Paludibacterium yongneupense gen. nov., sp. nov., isolated from a wetland, Yongneup, in Korea

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A cream-coloured bacterial strain, 5YN8-15^T, was isolated from a wetland, Yongneup, in the Inje region of the Republic of Korea. The bacterium was facultatively anaerobic, Gram-negative, motile with a single polar flagellum and curved-rod-shaped. Based on 16S rRNA gene sequence analysis, strain 5YN8-15^T is a member of the *Betaproteobacteria*. Closely related taxa were *Gulbenkiania mobilis* E4FC31^T (94.9 % sequence similarity), *Chromobacterium species* (94.1–94.4 %), *Aquitalea magnusonii* TRO-001DR8^T (93.2 %) and *Aquaspirillum serpens* IAM 13944^T (92.5 %). All other species with validly published names analysed showed sequence similarities of below 92 %. Strain 5YN8-15^T had ubiquinone 8 as the major isoprenoid quinone. The major fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. The DNA G + C content was 63.0 mol%. Based on the data from the polyphasic study, strain 5YN8-15^T represents a novel genus and species of the family *Neisseriaceae*, for which the name *Paludibacterium yongneupense* gen. nov., sp. nov. is proposed. The type strain is 5YN8-15^T (=KACC 11601^T=DSM 18731^T).

In *Bergey's Manual of Systematic Bacteriology* (Tønjum, 2005), 15 genera were included in the family *Neisseriaceae* with the type genus *Neisseria*. Since then, new genera such as *Aquitalea* (Lau *et al.*, 2006), *Bergeriella* (Xie & Yokota, 2005), *Chitinibacter* (Chern *et al.*, 2004), *Conchiformibius* (Xie & Yokota, 2005), *Gulbenkiania* (Vaz-Moreira *et al.*, 2007), *Silvimonas* (Yang *et al.*, 2005) and *Uruburuella* (Vela *et al.*, 2005) have been described and assigned to the family *Neisseriaceae*. These novel bacterial strains were isolated from soil, water and animals.

We isolated a bacterial strain that was phylogenetically closely related to members of the genera *Gulbenkiania*, *Chromobacterium*, *Aquitalea* and *Vogesella*. In this paper, we describe the characterization of a novel species in a new genus within the family *Neisseriaceae*.

Yongneup (38° 12' 53" N 128° 07' 30" E), the only high moor in Korea, is a wetland at 1200–1280 m above sea level. It is located around the top of Mount Daeam,

Seohwa-myōn, Inje-gun, Kangwon-do, Korea. The peat layers are about 150 cm thick and were formed over 4000–5000 years. This area has a special type of ecosystem in terms of weather, soil and vegetation.

In the course of a study of the bacterial diversity of Yongneup, a bacterial strain, 5YN8-15^T, was isolated. The wetland peat sample was serially diluted with 0.85 % (w/v) NaCl and suitable 10-fold dilutions were plated onto R2A agar (Difco). The plates were incubated at 28 °C for 4 days. Among the colonies formed, a cream-coloured colony was isolated and named strain 5YN8-15^T. After incubation for 1 day on R2A agar, cell morphology was examined using phase-contrast microscopy (Axio; Zeiss) as well as an electron microscope (model 912AB; Leo) after the cells had been negatively stained with uranyl acetate. For physiological and biochemical tests, the isolate was cultivated routinely on R2A medium at 28 °C. The temperature range for growth was determined at 4, 10, 15, 20, 25, 30, 33, 35, 40 and 45 °C on R2A agar medium. The pH range (pH 4.0–10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth that was buffered with citrate/phosphate or Tris/HCl buffer (Breznak & Costilow, 1994). Tolerance to various salinity levels was tested by determining growth in R2A broth supplemented with 0, 1, 2, 3 and 5 % (w/v) NaCl. Biochemical traits were determined using both

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5YN8-15^T is AM396358.

A maximum-parsimony tree based on 16S rRNA gene sequences of strain 5YN8-15^T and other members of the *Betaproteobacteria* is available as supplementary material with the online version of this paper.

Fig. 1. Transmission electron micrograph of a cell of strain 5YN8-15^T. Bar, 500 nm.

conventional methods and the API 20NE, API 20E, API ZYM and API ID 32 GN test strips (bioMérieux).

Genomic DNA was isolated by using the method described by Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified by using the universal primers fD1 and rP2 (Weisburg *et al.*, 1991) and its nucleotide sequence was determined automatically. Sequences were aligned using CLUSTAL W software (Thompson *et al.*, 1994) and phylogenetic analysis was performed using MEGA version 3 (Kumar *et al.*, 2004). Phylogenetic trees were obtained by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971). The robustness of the tree topologies was assessed using bootstrap analyses based on 1000 replications.

Isoprenoid quinones were analysed using HPLC as described by Groth *et al.* (1996). For quantitative analysis

Table 1. Phenotypic comparisons among strain 5YN8-15^T and closely related species

Strains: 1, 5YN8-15^T; 2, *G. mobilis* DSM 18507^T (data from Vaz-Moreira *et al.*, 2007); 3, *Chromobacterium violaceum* KCTC 2897^T (Gillis & Logan, 2005); 4, *Chromobacterium subtsugae* DSM 17043^T (Martin *et al.*, 2007); 5, *Aquitalea magnusonii* LMG 23054^T (Lau *et al.*, 2006); 6, *V. indigofera* DSM 3303^T (Krieg, 2005). All strains are catalase and oxidase-positive. According to the API 20NE test strips, all are negative for aesculin hydrolysis. According to the API 20NE test strips, all assimilate L-histidine, but not L-arabinose, D-mannitol, adipic acid or phenylacetic acid. +, Positive; –, negative; (+), weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6
Isolation source	Wetland	Municipal wastewater	Soil and water	Soil	Humic lake	Freshwater
DNA G + C content (mol%)	63.0	63.0	65–68	64.5	59.2	65.4
Colony colour	Cream	Cream*	Violet	Violet	Tan	Blue
Growth at:						
4 °C	+	–	–	+	–	+
40 °C	–	+	+	+	+	+
pH 4	+	–	–	–	–	–
Hydrolysis of:						
DNA	–	ND	+	ND	ND	+
Casein	–	ND	+	+	ND	–
Starch	+	ND	–	ND	ND	–
Tween 80	–	–	+	ND	ND	–
API 20NE*:						
Nitrate reduction	+	+	+	–	+	+
Indole production	–	+	–	–	+	+
D-Glucose fermentation	+	–	+	–	+	–
Arginine dihydrolase	–	+	+	+	+	–
Urease	–	+	(+)	+	–	–
Gelatin hydrolysis	–	–	+	+	–	–
Assimilation (API 20NE) of*:						
D-Glucose	+	–	+	+	+	+
D-Mannose	–	–	+	–	–	–
N-Acetylglucosamine	+	–	+	+	+	+
Maltose	+	–	–	–	–	–
Potassium gluconate	–	–	+	+	+	+
Capric acid	–	+	+	+	+	–
Malic acid	–	+	+	+	+	+
Trisodium citrate	–	–	–	–	+	–

*Data from this study.

of the whole-cell fatty acids, strains were cultivated in R2A medium at 28 °C for 2 days. Cellular fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990). The G+C content of the DNA was determined using a reversed-phase HPLC system with a C18 column (Mesbah *et al.*, 1989).

Cells of strain 5YN8-15^T were facultatively anaerobic, Gram-negative, motile and curved-rod-shaped (0.6–0.9 × 2.0–4.0 µm) (Fig. 1). The strain grew on R2A and nutrient agar (Difco), but not on tryptic soy agar (Difco) or MacConkey agar (Difco). Differential physiological characteristics for strain 5YN8-15^T and closely related taxa are given in Table 1.

Strain 5YN8-15^T contained ubiquinone 8 (Q-8) as the major isoprenoid quinone. The fatty acid composition was dominated by summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 32.1%), C_{16:0} (31.7%) and C_{18:1}ω7c (11.2%) (Table 2). The DNA G+C content was 63.0 mol%.

According to the comparison of the 16S rRNA gene sequence with those of other recognized species, strain 5YN8-15^T was most closely related to *Gulbenkiania mobilis* E4FC31^T (94.9% sequence similarity), *Chromobacterium subtsugae* PRAA4-1^T (94.4%), *Chromobacterium violaceum* ATCC 12472^T (94.1%), *Aquitalea magnusonii* TRO-001DR8^T (93.2%) and *Aquaspirillum serpens* IAM 13944^T (92.5%). All other species used showed sequence similarities of below 92%. In the neighbour-joining tree (Fig. 2), strain 5YN8-15^T formed a cluster with *G. mobilis* with

Table 2. Cellular fatty acid compositions (%) of strain 5YN8-15^T and closely related species

Strains: 1, 5YN8-15^T; 2, *G. mobilis* DSM 18507^T; 3, *Chromobacterium violaceum* KCTC 2897^T; 4, *Chromobacterium subtsugae* DSM 17043^T; 5, *Aquitalea magnusonii* LMG 23054^T; 6, *V. indigofera* DSM 3303^T. All strains were grown in R2A medium at 28 °C for 2 days. Fatty acids representing less than 1.0% have been omitted. –, Not detected.

Fatty acid	1	2	3	4	5	6
C _{10:0}	—	2.8	—	—	—	2.7
C _{10:0} 3-OH	3.0	4.0	4.0	3.8	3.4	5.7
C _{12:0}	7.9	4.2	5.1	4.4	6.7	3.0
C _{12:0} 2-OH	—	—	1.9	2.1	—	—
C _{12:0} 3-OH	3.8	3.4	4.2	3.1	2.2	—
C _{14:0}	4.3	—	1.8	1.3	1.5	1.0
C _{16:0}	31.7	25.0	27.3	26.7	26.9	25.4
C _{17:0}	—	1.5	—	—	—	—
C _{17:0} cyclo	4.5	—	11.8	—	—	—
C _{18:0}	—	1.3	—	—	3.0	1.3
iso-C _{18:0}	—	—	1.2	—	—	—
C _{18:1} ω7c	11.2	6.6	18.3	22.0	11.5	8.5
Summed feature 3*	32.1	47.8	21.8	35.6	41.0	49.4

*Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

moderate bootstrap support (55%), and this cluster was further related to other clusters including *Chromobacterium subtsugae*, *Chromobacterium violaceum*, *Aquitalea magnusonii* and *Vogesella indigofera* with 52% bootstrap support. The maximum-parsimony tree (see Supplementary Fig. S1 available in IJSEM Online) also showed the grouping of strain 5YN8-15^T with members of the genera *Gulbenkiania*, *Chromobacterium*, *Aquitalea* and *Vogesella*, despite small differences in the topologies between the two trees.

Phenotypic characteristics that supported the consideration of strain 5YN8-15^T as representing a genus different from *Gulbenkiania*, *Chromobacterium*, *Aquitalea* and *Vogesella* included pigmentation, temperature and pH ranges for growth, various biochemical properties and assimilation patterns of various substrates. In particular, strain 5YN8-15^T could be clearly differentiated from its closest relative, *G. mobilis*, based on the ability to grow at lower temperatures and pH, the inability to produce indole, arginine dihydrolase and urease, and the presence of the fatty acids C_{14:0} (4.3%) and C_{17:0} cyclo (4.5%). Although the two strains showed limited ranges of substrate utilization as sole carbon sources or of carbon source fermentation, strain 5YN8-15^T could be clearly differentiated from *G. mobilis* by the ability to use some sugars as sole carbon sources and to ferment glucose (Table 1; Vaz-Moreira *et al.*, 2007). Based on its phylogenetic, genetic and physiological properties, we propose the creation of a novel genus and species, *Paludibacterium yongneupense*, to accommodate strain 5YN8-15^T.

Description of *Paludibacterium* gen. nov.

Paludibacterium (Pa.lu'di.bac.te'ri.um. L. n. *palus* -udis a marsh; L. neut. n. *bacterium* a rod; N.L. neut. n. *Paludibacterium* a rod isolated from peat).

Cells are Gram-negative, non-spore forming and curved-rod-shaped. Motile by means of a single polar flagellum, facultatively anaerobic and catalase- and oxidase-positive. Reduce nitrate. Do not produce indole or acetoin. Predominant isoprenoid quinone is Q-8. Major fatty acids are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. Member of the family *Neisseriaceae*. The type species is *Paludibacterium yongneupense*.

Description of *Paludibacterium yongneupense* sp. nov.

Paludibacterium yongneupense (yong.ne.up.en'se. N.L. neut. adj. *yongneupense* pertaining to Yongneup, a wetland in Korea where the organism was first isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are approximately 0.6–0.9 µm wide and 2.0–4.0 µm long. On R2A medium, colonies are cream-coloured, round and convex with clear margins. Growth occurs at 4–35 °C and pH 4.0–8.0. Degrades CM-cellulose, starch and xanthine. Does not

Fig. 2. Neighbour-joining tree showing the relationships between strain 5YN8-15^T and some other members of the *Betaproteobacteria*, inferred by 16S rRNA gene sequence analysis. Bootstrap values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

degrade casein, chitin, DNA, hypoxanthine, gelatin, pectin, Tween 80, tyrosine or urea. Assimilates only D-glucose, N-acetylglucosamine, maltose and L-histidine (API 20NE and API ID 32 GN). Ferments only D-glucose (API 20E). Positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase, but not for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase (API ZYM) or arginine dihydrolase (API 20NE). The G + C content of the genomic DNA of the type strain is 63.0 mol%.

The type strain, 5YN8-15^T (=KACC 11601^T=DSM 18731^T), was isolated from a wetland, Yongneup, in Inje region, Republic of Korea.

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