide substitution per position.

quantities, especially for unsaturated ($C_{15:0}$) and mono-saturated ($C_{16:1}$) fatty acids (Table 2). The DNA G+C content of strain WPCB131^T was 65 mol%.

Formation of a distinctive phyletic line within the genus *Hymenobacter* indicates that strain WPCB131^T can be assigned as a representative of a novel species in the genus *Hymenobacter*. In addition, a number of physiological and chemotaxonomic characters clearly distinguish this isolate from other *Hymenobacter* species (Tables 1 and 2). Therefore, strain WPCB131^T should be classified as a member of a novel species within the genus *Hymenobacter*, for which the name *Hymenobacter rigui* sp. nov. is proposed.

Description of *Hymenobacter rigui* sp. nov.

Hymenobacter rigui (ri'gu.i. L. gen. n. *rigui* of a well-watered place).

Cells are rod-shaped, Gram-negative, aerobic, non-spore-forming and produce water-insoluble pinkish-red pigment. Motility is not observed. Cells grow best on R2A and TSA; weak growth is observed on NA. Colonies on TSA are translucent, low-convex, circular, smooth and slimy; diameter is up to 3.0 mm after 5 days at 25–30 °C (pH 7). Growth occurs in 0–2% (w/v) NaCl (optimum 0%), pH 5–11 (optimum pH 6) and at 4–37 °C (optimum 30 °C). Oxidase-negative and catalase-positive. Does not reduce nitrate to nitrite. Aesculin hydrolysis is weakly positive. Does not utilize citrate. Negative for fermentation of glucose. Produces gelatinase, but not arginine dihydro-lase, urease, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, H₂S, indole or acetoin. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, valine arylamidase and α -glucosidase, but not lipase (C14), trypsin, α -chymotrypsin, β -glucuronidase, α -galactosidase, β -galactosidase, β -glucosidase, α -mannosidase or α -fucosidase. Utilizes galactose, aesculin, starch, lactose, inulin, melezitose, raffinose, glycogen and gentiobiose as sole carbon and energy sources. Does not utilize glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xylopyranoside, sorbose, rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, melibiose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol or gluconate. Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are iso- $C_{15:0}$ (34.8%), $C_{16:1\omega5c}$ (15.0%), anteiso- $C_{17:1}$ B/iso- $C_{17:1}$ I (14.4%) and $C_{16:1\omega7c}$ /iso- $C_{15:0}$ 2-OH (13.8%); complete fatty acid data are given in Table 2.

The type strain is WPCB131^T (=IMSNU 14116^T=KCTC 12533^T=NBRC 101118^T), isolated from freshwater of Woopo wetland, Republic of Korea. The DNA G+C content of strain WPCB131^T is 65 mol%.

Table 2. Cellular fatty acid content (%) of strain WPCB131^T and the type strains of *Hymenobacter* species

Strains: 1, WPCB131^T; 2, *H. actinosclerus*; 3, *H. aerophilus*; 4, *H. roseosalivarius*. Data from Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002) and this study. Values are percentages of total fatty acids. Tr, <1.0%; ND, not detected.

Characteristic	1	2	3	4
Summed feature 2*	ND	2.3	Tr	ND
iso- $C_{15:0}$	34.8	22.3	10.8	8.3
anteiso- $C_{15:0}$	5.9	25.8	22.3	ND
$C_{15:1\omega6c}$	ND	Tr	Tr	ND
iso- $C_{16:1}$ H	ND	1.5	1.5	2.7
$C_{16:1\omega7c}$ alcohol	ND	ND	ND	Tr
iso- $C_{16:0}$	ND	ND	Tr	2.1
anteiso- $C_{16:0}$	1.9	ND	ND	ND
Summed feature 4†	13.8	13.1	21.4	29.8
$C_{16:1\omega5c}$	15.0	3.7	7.9	23.3
$C_{16:0}$	6.4	ND	1.7	1.1
iso- $C_{15:0}$ 3-OH	ND	1.6	1.6	2.7
iso- $C_{15:0}$ 2-OH	ND	Tr	Tr	ND
Summed feature 5‡	14.4	19.9	17.7	18.5
iso- $C_{17:0}$	5.0	1.8	4.5	1.7
anteiso- $C_{17:0}$	ND	Tr	2.3	ND
$C_{17:1\omega6c}$	ND	Tr	ND	1.1
iso- $C_{16:0}$ 3-OH	ND	ND	ND	1.0
$C_{16:0}$ 3-OH	ND	ND	ND	1.2
iso- $C_{17:0}$ 3-OH	3.1	2.1	3.5	3.9
$C_{17:0}$ 2-OH	ND	2.0	1.3	ND

*Summed feature 2 contains one or more of the following fatty acids: iso- $C_{15:1}$ I/ $C_{13:0}$ 3-OH.

†Summed feature 4 contains one or more of the following fatty acids: $C_{16:1\omega7c}$ /iso- $C_{15:0}$ 2-OH.

‡Summed feature 5 contains one or more of the following fatty acids: anteiso- $C_{17:1}$ B/iso I.

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