Uliginosibacterium gangwonense gen. nov., sp. nov., isolated from a wetland, Yongneup, in Korea

Hang-Yeon Weon,¹ Byung-Yong Kim,² Seung-Hee Yoo,²
Soon-Wo Kwon,² Seung-Joo Go² and Erko Stackebrandt³

Correspondence

Soon-Wo Kwon

swkwon@rda.go.kr

¹Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Republic of Korea

²KACC-Korean Agricultural Culture Collection, Microbial Genetics Division, National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Republic of Korea

³DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7b, 38124 Braunschweig, Germany

A yellow-pigmented, Gram-negative, aerobic bacterial strain, designated 5YN10-9^T, was isolated from a wetland, Yongneup, of the Inje region, Korea. Cells were motile by means of one polar flagellum. Based on 16S rRNA gene sequence analyses, strain 5YN10-9^T was shown to be related to the genera *Azoarcus*, *Azovibrio*, *Thauera* and *Zoogloea*, showing moderate sequence similarities of 90.2–93.3, 92.7, 90.9–93.3 and 92.4–92.6 % to members of these genera, respectively. Its distinct phylogenetic position and the low 16S rRNA gene sequence similarity values toward the closest related genera support the inclusion of this novel isolate in a new genus. The major isoprenoid quinone was ubiquinone 8 (Q-8). The predominant cellular fatty acids were C_{16:0} and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The DNA G + C content was 59.3 mol%. The results of phenotypic, chemotaxonomic and phylogenetic analyses indicate that strain 5YN10-9^T represents a novel species of a new genus within the family *Rhodocyclaceae*, class *Betaproteobacteria*, for which the name *Uliginosibacterium gangwonense* gen. nov., sp. nov. is proposed. The type strain is 5YN10-9^T (=KACC 11603^T=DSM 18521^T).

Garrity & Holt (2001) listed five genera, *Rhodocyclus*, *Azoarcus*, *Propionibacter*, *Thauera* and *Zoogloea*, within the family *Rhodocyclaceae*. Since then, many genera, for example *Azonexus*, *Azospira*, *Azovibrio* (Reinhold-Hurek & Hurek, 2000), *Dechloromonas* (Achenbach *et al.* 2001), *Ferribacterium* (Cummings *et al.*, 1999), *Propionivibrio* (Brune *et al.*, 2002), *Quadricoccus* (Maszenan *et al.*, 2002) and *Sterolibacterium* (Tarlera & Denner, 2003), have been newly classified within the family *Rhodocyclaceae*, class *Betaproteobacteria* (Garrity *et al.*, 2005). The type genus of the family *Rhodocyclaceae* is *Rhodocyclus*.

Yongneup (38° 12' 53" N 128° 07' 30" E) is a wetland located at over 1200 m above sea level and is the only high moor in Korea. Peat layers in the wetland are about 150 cm thick and have been formed over the past 4000–5000 years.

Wetland peat samples were serially diluted with 0.85 % NaCl (w/v) and suitable ten-fold dilutions were plated onto R2A agar (Difco). These plates were incubated at 28 °C for 4 days. Among the colonies subsequently formed, a yellow-coloured colony was isolated and designated 5YN10-9^T. This isolate was able to grow on R2A and nutrient agar

(NA; Difco), but not on trypticase soy agar (TSA; Difco) or MacConkey agar (Difco).

Cell morphology was observed by transmission electron microscopy (912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss) by using cells grown on R2A agar. The pH, temperature and NaCl ranges for growth were determined by using R2A medium. Strain 5YN10-9^T was analysed phenotypically by using the API 20NE, API ZYM and API ID 32 GN systems (bioMérieux) according to the manufacturer's instructions. Gram staining, presence of catalase, oxidase, poly-β-hydroxybutyrate, and hydrolysis of casein, DNA and starch were determined as described by Smibert & Krieg (1994). Hydrolysis of carboxymethyl-cellulose (CM-cellulose; Sigma) (0.1 %, w/v), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. Cells of strain 5YN10-9^T were Gram-negative, motile by means of a single, polar flagellum, rod-shaped and strictly aerobic (Fig. 1). Table 1 lists the phenotypic differences between strain 5YN10-9^T and other closely related genera.

The procedure for identification of isoprenoid quinones followed the HPLC method of Groth *et al.* (1996). DNA G + C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5YN10-9^T is DQ665916.

Fig. 1. Transmission electron micrograph of a cell of strain 5YN10-9^T, showing one polar flagellum. Bar, 500 nm.

(1989), by using a reversed-phase column (Supelcosil LC-18 S; Supelco). Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990). Strain 5YN10-9^T had ubiquinone 8 as the predominant isoprenoid quinone. The DNA G+C content was 59.3 mol%. Strain 5YN10-9^T contained C_{16:0} (35.2%) and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 28.5%) as the predominant cellular fatty acids. The following components were also present at >1% of the total fatty acids (Table 2): C_{18:1}ω7c (7.4%),

C_{17:0} cyclo (5.9%), C_{12:0} (5.7%), C_{14:0} (5.4%), C_{12:0} 3-OH (4.6%), C_{18:0} (2.3%) and summed feature 2 (comprising C_{14:0} 3-OH and/or iso-C_{16:1} I; 1.5%).

The 16S rRNA gene of strain 5YN10-9^T was amplified and sequenced as described by Kwon *et al.* (2003). This sequence was aligned and compared with those from the GenBank nucleotide database by using an online BLAST search. Phylogenetic trees were inferred by using the neighbour-joining method (Saitou & Nei, 1987). Evolutionary distance matrices for the neighbour-joining method were generated according to the Kimura two-parameter model (Kimura, 1980). Resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. According to 16S rRNA gene sequence analysis, strain 5YN10-9^T was closely related to members of the family *Rhodocyclaceae* within the class *Betaproteobacteria*, in particular to members of the genera *Azoarcus*, *Azovibrio*, *Thauera* and *Zoogloea* (Fig. 2). However, levels of 16S rRNA gene sequence similarity towards *Azoarcus* species (90.2–93.3%), *Azovibrio restrictus* LMG 9099^T (92.7%), *Thauera* species (90.9–93.3%) and *Zoogloea* species (92.4–92.6%) were low.

Strain 5YN10-9^T could be phenotypically differentiated from members of the above genera based on yellow colony colour, inability to reduce nitrate, ability to hydrolyse urea, differential assimilation patterns and a relatively low DNA G+C content (Table 1). Based on comparisons of fatty acid contents, all strains included in the present study contained C_{16:0} and summed feature 3 as major components, which was consistent with data from previous

Table 1. Phenotypic comparison of strain 5YN10-9^T and closely related genera

Taxa: 1, strain 5YN10-9^T; 2, *Azoarcus* (data from Garrity *et al.*, 2005); 3, *Azovibrio* (Garrity *et al.*, 2005; this study); 4, *Thauera* (Garrity *et al.*, 2005); 5, *Zoogloea* (Garrity *et al.*, 2005; Xie & Yokota, 2006). +, Positive result; –, negative result; v, variable among species; ND, not determined.

Characteristic	1	2	3	4	5
Cell width (μm)	0.6–1.0	0.4–1.0	0.6–0.8	0.7–1.0	0.5–1.2
Colony colour	Yellow	Yellow to beige	Beige	White–yellow	White
Nitrate reduction	–	+	–	+	v
Urease	+	ND	–*	–	v
Assimilation of:					
N-Acetylglucosamine	+	ND	–*	ND	ND
L-Arabinose	+	v	–*	ND	–
D-Glucose	+	v	–*	–	v
4-Hydroxybenzoic acid	–	+	–	+	v
Malic acid	–	v	+	–	v
Maltose	+	v	–*	–	–
D-Mannitol	–	ND	–*	ND	–
D-Mannose	+	–	–*	ND	–
Phenylacetic acid	–	+	–*	+	v
L-Proline	–	v	–*	+	ND
Propionic acid	–	v	+	+	v
DNA G+C content (mol%)	59.3	62–68	64.8	64–69	67.3–69.0

*Data from the present study.

Table 2. Comparison of the fatty acid contents of strain 5YN10-9^T and related species

Strain: 1, 5YN10-9^T; 2, *Azoarcus communis* LMG 9095^T; 3, *Azoarcus evansii* DSM 6898^T; 4, *Azoarcus indigens* LMG 9092^T; 5, *Azoarcus toluvorans* DSM 15124^T; 6, *Azovibrio restrictus* LMG 2218^T; 7, *Thauera aminoaromatica* DSM 14742^T; 8, *Thauera aromatica* DSM 6984^T; 9, *Thauera chlorobenzoica* DSM 18012^T; 10, *Thauera mechernichensis* DSM 12266^T; 11, *Thauera selenatis* ATCC 55363^T; 12, *Thauera terpenica* DSM 12139^T; 13, *Thauera phenylacetica* DSM 14743^T; 14, *Zoogloea oryzae* A-7^T; 15, *Zoogloea ramigera* CIP 107119^T. Data for all strains except *Z. oryzae* A-7^T (Xie & Yokota, 2006) were obtained in the present study and cells of these strains were harvested after growth on R2A at 30 °C. Values are percentages of the total fatty acids; –, fatty acids representing <1.0 % of the total.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C _{10:0}	–	–	–	–	–	–	–	–	–	–	–	1.5	–	–	–
C _{10:0} 3-OH	–	6.4	7.3	6.0	6.5	7.8	6.7	12.5	8.9	4.0	6.0	19.3	8.0	2.8	4.7
iso-C _{11:0} 3-OH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.1
C _{12:0}	5.7	–	7.4	7.7	5.4	–	10.1	9.7	8.7	6.4	8.0	14.3	10.2	3.1	3.0
C _{12:0} 3-OH	4.6	–	–	–	–	–	4.6	–	–	2.5	3.9	–	5.5	1.8	2.4
C _{14:0}	5.4	4.7	2.2	–	–	3.9	1.0	1.2	1.6	–	–	1.1	1.2	–	2.2
C _{16:0}	35.2	33.4	30.6	35.5	28.5	27.7	22.2	17.1	24.5	28.3	21.9	21.5	26.7	25.9	27.6
C _{16:0} 2-OH	–	–	–	–	–	–	–	–	1.6	–	–	–	–	–	1.9
C _{17:0} cyclo	5.9	–	–	9.0	–	–	–	1.3	–	–	–	–	–	–	–
C _{18:0}	2.3	–	–	–	–	–	–	–	2.4	–	–	3.1	–	–	8.5
C _{18:1} ω7c	7.4	9.9	1.8	8.0	11.4	11.2	5.8	9.9	12.6	13.0	11.4	5.2	9.5	2.5	12.6
C _{18:1} ω9c	–	–	–	–	–	–	–	–	1.2	–	–	–	–	–	2.2
Summed feature 3*	28.5	42.8	49.5	32.3	45.7	45.6	44.8	45.9	37.9	44.2	45.5	32.5	35.6	61.6	32.0
Summed feature 5*	–	–	–	–	–	–	–	–	–	–	–	–	–	1.1	1.1

*Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 5 comprises C_{18:2}ω6,9c and/or anteiso-C_{18:0}.

Fig. 2. Neighbour-joining phylogenetic tree derived from analysis of the 16S rRNA gene sequences of strain 5YN10-9^T and other members of the family *Rhodocyclaceae*, class *Betaproteobacteria*. Numbers at nodes indicate levels of bootstrap support based on 1000 resamplings; only values above 40 % are indicated. Bar, 0.01 substitutions per nucleotide position.

studies (Song *et al.*, 2001; Xie & Yokota, 2006). However, strain 5YN10-9^T was unique in that it lacked C_{10:0} 3-OH and had a low proportion of summed feature 3. C_{12:0} 3-OH was found only in strain 5YN10-9^T and members of the genera *Thauera* and *Zoogloea*, and C_{17:0} cyclo was present only in strain 5YN10-9^T, *Azoarcus indigens* LMG 9092^T and *Thauera aromatica* DSM 6984^T (Table 2). These phenotypic differences were further supported by its phylogenetic position based on 16S rRNA gene sequence data (Fig. 2); the distinct monophyletic line of strain 5YN10-9^T and its low 16S rRNA gene sequence similarity values with its closest related genera support the inclusion of this isolate in a new genus.

Based on combined phylogenetic and phenotypic data, strain 5YN10-9^T is considered to represent a novel species in a new genus, for which the name *Uliginosibacterium gangwonense* gen. nov., sp. nov. is proposed

Description of *Uliginosibacterium* gen. nov.

Uliginosibacterium (Uli.gi.no.si.bac.te'ri.um. L. adj. *uliginosus* wet, moist, marshy; L. neut. n. *bacterium* a rod; N.L. neut. n. *Uliginosibacterium* a rod isolated from peat).

Gram-negative, strictly aerobic rods. Motile by means of a single polar flagellum. The major isoprenoid quinone is Q-8. The major fatty acids are C_{16:0} and summed feature 3; C_{18:1}ω7c, C_{17:0} cyclo, C_{12:0}, C_{14:0} and C_{12:0} 3-OH are present as minor components. On the basis of the results of 16S rRNA gene sequence comparisons, the genus belongs to the family *Rhodocyclaceae*, class *Betaproteobacteria*. The type species is *Uliginosibacterium gangwonense*.

Description of *Uliginosibacterium gangwonense* sp. nov.

Uliginosibacterium gangwonense (gang.won.en'se. N.L. neut. adj. *gangwonense* named after Gangwon Province in Korea, the geographical origin of the type strain of the species).

Has the following characteristics in addition to those given for the genus above. Colonies are irregular and yellow-coloured on R2A agar plates. Cells are 0.6–1.0 µm wide and 3.0–7.0 µm long. Temperature range for growth is 4–35 °C (optimum, 25–30 °C) and pH range for growth is 4.0–8.0 (optimum, pH 6.0–7.0). Unable to grow in the presence of 2% NaCl. Catalase- and oxidase-positive. Accumulates poly-β-hydroxybutyrate. CM-cellulose, starch and Tween 80 are degraded, but casein, chitin, DNA, pectin and tyrosine are not. Positive for urease, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase (API 20NE). Positive for esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase, but negative for alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase,

cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, β-mannosidase and α-fucosidase (API ZYM). Assimilates D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, maltose, sucrose, glycogen and L-serine, but not D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32 GN). The DNA G + C content is 59.3 mol%.

The type strain, 5YN10-9^T (=KACC 11603^T=DSM 18521^T), was isolated from a wetland in Yongneup, Republic of Korea.

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