Gaetbulibacter marinus sp. nov., isolated from coastal seawater, and emended description of the genus *Gaetbulibacter*

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A Gram-negative, yellow-coloured, chemoheterotrophic, non-motile, strictly aerobic, rod-shaped bacterium, designated IMCC1914^T, was isolated from coastal surface seawater of the Yellow Sea, Korea. The temperature, pH and NaCl ranges for growth were 3–37 °C, pH 8.0–11.0 and 0.5–4.0 %. The DNA G+C content of the strain was 38.1 mol% and the major cellular fatty acids were iso-$C_{15:1}$ (32.1 %), iso-$C_{15:0}$ (20.6 %) and iso-$C_{17:0}$ 3-OH (7.8 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain IMCC1914^T was related most closely to *Gaetbulibacter saemankumensis* SMK-12^T, with a sequence similarity of 96.2 %. On the basis of phylogenetic data and several distinct phenotypic characteristics, strain IMCC1914^T (=KCCM 42380^T =NBRC 102040^T) could be assigned to the genus *Gaetbulibacter* as the type strain of a novel species, for which the name *Gaetbulibacter marinus* sp. nov. is proposed. In addition, an emended description of the genus *Gaetbulibacter* is presented.

The genus *Gaetbulibacter* (Jung *et al.*, 2005) is a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Bernardet & Nakagawa, 2006; Reichenbach, 1989) and, at the time of writing, comprises one recognized species, *Gaetbulibacter saemankumensis*, isolated from a tidal flat sediment. *G. saemankumensis* is characterized by Gram-negative, yellow-pigmented, non-flagellated, rod-shaped cells that show gliding motility. In the present study, we describe a seawater strain, designated IMCC1914^T, that represents a novel species of the genus *Gaetbulibacter*.

Strain IMCC1914^T was isolated from a surface seawater sample collected off the coast of Deokjeok island, Yellow Sea (37° 04′ N 127° 05′ E), Korea, by using a standard dilution-plating method. The sample was diluted in filtered (0.2-μm mesh) and autoclaved seawater and spread on R2A agar (Difco) diluted in aged seawater (1 : 10, v/v, 1/10R2A). The agar plates were incubated aerobically at 20 °C for 1 month. Strain IMCC1914^T was grown on 1/10R2A and further purified on marine agar 2216 (MA; Difco) and stored as a suspension in 10 % glycerol marine broth 2216 (MB; Difco) at −80 °C. After the optimum growth temperature had been determined, cultures were maintained on MA or in MB at 25 °C. Unless otherwise indicated, phenotypic and biochemical characterizations were performed on MA at 25 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC1914^T is EF108219.

Growth temperature range and optimum were measured at 3–42 °C (at 3 °C, 10–30 °C at 5 °C intervals, 37 and 42 °C) in MB. The pH range and optimum for growth were examined at pH 4.0–12.0 (at 0.5 pH unit intervals). The pH was adjusted with 0.1 M HCl or 0.1 M NaOH. The NaCl concentration range and optimum for growth were determined in NaCl-free artificial seawater medium (ASW; per litre distilled water: 5.9 g $MgCl_2.6H_2O$, 3.24 g $MgSO_4.7H_2O$, 1.8 g $CaCl_2.2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 0.034 g $SrCl_2.6H_2O$, 0.022 g H_3BO_3, 0.008 g $Na_2H_2PO_4$, 0.004 g Na_2SiO_3, 0.0024 g NaF and 0.0016 g NH_4NO_3), supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (0–5.0 % at 0.5 % intervals and 5.0–15.0 % at 2.5 % intervals; w/v). Cell morphology and size were examined by transmission electron microscopy (CM200; Philips) and phase-contrast microscopy (Nikon 80i) by using a 4-day MB culture. Colony morphology, size and colour were examined from cultures grown aerobically on MA for 4 days. The MGC anaerobic system and an AnaeroPACK Anaero (Mitsubishi Gas Chemical company, Inc.) were used for anaerobic growth tests. Flagellar motility was examined by using wet mounts made from fresh cultures grown both on MA and in MB at 25 °C for 3 days. Gliding motility was determined by phase-contrast microscopy of cells incubated for 17 h on microscope slides coated with MA (0.7 % agar), initially grown in MB at 25 °C for 2 days, according to the method described by Bowman (2000). Gliding motility was also examined by using wet mounts, made from cultures grown in MB at 25 °C for 3 days. The

presence of flexirubin-type pigments was determined by the bathochromatic shift test by using 20 % (w/v) KOH solution (Bernardet *et al.*, 2002; McCammon & Bowman, 2000). Cellular pigments were extracted with acetone/methanol (1 : 1, v/v) and their absorption spectra were determined by using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis Co.). The catalase test was performed by addition of 3 % hydrogen peroxide to exponential-phase colonies, and oxidase activity was determined by using oxidase reagent (bioMérieux). Other biochemical tests were carried out on API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions and inoculated with bacterial suspensions in ASW. Carbon source oxidation tests were performed on GN2 microplates (Biolog) by using bacterial suspensions in filtered (0.2-μm mesh) and autoclaved seawater. Degradation of macromolecules was tested by incubating bacterial cultures on MA containing macromolecules at 25 °C for 14 days. The following macromolecules were tested (all w/v): starch (0.2 %), casein (1 % skimmed milk), elastin (0.5 %), chitin (0.5 %) and CM-cellulose (0.2 %). Hydrolysis was revealed by formation of clear zones around the colonies either directly or after flooding with adequate staining solutions (Teather & Wood, 1982). Susceptibility to antimicrobial agents (see the species description below for list and concentrations) was determined by using the disc diffusion method (Jorgensen *et al.*, 1999). The DNA G+C content of strain IMCC1914^T was analysed by using HPLC according to Mesbah *et al.* (1989) with a Discovery C18 column (5 μm, 15 cm × 4.6 mm; Supelco). Cellular fatty acid methyl esters were extracted and prepared from cultures grown on MA at 25 °C for 4 days, and analysed according to the MIDI Microbial Identification System by the Korean Culture Center of Micro-organisms (KCCM).

The nearly full-length sequence of the 16S rRNA gene (1466 bp) of strain IMCC1914^T was obtained as described by Cho & Giovannoni (2003). Comparative analysis of the 16S rRNA gene sequence of this strain with sequences held in GenBank showed that it belonged to the family *Flavobacteriaceae*. The 16S rRNA gene sequence of strain IMCC1914^T was aligned with those of its nearest neighbours in the family *Flavobacteriaceae* by using the ARB software package (Ludwig *et al.*, 2004) and 16S rRNA gene sequence similarity values were calculated based on this alignment in the ARB software. Comparative sequence analyses in the ARB database showed that strain IMCC1914^T was related most closely to *G. saemankumensis* SMK-12^T (96.2 % similarity), followed by *Flaviramulus basaltis* H35^T (96.0 %) and *Algibacter lectus* KMM 3902^T (95.7 %). To clarify the phylogenetic position of strain IMCC1914^T further, 1151 unambiguously aligned nucleotide positions were employed for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were inferred by three tree-generating algorithms, neighbour joining (Saitou & Nei, 1987) with the Jukes–Cantor model (Jukes & Cantor, 1969), maximum parsimony (Fitch, 1971) and maximum likelihood (Felsenstein, 1981). Robustness of the resultant neighbour-joining and maximum-parsimony trees was evaluated by bootstrap analysis based on 1000 resamplings. In all of the phylogenetic trees generated in the present study (Fig. 1), strain IMCC1914^T formed a clade together with *G. saemankumensis*, although levels of bootstrap support for the clade were relatively low (62 % in the neighbour-joining tree and 51 % in the maximum-parsimony tree). This *Gaetbulibacter* clade formed a larger clade with *F. basaltis* in the neighbour-joining and maximum-likelihood trees, but bootstrap values for the clade did not support the relationship. DNA–DNA hybridization studies as used for demarcating bacterial species (Wayne *et al.*, 1987) were not performed because the level of 16S rRNA gene sequence similarity between strain IMCC1914^T and *G. saemankumensis* was lower than 97 % (Stackebrandt & Goebel, 1994). The phylogenetic results coupled with 16S rRNA gene sequence similarity (<97 %) between strain IMCC1914^T and *G. saemankumensis* suggested that the strain should be assigned to the genus *Gaetbulibacter* as representing a novel species.

The phenotypic characteristics of strain IMCC1914^T are summarized in Table 1 and in the species description. In

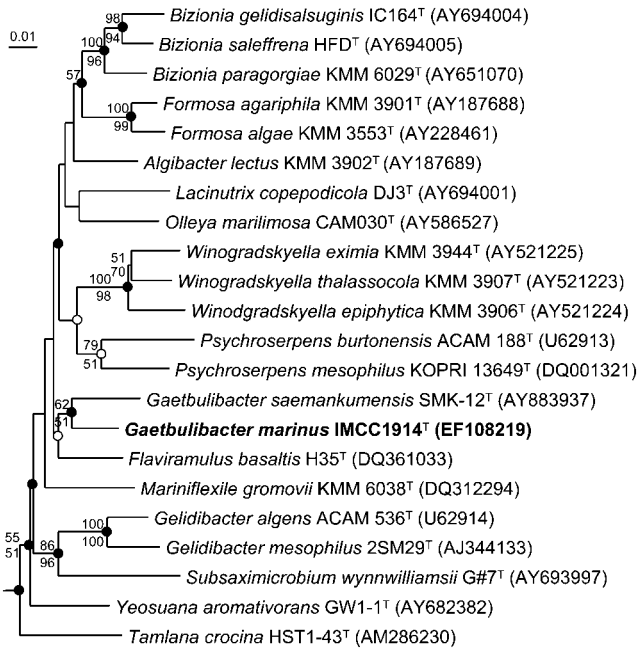

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing relationships between strain IMCC1914^T and representatives of the family *Flavobacteriaceae*. Bootstrap percentages (>50 %) from both neighbour joining (above nodes) and maximum parsimony (below nodes) are shown. Filled and open circles indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. *Bacteroides fragilis* Bfr901 (GenBank accession no. X83945) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics between strain IMCC1914^T and *G. saemankumensis*

Data for *G. saemankumensis* strains are from Jung *et al.* (2005). +, Positive; −, negative; w, weakly positive; v, variable. All strains were positive for hydrolysis of aesculin and for catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and acid phosphatase activities, but negative for production of flexirubin-type pigments, gelatin liquefaction and α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities.

Characteristic	IMCC1914^T	*G. saemankumensis* (*n*=3)
Gliding motility	−	+
Facultatively anaerobic growth	−	+
Temperature range for growth (°C)	3–37	13–40
Growth with 7 % NaCl	−	+
Oxidase activity	−	+
Nitrate reduction	−	+
Enzyme activities (API ZYM)		
Lipase (C14)	−	+
α-Glucosidase	−	+
N-Acetyl-β-glucosaminidase	−	+
Trypsin	+	−
Naphthol-AS-BI-phosphohydrolase	+	−
α-Chymotrypsin	+	v*
β-Glucosidase	w	v*
Hydrolysis of:		
Casein	+	−
Starch	−	+
DNA G+C content (mol%)	38.1	34.7–34.9

*The type strain is negative, whereas the other two strains described by Jung *et al.* (2005) are positive.

summary, cells of strain IMCC1914^T were Gram-negative, chemoheterotrophic, non-motile, obligately aerobic and lacked flexirubin-type pigments. The DNA G+C content (Table 1) and predominant cellular fatty acids (Table 2) of the strain were generally in good agreement with those of *G. saemankumensis*. However, several phenotypic properties, including gliding motility, oxidase activity, anaerobic growth and hydrolysis of macromolecules, differentiated strain IMCC1914^T from *G. saemankumensis*. 16S rRNA gene sequence similarity and phylogenetic analyses, together with phenotypic characteristics, indicate that strain IMCC1914^T can be assigned to a novel species of the genus *Gaetbulibacter*, for which the name *Gaetbulibacter marinus* sp. nov. is proposed. Based on differential phenotypic characteristics of strain IMCC1914^T not reported in the description of the genus *Gaetbulibacter* by Jung *et al.* (2005), an emended description of the genus is presented.

Table 2. Cellular fatty acid composition of strain IMCC1914^T and *G. saemankumensis*

Data for *G. saemankumensis* strains are from Jung *et al.* (2005) and are given as values for the type strain, with values for the two other strains in parentheses. −, Not detected. Data are percentages of the total fatty acids; components that represent >1.0 % of the total cellular fatty acids of at least one of the strains are shown. All strains were grown on MA. Strain IMCC1914^T was grown at 25 °C for 4 days, whereas strains of *G. saemankumensis* were grown at 30 °C for 3 days.

Fatty acid	IMCC1914^T	*G. saemankumensis* (*n*=3)
$C_{15:0}$	3.9	1.5 (1.3, 1.6)
$C_{16:0}$	1.2	1.3 (0.9, 1.3)
iso-$C_{13:0}$	0.6	2.0 (1.9, 2.4)
iso-$C_{14:0}$	1.0	−
iso-$C_{15:0}$	20.6	23.0 (22.7, 24.3)
iso-$C_{15:1}$	32.1	12.5 (13.0, 13.7)
iso-$C_{16:0}$	1.2	−
anteiso-$C_{15:0}$	4.0	10.8 (8.9, 9.8)
anteiso-$C_{15:1}$	3.4	1.5 (1.4, 1.6)
iso-$C_{17:1}\omega 9c$	0.3	2.1 (2.2, 2.4)
anteiso-$C_{17:1}\omega 9c$	−	1.3 (1.0, 1.2)
$C_{17:1}\omega 6c$	0.1	1.0 (0.9, 1.1)
iso-$C_{15:0}$ 3-OH	4.8	8.2 (7.6, 8.6)
iso-$C_{16:0}$ 3-OH	5.5	2.0 (2.4, 2.5)
iso-$C_{17:0}$ 3-OH	7.8	15.2 (12.9, 16.0)
$C_{15:0}$ 2-OH	0.6	1.6 (1.4, 1.6)
$C_{17:0}$ 2-OH	1.0	2.4 (2.1, 2.4)
$C_{16:0}$ 3-OH	1.2	−
Summed feature 3*	4.5	10.4 (8.2, 10.4)

*Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

Emended description of *Gaetbulibacter* Jung *et al.* 2005

The description of the genus *Gaetbulibacter* is as given by Jung *et al.* (2005) with the following amendments. Cells are obligately aerobic or facultatively anaerobic. Oxidase activity and gliding motility are species-dependent. The DNA G+C content is 34.7–38.1 mol%.

Description of *Gaetbulibacter marinus* sp. nov.

Gaetbulibacter marinus (ma.ri′nus. L. masc. adj. *marinus* of the sea, marine).

Cells are Gram-negative, chemoheterotrophic and obligately aerobic and show no flagellar or gliding motility. Cells in exponential phase are rods, 0.8–1.9 μm long and 0.6–0.7 μm wide. Flexirubin-type pigments are absent (KOH test-negative). The yellow pigments (absorption peaks at 478 and 451 nm) are probably of carotenoid type. After 5 days incubation, colonies on MA are circular, convex, entire, smooth, yellow-coloured and 1.0–3.0 mm in diameter. Growth occurs at 3–37 °C (optimum, 25 °C), at pH 8–11 (optimum, pH 9) and with 0.5–4.0 % NaCl

(optimum, 1.0–1.5 %). Catalase-positive. Negative for nitrate reduction, indole production, acid production from glucose, hydrolysis of urea, oxidase, arginine dihydrolase and β-galactosidase activities and gelatin liquefaction (API 20NE strips). Casein is hydrolysed, but starch, elastin, CM-cellulose and chitin are not. Other enzyme activities determined by using API ZYM strips are given in Table 1. The following carbon substrates are oxidized (Biolog GN2 microplates): α-cyclodextrin, dextrin, D-cellobiose, D-fructose, gentiobiose, α-D-glucose, α-D-lactose, maltose, D-mannose, methyl β-D-glucoside, sucrose, trehalose, turanose, succinic acid monomethyl ester, acetic acid, propionic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-ornithine, L-proline, L-serine, L-threonine, α-D-glucose 1-phosphate and D-glucose 6-phosphate. The following carbon substrates are not oxidized: glycogen, Tweens 40 and 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, L-fucose, D-galactose, *myo*-inositol, lactulose, D-mannitol, melibiose, D-psicose, raffinose, L-rhamnose, D-sorbitol, xylitol, pyruvic acid methyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-histidine, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. Susceptible to chloramphenicol (25 μg), erythromycin (15 μg), penicillin G (10 μg), tetracycline (30 μg) and rifampicin (50 μg), but resistant to vancomycin (30 μg), ampicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg) and streptomycin (10 μg). The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ and iso-$C_{17:0}$ 3-OH (Table 2). The DNA G+C content is 38.1 mol%.

The type strain, IMCC1914^T (=KCCM 42380^T =NBRC 102040^T), was isolated from seawater off Deokjeok-do, Yellow Sea, Republic of Korea.

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