Gaetbulimicrobium brevivitae gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a tidal flat of the Yellow Sea in Korea

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A Gram-negative, non-spore-forming and rod-shaped gliding bacterium, designated strain SMK-19^T, was isolated from a tidal flat sediment of the Yellow Sea, Korea, and its taxonomic position was investigated by using a polyphasic approach. Strain SMK-19^T grew optimally at 37 °C, in the presence of 2–3% (w/v) NaCl and at pH 7.0–8.0. It contained MK-6 as the predominant menaquinone, and iso-C_{17:0} 3-OH and iso-C_{15:0} as the major fatty acids. Major polar lipids were phosphatidylethanolamine, unidentified phospholipids and an amino-group-containing lipid that is ninhydrin-positive. The DNA G + C content was 36.0 mol%. Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that strain SMK-19^T formed a distinct evolutionary lineage within the family *Flavobacteriaceae*. The 16S rRNA gene sequence of strain SMK-19^T exhibited similarity values of <94.4% to those of other members of the family *Flavobacteriaceae*. Strain SMK-19^T was distinguished from phylogenetically related genera by differences in several phenotypic properties. On the basis of phenotypic, phylogenetic and chemotaxonomic data, SMK-19^T (=KCTC 12390^T = DSM 17196^T) was classified at the type strain of a novel genus and species, *Gaetbulimicrobium brevivitae* gen. nov., sp. nov.

In recent years, many novel bacterial taxa have been isolated from a variety of regions of the west coast of the Korean peninsula (Yi *et al.*, 2003, 2004; Yi & Chun, 2004; Yoon *et al.*, 2004, 2005a, b; Kim *et al.*, 2005). In the course of screening novel micro-organisms from a tidal flat of Saemankum, on the Yellow Sea of Korea, many moderately halophilic or halotolerant bacteria have been isolated and characterized taxonomically. This study focuses on one of these isolates, designated strain SMK-19^T. Comparative 16S rRNA gene sequence analysis indicated that strain SMK-19^T forms a deep branch within the family *Flavobacteriaceae*. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain SMK-19^T by using a polyphasic characterization that included phenotypic properties and detailed phylogenetic analysis based on 16S rRNA gene sequences.

Tidal flat sediment collected from Saemankum, Pyunsan, Korea, provided the source for isolation of bacterial strains. Strain SMK-19^T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C.

Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells at the exponential growth stage. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer's instructions. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (initial pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization to various levels by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl was investigated in R2A agar (Difco) and trypticase soy broth prepared according to the formula for the Difco medium except that no NaCl was used. Growth at various NaCl concentrations was investigated in MB or trypticase soy broth. Growth at various temperatures (4–45 °C) was measured on MA. 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. Catalase and oxidase activities and hydrolysis of casein and starch were determined as described by Cowan & Steel (1965). Hydrolysis of Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965) with a modification that artificial sea water was used instead of distilled water.

Published online ahead of print on 26 August 2005 as DOI 10.1099/ijs.0.63795-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SMK-19^T is AY987367.

Hydrolysis of aesculin, gelatin and urea and nitrate reduction were determined as described by Lanyi (1987) with a modification that artificial sea water was used instead of distilled water. The artificial sea water contained (per litre distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Bruns *et al.*, 2001). Hydrolysis of hypoxanthine, tyrosine and xanthine was investigated on MA with the substrate concentrations described by Cowan & Steel (1965), i.e. 0.4, 0.5 and 0.4% (w/v), respectively. H_2S production was

tested as described by Bruns *et al.* (2001). Presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Freeze-dried cells were extracted with acetone/methanol (1:1, v/v) to investigate the presence of carotenoids. Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) using supplementation with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957), 1% (v/v) vitamin solution (Staley, 1968)

Table 1. Differential phenotypic characteristics of *Gaetbulimicrobium* gen. nov. and related genera

Genera: 1, *Gaetbulimicrobium* gen. nov.; 2, *Aquimarina* (data from Nedashkovskaya *et al.*, 2005a); 3, *Stanierella* (Reichenbach, 1989; Nedashkovskaya *et al.*, 2005a); 4, *Gillisia* (Van Trappen *et al.*, 2004; Nedashkovskaya *et al.*, 2005c); 5, *Salegentibacter* (Nedashkovskaya *et al.*, 2004, 2005b); 6, *Mesonina* (Nedashkovskaya *et al.*, 2003); 7, *Psychroflexus* (Bowman *et al.*, 1998; Donachie *et al.*, 2004). All genera are positive for NaCl requirement (variable for *Gillisia*, *Salegentibacter* and *Psychroflexus*), alkaline phosphatase (not determined for *Stanierella*), oxidase (variable for *Psychroflexus*), hydrolysis of Tweens 20 and 40 (variable for *Salegentibacter* and not determined for *Psychroflexus*) and hydrolysis of gelatin and Tween 80 (variable for *Psychroflexus*). All genera are negative for urease (variable for *Salegentibacter* and *Psychroflexus*), nitrate reduction (variable for *Salegentibacter* and *Psychroflexus*) and acid production from D-glucose (variable for *Gillisia*, *Salegentibacter* and *Psychroflexus*). +, Positive; –, negative; ND, not determined; V, variable.

Characteristic	1	2	3	4	5	6	7
Catalase	+	+	–	+	+	+	+
Gliding motility	+	+	–	–	–	–	V
Pigmentation	Orange	Dark yellow to brown	Orange–red	Yellow	Yellow	Yellow	Orange
Flexirubins	–	+	–	–	–	–	–*
Growth at >10% NaCl	–	–	–	V	V	+	V
Growth at 37 °C	+	–	–	–	V	–	V
H_2S production	–	–	+	–	+	+	–*
β -Galactosidase	–	ND	+	–†	+	ND	–*
Hydrolysis of:							
Agar	–	–	+	–	–	–	–*
Casein	+	+	+‡	V	V	+	V
Starch	+	+	–	–	+	–	+*
Acid production from:							
D-Cellobiose	+	–	–	–	–	–	–
Maltose	–	–	–	–	+	–	V
Utilization of:							
D-Glucose	+	–	ND	+	+	–	ND
D-Mannose	–	–	ND	–	+	–	ND
Sucrose	–	–	ND	+	V	–	ND
Susceptibility to:							
Benzylpenicillin	+	–	–	–	+	ND	ND
Carbenicillin	+	+	–	+	V	ND	ND
Tetracycline	–	ND	ND	V	+	ND	ND
Major fatty acids§ (>10% of total)	i-C _{17:0} 3-OH, i-C _{15:0}	i-C _{17:0} 3-OH, i-C _{15:0} , i-C _{15:1} , i-C _{17:1} ω9c	i-C _{17:0} 3-OH, i-C _{15:0} , i-C _{17:1} ω7c	C _{17:0} 2-OH or i-C _{15:1}	i-C _{15:0} , i-C _{15:1} , C _{16:1} ω7c or i-C _{15:1}	i-C _{15:0} , i-C _{17:0} 3-OH	ai-C _{15:0} , ai-C _{15:1} ω10c, i-C _{16:0} 3-OH
DNA G+C content (mol%)	36.0	31.6–32.5	34	36–39	37–38	32.7–34.0	32–36

*Data for *Psychroflexus torquis* and *Psychroflexus gondwanensis*.

†Data for *Gillisia limnaea*.

‡Data taken from Nedashkovskaya *et al.* (2005a); the opposite result was reported by Reichenbach (1989).

§Abbreviations: i, iso; ai, anteiso. Data for the genus *Salegentibacter* were taken from *Salegentibacter holothuriorum* and *Salegentibacter mishustiniae*.

and 0.005 % (w/v) yeast extract. Susceptibility to antibiotics was tested on MA plates using discs containing the following antibiotics: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) or carbenicillin (100 µg). Other physiological and biochemical tests were performed with the API ZYM and API 20E systems (bioMérieux); cell suspension to inoculate the systems was prepared by using cells cultivated for 2 days at 37 °C on MA and artificial sea water (Bruns *et al.*, 2001).

Cell biomass of strain SMK-19^T for DNA extraction and for isoprenoid quinone and polar lipid analyses was obtained by cultivation for 2 days in MB at 37 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain SMK-19^T was harvested from agar plates after incubation for 3 days on MA at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resulting nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain SMK-19^T are given in the species description (see below) or are detailed in Table 1, together

with those of several phylogenetically related genera. The almost complete 16S rRNA gene sequence of strain SMK-19^T was determined, comprising 1480 nt (approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence). Phylogenetic trees based on 16S rRNA gene sequences showed that strain SMK-19^T forms a distinct phylogenetic lineage within the family *Flavobacteriaceae* (Fig. 1). In phylogenetic trees based on the neighbour-joining and maximum-likelihood algorithms, strain SMK-19^T joined the phylogenetic clade comprising the genera *Aquimarina* and *Stanierella*, with which it exhibited the highest 16S rRNA gene sequence similarity values (94.0–94.4 %); its sequence similarity values with the other species used in the phylogenetic analysis were lower (e.g. 92.6 % with *Gillisia limnaea*) (Fig. 1).

The predominant isoprenoid quinone detected in strain SMK-19^T was menaquinone-6 (MK-6) at a peak area ratio of approximately 94 %. Strain SMK-19^T contained large amounts of branched, hydroxy, straight-chain and unsaturated fatty acids; components making up ≥ 1 % of the total were iso-C_{17:0} 3-OH (31.9 %), iso-C_{15:0} (20.2 %), iso-C_{15:0} 3-OH (6.9 %), iso-C_{15:1} (5.2 %), C_{15:0} (4.5 %), iso-C_{16:0} (4.0 %), unknown fatty acids (3.9 %, sum of ECLs 11.543, 13.565 and 16.582), iso-C_{15:0} 2-OH and/or C_{16:1} ω7c (3.7 %), iso-C_{17:1} ω9c (3.7 %), iso-C_{16:0} 3-OH (2.9 %), C_{17:1} ω6c (1.4 %), iso-C_{16:1} (1.4 %), iso-C_{14:0} (1.3 %), C_{16:0} (1.2 %) and C_{17:0} 3-OH (1.0 %). Phosphatidylethanolamine was the only phospholipid identified in strain SMK-19^T, and other major polar lipids were three unidentified phospholipids and an amino-group-containing lipid that was ninhydrin-positive. The DNA G + C content of strain SMK-19^T was 36.0 mol%.

Strain SMK-19^T could be differentiated from its closest phylogenetic neighbours in the family *Flavobacteriaceae* based on the phenotypic properties listed in Table 1. It is noteworthy that growth of strain SMK-19^T persisted for a short period only (approximately 4–5 days on MA at 37 °C). Acetone/methanol extract of the freeze-dried cells showed absorption maxima at 474 and 505 nm, demonstrating the

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain SMK-19^T and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) > 50 % are shown at the branch points. *Bacteroides fragilis* ATCC 25285^T was used as an outgroup. Dots indicate that the corresponding nodes are also recovered in the tree generated with the maximum-likelihood algorithm. Bar, 0.01 substitutions per nucleotide position.

presence of carotenoids. The nature and concentration of the major fatty acids also helped to differentiate strain SMK-19^T from the members of related genera (Table 1), although these differences may result in part from the use of different culture conditions. On the basis of the phenotypic and phylogenetic data presented, we propose the creation of a novel genus and species in the family *Flavobacteriaceae*, *Gaetbulimicrobium brevivitae* gen. nov., sp. nov., to accommodate strain SMK-19^T.

Description of *Gaetbulimicrobium* gen. nov.

Gaetbulimicrobium (Gaet.bu.li.mi.cro'bi.um. N.L. n. *gaetbulum* -i gaetbul, the Korean name for a tidal flat; N.L. neut. n. *microbium* a microbe; N.L. neut. n. *Gaetbulimicrobium* a microbe isolated from a tidal flat).

Cells are Gram-negative, non-spore-forming, non-flagellated and rod-shaped. Strictly aerobic. The predominant menaquinone is MK-6. Major polar lipids are phosphatidylethanolamine, unidentified phospholipids and an amino-group-containing lipid that is ninhydrin-positive. The type species is *Gaetbulimicrobium brevivitae*.

Description of *Gaetbulimicrobium brevivitae* sp. nov.

Gaetbulimicrobium brevivitae (bre.vi.vi'tae. L. adj. *brevis* short; L. gen. n. *vitae* of life; N.L. gen. n. *brevivitae* of a short life, referring to the short-lived cultures of the type strain).

Exhibits the following properties in addition to those given in the genus description. Cells are rods, 0.2–0.3 × 1.0–15.0 µm, motile by gliding. Colonies on MA are circular to irregular, slightly raised, smooth, orange-coloured and 1.0–2.0 mm in diameter after incubation for 2 days at 37 °C. Carotenoid pigments are produced. Growth occurs at 10 and 41 °C with an optimum temperature of 37 °C; growth does not occur at 4 or 42 °C. Optimal pH for growth is 7.0–8.0; growth is observed at pH 5.5 but not at pH 5.0. Optimal growth occurs in the presence of 2–3 % (w/v) NaCl; growth does not occur in the absence of NaCl and in the presence of > 10 % (w/v) NaCl. Aesculin, hypoxanthine, Tween 60 and tyrosine are hydrolysed, but xanthine is not. Indole is not produced. Acetone/methanol extract shows absorption maxima at 474 and 505 nm. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present and β-glucosidase is weakly present, but lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Maltose and pyruvate are utilized, but D-fructose, D-galactose, D-cellobiose, D-trehalose, D-xylose, L-arabinose, acetate, citrate, succinate, benzoate, L-malate, salicin, formate and L-glutamate are not utilized. Acid is not produced from L-arabinose, D-fructose, D-galactose, lactose, D-mannose,

D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose, *myo*-inositol, D-mannitol or D-sorbitol. Susceptible to ampicillin, cephalothin, chloramphenicol, novobiocin, lincomycin, streptomycin and oleandomycin, but not to gentamicin, kanamycin, polymyxin B or neomycin. The major fatty acids are iso-C_{17:0} 3-OH (31.9 %) and iso-C_{15:0} (20.2 %). The DNA G + C content is 36.0 mol%. Other characteristics are detailed in Table 1.

The type strain, SMK-19^T (= KCTC 12390^T = DSM 17196^T), was isolated from a tidal flat sediment at Saemankum, Pyunsan, Korea.

Acknowledgements

This work was supported by the 21C Frontier programme of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea. We are grateful to Professor Hans Trüper for help with nomenclature of the novel species.

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