Owenweeksia hongkongensis gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes'

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An aerobic, Gram-negative, non-fermentative, rod-shaped, motile, orange-pigmented bacterium, UST20020801^T, was isolated from sea-water samples collected from Port Shelter, Hong Kong, S.A.R., China, in August 2002. The full 16S rRNA gene sequence of this strain shared only 87.5% similarity with its nearest relative, *Crocinitomix catalasitica*, a species of the family *Cryomorphaceae*. However, strain UST20020801^T possessed menaquinone-6, a major respiratory quinone of members of the family *Flavobacteriaceae*. This strain contains unique fatty acids such as i15:1G, i17:1 ω 9c, 2-OH 15:0, 15:1 ω 6c and three unknown fatty acids of equivalent chain-length of 11.543, 13.565 and 16.582. Further analysis of its ecophysiology and biochemistry suggests that this strain represents a new genus in the phylum 'Bacteroidetes'. The name *Owenweeksia hongkongensis* gen. nov., sp. nov. is proposed. The type strain is UST20020801^T (=NRRL B-23963^T=JCM 12287^T).

Members of the phylum 'Bacteroidetes' are important decomposers of high-molecular-mass organic matter in sea water (Cottrell & Kirchman, 2000). Recently, the family *Cryomorphaceae* was proposed as a member of the phylum 'Bacteroidetes'. This family encompasses three genera, *Brumimicrobium*, *Cryomorpha* and *Crocinitomix*, which form a distinct clade in the class 'Flavobacteria', branching between the families *Flavobacteriaceae* and *Bacteroidaceae* (Bowman *et al.*, 2003). Current members of the family *Cryomorphaceae* are psychrotolerant, rod- to filamentous-shaped and possess carotenoid pigments. They also require sea-water salts and complex organic compounds for growth (Bowman *et al.*, 2003). In a study of the bacterial diversity in Hong Kong coastal sea water, a mesophilic, orange-pigmented, rod-shaped, moderately halophilic bacterium (strain UST20020801^T) was isolated and characterized by polyphasic taxonomy. Analysis of the 16S rRNA gene sequence confirmed that this bacterium was

affiliated to the family *Cryomorphaceae* in the 'Bacteroidetes'; sequence similarity with its nearest phylogenetic relative, *Crocinitomix catalasitica*, was 87.5%. On the basis of phenotypic and genotypic characteristics, fatty acid composition and phylogenetic position, it is proposed that strain UST20020801^T represents a novel species of a new genus, *Owenweeksia hongkongensis* gen. nov., sp. nov., of the family *Cryomorphaceae* in the phylum 'Bacteroidetes'.

Strain UST20020801^T was isolated from a sea-water sample collected from the outlet of a tank storing sand-filtered sea water that was pumped from a depth of 5 m adjacent to the Coastal Marine Laboratory of the Hong Kong University of Science and Technology. Aliquots of 100 μ l were spread onto agar plates containing YPS-SW medium (0.4% yeast extract, 0.2% peptone, 1% starch, 75% sea water filtered through a 0.45 μ m filter and 1.5% agar) and incubated at 30 °C for 3 days. The isolate was cultivated aerobically on YP-SW medium (YPS-SW without starch), marine agar 2216 (Difco) or artificial sea water (ASW; 0.1% CaCl₂·2H₂O, 0.1% KCl, 0.5% MgSO₄·7H₂O, 0.25% NaCl) (Lewin & Lounsbery, 1969) with 0.4% yeast extract and stored at –80 °C in YP-SW supplemented with 20% glycerol. Colony morphology was examined on YP-SW agar plates that had been incubated at 30 °C for 5 days. Cell morphology under various growth conditions and gliding motility were elucidated by using an Olympus

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of UST20020801^T is AB125062.

Micrographs of *Owenweeksia hongkongensis* UST20020801^T and a phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain UST20020801^T and related taxa are available as supplementary material in IJSEM Online.

light microscope at 1000× magnification. Cell surface morphology was examined by scanning electron microscopy (Philips XL30) using a conventional method (Robinson *et al.*, 1987). Gram-reaction was assessed according to Collins *et al.* (1989). The absorption spectrum of pigment extracted with 7:3 (v/v) acetone:methanol was determined at 300–700 nm with a Beckman DU650 spectrophotometer. Bathochromatic shift test of flexirubin was performed by addition of 20 % KOH (Fautz & Reichenbach, 1980). Growth was evaluated at various temperatures (4, 16, 20, 25, 30, 33, 37, 40 and 42 °C) for up to 1 month in YP-SW broth. Growth at various pH values (pH 3.0–10.0) was evaluated in YP-SW broth adjusted with HCl or NaOH. Salinity tolerance was determined in YP-SW broth prepared with 0, 5, 15, 20, 40, 75 or 100 % filtered sea water. Salt requirement and tolerance were tested in modified ASW supplemented with 0.4 % yeast extract and with NaCl added at 0, 1, 2, 5, 7.5, 10 or 15 %. Anaerobic growth was examined by using the Oxoid anaerobic system. Acid production from carbohydrates was determined by using API 50CH strips (bioMérieux) and cells grown on a medium composed of 50 % CHB/E medium (bioMérieux) with 0.075 % CaCl₂·2H₂O, 1.875 % NaCl and 0.375 % MgCl₂. Carbohydrate assimilation was determined using API 50CH strips and cells grown on ASW supplemented with 0.05 % yeast extract. Fermentation of (+)-D-glucose, D-galactose, raffinose, sucrose, maltose, (–)-D-mannitol, D-sorbitol, inositol and dextran and hydrolysis of alginate, chitin and Tween 20 were tested according to Baumann & Baumann (1981). Catalase, oxidase, alkaline phosphatase and lecithinase activities, nitrate reduction, indole production, H₂S generation from thiosulfate or cysteine, and hydrolysis of cellulose, starch and gelatin were tested according to Smibert & Krieg (1994). β -Galactosidase activity and casein hydrolysis were examined according to Gosink *et al.* (1998) and Norris *et al.* (1985), respectively. Haemolytic activity was investigated using defibrinated rabbit blood (5 %, v/v) agar prepared with blood agar base (BBL). Degradation of dead yeast cells was tested on VY/2 agar prepared with 20 % filtered sea water (Reichenbach, 1989). DNA hydrolysis was examined by culturing cells in YP-SW broth supplemented with 100 µg salmon sperm DNA ml^{–1} for 36 h and monitoring DNA degradation by agarose-gel electrophoresis. Susceptibility to antibiotics was tested by the disc-diffusion plate method on YP-SW agar. Isoprenoid quinone analysis was performed by the HPLC method (Collins, 1994) using menaquinones extracted from *Cytophaga lytica* (Nakagawa & Yamasato, 1993) and *Sphingobacterium heparinum* (Steyn *et al.*, 1998) as the MK-6 and MK-7 references, respectively. The whole-cell fatty acid methyl ester profile was determined by using the MIDI Sherlock Microbial Identification system (Microbial ID) with cells of UST20020801^T grown at 10 °C for 5 days in marine agar 2216 (Difco). Genomic DNA was extracted by using the Invisorb spin tissue kit (Invitex) and DNA base composition was determined by the HPLC method (Johnson, 1985), calibrated and calculated according to Mesbah *et al.* (1989). The 16S rRNA gene was amplified

by using primer pair 27F (5'-AGAGTTTGATCCTGGC-TCAG-3') and 1525R (5'-AAGGAGTGTCCARCC-3') (Lane, 1991) with *Taq* polymerase (Promega) and the amplicon was purified with the ConcerT-PCR purification kit (Life Technologies), treated with the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit version 3.0 and sequenced using an Applied Biosystems 3100 automated DNA sequencer. The sequences were aligned and assembled before being compared with sequences from GenBank using the programs BLASTN (Altschul *et al.*, 1997) and WU-BLAST 2.0 (<http://blast.wustl.edu>). The sequences of UST20020801^T and related species were then aligned by using CLUSTAL_X (Thompson *et al.*, 1997) and the alignment file was edited with the BIOEDIT sequence alignment editor V5.0.9 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>)

Table 1. Phenotypic features of strain UST20020801^T

This strain cannot form acid from or utilize glucose, dextran, glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β -D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol or potassium gluconate. It is unable to ferment glucose, D-galactose, raffinose, sucrose, maltose, (–)-D-mannitol, D-sorbitol, inositol or dextran.

Characteristic	UST20020801 ^T
Salinity range (%)	
NaCl	1.0–7.5
Sea water	15–100
Oxidase	+
Catalase	+
Nitrate reduction	–
Alkaline phosphatase	+
β -Galactosidase	–
Lecithinase	–
Utilization of:	
Tween 20	+
Gelatin	+
Alginate	–
Agar	–
Casein	–
Cellulose	–
Chitin	–
DNA	–
Starch	–
Production of:	
H ₂ S	–
Indole	–
Hydrolysis of dead yeast cells	–
Haemolysis in rabbit blood agar	–

with gaps and missing nucleotide positions removed. Evolutionary distances of 1139 aligned positions were computed using the Kimura two-parameter model (Kimura, 1980) and the phylogenetic tree was generated by TREECON v1.3b (Van de Peer & De Wachter, 1994) using the neighbour-joining method (Saitou & Nei, 1987) and evaluated by bootstrap analyses (Felsenstein, 1985) based on 300 resamplings. Maximum-parsimony analysis was conducted by using MEGA version 2.1 (Kumar *et al.*, 2001).

Morphological properties of UST20020801^T are listed in the genus and species descriptions. A photograph of its colony morphology and phase-contrast and scanning electron micrographs are available as supplementary material in IJSEM Online (Figs a–e). Table 1 lists all the physiological and biochemical properties that were analysed. The more conserved characteristics of this strain were also investigated. The isoprenoid quinone of UST20020801^T is MK-6, a major respiratory quinone detected in members of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002).

The fatty acid profile of UST20020801^T and those of phylogenetically related members of the family *Cryomorphaceae* are given in Table 2. UST20020801^T contained a high percentage of the following branched-chain fatty acids (77.8% total fatty acids): i15:1G, i15:1, 3-OH i17:0, i17:1 ω 9c and 3-OH i15:1. The fatty acid profile of UST20020801^T differed significantly from those of other members of the family *Cryomorphaceae*. The strain possessed several unique fatty acids: i15:1G, i17:1 ω 9c, 2-OH 15:0, 15:1 ω 6c and three unknown fatty acids with equivalent chain-lengths of 11:543, 13:565 and 16:582. Strain UST20020801^T had the highest level of 3-OH i17:0 among members of the family *Cryomorphaceae*. Compared with other members of the family *Cryomorphaceae*,

UST20020801^T most resembled *Cryomorpha ignava* 1-22^T in terms of its relatively low content of straight-chain fatty acids and relatively high content of branched-chain hydroxy fatty acids.

The 16S rRNA gene sequence of strain UST20020801^T (1482 nt positions) showed 92.4% similarity to an uncultured clone, ARKIA-105 (GenBank/EMBL/DBJ accession no. AF468278), isolated from Arctic pack ice located at Northern Fram Strait (Brinkmeyer *et al.*, 2003), and 87.5% similarity to '*Microscilla aggregans* var. *catalatica*' strain IFO 15977, which has been reclassified as the type strain of *Crocinitomix catalasitica* (Bowman *et al.*, 2003). The 16S rRNA gene sequences of relatives of *Crocinitomix catalasitica* and other members of the phylum '*Bacteroidetes*' were retrieved from GenBank. Phylogenetic analysis showed that strain UST20020801^T and clone ARKIA-105 formed a distinct lineage within the family *Cryomorphaceae*, linked to *Cryomorpha ignava* 1-22^T with bootstrap support of 85% by the neighbour-joining method (Fig. 1) and 62% by maximum-parsimony analysis (data not shown). Sequence similarities to other members of the family *Cryomorphaceae* were less than 90% (*Cryomorpha ignava* 1-22^T, 87.2%; *Brumimicrobium glaciale* IC156^T, 86.1%), indicating that strain UST20020801^T is distantly related to them at the genus level. Phenotypically, strain UST20020801^T displayed some traits in common with members of the family *Cryomorphaceae*: rod- to filamentous-shaped cells; possession of carotenoid pigments; the ability to perform aerobic respiration; the requirement of sea-salts and complex organic compounds such as yeast extract or peptone for growth; no acid production from carbohydrate; and an inability to utilize starch, casein or chitin. The DNA G+C content of UST20020801^T was 39.6 ± 0.5 mol%, which is within the range of values for members of the family

Fig. 1. Phylogenetic relationship of strain UST20020801^T and related taxa within the phylum '*Bacteroidetes*' based on 16S rRNA gene sequences. The tree was created by using the neighbour-joining method and the numbers at the nodes represent levels of bootstrap support (as percentages) from 300 resampled datasets. *Escherichia coli* ATCC 11775^T (GenBank/EMBL/DBJ accession no. X80725) was used as the outgroup. Bar, 0.02 nt substitutions per position.

Table 2. Whole-cell fatty acid profiles of strain UST20020801^T and the members of the family *Cryomorphaceae*

Strains: 1, UST20020801^T; 2, *Brumimicrobium glaciale* IC156^T; 3, *Cryomorpha ignava* 1-22^T; 4, *Crocinitomix catalasitica* NCIMB 1418^T. Values given are percentages of total fatty acids. Fatty acids are designated as follows: total number of carbon atoms:number of double bonds, followed by the position of the double bond from the aliphatic end of the molecule. Prefixes i, a and OH represent iso-branched, anteiso-branched and hydroxy fatty acids, respectively. The suffix *c* represents a *cis* isomer. ECL, equivalent chain-length; TR, less than 0.1%. Data for UST20020801^T are from this study; data for strains 2–4 are from Bowman *et al.* (2003).

Fatty acid	1	2	3	4
13:0	—	0.5	—	—
14:0	—	1.5	—	0.3
15:0	—	2.9	0.8	29.3
16:0	0.3	1.9	0.9	1.8
18:0	—	0.3	—	—
i10:0	TR	—	—	—
i11:0	0.3	—	—	—
i13:0	—	5.6	—	—
a13:0	—	TR	—	—
i14:0	—	0.8	13.8	—
i15:0	18.7	36.4	7.8	36.3
i15:1G	28.0	—	—	—
a15:0	—	TR	5.8	—
i16:0	TR	0.6	7.8	0.4
a16:0	0.2	—	—	—
i14:1 ω 9 <i>c</i>	—	0.4	6.8	0.7
i15:1 ω 10 <i>c</i>	—	—	7.1	10.9
a15:1 ω 10 <i>c</i>	—	45	8.2	—
i16:1 ω 6 <i>c</i>	—	0.5	—	—
i16:1 ω 11 <i>c</i>	—	—	2.9	0.3
i17:1 ω 9 <i>c</i>	7.3	—	—	—
i17:1 ω 12 <i>c</i>	—	—	—	0.7
i17:1 ω 13 <i>c</i>	—	—	—	0.2
a17:1 ω 13 <i>c</i>	—	—	—	TR
15:1 ω 4 <i>c</i>	—	—	—	TR
15:1 ω 6 <i>c</i>	1.3	—	—	—
15:1 ω 8 <i>c</i>	—	1.4	—	—
15:1 ω 11 <i>c</i>	—	—	—	8.3
16:1 ω 5 <i>c</i>	—	—	—	TR
16:1 ω 7 <i>c</i>	ND	1.5	—	—
16:1 ω 12 <i>c</i>	—	—	—	4.4
17:1 ω 13 <i>c</i>	—	—	—	2.1
18:1 ω 5 <i>c</i>	0.3	—	—	—
18:1 ω 7 <i>c</i>	—	0.3	—	—
18:1 ω 9 <i>c</i>	—	0.4	—	—
2-OH i14:0	—	—	6.7	—
2-OH 15:0	3.0	—	—	—
2-OH a15:0	—	—	18.4	—
3-OH 15:0	1.0	—	—	TR
3-OH i15:0	4.9	—	—	1.0
3-OH 16:0	—	—	2.9	—
3-OH i16:0	0.2	—	6.8	TR
3-OH 17:0	1.2	—	—	TR
3-OH i17:0	18.1	—	—	3.3
Sum (2-OH i15:0/16:1 ω 7 <i>c</i>)	10.0	1.5	1.6	—
Unknown fatty acid, ECL 11.543	0.4	—	—	—
Unknown fatty acid, ECL 13.565	4.5	—	—	—
Unknown fatty acid, ECL 16.582	0.2	—	—	—

Cryomorphaceae (35–40 mol%) and close to that of its nearest phylogenetic relative, *Cryomorpha ignava* (36–37 mol%). Strain UST20020801^T differed from other members of the family *Cryomorphaceae* by possessing oxidase and gelatinase activities and growing well at 37 °C. Strain UST20020801^T can be distinguished from related genera of the ‘*Bacteroidetes*’ by several phenotypic tests (Table 3). On the basis of all characteristics described above, it is proposed that strain UST20020801^T should be placed in a new genus as a representative of a novel species, *Owenweeksia hongkongensis* gen. nov., sp. nov.

Description of *Owenweeksia* gen. nov.

Owenweeksia (Ow.en.week'si.a. N.L. fem. n. *Owenweeksia* bacterial genus named after Owen B. Weeks, who did a lot of work in the 1950s, 1960s and 1970s on *Flavobacterium*, *Cytophaga* and related species).

Cells are Gram-negative, halophilic, non-flagellated, motile, flexible, short rods with slightly tapered ends. Cells do not form spores and are strictly aerobic heterotrophs requiring Na⁺, Mg²⁺, sea-salts and either yeast extract or peptone for growth. Cells contain oxidase, catalase and alkaline phosphatase. Cellular fatty acids include large amounts of branched-chain fatty acids: i15:0G, i15:0, 3-OH i17:0, 2-OH i15:0/16:1 ω 7c, i17:1 ω 9c, 3-OH i15:0 and an unknown fatty acid of equivalent chain-length 13:565. The major respiratory quinone is MK-6. Phylogenetically, the genus *Owenweeksia* is a member of the family *Cryomorphaceae* in the phylum ‘*Bacteroidetes*’.

The type species is *Owenweeksia hongkongensis*.

Description of *Owenweeksia hongkongensis* sp. nov.

Owenweeksia hongkongensis (hong.kong.en'sis. N.L. fem. adj. *hongkongensis* pertaining to Hong Kong, S. A. R., P. R. China).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.3–0.5 µm in diameter and 0.5–4.0 µm in length. Filamentous cells detected in cultures supplemented with 0.1 % Tween 20. In YP-SW broth incubated at 4 °C or room temperature for 1 week, cells with one end, both ends or the whole body greatly enlarged are observed. Colonies are orange, smooth, convex, glistening and translucent with an entire margin. Growth occurs at 4–37 °C and pH 5.2–9.0 with optimum growth at 25–33 °C and pH 6.0–8.0. Cells possess carotenoid pigments with maximum absorption at 470 nm and two shoulder peaks around 448 nm and 501 nm. Physiological and biochemical properties are listed in Table 1. The fatty acid profile is given in Table 2. Sensitive to ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (10 µg), penicillin G (2 U), rifampicin (10 µg), streptomycin (10 µg), tetracycline (30 µg) and polymyxin B (300 U), but resistant to kanamycin (10 µg), gentamicin sulfate (10 µg) and spectinomycin (10 µg).

The type strain is UST20020801^T (=NRRL B-23963^T=JCM 12287^T), which was isolated from a sea-water sample

Table 3. Characteristics that differentiate strain UST20020801^T from other related members in the phylum ‘*Bacteroidetes*’

Strain/taxa: 1, *Brumimicrobium glaciale*; 2, *Cryomorpha ignava*; 3, *Crocinitomix catalasitica*; 4, the genus *Cellulophaga*; 5, [*Cytophaga*] *marinoflava*; 6, *Croceibacter atlanticus*; 7, *Tenacibaculum maritimum*; 8, [*Cytophaga*] *latercula*; 9, *Muricauda ruestringensis*; 10, *Tenacibaculum amylo-lyticum*; 11, UST20020801^T; 12, *Vitellibacter vladvostokensis*; 13, *Arenibacter latericius*; 14, the genus *Zobellia*; 15, *Tenacibaculum mesophilum*. Data are from Bowman *et al.* (2003), Johansen *et al.* (1999), Reichenbach (1989), Cho & Giovannoni (2003), Suzuki *et al.* (2001), Bruns *et al.* (2001), Nedashkovskaya *et al.* (2003), Ivanova *et al.* (2001), Barbeyron *et al.* (2001) and this study. ND, Not determined; +, positive; –, negative; v, variable; w, weak; A, strict aerobic; F, facultative anaerobe; O, orange; R, red; Y, yellow.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Growth at 4 °C/37 °C	+/-	+/-	+/-	+/-	+/-	-/-	-/-	ND/-	-/+	-/-	+/+	+/+	-/+	-/+	-/+
Oxidase/catalase	-/+	-/+	-/+	v/+	+/+	-/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Metabolism	F	A	A	A	A	A	A	A	F	A	A	A	A	A	A
Colour of cell mass	O	O	Y	O-Y	Y	O	Y	O-R	Y	Y	O	Y-O	O	Y-O	Y
Flexirubin reaction	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–
Gliding motility	+	–	+	+	+	–	+	–	–	+	+	–	–	+	+
Nitrate reductase	+	–	–	ND	+	–	–	–	–	W	–	–	+	+	–
Phosphatase	+	–	+	ND	ND	ND	ND	ND	ND	ND	+	+	+	ND	ND
Hydrolysis of:															
Agar	–	–	–	+	–	–	–	+	–	–	–	–	–	+	–
Gelatin	–	–	–	V	ND	+	–	+	–	+	+	+	–	+	+
Starch	–	–	–	+	+	+	–	–	–	+	–	–	–	V	–
DNA	V	–	–	ND	ND	+	+	ND	ND	+	–	+	–	+	+
DNA G+C content (mol%)	38–40	36–37	35	32–33	37–39	35	31–33	34	41	30.9	39–40	41.3	37–38	42–44	31–32

collected in Port Shelter, Hong Kong, S. A. R., P. R. China. DNA G + C content of the type strain is 39.6 ± 0.5 mol%.

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References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B. & Potin, P. (2001). *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 985–997.
- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. 1, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.
- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Bowman, J. P., Mancuso Nichols, C. & Gibson, J. A. E. (2003). *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol* **53**, 1343–1355.
- Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R. & Helmke, E. (2003). Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl Environ Microbiol* **69**, 6610–6619.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cho, J. C. & Giovannoni, S. J. (2003). *Croceibacter atlanticus* gen. nov., sp. nov., a novel marine bacterium in the family *Flavobacteriaceae*. *Syst Appl Microbiol* **26**, 76–83.
- Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–310. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Collins, C. H., Lyne, P. M. & Grange, J. M. (1989). *Collins and Lyne's Microbiological Methods*. London/Boston: Butterworths.
- Cottrell, M. T. & Kirchman, D. L. (2000). Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular weight dissolved organic matter. *Appl Environ Microbiol* **66**, 1692–1697.
- Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gosink, J. J., Woese, C. R. & Staley, J. T. (1998). *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of '*Flectobacillus glomeratus*' as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.
- Ivanova, E. P., Nedashkovskaya, O. I., Chun, J. & 7 other authors (2001). *Arenibacter* gen. nov., new genus of the family *Flavobacteriaceae* and description of a new species, *Arenibacter latericius* sp. nov. *Int J Syst Evol Microbiol* **51**, 1987–1995.
- Johansen, J. E., Nielsen, P. & Sjøholm, C. (1999). Description of *Cellulophaga baltica* gen. nov., sp. nov. and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *lytica* to *Cellulophaga lytica* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 1231–1240.
- Johnson, J. L. (1985). Determination of DNA base composition. *Methods Microbiol* **18**, 1–31.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: John Wiley.
- Lewin, R. A. & Lounsbury, D. M. (1969). Isolation, cultivation and characterization of flexibacteria. *J Gen Microbiol* **58**, 145–170.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Vitellibacter vladivostokensis* gen. nov., sp. nov., a new member of the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 1281–1286.
- Norris, J. R., Ribbons, D. W. & Varma, A. K. (editors) (1985). *Methods in Microbiology*, vol. 18. London: Academic Press.
- Reichenbach, H. (1989). Family 1. *Cytophagaceae* Stanier 1940, 630^{AL} emend. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2082. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Robinson, D. G., Ehlers, U., Herken, R., Herrmann, B., Mayer, F. & Schürmann, F.-W. (1987). Methods for SEM. In *Methods of Preparation for Electron Microscopy: an Introduction for the Biomedical Sciences*, pp. 145–165. Berlin: Springer.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stein, P. L., Segers, P., Vancanney, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus*

sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae*. *Int J Syst Bacteriol* **48**, 165–177.

Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (2001). Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amyolyticum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1639–1652.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Van de Peer, Y. & De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.