***Arenibacter* gen. nov., new genus of the family Flavobacteriaceae and description of a new species, *Arenibacter latericius* sp. nov.**

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Five dark-orange-pigmented, Gram-negative, rod-shaped, non-motile, aerobic bacterial strains were isolated from sandy sediment samples collected in the South China Sea in the Indian Ocean, from a holothurian, *Apostichopus japonicus*, in the Sea of Japan and from a brown alga, *Chorda filum*, from the Sea of Okhotsk in the Pacific Ocean. Phenotypic data were collected, demonstrating that the bacteria are chemo-organotrophic and require seawater-based media for growth. Polar lipids were analysed and 27% of the total extract comprised phosphatidylethanolamine as the major component. The predominant cellular fatty acids were branched-chain saturated and unsaturated [i-C15:0, i-C15:1, a-C15:0, C15:0, C16:1(n-7)]. The DNA base composition was 37.5–38.2 mol% G+C. The level of DNA homology of the five isolates was 83–94%, indicating that these isolates belong to the same species. A 16S rDNA sequence of the type strain KMM 426^T was determined and phylogenetic analysis, based on neighbour-joining and Fitch–Margoliash methods, revealed that the type strain formed a distinct phyletic line in a clade corresponding to the family Flavobacteriaceae and represented a new genus. From the results of this polyphasic taxonomic analysis, it is proposed that the bacterial strains be classified in a new genus, *Arenibacter* gen. nov., and species, *Arenibacter latericius* sp. nov. The type strain is KMM 426^T (VKM B 2137D^T = LMG 19694^T = CIP 106861^T).

Keywords: *Flavobacteriaceae*, *Arenibacter latericius*

INTRODUCTION

A large number of representatives of the *Cytophaga–Flavobacterium–Bacteroides* group, rRNA superfamily V (Segers *et al.*, 1993) or the ‘*Flavobacterium–Bacteroides*’ phylum (Gherna & Woese, 1992) were described during the premolecular era in bacterial systematics. Based mainly on limited species definition and phenotypic similarities, the differentiation of species within this group was difficult. Recent genetic and phylogenetic advances,

along with development and application of polyphasic taxonomy to flavobacteria systematics has changed the situation dramatically (Holmes, 1993; Vandamme *et al.*, 1994; Bernardet *et al.*, 1996; Nakagawa *et al.*, 1997). Reclassification of misnamed species, emendation of the description for the family *Flavobacteriaceae* and genera within the family, and descriptions of the new species have led to the creation of novel taxa composed of bacteria from diverse environments (McGuire *et al.*, 1987; Naganuma *et al.*, 1993; Mudarris *et al.*, 1994; Bernardet *et al.*, 1996; Nakagawa & Yamasato, 1996; Nakagawa *et al.*, 1997; Bowman *et al.*, 1997b, 1998; Gosink *et al.*, 1998; Johansen *et al.*, 1999).

We report here a polyphasic study (phenotypic, chemo-

The GenBank accession number for the 16S rDNA sequence of *Arenibacter latericius* KMM 426^T is AF052740.

taxonomic, genetic and phylogenetic) of a new bacterial species isolated from different geographic areas: sandy sediments of the South China Sea, a holothurian from the Sea of Japan and a brown alga from the Sea of Okhotsk. On the basis of the results presented in this paper, we propose that the bacterial species be classified in a new genus, *Arenibacter* gen. nov., in the family *Flavobacteriaceae*, and describe the new species as *Arenibacter latericius* sp. nov.

METHODS

Bacterial cultures, sampling and isolation procedure. Sediment samples were collected from a depth of 20 m (salinity, 32‰; temperature, 18 °C) in the South China Sea, near the island of Ku-Lao-Re (lat 15°21'08"N, long 109°07'02"E) during December 1988. The bacterial strain KMM 426^T was isolated from sandy sediment samples by plating 0.1 ml of 1 g sediment suspended in 10 ml natural sea water on medium B agar plates containing 0.2% (w/v) Bacto Peptone (Difco), 0.2% (w/v) casein hydrolysate (Merck), 0.2% (w/v) Bacto Yeast Extract (Difco), 0.1% (w/v) glucose, 0.002% (w/v) KH₂PO₄, 0.005% (w/v) MgSO₄·7H₂O and 1.5% (w/v) Bacto Agar (Difco) in 50% (v/v) natural seawater and 50% (v/v) distilled water at pH 7.5–7.8, as described elsewhere (Ivanova *et al.*, 1996). The holothurians, *Apostichopus japonicus*, were collected in November 1997 at a depth of 8 m (salinity, 33‰, temperature, 12 °C) at the Pacific Institute Bioorganic Chemistry Marine Experimental Station, Troitsa Bay, Gulf of Peter the Great, Sea of Japan. The brown alga, *Chorda filum*, was collected in August 1997 at a depth of 10 m (salinity, 31‰, temperature, 12 °C) near the island of Iturup, one of the Kuril Island chain, in the Sea of Okhotsk, Pacific Ocean. Isolates from invertebrates and algae were prepared aseptically as follows. The strains were isolated from tissue homogenates by plating (0.1 ml of homogenate) on marine agar 2216 (Difco) agar plates and on medium B plates. After initial isolation, the strains were purified on medium B, maintained on the same semisolid medium B in tubes under mineral oil at 4 °C and stored at –80 °C in marine broth (Difco) supplemented with 30% (v/v) glycerol.

All isolates were streaked on agar plates from broth culture every six months to ensure purity and viability.

Phenotypic analysis. Unless otherwise indicated, the phenotypic properties used for the characterization of *Flavobacterium*-related species were tested following established procedures (McMeekin *et al.*, 1971; Smibert & Krieg, 1994; Ivanova *et al.*, 1996). To test for spreading growth and gliding motility, strains were grown on medium B with the peptone content reduced to 0.2 g l⁻¹.

Gliding motility was verified by phase-contrast microscopy of hanging drop preparations. The bathochromic shift test with 20% (w/v) KOH was performed to detect flexirubin pigmentation (Fautz & Reichenbach, 1980).

Electron micrographs of negatively stained cells were prepared with a Zeiss EM 10 CA electron microscope (80 kV). A drop of particle-free (autoclaved and ultracentrifuged) distilled water was placed on the bacterial growth of a 24 h culture. A sample (30 µl) of the resulting bacterial suspension was applied to carbon- and Formvar-coated 400-mesh copper grids, a drop of 1.25% uranyl acetate was added and the bacteria were allowed to adhere for 1 min at room

temperature. Superfluous liquid was gently removed by using a piece of filter paper.

Substrate utilization tests. Starch, casein and gelatin hydrolysis was tested by the methods of Smibert & Krieg (1994). Degradation of macromolecules by the strains was tested using medium B. Chitin (1%, w/v), elastin (0.1%, w/v) and alginate (sodium salt; 0.1%, w/v) hydrolysis was determined by development of clear zones around the colonies. Cellulose hydrolysis was tested both by the cellulose overlay plate method (1% carboxymethylcellulose) and by examining strips of filter paper in liquid culture for dissolution (Smibert & Krieg, 1994). The presence of α -galactosidase and α -N-acetylgalactosaminidase was determined as described elsewhere (Bakunina *et al.*, 1996). Briefly, glycosidase activities were tested with the *p*-nitrophenyl derivatives of relevant monosaccharides (Sigma): *p*-nitrophenyl- α -D-galactopyranoside and *p*-nitrophenyl- α -Nac-D-galactosaminide. Glycosidase activities of bacterial (cell-free) extracts were measured in 0.1 M phosphate buffer, pH 7.0 at 20 °C. The reaction mixture consisted of 0.05 ml bacterial extract and 0.05 ml of the corresponding *p*-nitrophenyl glycoside (1 mg ml⁻¹ in 50 mM Tris/phosphate buffer, pH 7.5). The reaction was stopped by adding 0.1 ml 1 M Na₂CO₃. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 nmol *p*-nitrophenol h⁻¹. Oxidative utilization of 95 carbon sources was tested using BIOLOG GN Microplates (Ruger & Krambeck, 1994; Ivanova *et al.*, 1998).

Growth at different temperatures, NaCl concentrations or pH was measured using OD₆₆₀ after incubation for 24 h in medium B. Incubation temperatures employed ranged from 4 to 42 °C. NaCl concentrations used were 0 to 15%. The pH was adjusted, using HCl and NaOH, in the range 4.5 to 12.0. Cultures were incubated on a rotary shaker at 160 r.p.m. for 24–36 h at 28 °C.

Antibiotic susceptibility. Susceptibility to antibiotics was tested by the diffusion plate method, employing medium B agar and disks impregnated with the following antibiotics (µg): kanamycin, 10; ampicillin, 10; benzylpenicillin, 10; streptomycin, 10; erythromycin, 15; gentamicin, 10; oxacillin, 20; lincomycin, 15; ristomycin, 25; rifampicin, 25; neomycin, 25; cephaloridin, 10; and polymyxin B, 25.

Characterization of pigment. The strains were grown on medium B for 2 d at 28 °C. Pigments were extracted using methanol. Absorption spectra were determined between 250 and 700 nm (spectrophotometer, Specord M40).

Polar lipids. Polar lipids were extracted from wet cells, using the Bligh & Dyer (1953) method. Column chromatography was carried out using silica gel L 100/160 (Lachema) and DEAE cellulose (Reanal). Polar lipids were subjected to two-dimensional TLC on silica gel (KSK) thin layer plates (10 × 10 cm).

Following treatment with solvents [chloroform/methanol/ammonium hydrate/benzene (65:30:6:10, by vol.) (first dimension) and chloroform/methanol/acetone/acetic acid/benzol/water (70:30:5:4:1:10, by vol.) (second dimension)], compounds were detected on chromatograms by spraying with non-specific reagents (50% sulfuric acid in methanol by heating at 180 °C) and with specific reagents (ninhydrin, Dragendorff and periodate–Schiff). Quantification of phospholipids on two-dimensional chromatograms was performed by the method of Vaskovsky *et al.* (1975).

Fatty acids methyl ester (FAME) analysis. The analysis of FAMES was performed by GLC (30 m × 0.25 mm Supelcowax 10 column, 210 °C) as described by Svetashev *et al.* (1995).

DNA base composition. DNA was isolated following the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method of Marmur & Doty (1962). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley *et al.* (1970).

Phylogenetic analysis. PCR, cloning and sequencing of 16S rDNA were carried out using the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer, as described previously by Chun & Goodfellow (1995). The resultant 16S rDNA sequence was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were constructed using the Fitch–Margoliash (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1972) methods. Evolutionary distance matrices were calculated according to Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant unrooted tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining tree based on 1000 resamplings.

RESULTS

Phenotypic characteristics of the isolates

The bacteria isolated in this study were Gram-negative, strictly aerobic, non-motile and non-gliding rods with parallel or slightly irregular sides, 0.4–0.6 µm in diameter and 3–5 µm long (Fig. 1). On marine agar and B medium, colonies were uniformly round, 2–3 mm in diameter, convex, translucent and yellow to reddish-orange. The bacteria did not form endospores, nor did they accumulate poly-β-hydroxybutyrate as an intracellular reserve product, and they did not possess an arginine dihydrolase system. They were oxidase-

catalase- and alkaline phosphatase-positive, and required Na⁺ for growth. Growth occurred in media containing 1–7% NaCl. The temperature range for growth was 10–37 °C, with optimum growth occurring at 28 °C. No growth was detected at 4 or 42 °C. The pH range for growth was 6.0–10.0, with optimum growth occurring between pH 7.5 and 8.5.

Agar, chitin, starch, alginate, gelatin, cellulose, Tween-20, -40, -60, and -80, and DNA were not hydrolysed. α-N-Acetylgalactosaminidase was produced. α-Galactosidase was produced by only one strain, KMM 426^T; elastin was hydrolysed by two strains (Table 1). α-D-Glucose, L-glutamic acid, L-ornithine, uridine, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate were utilized according to BIOLOG results. Dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, cellobiose, D-fructose, α-lactose, α-D-lactose lactulose, glycogen, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-raffinose, sucrose, D-trehalose, DL-lactic acid, turanose, succinamic acid, glucuronamide, alaninamide, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, D-serine, L-serine and L-threonine were weakly utilized. None of the bacterial species in the GN Microplate database (BIOLOG) matched the utilization pattern of KMM 426^T.

All strains were susceptible to erythromycin, ampicillin, lincomycin, ristomycin, rifampicin, cephaloridin, oleandomycin and polymyxin B. (Table 1).

The bacteria produced carotenoid pigments with wavelength absorbance spectral peaks at 338, 408, 434, 459 and 488 nm. The bathochromic shift test with 20% KOH revealed no flexirubin pigments. The polar lipids A in the lipid extract of strain KMM 426^T accounted for 27% of the polar lipids, but about 50% of those were phospholipids. The overall polar lipid pattern included phosphatidylethanolamine as the major compound and two unidentified polar lipids reacting with ninhydrin, but negative for phosphorus-containing compounds.

Fatty acid composition

The most relevant cellular fatty acids were branched-chain saturated and unsaturated, namely i15:0, i15:1, a15:0, 15:0 and 16:1(n-7) (Table 2). In addition, about 11.34% of fatty acids could not be identified.

DNA base composition and DNA relatedness

The G+C content of the DNA ranged from 37.5 to 38.2 mol% (determined by the thermal denaturation method). The levels of DNA relatedness of the strains isolated from sandy sediment, a holothurian and an alga are presented in Table 3. Since the level of DNA homology of five of the strains was 83–94%, all five

Fig. 1. Electron micrograph of a negatively stained cell of *Arenibacter latericius* KMM 426^T. Bar, 1 µm.

Table 1. Major phenotypic characteristics of *Arenibacter latericius*

Characteristic	<i>Arenibacter latericius</i>	
	KMM 426 ^T	<i>n</i> = 4*
Flagellation	—	0
Gliding motility	—	0
Oxidase, catalase activity	+	100
Na ⁺ required for growth	+	100
Requirement for organic growth factors	—	0
Arginine dihydrolase activity	—	0
Denitrification	+	100
Indole production	—	0
Urease	+	100
Phenylalanine deaminase	—	0
Haemolytic activity	+	100
Degradation of:		
Agar, alginic acids, starch, gelatin, cellulose, chitin, Tween-20, -40, -60, -80, DNA	—	0
Elastin	—	50
Production of:		
α -Galactosidase	+	0
α -N-Acetylgalactosaminidase, alkaline phosphatase	+	100
Growth at:		
10–37 °C	+	100
1–7% NaCl	+	100
Utilization of:		
Melibiose, citrate, trehalose	—	0
Lactose	+	100
Glycerol	+	75
L-Arginine, L-tyrosine	+	100
Susceptibility to:		
Kanamycin (30 µg)	—	0
Ampicillin (10 µg)	+	100
Benzylpenicillin (10 µg)	—	0
Streptomycin (10 µg)	—	0
Erythromycin (15 µg)	+	100
Gentamicin (10 µg)	—	0
Oxacillin (20 µg)	—	0
Lincomycin (15 µg)	+	100
Ristomycin (25 µg)	+	100
Rifampicin (25 µg)	+	100
Neomycin (25 µg)	—	0
Cephaloridin (10 µg)	+	100
Polymyxin B (25 µg)	—	0

* Values are the percentages of the four strains tested that are positive.

strains were assigned to a single species genotypically (Wayne *et al.*, 1987).

Phylogeny

An almost complete 16S rDNA sequence (1444 nt) was obtained for strain KMM 426^T. Phylogenetic analyses revealed that the organism is an authentic member of the *Cytophaga*–*Flavobacterium*–*Bacteroides* phylum (Fig. 2). *Zobellia* (formerly *Cytophaga*) *uliginosa* formed a monophyletic clade with strain KMM 426^T

at a low nucleotide sequence similarity (91.4%). The relationship was confirmed in all three tree-making analyses and by the high bootstrap value (97%), based on the neighbour-joining algorithm. The next closest neighbour was *Cellulophaga lytica* (Johansen *et al.*, 1999), sharing 90.2% 16S rDNA sequence similarity. Phylogenetic affiliation of *Cellulophaga lytica* to a monophyletic clade encompassing strain KMM 426^T and *Cytophaga uliginosa* was unclear, as the relationship was not supported by a significant bootstrap value (51%) and was not observed in the maximum-parsimony analysis (results not shown).

Table 2. Cellular fatty acid composition of *Arenibacter latericius* KMM 426^T

Fatty acid	<i>Arenibacter latericius</i> KMM 426 ^T
Saturated acids	
13:0	0.07
14:0	1.03
15:0	13.26
16:0	3.30
17:0	0.18
18:0	0.10
Total	17.96
Branched chain acids	
i13:0	0.30
a13:0	0.13
i14:0	0.17
a14:0	0.17
i15:0	17.26
a15:0	6.60
i16:0	0.92
a16:0	0.13
i17:0	0.75
a17:0	0.28
Total	26.71
Unsaturated acids	
14:1	0.15
15:1(n-8)	0.76
15:1(n-6)	2.33
16:1(n-7)	10.96
16:1(n-5)	0.34
17:1(n-8)	0.57
17:1(n-6)	1.23
Total	16.34
Branched chain unsaturated acids	
i15:1	19.27
a15:1	1.73
i16:1	0.32
a16:1	–
i17:1	5.79
a17:1	0.56
Total	27.67
Sum	88.66

DISCUSSION

The flavobacteria-like organisms isolated from sandy sediment samples collected in the South China Sea, near the island of Ku-Lao-Re, from a holothurian, *Apostichopus japonicus*, and from a brown alga, *Chorda filum*, were subjected to a polyphasic taxonomy analysis. The bacteria were aerobic, non-motile, Gram-negative, non-spore-forming rods without

resting stages. The G + C content of the DNA ranged from 37.5 to 38.2 mol %. All strains studied produced oxidase, catalase and phosphatase, were not proteolytic and did not hydrolyse starch, agar, gelatin or cellulose. The strains synthesized carotenoid pigments, but not flexirubin, as the basis for the colour of the colonies. The presence of branched-chain saturated and unsaturated fatty acids, namely i15:0, i15:1, a15:0, 15:0 and 16:1(n-7), and the absence of hydroxy fatty acids were characteristic for the bacteria. In spite of the intraspecific heterogeneity of the fatty acid content of the micro-organisms belonging to rRNA superfamily V, the high levels of i15:0 and i17:0 3OH are diagnostic features (Holmes *et al.*, 1984; Holmes 1993; Bernardet *et al.*, 1996). In contrast to other *Flavobacterium*-related bacteria, the bacteria included in this study were distinct by the lack of hydroxy fatty acids.

Taken together, all of the features lead to the conclusion that these strains bear sufficient resemblance to species and genera included in the emended family *Flavobacteriaceae* (Bernardet *et al.*, 1996). Though there are problems concerning the definition of the families *Flavobacteriaceae* and *Cytophagaceae*, the strains described in this study can easily be differentiated from representatives of the family *Cytophagaceae* Stanier 1940, which includes the genera *Cytophaga* [emended genus restricted to *Cytophaga hutchinsonii* and *Cytophaga aurantiaca* (Nakagawa & Yamasato, 1996), *Flexithrix* and *Sporocytophaga*] by lack of gliding motility and lack of ability to decompose agar and cellulose. These features, together with the lack of ability to hydrolyse starch, make it relatively easy to differentiate new strains from *Zobellia* (*Cytophaga*) *uliginosa* and *Cellulophaga lytica*, the closest phylogenetic neighbours (Table 4). The genera *Capnocytophaga*, *Ornithobacterium* and *Riemerella*, members of the *Flavobacteriaceae*, differ from the bacteria studied by their capnophilic metabolism. Bacteria of the genera *Bergeyella*, *Ornithobacterium*, *Riemerella* and *Weeksella* (Botha *et al.*, 1989) represent solitary rRNA branches that are non-pigmented, while *Arenibacter latericius* is pigmented. The genera *Empedobacter* and *Chryseobacterium* also belong to separate rRNA branches, distinct by several phenotypic properties and a low DNA G + C content (Table 4).

Placement of the isolates studied in the genus *Flavobacterium*, specifically KMM 426^T, is not appropriate because of the lack of ability to decompose gelatin, casein and Tween compounds. One of the two new *Flavobacterium*-like taxa, described recently for marine bacteria (Bowman *et al.*, 1997a) and related to the *Flexibacter maritimus* rRNA branch, namely *Psychroserpens burtonensis*, has a characteristic cell morphology, non-saccharolytic metabolism and low DNA G + C content. Strains of the second taxon, *Gelidibacter algens*, exhibited gliding motility and significant levels of characteristic a15:1 ω 10c and a17:1 ω 7c lipids. To establish the precise phylogenetic affiliation

Table 3. DNA relatedness among tested strains of *Arenibacter latericius*

Strain	G + C content (mol %)	Percentage hybridization with DNA from <i>A. latericius</i> KMM 426 ^T
KMM 426 ^T	37.5	100
KMM 3522	38.0	83
KMM 3557	37.9	94
KMM 3523	38.2	84
KMM 3527	37.9	94
KMM 3528	37.7	83

Fig. 2. Neighbour-joining tree, based on 1287 unambiguously aligned 16S rRNA nucleotide positions. Numbers at the nodes exhibit the levels of bootstrap support, based on neighbour-joining analyses of 1000 resampled datasets. The scale bar represents 0.1 nt substitutions per position.

of strain KMM 426^T, almost the entire 16S rDNA gene sequence was determined. A phylogenetic analysis was done, including all of the taxa from rRNA superfamily V. As defined by Gherna & Woese (1992), the phylum *Cytophaga-Flavobacterium-Bacteroides* is composed of five major phylogenetic subgroups: *Cytophaga*, *Flavobacterium*, *Sphingobacterium*, *Saprospira* and *Bacteroides*. Our analysis showed that strain KMM 426^T formed a related but distinct lineage within the *Cytophaga-Flavobacterium-Bacteroides* phylum and its 16S rDNA sequence was most similar to *Zobellia (Cytophaga) uliginosa*. The level of 16S rDNA sequence similarity with other members of the phylum was 91.4% and clearly indicates that the bacteria isolated in this study represent a novel genus. Bootstrap resampling showed this lineage to be statistically reliable.

Taking into consideration all the data presented, the environmental isolates could not be placed in any of the species or genera currently included in the family *Flavobacteriaceae*. Consequently, we propose that strain KMM 426^T be placed in a new genus, *Arenibacter* gen. nov., as *Arenibacter latericius* sp. nov.

Description of *Arenibacter* gen. nov.

Arenibacter (A.re.ni.bac'ter. L. fem. n. *arena* sand; N.L. masc. n. *bacter* rod; N.L. masc. n. *Arenibacter* sand-dwelling rod).

Rod-shaped cells with pearled or slightly irregular sides and rounded ends, about 3–5 µm long and 0.4–0.6 µm diameter. Non-motile. Does not exhibit gliding motility. Gram-negative. Does not form endospores or resting stages. Does not accumulate

Table 4. Differential characteristics of *Arenibacter latericius* of the family *Flavobacteriaceae* and other allied marine genera

ND, Not determined; A, strict aerobes; F, facultative anaerobes; M, microaerophiles; —, negative; +, positive; v, variable; ±, previously reported results differ; Cad, cadaverine; Spd, spermidine; Hspd, homospermidine. Data from Bernardet *et al.* (1996), Nakagawa *et al.* (1997), Bowman *et al.* (1997), Mudarris *et al.* (1994), Botha *et al.* (1989), McGuire *et al.* (1987) and this study.

Taxon/species	Habitat	Reaction to oxygen	Pigmentation/ flexirubin	Gliding motility	Seawater requirement	Amino acid requirement	Carbohydrate utilization	Starch	Sphingolipid	Polyamine	Quinone	GC content (mol %)
<i>Arenibacter latericius</i>	Marine	A	+ / —	—	+	—	+	—	—	ND	ND	38–39
<i>Bergeyella</i>	Parasitic saprophytic	A	— / —	—	—	—	—	v	—	ND	MK-6	35–37
<i>Capnocytophaga</i>	Parasitic saprophytic	F	+ / —	+	—	+	+	ND	MK-6	33–41	—	—
<i>Chryseobacterium</i>	Free-living, parasitic	A	+ / +	v	—	—	+	—	—	Hspd	MK-6	33–38
<i>Cellulophaga lytica</i>	Free-living	A	+ / —	+	v	—	+	+	ND	ND	ND	31–0
<i>Cytophaga hutchinsonii</i>	Free-living, soil	A	+ / —	+	v	—	v	+	ND	Hspd	MK-7	39–42
[<i>Cytophaga</i>] <i>marinoflava</i>	Marine	A	+ / —	+	—	—	+	+	ND	ND	ND	33–0
<i>Zobellia</i> (<i>Cytophaga</i>) <i>uliginosa</i>	Free-living	A	+ / ±	+	+	—	+	+	ND	ND	ND	32–0
<i>Empedobacter</i>	Free-living, parasitic	A	+ / —	—	—	—	—	—	—	Hspd	MK-6	31–33
<i>Flammeovirga</i>	Marine	A	+ / —	+	+	—	+	+	ND	Cad	MK-6	35–37
<i>Flavobacterium</i>	Free-living, saprophytic	A	+ / v	v	v	—	+	+	—	Hspd	MK-6	32–37
<i>Psychroflexus gondwanensis</i>	Hypersaline lake	A	+ / —	ND	—	ND	+	+	ND	ND	ND	ND
<i>Salegentibacter salegens</i>	Hypersaline lake	A	+ / —	ND	—	ND	+	+	ND	ND	ND	ND
<i>Flectobacillus</i>	Marine	A	+ / —	—	+	—	—	v	ND	ND	ND	34–40
<i>Flexibacter flexilis</i>	Fresh water	A	+ / —	+	—	—	—	ND	Hspd	MK-7	29–48	—
<i>Flexibacter maritimus</i>	Parasitic, saprophytic	A	+ / —	+	+	+	—	—	ND	ND	ND	31–0
<i>Gelidibacter</i>	Marine	A	+ / —	+	+	+	—	+	ND	ND	ND	36–38
<i>Marinilabilia</i>	Marine	F	+ / —	—	+	ND	Spd	MK-7	37–41	—	—	—
<i>Microscilla</i>	Marine	A	+ / —	v	+	—	+	+	ND	ND	ND	32–44
<i>Ornithobacterium</i>	Parasitic	F	— / —	—	—	ND	N	MK-6	37–39	—	—	—
<i>Persicobacter</i>	Marine	A	+ / —	+	+	—	+	+	ND	Spd	MK-7	40–42
<i>Psychroserpens</i>	Marine	A	+ / —	—	+	+	—	—	ND	ND	ND	27–29
<i>Riemerella</i>	Parasitic	M	— / —	—	—	+	ND	ND	MK-6	29–35	—	—
<i>Sphingobacterium</i>	Free-living	A	+ / —	+	—	+	+	ND	+	Hspd	MK-7	39–45
<i>Weeksella</i>	Parasitic, saprophytic	A	— / —	—	—	—	—	v	—	ND	MK-6	35–38

poly- β -hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. Requires Na^+ for growth. Strictly aerobic. Chemo-organotroph. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Does not decompose agar, starch, alginate, gelatin and chitinase. Phylogenetically is a member of the 'Flavobacterium-Bacteroides' phylum. Contains one species, *Arenibacter latericius*, the type strain of which is KMM 426^T.

Description of *Arenibacter latericius* gen. nov., sp. nov.

Arenibacter latericius (la.te.ri'ci.us. L. adj. *latericius* of brick, here pertaining to the dark orange pigmentation).

Description as for the genus. In addition, colonies are circular, low convex, shiny with entire edges on solid media containing high nutrient components. Colonies are dark orange in colour because of non-diffusible carotenoid pigments. Flexirubin pigments are absent. No growth is detected at 4 and 42 °C. Alkalitolerant. Does not hydrolyse gelatin or casein. Produces α -N-acetylgalactosaminidase. Utilizes α -D-glucose, L-glutamic acid, L-ornithine, uridine, glycerol, DL- α -glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate, according to the BIOLOG system. Dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, cellobiose, D-fructose, α -lactose, α -D-lactose lactulose, glycogen, maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, D-raffinose, sucrose, D-trehalose, DL-lactic acid, turanose, succinamic acid, glucuronamide, alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, D-serine, L-serine and L-threonine are weakly utilized. Susceptible to erythromycin, ampicillin, cephaloridin and lincomycin. Not susceptible to kanamycin, benzylpenicillin, oxacillin, neomycin, streptomycin, gentamicin and polymyxin B. The major polar lipid is phosphatidylethanolamine. Sphingophospholipids are absent. The predominant cellular fatty acids are odd-numbered, iso-branched (about 70%): 15:0, i15:0, a15:0, i15:1, i17:0; 16:0, 16:1(n-7), 18:0, 18:1(n-7) in minor quantities. The G + C content of the DNA ranges from 37 to 38 mol%. Type strain is KMM 426^T (VKM B 2137D^T = LMG 19694^T = CIP 106861^T), deposited in the Collection of Marine Micro-organisms (KMM) of the Pacific Institute of Bioorganic Chemistry, Vladivostok, the All-Russian Collection of Micro-organisms, Moscow, Russia, and the Culture Collection of the Laboratory of Microbiology, University of Gent, Belgium. Isolated from the marine environment (sandy sediments, holothurian, brown alga).

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