Aestuariibaculum suncheonense gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from a tidal flat and emended descriptions of the genera *Gaetbulibacter* and *Tamlana*

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A Gram-staining-negative, yellow-pigmented, strictly aerobic bacterium, designated strain SC17^T, was isolated from sediment of a tidal flat of Suncheon bay in South Korea. Cells were halotolerant, catalase- and oxidase-positive and non-motile rods. Growth of strain SC17^T was observed at 5–40 °C (optimum, 25–30 °C), at pH 6.0–8.5 (optimum, pH 7.0) and in the presence of 1–8 % (w/v) NaCl (optimum, 1–2 %). The major cellular fatty acids were iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G and anteiso-$C_{15:0}$. The polar lipid content consisted of phosphatidylethanolamine and unidentified amino lipids and lipids. The G+C content of the genomic DNA was 46.4 mol% and the only respiratory quinone detected was menaquinone-6 (MK-6). Phylogenetic inference based on 16S rRNA gene sequences showed that strain SC17^T formed a distinct phyletic lineage within the family *Flavobacteriaceae* and was most closely related to members of the genera *Gaetbulibacter* and *Tamlana* with 95.0–95.8 % sequence similarity. On the basis of phenotypic and molecular features, strain SC17^T represents a novel genus of the family *Flavobacteriaceae*, for which the name *Aestuariibaculum suncheonense* gen. nov., sp. nov. is proposed. The type strain is SC17^T (=KACC 16186^T=JCM 17789^T). Emended descriptions of the genera *Gaetbulibacter* and *Tamlana* are also proposed.

The families *Flavobacteriaceae*, *Bacteroidaceae*, *Cryomorphaceae*, *Cytophagaceae*, *Chitinophagaceae* and *Sphingobacteriaceae* consist of very diverse taxa and they are affiliated with the different classes of the phylum *Bacteroidetes*: the class *Flavobacteriia* (families *Flavobacteriaceae* and *Cryomorphaceae*), the class *Bacteroidia* (family *Bacteroidaceae*), the class *Cytophagia* (family *Cytophagaceae*) and the class *Sphingobacteriia* (families *Chitinophagaceae* and *Sphingobacteriaceae*) (Ludwig *et al.*, 2010). The family *Flavobacteriaceae* contains Gram-stain-negative, rod-shaped, chemoheterotrophic bacteria that are non-motile or motile by gliding and contain menaquinone-6 (MK-6) as the major isoprenoid quinone (Bernardet *et al.*, 2002; Bowman & Nichols, 2005; Bernardet & Nakagawa, 2006; Bernardet, 2011). Currently, the family *Flavobacteriaceae* consists of more than 90 genera with validly published names. Sea tidal flats are broad, plain marshes with low-gradients, or muddy coast areas that experience exposure and flooding by seawater between low and high tides. The west and south-west coast of the Korean peninsula largely consists of sea tidal flats, called getbol, that contain a wealth of valuable biological resources such as micro-organisms and marine animals. Recently, numerous microbial species have been isolated from sea tidal flats. Therefore, efforts have been made in our laboratory to isolate and characterize marine bacteria from sea tidal flats (Jin *et al.*, 2011a, b; Jung *et al.*, 2011; Lee *et al.*, 2011; Park *et al.*, 2011). In the course of this study, a novel yellow-pigmented, Gram-staining-negative, rod-shaped bacterium was isolated. Here, we describe its taxonomic characterization and propose a novel genus and species within the family *Flavobacteriaceae*.

Strain SC17^T was isolated from a surface tidal flat (less than 5 cm depth) of Suncheon bay (34° 52′ 56.35″ N

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank accession number for the 16S rRNA gene sequence of strain SC17^T is JF751043.

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

127° 30′ 48.94″ E) in South Korea using a previously described procedure with some modifications (Kim *et al.*, 2010; Jung *et al.*, 2011). Briefly, a tidal flat sediment sample was serially diluted with artificial seawater (ASW; 20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2 . 6H_2O$, 0.64 g KCl, 1.75 g $CaCl_2 . 2H_2O$ per litre), spread on marine agar 2216 (MA; Becton, Dickinson and Company) and incubated at 25 °C for 5 days. Colonies were randomly selected and crude genomic DNA was prepared as described previously (Lu *et al.*, 2006). PCR amplification of 16S rRNA genes was performed using universal primers (Jin *et al.*, 2011b) and the amplicons were double-digested with *Hae*III and *Hha*I. Based on their restriction fragment patterns, PCR products with distinct fragment patterns were selected and sequenced. The resulting 16S rRNA gene sequences were analysed using the BLAST program (http://www.ncbi.nlm.nih.gov/Blast.cgi) in GenBank, and the search results were used as a guide to classify the strains. From the analysis, a novel strain belonging to the family *Flavobacteriaceae*, designated strain SC17^T, was selected for the performance of further phenotypic and phylogenetic analyses. Strain SC17^T was routinely grown aerobically on MA at 25 °C for 3 days, except where indicated otherwise, and stored at −80 °C in marine broth (MB; Becton, Dickinson and Company) supplemented with 10 % (v/v) glycerol. *Gaetbulibacter saemankumensis* KCTC 12379^T, *Gaetbulibacter marinus* KCTC 23046^T, *Tamlana crocina* KCTC 12721^T and *Tamlana agarivorans* KCTC 22176^T (purchased from KCTC, South Korea) were used as reference strains for phenotypic characterization.

The 16S rRNA gene amplicon of strain SC17^T was ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer's instructions for almost full sequencing. The inserted 16S rRNA gene was sequenced with the M13 reverse and T7 primers of the TOPO cloning kit. The resulting almost complete 16S rRNA gene sequence (1482 nt) of strain SC17^T was checked manually for the evaluation of quality and gaps. Sequence similarity values between strain SC17^T and closely related taxa were evaluated using the EzTaxon server (http://147.47.212.35:8080/; Chun *et al.*, 2007) and aligned using the greengenes alignment program (http://greengenes.lbl.gov/). Phylogenetic trees using the neighbour-joining (NJ) and maximum-parsimony (MP) algorithms were constructed using the PHYLIP software (version 3.6, Felsenstein, 2002). The topology of the resulting trees was evaluated using bootstrap analyses based on 1000 resampled datasets within the PHYLIP package. Maximum-likelihood (ML) analysis with bootstrap values was performed using RAxML-HPC BlackBox (version 7 . 2. 6) of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org; Stamatakis *et al.*, 2005) at the San Diego Supercomputer Center. An additional taxonomic assignment was performed using the Ribosomal Database Project (RDP) naïve Bayesian rRNA Classifier tool (http://rdp.cme.msu.edu/classifier; Wang *et al.*, 2007).

Comparative analysis of the 16S rRNA gene sequences showed that strain SC17^T was closely related to *Gaetbulibacter saemankumensis* SMK-12^T, *Gaetbulibacter marinus* IMCC1914^T and *Tamlana crocina* HST1-43^T with similarities of 95.8, 95.5 and 95.0 %, respectively. In the NJ and MP trees (Fig. 1), strain SC17^T formed a clade with members of the genus *Tamlana*. The bootstrap values, however, were very low (25 % in the NJ tree and 32 % in the MP tree), which means that tree topology was not stable. In the ML tree (data not shown), strain SC17^T formed a phyletic lineage distinct from other related genera including *Tamlana*, *Gaetbulibacter*, *Mariniflexile* and *Bizionia* within the family *Flavobacteriaceae*. This was also confirmed by the RDP Classifier program (90 % confidence threshold).

Growth of strain SC17^T was assessed on MA at 0–45 °C (at 5 °C intervals) and in MB adjusted to pH 4.5–10.0 (at 0.5 pH unit intervals) prior to sterilization using the following biological buffers; Na_2HPO_4-NaH_2PO_4 and Na_2CO_3-$NaHCO_3$ were used for pH <8.0 and pH 8.0–10.0, respectively (Gomori, 1955). The pH values were verified after sterilization and adjusted again when necessary. Growth in the presence of 0–10 % NaCl (w/v, at 1 % intervals) was investigated using MB prepared in the

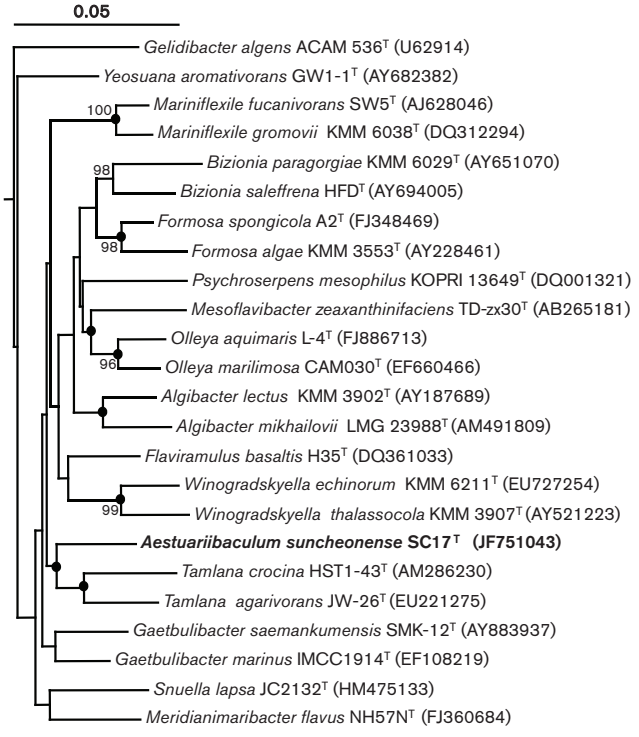

Fig. 1. NJ tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain SC17^T and representative members of the family *Flavobacteriaceae*. Bootstrap values are shown as percentages of 1000 replicates, when >70 %. Filled circles indicate that the corresponding nodes were also recovered in tree generated with the MP algorithm. *Bacteroides fragilis* ATCC 25285^T (GenBank accession no. X83945) was used as an outgroup (not shown). Bar, 0.05 changes per nucleotide position.

laboratory according to the formula of the Difco medium. Gram staining was performed using the bioMérieux Gram Stain kit according to the instructions of the manufacturer. Anaerobic growth was assessed on MA and on MA supplemented with potassium nitrate (0.1 %, w/v) under anaerobic (with 4–10 % CO_2) conditions using the GasPak Plus system (BBL) at 25 °C for 20 days. Cell morphology, motility and the presence of flagella were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) with 2-day-old cells grown on MA as described previously (Jeon *et al.*, 2004). Gliding motility was assessed by phase-contrast microscopy (Axio Lab.A1; Carl Zeiss) as described by Bowman (2000). The presence of flexirubin-type pigments was investigated using two different procedures of the KOH test as described by previously (Bernardet *et al.*, 2002; McCammon & Bowman 2000). In addition, cellular pigments were extracted in a mixture of acetone/methanol (7 : 2, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (SynergyMx; BioTek) (Biebl *et al.*, 2005). Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994). Hydrolysis of casein, Tween 80, Tween 20, tyrosine, starch and xylan was investigated on MA according to the methods described previously (Lányí, 1987; Smibert & Krieg, 1994). Nitrate reduction was assessed according to the method of Lányí (1987). The Voges–Proskauer reaction was determined as described by Cowan & Steel (1965). H_2S production was tested as described previously (Bruns *et al.*, 2001). Acid production from carbohydrates (D-glucose, D-fructose, lactose, D-galactose, D-mannose, sucrose, melibiose and raffinose) was determined as described by Leifson (1963). Additional enzymic activities, biochemical features and utilization of carbon sources by strain SC17^T and the four reference strains were determined using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog) according to the manufacturers' instructions, except that inocula were prepared by suspending cells in ASW and that kits were incubated at 25 °C for 2 days. Antibiotic susceptibility tests were performed using 6 mm filter-paper discs (Whatman) impregnated with the following antibiotics (μg per disc unless stated otherwise): ampicillin (10), polymyxin B (100 U), streptomycin (50), penicillin G (20 U), gentamicin (30), chloramphenicol (100), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), carbenicillin (100), neomycin (30) and novobiocin (5).

Cells of strain SC17^T were Gram-staining-negative, obligately aerobic and straight or slightly curved non-motile rods. Cells contained non-diffusible yellow pigments with a typical carotenoid type absorption spectrum (absorption peaks at 474, 504 and 445 nm) (Asker *et al.*, 2007), but flexirubin pigments were absent (KOH test-negative). Other biochemical characteristics of strain SC17^T are presented in Table 1 and in the genus and species descriptions. Some of them were in accordance with the characteristics of related genera, whereas others allowed the differentiation of strain SC17^T from closely related genera (Table 1 and Table S1 in IJSEM Online).

Isoprenoid quinones of strain SC17^T were analysed using a HPLC (LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 × 4.6 mm; Kromasil, Akzo Nobel) as described previously (Komagata & Suzuki, 1987). Methanol-2-propanol (2 : 1, v/v) was used as an eluent at a flow rate of 1 ml min^{-1}. Analysis of fatty acid methyl esters of strain SC17^T and the four reference strains was performed according to the instructions of the Sherlock Microbial Identification System (MIDI). Cells with similar physiological age were obtained by harvesting colonies approximately 1 mm in diameter from quadrant sectors on MA after about 3 days of incubation at 25 °C, except for *G. marinus* KCTC 23046^T that needed about 6 days to produce colonies of the required diameter. Fatty acids were saponified, methylated and extracted using the standard MIDI protocol, version 4.0. The fatty acids were analysed by GC (6890; Hewlett Packard) and identified by using the TSBA40 database of the Microbial Identification System (Sasser, 1990). The polar lipids of strain SC17^T and two reference strains (*G. saemankumensis* KCTC 12379^T and *T. crocina* KCTC 12721^T) were analysed by TLC as described by Minnikin *et al.* (1977) using cells harvested on the exponential growth phase. The following reagents were used to detect the different polar lipids: 10 % ethanolic phosphomolybdic acid (for the total polar lipids), ninhydrin (for amino lipids), α-naphthol/sulfuric acid (for glycolipids) and the Dittmer–Lester reagent (for phospholipids). The DNA G+C content of strain SC17^T was determined by the fluorometric method (Gonzalez & Saiz-Jimenez, 2002) using SYBR Green I and a real-time PCR thermocycler (Bio-Rad).

The only respiratory lipoquinone detected in strain SC17^T was menaquinone-6 (MK-6), in line with related members of the family *Flavobacteriaceae* (Bernardet, 2011). The polar lipid profile of strain SC17^T consisted of phosphatidylethanolamine, one unidentified aminolipid and two unidentified lipids as the major components; one unidentified aminolipid and two unidentified lipids were also detected as minor components (Fig. 2). The type strains of the type species of the genera *Gaetbulibacter* and *Tamlana* had very similar polar lipid patterns, but contained one more unidentified amino lipid, which was not detected in strain SC17^T (Fig. 2). The major cellular fatty acids (>5 % of the total fatty acids) of strain SC17^T were iso-$C_{15:0}$ (26.0 %), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH; 11.7 %), iso-$C_{17:0}$ 3-OH (10.1 %), iso-$C_{15:1}$ G (9.6 %) and anteiso-$C_{15:0}$ (9.5 %). The overall fatty acid profile of strain SC17^T was similar to those of the reference strains grown under the same conditions; there were only limited differences in the respective proportions of some components (Table 2). The DNA G+C content of strain SC17^T was 46.4 mol%, a

Table 1. Differential characteristics of strain SC17^T and phylogenetic neighbours of the family *Flavobacteriaceae*

Strains: 1, SC17^T; 2, *G. saemankumensis* KCTC 12379^T; 3, *G. marinus* KCTC 23046^T; 4. *T. crocina* KCTC 12721^T; 5. *T. agarivorans* KCTC 22176^T. All data are from this study except for the DNA G+C contents of the reference strains. All strains are Gram-staining-negative, strictly aerobic, rod-shaped, and positive for the following characteristics: hydrolysis of aesculin; catalase, esterase lipase (C8), alkaline phosphatase, acid phosphatase and leucine arylamidase activities; production of acetoin. All strains are negative for the following characteristics: hydrolysis of casein, Tween 80, urea, gelatin and xylan; β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities; productions of indole and H_2S. +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Cell size (μm)	0.4–0.5 × 2.5–3.0	0.4–0.5 × 3.5–4.6	0.5–0.7 × 1.0–2.2	0.4–0.5 × 1.2–2.3	0.5–0.8 × 1.2–2.8
Colony pigmentation	Yellow	Yellow	Yellow	Saffron	Yellow
Gliding motility	−	+	−	−	−
Growth with 7 % NaCl	+	+	−	−	−
Temperature range (optimal) (°C)	5–40 (25–30)	10–40 (25–30)	10–35 (25)	15–40 (25–30)	10–35 (25–30)
Flexirubin-type pigments	−	−	−	−	+
Oxidase	+	+	−	+	+
Nitrate reduction	+	+	−	+	+
Acid from glucose	+	+	−	+	+
Hydrolysis of:					
Starch	+	+	−	−	−
Tyrosine	+	+	−	+	+
Tween 20	+	+	+	+	−
Enzyme activities (API ZYM)					
Esterase (C4)	−	w	w	−	w
Lipase (C14)	−	+	−	−	−
Valine arylamidase	+	+	+	−	−
Cystine arylamidase	w	+	+	w	−
Trypsin	−	−	+	w	−
α-Chymotrypsin	−	+	+	−	+
Naphthol-AS-BI-phosphohydrolase	+	−	+	+	+
α-Galactosidase	+	−	−	−	−
β-Galactosidase	+	−	−	−	+
α-Glucosidase	+	+	+	+	−
N-Acetyl-β-glucosaminidase	w	+	w	−	−
DNA G+C content (mol%)	46.4	34.8*	38.1*	36.2±0.4*	36.8*

*Data from Jung *et al.* (2005); Yang & Cho (2008); Lee (2007) and Yoon *et al.* (2008).

value readily distinguished from those reported for species of the genera *Gaetbulibacter* and *Tamlana* (Table 1). Therefore, the phenotypic and DNA features of strain SC17^T, as well as phylogenetic inference, support its description as a novel genus and species within the family *Flavobacteriaceae*, for which the name *Aestuariibaculum*

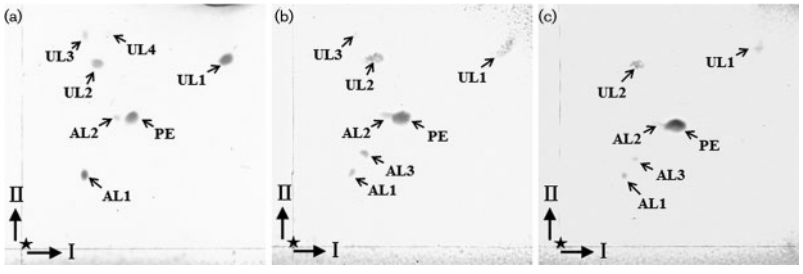

Fig. 2. Thin-layer chromatograms of the total polar lipids of (a) strain SC17^T, (b) *G. saemankumensis* KCTC 12379^T and (c) *T. crocina* KCTC 12721^T following separation by 2D TLC and spraying with 10 % ethanolic phosphomolybdic acid. Solvent system: (I) chloroform-methanol-water (65 : 25 : 4, by vol.); (II) chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.). PE, phosphatidylethanolamine; AL1–3, unidentified aminolipids; UL1–4, unidentified lipids.

Table 2. Cellular fatty acid compositions of strain SC17^T and related type strains of the family *Flavobacteriaceae*

Strains: 1, SC17^T; 2, *G. saemankumensis* KCTC 12379^T; 3, *G. marinus* KCTC 23046^T; 4. *T. crocina* KCTC 12721^T; 5. *T. agarivorans* KCTC 22176^T. All data from this study. Data are expressed as percentages of the total fatty acids. Fatty acids amounting to <0.5 % in all strains are not shown. tr, Trace amount (<0.5 %); −, not detected. Major components (>5.0 %) are highlighted in bold.

Fatty acid	1	2	3	4	5
Saturated					
$C_{14:0}$	−	tr	tr	−	2.0
$C_{15:0}$	1.8	2.3	**5.3**	**5.0**	**7.3**
$C_{16:0}$	4.1	2.8	1.6	2.7	−
Unsaturated					
$C_{15:1}\omega6c$	1.1	0.5	−	1.3	2.1
$C_{17:1}\omega6c$	tr	0.8	0.5	1.3	0.7
Branched					
iso-$C_{13:0}$	tr	1.8	0.6	−	tr
iso-$C_{14:0}$	0.8	0.6	1.8	1.4	1.2
iso-$C_{15:0}$	**26.0**	**29.9**	**21.9**	**27.4**	**21.6**
iso-$C_{15:1}$ G	**9.6**	**6.4**	**23.6**	**11.5**	**13.9**
iso-$C_{16:0}$	1.5	1.2	3.2	1.7	1.7
iso-$C_{16:1}$	0.7	tr	1.6	−	0.5
iso-$C_{17:0}$	−	0.6	tr	tr	−
iso-$C_{17:1}\omega9c$	1.3	2.5	0.7	0.7	0.6
anteiso-$C_{15:0}$	**9.5**	**7.7**	3.6	**6.3**	3.9
anteiso-$C_{15:1}$	1.1	tr	1.3	1.1	1.4
anteiso-$C_{17:1}\omega9c$	1.2	0.8	−	−	−
Hydroxyl					
$C_{12:0}$ 3-OH	−	−	0.9	−	−
$C_{15:0}$ 2-OH	1.2	1.0	0.7	−	0.8
$C_{15:0}$ 3-OH	−	tr	tr	−	0.8
$C_{16:0}$ 3-OH	2.7	1.4	0.7	2.4	**5.8**
$C_{17:0}$ 2-OH	1.6	2.7	0.8	1.6	0.7
iso-$C_{14:0}$ 3-OH	tr	tr	0.5	tr	−
iso-$C_{15:0}$ 3-OH	4.0	**5.4**	**5.0**	**5.0**	3.4
iso-$C_{16:0}$ 3-OH	2.4	1.5	**6.5**	**5.7**	1.3
iso-$C_{17:0}$ 3-OH	**10.1**	**18.8**	**9.6**	**12.1**	**6.9**
ECL					
11.543*	tr	−	0.8	−	0.5
13.565*	2.8	1.5	2.9	−	2.3
16.582*	0.5	0.5	−	0.5	−
Summed features†					
2	tr	3.2	−	−	0.7
3	**11.7**	**5.5**	4.4	**7.6**	**13.0**

*Unknown fatty acids are designated by their equivalent chain-length (ECL).

†Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 2 contains iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH. Summed feature 3 contains $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH.

suncheonense gen. nov., sp. nov. is proposed. On the basis of new data obtained in this study, emended descriptions of the genera *Gaetbulibacter* and *Tamlana* are also proposed.

Emended description of the genus *Gaetbulibacter* Jung *et al.* 2005 emend. Yang and 2008

The description of the genus *Gaetbulibacter* is as given by Jung *et al.* (2005) and emended by Yang & Cho (2008). In addition, the polar lipid profile of the type strain of the type species consists of phosphatidylethanolamine, three unidentified aminolipids and three unidentified lipids.

Emended description of the genus *Tamlana* Lee 2007

The description of the genus *Tamlana* is as given by Lee (2007). In addition, the polar lipid profile of the type strain of the type species consists of phosphatidylethanolamine, three unidentified aminolipids and two unidentified lipids.

Description of *Aestuariibaculum* gen. nov.

Aestuariibaculum (Aes.tu.ar.i.i.ba′cu.lum. L. neut. n. *aestuarium -i* tidal flat; L. neut. n. *baculum* stick; N.L. neut. n. *Aestuariibaculum* a rod-shaped bacterium isolated from a tidal flat).

Cells are Gram-reaction-negative, obligately aerobic rods that are devoid of flagellar or gliding motility. Chemoheterotrophic. Oxidase- and catalase-positive. Cells contain carotenoid pigments, but flexirubin pigments are absent. The only isoprenoid quinone detected is menaquinone-6 (MK-6). The major cellular fatty acids are iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$ and/or iso-$C_{15:0}$ 2-OH) and iso-$C_{17:0}$ 3-OH. Phosphatidylethanolamine is the major polar lipid. The DNA G+C content of the type strain of the type species is 46.4 mol%. Phylogenetically, the genus is a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. The type species is *Aestuariibaculum suncheonense*.

Description of *Aestuariibaculum suncheonense* sp. nov.

Aestuariibaculum suncheonense (sun.che.on.en′se. N.L. neut. adj. *suncheonense* pertaining to Suncheon, South Korea, from where the type strain was isolated).

In addition to the characteristics listed for the genus above, the species is characterized by the following features. Cells are straight or slightly curved rods approximately 0.4–0.5 μm in diameter and 2.0–3.0 μm in length. Colonies on MA are yellow, circular and convex with regular edges. Growth occurs at 5–40 °C (optimum, 25–30 °C), at pH 6.0–8.5 (optimum, pH 7.0) and in the presence of 1.0–8.0 % (w/v) NaCl (optimum, 1.0–2.0 %). Anaerobic growth is not observed after 20 days at 25 °C on MA or MA supplemented with nitrate. Starch, tyrosine and Tween 20 are hydrolysed, while casein, xylan and Tween 80 are not. Acid is produced from D-glucose, D-fructose, lactose, D-galactose, D-mannose and sucrose, but not from

melibiose and raffinose. Voges–Proskauer reaction is positive. H_2S is not produced. Nitrate is reduced to nitrite, but nitrogen gas is not produced. The following tests are negative in the API 20NE kit: indole production, acid production from glucose, arginine dihydrolase and urease activities; and hydrolysis of aesculin and gelatin. In the API ZYM kit, alkaline phosphatase, esterase lipase (C8), valine arylamidase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and α-glucosidase activities are present; weak cystine arylamidase and *N*-acetyl-β-glucosaminidase activities are present; and esterase (C4), lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities are absent. The following substrates in the GN2 MicroPlate are utilized: α–cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, gentiobiose, cellobiose, D-fructose, L-fucose, D-galactose, α-D-glucose, maltose, α-lactose, D-mannose, melibiose, D-psicose, raffinose, sucrose, turanose, acetic acid, D-galacturonic acid, D-glucuronic acid, DL-lactic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, urocanic acid, uridine, α-D-glucose 1-phosphate and D-glucose 6-phosphate. The other substrates in the GN2 MicroPlate are not utilized. Resistant to polymyxin B, streptomycin, penicillin G, ampicillin, gentamicin, kanamycin, neomycin and carbenicillin, but sensitive to chloramphenicol, novobiocin, tetracycline, lincomycin and oleandomycin. In addition to phosphatidylethanolamine, two unidentified aminolipids and four unidentified lipids are also present. In addition to the major fatty acids listed in the genus description, significant amounts of iso-$C_{15:1}$ G and anteiso-$C_{15:0}$ are also present. The complete fatty acid composition is given in Table 2.

The type strain is SC17^T (=KACC 16186^T=JCM 17789^T), isolated from sediment of a tidal flat in the Suncheon bay in South Korea. The DNA G+C content of the type strain is 46.4 mol%.

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