Gaetbulibacter saemankumensis gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a tidal flat sediment in Korea

Seo-Youn Jung, So-Jung Kang, Mi-Hwa Lee, Soo-Young Lee, Tae-Kwang Oh and Jung-Hoon Yoon

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, South Korea

Three Gram-negative, yellow-pigmented, rod-shaped bacterial strains, SMK-12^T, SMK-36 and SMK-45, were isolated from a tidal flat sediment of the Yellow Sea in Korea, and their taxonomic positions were investigated by a polyphasic approach. The three strains grew optimally at 25–30 °C and in the presence of 2–3 % (w/v) NaCl. They contained MK-6 as the predominant menaquinone. The major cellular fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{15:0} 3-OH and C_{16:1} ω7c and/or iso-C_{15:0} 2-OH. The DNA G+C contents of the three strains were 34.7–34.9 mol%. The phylogenetic tree based on 16S rRNA gene sequences revealed that the three strains form one distinct evolutionary lineage supported by a bootstrap value of 100 % within the family *Flavobacteriaceae*. The three strains exhibited 16S rRNA gene sequence similarity levels of 93.8–94.9 % to the nearest phylogenetic neighbours, the genera *Algibacter*, *Bizionia* and *Formosa*. On the basis of differences in phenotypic characteristics and phylogenetic distinctiveness, strains SMK-12^T, SMK-36 and SMK-45 were classified in a novel genus and species, for which the name *Gaetbulibacter saemankumensis* gen. nov., sp. nov. is proposed. The type strain for the novel species is SMK-12^T (=KCTC 12379^T = DSM 17032^T).

The family *Flavobacteriaceae* was proposed by Jooste (1985) and included in the first edition of *Bergey's Manual of Systematic Bacteriology* (Reichenbach, 1989). The family *Flavobacteriaceae* forms an evolutionary lineage of descent within the domain *Bacteria*, together with the families *Bacteroidaceae*, *Cytophagaceae*, *Sphingobacteriaceae*, *Spirosomaceae* and several taxa unaffiliated to any family (Woese *et al.*, 1985; Bernardet *et al.*, 1996; Bernardet *et al.*, 2002). The family *Flavobacteriaceae* accommodates bacteria that are motile by gliding or non-motile, chemo-organotrophic, Gram-negative and rod-shaped, and contain menaquinone-6 as the major isoprenoid quinone (Bernardet *et al.*, 1996, 2002; Nedashkovskaya *et al.*, 2004b). Currently, the family *Flavobacteriaceae* comprises more than 20 genera, including the recently described genera *Formosa* (Ivanova *et al.*, 2004), *Ulvibacter* (Nedashkovskaya *et al.*, 2004a),

Algibacter (Nedashkovskaya *et al.*, 2004b), *Winogradskyella* (Nedashkovskaya *et al.*, 2005a) and *Bizionia* (Nedashkovskaya *et al.*, 2005b). Recently, three Gram-negative, slightly halophilic, yellow-pigmented bacterial strains, SMK-12^T, SMK-36 and SMK-45, were isolated from a tidal flat sediment in Korea. Comparison of 16S rRNA gene sequence revealed that the three strains were phylogenetically affiliated to the family *Flavobacteriaceae*. The aim of the present work is to establish the taxonomic positions of strains SMK-12^T, SMK-36 and SMK-45 by using a polyphasic taxonomic approach.

Tidal flat sediment collected from Saemankum, Pyunsan, Korea, was used as the source for isolation of bacterial strains. Strains SMK-12^T, SMK-36 and SMK-45 were isolated by the usual dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. Growth at various temperatures from 4 to 45 °C was measured on MA, and growth at various pH and tolerance to various NaCl concentrations were measured in marine broth 2216 (MB; Difco). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Cell morphology and presence

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Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SMK-12^T, SMK-36 and SMK-45 are AY883937, AY883938 and AY883939, respectively.

of flagella were examined by light microscopy (Nikon E600) and transmission electron microscopy using cells grown on MA. Gliding motility was determined as described by Bowman (2000). Presence of flexirubin pigment was investigated as described by Reichenbach (1992). Gram reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution on MA. Oxidase activity was determined by oxidation of 1% (w/v) *p*-aminodimethyl-aniline oxalate. Hydrolysis of casein and starch was determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was performed on MA with the substrate concentrations described previously (Cowan & Steel, 1965). Hydrolysis of Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965) with the modification that artificial sea water (containing 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O per litre distilled water; Bruns *et al.*, 2001) was used for the preparation of media. Hydrolysis of aesculin and urea and nitrate reduction were studied as described by Lanyi (1987) with the modification that artificial sea water was used for the preparation of media. The production of H₂S was tested as

described previously (Bruns *et al.*, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994). The API ZYM system (bioMérieux) was used to determine enzyme activity. Antibiotic sensitivity was tested by spreading bacterial suspension on MA and applying discs impregnated with the following antibiotics (concentration per disc): ampicillin (10 µg), carbenicillin (25 µg), lincomycin (15 µg), gentamicin (10 µg), oleandomycin (15 µg), benzylpenicillin (10 U), polymyxin B (300 U), streptomycin (30 µg), tetracycline (30 µg) and neomycin (15 µg).

Cell biomass for isoprenoid quinone analysis and for DNA extraction was obtained after cultivation for 3 days in MB at 30 °C. Isoprenoid quinones were analysed as described previously (Komagata & Suzuki, 1987), using reversed-phase HPLC. For fatty acid methyl ester (FAME) analysis, cell biomass of strains SMK-12^T, SMK-36 and SMK-45 was harvested from agar plates after cultivation for 3 days on MA at 30 °C. The FAMEs were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified by the

Table 1. Differential characteristics of *Gaetbulibacter saemankumensis* gen. nov., sp. nov. and phylogenetically related genera of the family *Flavobacteriaceae*

Genera: 1, *Gaetbulibacter* gen. nov.; 2, *Algibacter* (data from Nedashkovskaya *et al.*, 2004b); 3, *Formosa* (Ivanova *et al.* 2004; Nedashkovskaya *et al.* 2004b, 2005b); 4, *Bizionia* (Nedashkovskaya *et al.* 2005b). +, Positive; –, negative; V, variable; ND, not determined. Members of all genera are rod-shaped and positive for catalase and negative for utilization of L-arabinose, citric acid, D-mannitol and *myo*-inositol.

Characteristic	1	2	3	4
Cell size (µm)	0.4–0.5 × 3.0–4.5	0.4–0.5 × 2.0–3.0	0.8–1.8 × 0.4–0.9	0.4–0.5 × 1.9–2.3
Growth at 7% (w/v) NaCl	+	–	–	+
Gliding motility	+	+	+	–
Urease	–	ND	+	–
Oxidase	+	+	V*	+
Nitrate reduction	+	–	–	–
Acid production from carbohydrates	+	+	+	–
Temperature range for growth (°C)	13–40	4–35	<37	4–36
Optimal growth temperature (°C)	25–30	21–23	23	23–25
Hydrolysis of:				
Casein	–	–	ND	+
Gelatin	–	+	V	+
Starch	+	+	V	–
H ₂ S production	–	–	–	+
Sensitivity to antibiotics:				
Ampicillin (10 µg), gentamicin (10 µg), polymyxin B (300 U), streptomycin (30 µg), tetracycline (30 µg), neomycin (15 µg)	–	–	–	ND
Lincomycin (15 µg), oleandomycin (15 µg)	+	+	–	ND
Benzylpenicillin (10 U)	+	–	–	ND
Carbenicillin (25 µg)	+	+	ND	ND
DNA G + C content (mol%)	34.7–34.9	31.0–33.0	34.0–34.4	37.6

*Reported as negative by Ivanova *et al.* (2004) and positive by Nedashkovskaya *et al.* (2004b, 2005b).

procedure described previously (Yoon *et al.*, 1996). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA relatedness was determined by the microplate hybridization method (Ezaki *et al.*, 1989) using photobiotin-labelled DNA probes.

16S rRNA gene amplification was performed according to the method described previously using two universal primers (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with CLUSTAL W program (Thompson *et al.*, 1994) and gaps at the 5' and 3' ends of the alignment were omitted from further analysis. The evolutionary distances were calculated using the Kimura two-parameter correction with the CLUSTAL W package (Thompson *et al.*, 1994). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) on the basis of distance matrix data. The reliability of grouping was assessed by 1000 bootstrap resamplings of the neighbour-joining dataset by using the CLUSTAL W package.

Morphological, cultural, physiological and biochemical characteristics of strains SMK-12^T, SMK-36 and SMK-45 are shown in Table 1 or are given in the genus and species descriptions. The predominant menaquinone detected in the three strains was MK-6, at peak area ratios of greater than 90%. Cellular fatty acid profiles of strains SMK-12^T, SMK-36 and SMK-45 are summarized in Table 2. The fatty acids profiles were characterized by a common core of straight-chain, branched, unsaturated and hydroxy fatty acids in similar amounts; the major components were iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{15:0} 3-OH and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. The DNA G+C contents of strains SMK-12^T, SMK-36 and SMK-45 were 34.8, 34.7 and 34.9 mol%, respectively.

The 16S rRNA gene sequences of the three strains determined in this study each comprised 1474 nucleotides, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. The 16S rRNA gene sequences of strains SMK-36 and SMK-45 were identical, but had 4 bp differences from the 16S rRNA gene sequence of strain SMK-12^T. Comparative 16S rRNA gene sequence analysis and estimation of phylogenetic relationships showed that strains SMK-12^T, SMK-36 and SMK-45 formed a distinct evolutionary lineage within the family *Flavobacteriaceae* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, the cluster comprising strains SMK-12^T, SMK-36 and SMK-45 joined the phylogenetic lineage comprising the genus *Algibacter* by a bootstrap resampling value of 82.3% (Fig. 1). The 16S rRNA gene sequence similarity values between strains SMK-12^T, SMK-36, SMK-45 and members of three phylogenetically related genera were 94.9% (*Algibacter lectus* KMM 3902^T), 94.5% (*Bizionia paragorgiae* KMM 6029^T) and 93.8% (*Formosa algae* KMM 3553^T). Sequence similarities to all other species

Table 2. Cellular fatty acid compositions of *Gaetbulibacter saemankumensis* gen. nov., sp. nov. and phylogenetically related genera

Strains: 1, *Gaetbulibacter saemankumensis* SMK-12^T (ranges for the other two strains are given in parentheses); 2, *Algibacter lectus* KMM 3902^T (data from Nedashkovskaya *et al.*, 2004b); 3, *Formosa algae* KMM 3553^T (Ivanova *et al.*, 2004); 4, *Bizionia paragorgiae* KMM 6029^T (Nedashkovskaya *et al.*, 2005b). Values are percentage of total fatty acids. —, Not detected or not described. Fatty acids that represented <1.0% in *Gaetbulibacter saemankumensis* were not indicated.

Fatty acid	1	2	3	4
Straight-chain				
C _{15:0}	1.5 (1.3–1.6)	13.4	27.2	4.3
C _{16:0}	1.3 (0.9–1.3)	—	1.0	3.4
Branched				
iso-C _{13:0}	2.0 (1.9–2.4)	—	—	—
iso-C _{15:0}	23.0 (22.7–24.3)	12.5	13.3	13.2
iso-C _{15:1}	12.5 (13.0–13.7)	13.4	15.3	9.4
iso-C _{17:1}	—	—	1.4	—
anteiso-C _{15:0}	10.8 (8.9–9.8)	7.2	7.2	12.3
anteiso-C _{15:1}	1.5 (1.4–1.6)	—	—	5.8
anteiso-C _{16:1}	—	—	1.0	—
anteiso-C _{17:0}	—	—	—	3.7
iso-C _{17:1} ω9c	2.1 (2.2–2.4)	—	—	1.7
anteiso-C _{17:1} ω9c	1.3 (1.0–1.2)	—	—	1.5
Unsaturated				
C _{17:1} ω6c	1.0 (0.9–1.1)	—	—	1.9
Hydroxy				
iso-C _{15:0} 3-OH	8.2 (7.6–8.6)	9.4	—	3.4
iso-C _{16:0} 3-OH	2.0 (2.4–2.5)	—	—	6.8
iso-C _{17:0} 3-OH	15.2 (12.9–16.0)	9.1	—	4.3
C _{15:0} 2-OH	1.6 (1.4–1.6)	—	—	—
C _{17:0} 2-OH	2.4 (2.1–2.4)	—	—	—
C _{17:0} cyclo	—	—	7.4	—
Summed feature 3*	10.4 (8.2–10.4)	—	—	6.3

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

included in the phylogenetic analysis were lower than 93.1%. Mean DNA–DNA relatedness values of strains SMK-12^T, SMK-36 and SMK-45 were 83–106% when their DNAs were used individually as labelled DNA probes for cross-hybridization. These values indicated that strains SMK-12^T, SMK-36 and SMK-45 were members of the same genomic species (Wayne *et al.*, 1987).

The phenotypic characteristics of strains SMK-12^T, SMK-36 and SMK-45 were different from those of three related genera within the family *Flavobacteriaceae* (Table 1). In particular, nitrate reduction and hydrolysis of gelatin distinguish the three strains from the genera *Algibacter*, *Bizionia* and *Formosa*. Sensitivity of these three strains to

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains SMK-12^T, SMK-36, SMK-45 and other related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branching points. The 16S rRNA gene sequence of *Escherichia coli* ATCC 11775^T (accession no. X80725) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

several antibiotics differed from that of members of the genera *Algibacter* and *Formosa* (Table 1). The three strains were clearly distinguishable from the genus *Bizionia* in phenotypic properties, including gliding motility, acid production from carbohydrates, H₂S production and hydrolysis of some substrates (Table 1). There were noteworthy differences between the fatty acid profiles of the three strains and the three phylogenetically related genera *Algibacter*, *Bizionia* and *Formosa*, particularly in the proportion of some fatty acids (Table 2). Therefore, on the basis of phenotypic, phylogenetic and genetic data, strains SMK-12^T, SMK-36 and SMK-45 should be classified as members of a novel genus and species, for which the name *Gaetbulibacter saemankumensis* gen. nov., sp. nov. is proposed.

Description of *Gaetbulibacter* gen. nov.

Gaetbulibacter (Gaet.bu.li.bac'ter. N.L. n. *gaetbulum* -i gaetbul, the Korean name for a tidal flat; N.L. masc. n. *bacter* from Gr. neut. n. *baktron* rod; N.L. masc. n. *Gaetbulibacter* rod isolated from a tidal flat).

Cells are aerobic, Gram-negative, non-flagellated, non-spore-forming and rod-shaped. Growth also occurs under anaerobic conditions on MA and on MA with nitrate. Motile by means of gliding. Catalase- and oxidase-positive. Flexirubin pigments are absent. The predominant menaquinone is MK-6. Phylogenetically, the genus is a member of the family *Flavobacteriaceae*. The type species is *Gaetbulibacter saemankumensis*.

Description of *Gaetbulibacter saemankumensis* sp. nov.

Gaetbulibacter saemankumensis (sae.man.kum.en'sis. N.L. masc. adj. *saemankumensis* of Saemankum, from where the organism was originally isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.4–0.5 × 3.0–4.5 µm. Optimal pH for growth is 7.0–8.0; growth occurs weakly at pH 5.5, but not at 5.0. Optimal growth occurs in the presence of 2–5% (w/v) NaCl; growth occurs in the presence

of 7% (w/v) NaCl, but not in the presence of >8% NaCl. Aesculin, tyrosine and Tween 20 are hydrolysed, but hypoxanthine, xanthine and Tweens 40, 60 and 80 are not. Using the API ZYM system (bioMérieux), alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase and N-acetyl-β-glucosaminidase are present. Naphthol-AS-BI phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase and trypsin are absent; α-chymotrypsin and β-glucosidase activities are variable (absent for type strain). D-Cellobiose, D-fructose, D-galactose, D-glucose, D-xylose and L-rhamnose are utilized as sole carbon and energy sources. L-Alanine, L-asparagine, D-gluconic acid, glycerol, L-malic acid, melibiose, propionic acid, pyruvic acid, D-raffinose, succinic acid, D-sorbitol, L-serine and D-trehalose are not utilized. Utilization of maltose, L-proline and sucrose is variable (positive for type strain). Acid is produced from D-cellobiose, D-galactose, lactose and maltose. Acid is not produced from L-arabinose, D-fructose, D-mannitol, D-melezitose, melibiose, myo-inositol, D-raffinose, D-ribose, D-sorbitol, D-trehalose and D-xylose. Acid production from D-glucose and sucrose (positive for type strain), D-mannose and L-rhamnose (negative for type strain) is variable. The major cellular fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{15:0} 3-OH and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. The DNA G + C content is 34.7–34.9 mol%.

The type strain, SMK-12^T (=KCTC 12379^T=DSM 17032^T), was isolated from a tidal flat sediment at Saemankum, Pyunsan, Korea.

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