Gangjinia marincola gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae*

Dong-Heon Lee,¹ Sung-Ran Moon,¹ Young-Hyun Park,¹ Young Sun Lee,² Jae Sung Jung,² Jung Ho Kim,³ Hoon Kim³ and Hyung-Yeel Kahng¹

Correspondence
Hyung-Yeel Kahng
kahng@sunchon.ac.kr

¹Department of Environmental Education, Sunchon National University, Sunchon 540-742, Republic of Korea

²Department of Biology, Sunchon National University, Sunchon 540-742, Republic of Korea

³Department of Biological Environment, Sunchon National University, Sunchon 540-742, Republic of Korea

A novel strictly aerobic, orange-pigmented, Gram-stain-negative bacterium, designated strain GJ16^T, was isolated from coastal seawater of Gangjin Bay, the southernmost part of the Korean peninsula, and subjected to a polyphasic taxonomic study. It grew optimally at 25–30 °C, at pH 7.0–8.0 and in the presence of 3 % NaCl. Comparative 16S rRNA gene sequence analysis revealed that strain GJ16^T formed a distinct lineage within the family *Flavobacteriaceae* and shared less than 91.2 % 16S rRNA gene sequence similarity with members of the genera *Leptobacterium*, *Zhouia*, *Winogradskyella*, *Dokdonia* and *Krokinobacter*. The predominant cellular fatty acids were iso-C_{15:0} (40.2 %), iso-C_{15:1} G (12.8 %), iso-C_{17:0} 3-OH (11.2 %) and C_{15:0} (6.6 %). The G+C content of the genomic DNA was 39.4 mol% and the major respiratory isoprenoid quinone was MK-6. On the basis of phenotypic and genotypic data, strain GJ16^T represents a novel species in a new genus in the family *Flavobacteriaceae*, for which the name *Gangjinia marincola* gen. nov., sp. nov. is proposed; the type strain is GJ16^T (=KCTC 22649^T =JCM 16082^T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 2002) is one of the major branches of the phylum ‘*Bacteroidetes*’ and contains 90 genera with validly published names (<http://www.bacterio.cict.fr/>) at the time of writing. Members of the family occur in a variety of soil, freshwater and marine environments (Bernardet & Nakagawa, 2006), with those of marine origin forming a distinct clade in the phylogenetic tree (Bowman, 2006). A novel Gram-stain-negative bacterium that produced orange pigments was isolated from seawater of Gangjin Bay and investigated using a polyphasic approach. This organism is described below as a member of a novel species in a new genus within the family *Flavobacteriaceae*, for which the name *Gangjinia marincola* gen. nov., sp. nov. is proposed.

Strain GJ16^T was isolated from seawater collected from Gangjin Bay (34° 27′ 44″ N 126° 47′ 20″ E), Korea, by using a standard dilution plating technique on marine agar 2216 (MA; Difco) at 28 °C for 5 days. Subcultivation was performed routinely on MA at 30 °C for 3 days under aerobic conditions and the strain was preserved at –80 °C

in marine broth (MB; Difco) supplemented with 20 % glycerol.

Genomic DNA was extracted and purified by using a commercial genomic DNA extraction kit (Bioneer) and the nearly complete 16S rRNA gene sequence was amplified using bacterial universal primers (Weisburg *et al.*, 1991). Sequencing of the 16S rRNA gene of strain GJ16^T was carried out as described previously (Lane, 1991). The resulting 16S rRNA gene sequence was compared with 16S rRNA gene sequences available in GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast/>) to determine the approximate phylogenetic affiliation and using CLUSTAL W software (Thompson *et al.*, 1994) to align the sequences of closely related organisms. Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; <http://rdp.cme.msu.edu/index.jsp>; Cole *et al.*, 2003) and the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Gaps at the 5′ and 3′ ends of the alignment were omitted for further analyses. Phylogenetic trees were reconstructed by using the neighbour-joining and maximum-parsimony algorithms in the MEGA4.0 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications (Felsenstein, 1985). Evolutionary distance matrices were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1983).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GJ16^T is FJ463655.

A supplementary figure is available with the online version of this paper.

Strain GJ16^T formed a clade with the type strains of *Leptobacterium flavescens* (Mitra *et al.*, 2009) and *Zhouia amylytica* (Liu *et al.*, 2006) with levels of 16S rRNA gene sequence similarity of 91.2 and 90.4%, respectively (Fig. 1). Strain GJ16^T also showed significant 16S rRNA gene sequence similarity to members of the genera *Winogradskyella* (Lau *et al.*, 2005; 90.1–91.1%), *Dokdonia* (Yoon *et al.*, 2005; 91.1%) and *Krokinobacter* (Khan *et al.*, 2006; 90.8%). The three algorithms used for reconstruction of the phylogenetic trees generated the same branching of the *Leptobacterium*–*Zhouia* clade to which strain GJ16^T was affiliated with high bootstrap values. These data suggest that GJ16^T represents a member of a new genus within the family *Flavobacteriaceae*.

The following tests were performed on strains GJ16^T, *L. flavescens* KCTC 22160^T and *Z. amylytica* JCM 14016^T. Cells grown aerobically on MA at 30 °C for 5 days were used for the examination of colony morphology, size and colour. Cell morphology and size were examined by light (FDX-35; Nikon) and transmission electron (JEM-1010; JEOL) microscopy using cells from exponentially growing cultures. The optimal temperature and temperature range for growth were tested on MA at 4, 10, 15, 20, 25, 30, 35, 37 and 42 °C. NaCl requirements and tolerance were determined in the presence of 0, 2, 4, 6, 8 and 10% (w/v) NaCl on laboratory-prepared ZoBell agar [5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate and 15 g Bacto agar (ZoBell, 1941) in 1 l modified artificial seawater containing 0–10% (w/v) NaCl, 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl and 1.3 g CaCl₂]. The

pH range for growth was determined in MB 2216 (Difco) adjusted to pH 4.0–11.0 (at intervals of 0.5 pH unit) with HCl or NaOH after sterilization. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Gliding motility was investigated using phase-contrast light microscopy by the hanging drop method as described by Bernardet *et al.* (2002). Anaerobic growth was tested on MA incubated for up to 15 days in the AnaeroPak-Anaero system (Mitsubishi Gas Chemical). Catalase and oxidase activities were assessed using standard methods (Tindall *et al.*, 2007). Degradation of agar, DNA, starch and carboxymethyl-cellulose were assessed according to the methods of Smibert & Krieg (1994). Casein hydrolysis was assessed according to the method of Norris *et al.* (1985); hydrolysis of chitin and Tweens 20, 40, 60 and 80 was assessed according to Baumann & Baumann (1981). To detect flexirubin-type pigments, cell mass of the three strains was subjected to the KOH test as described by Bernardet *et al.* (2002). Antibiotic sensitivity was assessed after 3 days incubation at 30 °C on MA by the disc diffusion method and the following commercial antibiotic-impregnated discs (BBL Becton Dickson): ampicillin (10 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), penicillin (10 IU), polymyxin B (300 µg), streptomycin (10 µg) and tetracycline (30 µg). The results were interpreted according to the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI, 2003). Other phenotypic and enzymic characterizations of strain GJ16^T and the two reference strains were conducted using API 20E, API 20NE and API

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain GJ16^T and representative members of the marine clade of the family *Flavobacteriaceae*. Bootstrap percentages (based on 1000 replicates) greater than 70% are shown at branch points. *Bacteroides fragilis* ATCC 25285^T (accession no. X83935) was used as an outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

50CH kits (bioMérieux) incubated at 30 °C for 3 days and API ZYM kits incubated at 30 °C for 8 h. The utilization of different carbon sources was tested with GN2 MicroPlates (Biolog) according to the manufacturer's instructions. All commercial kits were inoculated with bacterial suspensions in 3 % sea salts. Detailed results of morphological, physiological and biochemical tests are given in the genus and species descriptions and in Table 1.

Pigments were extracted from cells of strain GJ16^T grown in the dark in MB using acetone/methanol (1:1, v/v). Cellular residues were removed by centrifugation. An adsorption spectrum (200–800 nm) was obtained from the crude cell extract using a Beckman Coulter DU 800 UV/visible spectrophotometer (Varian).

The whole-cell fatty acid compositions of strain GJ16^T, *L. flavescens* KCTC 22160^T and *Z. amylolytica* JCM 14016^T were analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID)

using cells grown on MA at 30 °C for 3 days. The major respiratory quinones of strain GJ16^T were analysed by the Korean Culture Center of Microorganisms (KCCM, Seoul, Republic of Korea) using reverse-phase HPLC (Komagata & Suzuki, 1987). The G + C content of the DNA of strain GJ16^T was determined using the thermal denaturation method (Marmur & Doty, 1962) using an Ultrospec 2100 spectrophotometer (Pharmacia Biotech) and DNA from *Escherichia coli* K-12 as a control.

The major cellular fatty acids of strain GJ16^T were iso-C_{15:0} (40.2 %), iso-C_{15:1} G (12.8 %), iso-C_{17:0} 3-OH (11.2 %) and C_{15:0} (6.6 %). The detailed fatty acid compositions of the three strains are compared in Table 2. Although the three strains grown under the same conditions shared similar overall fatty acid compositions, strain GJ16^T differed from the two reference strains by a significantly higher proportion of iso-C_{15:0}. The major respiratory quinone of strain GJ16^T was menaquinone 6 (MK-6), which is in line with *L. flavescens* KCTC 22160^T

Table 1. Phenotypic characteristics that differentiate strain GJ16^T from the type species of related genera

Strains: 1, GJ16^T; 2, *Leptobacterium flavescens* KCTC 22160^T; 3, *Zhouia amylolytica* JCM 14016^T. +, Positive; –, negative; w, weakly positive. All data are from this study except where marked.

Characteristic	1	2	3
Colony pigmentation on MA	Orange	Pale yellow	Yellow
Salinity range for growth (NaCl, %)	2–4	1–4	1–7
Temperature range for growth (°C)	25–30	15–30	10–40
pH range for growth	6–9	6–9	6–8
Hydrolysis of:			
Casein	+	–	–
Gelatin	–	+	+
Starch	+	–	–
Tween 80	–	+	+
Utilization of (GN2 MicroPlate):			
D-Galactose	–	–	+
D-Glucose	–	–	+
D-Mannose	–	–	+
Melibiose	+	–	+
Raffinose	+	–	+
L-Rhamnose	+	–	–
Aerobic acid production from (API 50CH):			
D-Arabinose	–	+	–
Cellobiose	–	+	+
Gentiobiose	–	+	–
Maltose	–	+	+
Enzyme activity (API ZYM)			
Esterase (C4)	–	+	–
Esterase lipase (C8)	–	+	w
Trypsin	–	+	–
α-Chymotrypsin	–	+	–
β-Glucosidase	+	–	–
DNA G + C content (mol%)	39.4	42.0*	34.5†

*Data from Mitra *et al.* (2009).

†Data from Liu *et al.* (2006).

Table 2. Fatty acid composition (%) of strain GJ16^T and the type species of related genera

Taxa: 1, strain GJ16^T; 2, *Leptobacterium flavescens* KCTC 22160^T; 3, *Zhouia amylolytica* JCM 14016^T. Fatty acids amounting to less than 1.0% in all strains tested are not listed. tr, Trace (<1.0%); –, not detected; ECL, equivalent chain-length. All data are from this study.

Fatty acid	1	2	3
Straight-chain			
C _{15:0}	6.6	6.9	3.5
C _{16:0}	tr	2.9	1.2
Branched			
iso-C _{14:0}	1.2	–	–
iso-C _{15:0}	40.2	27.4	31.3
iso-C _{16:0}	2.2	1.8	1.3
iso-C _{17:0}	tr	1.3	tr
anteiso-C _{15:0}	3.7	tr	1.6
iso-C _{15:1} G	12.8	15.5	17.6
iso-C _{16:1}	1.5	tr	tr
iso-C _{17:1} ω9c	2.2	–	tr
Unsaturated			
C _{15:1} ω6c	1.3	–	–
Hydroxy			
C _{15:0} 3-OH	tr	1.0	tr
iso-C _{15:0} 3-OH	4.4	6.6	7.0
C _{16:0} 3-OH	tr	3.4	tr
iso-C _{16:0} 3-OH	1.5	tr	tr
C _{17:0} 3-OH	tr	1.9	tr
iso-C _{17:0} 3-OH	11.2	14.6	15.1
Summed feature 3*	1.1	3.0	6.5
ECL 13.565	3.3	6.9	6.6

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1} ω7c.

(Mitra *et al.*, 2009) and *Z. amylolytica* JCM 14016^T (Liu *et al.*, 2006) and all other members of the family *Flavobacteriaceae*. Flexirubin-type pigments were not produced by any of the three strains. Cells of strain GJ16^T produced orange-coloured carotenoid-type pigments with absorption peaks at 451 nm, 474 nm (major peak) and 503 nm (Schmidt *et al.*, 1994). The G+C content of the genomic DNA of strain GJ16^T was 39.4 mol%, a value between those reported for the two reference strains (Mitra *et al.*, 2009; Liu *et al.*, 2006).

In conclusion, on the basis of phylogenetic analysis and differential phenotypic properties, strain GJ16^T represents a novel species in a new genus in the family *Flavobacteriaceae*, for which the name *Gangjinia marincola* gen. nov., sp. nov. is proposed.

Description of *Gangjinia* gen. nov.

Gangjinia (Gang.ji'ni.a. N.L. fem. n. *Gangjinia* named after Gangjin Bay, located on the South Sea in Korea, from where the organism was isolated).

Cells are strictly aerobic, Gram-stain-negative, orange-pigmented, non-flagellated and rod-shaped (Supplementary Fig. S1, available in IJSEM Online). Gliding motility is absent. Oxidase and catalase activities are present. Non-diffusible carotenoid-type pigments are produced, but flexirubin pigments are absent. The major respiratory quinone is MK-6. The cellular fatty acid composition is dominated by iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and C_{15:0}. The genus is a member of the family *Flavobacteriaceae*, phylum 'Bacteroidetes'. The type species is *Gangjinia marincola*.

Description of *Gangjinia marincola* sp. nov.

Gangjinia marincola (ma.rin.co'la. L. n. *mare* the sea; L. n. *incola* inhabitant; N.L. n. *marincola* inhabitant of the sea).

Displays the following characteristics in addition to those given in the genus description. Colonies on MA are orange in colour, circular, 1.0–3.0 mm in diameter and convex with smooth surfaces and entire margins. Non-diffusible carotenoid-type pigments with absorption peaks at 451 nm, 474 nm and 503 nm are produced. Growth occurs at 20–30 °C (optimum at 25–30 °C), at pH 6.0–9.0 (optimum at pH 7.0–8.0) and with 2–4% NaCl (optimum in 3% NaCl). However, no growth is observed at 15 °C or 30 °C, at pH 5.5 or 9.5, or in the presence of <1% or >5% NaCl. Anaerobic growth does not occur on marine agar. Acetoin, indole and H₂S are not produced. Nitrate is not reduced. Hydrolyses casein, starch and DNA, but not agar, carboxymethyl-cellulose, chitin or Tween compounds. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities are absent. In the API ZYM kit, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and *N*-acetyl-β-glucosaminidase activities are present, but esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities are absent. In the API 50CH kit, acid is only produced aerobically from aesculin-ferric citrate and 5-ketogluconate. In the GN2 MicroPlate, the following substrates are utilized as sole carbon sources: melibiose, raffinose, L-rhamnose, methyl pyruvate, α-ketoglutaric acid, α-ketovaleric acid, propionic acid, L-alanyl glycine, L-glutamic acid, L-leucine, L-ornithine, L-phenylalanine, thymidine, putrescine, α-cyclodextrin, dextrin, glycogen, acetic acid, γ-hydroxybutyric acid, glucuronamide, L-alanine, glycyl L-glutamic acid, L-proline and phenylethylamine. None of the other carbon sources in the GN2 Microplate are utilized. Resistant to kanamycin (30 µg), gentamicin (10 µg), neomycin (30 µg) and polymyxin B (300 µg), but sensitive to ampicillin (10 µg), penicillin (10 IU), streptomycin (10 µg) and tetracycline (30 µg).

The type strain is GJ16^T (=KCTC 22649^T =JCM 16082^T), isolated from coastal seawater of Gangjin Bay, the southernmost part of the Korean peninsula. The DNA G+C content of the type strain is 39.4 mol%.

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