Fluviicola taffensis gen. nov., sp. nov., a novel freshwater bacterium of the family *Cryomorphaceae* in the phylum 'Bacteroidetes'

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A novel, strictly aerobic, Gram-negative, yellow–orange-pigmented, motile, catalase-positive, oxidase-negative bacterium, RW262^T, was isolated from water of the River Taff, Cardiff, UK, during January 2000. Phylogenetic analysis of the 16S rRNA gene indicated that strain RW262^T was a member of the family *Cryomorphaceae* within the phylum 'Bacteroidetes'. The DNA G+C content of strain RW262^T was 37.2 mol%. The predominant fatty acid was the branched-chain saturated fatty acid i15:0 (44.2%). On the basis of polyphasic analysis of phenotypic, chemotaxonomic, genotypic and phylogenetic characteristics, it is proposed that this freshwater bacterium represents a novel genus and species within the family *Cryomorphaceae*, *Fluviicola taffensis* gen. nov., sp. nov. The type strain is RW262^T (= NCIMB 13979^T = DSM 16823^T).

Molecular phylogenetic approaches based on analysis of the 16S rRNA gene sequence have revealed that members of the phylum 'Bacteroidetes' are highly abundant in aquatic environments (DeLong *et al.*, 1993; Glöckner *et al.*, 1999; Cottrell & Kirchman, 2000; O'Sullivan *et al.*, 2002, 2004). However, the majority of the 16S rRNA gene sequences of members of 'Bacteroidetes' isolated from aquatic habitats are most closely related to those of uncultivated bacteria, indicating that a large amount of potentially interesting bacterial diversity has yet to be cultured (O'Sullivan *et al.*, 2004). In the past few years there has been an increase in the descriptions of new genera of aquatic origin within the phylum 'Bacteroidetes', including *Reichenbachia*, *Vitellibacter*, *Mesonina* and *Ulvibacter* (Nedashkovskaya *et al.*, 2003a, b, c, 2004, respectively), *Belliella* (Brettar *et al.*, 2004), *Gillisia* (Van Trappen *et al.*, 2004), *Kordia* (Sohn *et al.*, 2004), *Arcicella* (Nikitin *et al.*, 2004) and *Formosa* (Ivanova *et al.*, 2004).

The novel family *Cryomorphaceae* has recently been described as containing four genera of marine origin: *Brumimicrobium*, *Cryomorpha* and *Crocinitomix* (Bowman *et al.*, 2003), and *Owenweeksia* (Lau *et al.*, 2005). According to 16S rRNA gene phylogeny, the family *Cryomorphaceae* branches between the families *Flavobacteriaceae* and *Bacteroidaceae* (Bowman *et al.*, 2003). It also exhibits greatest phenotypic similarity to the well-defined family *Flavobacteriaceae* (Bernardet *et al.*, 1996). On the basis of polyphasic taxonomic data and in adherence with the proposed minimal standards for describing new taxa of the closely related family *Flavobacteriaceae* (Bernardet *et al.*, 2002), we describe a novel freshwater member of the family *Cryomorphaceae*, *Fluviicola taffensis* gen. nov., sp. nov.

Strain RW262^T was isolated from water of the River Taff (Cardiff, UK; Ordnance Survey map reference SO 783 165) in January 2000, after 6 days incubation at 20 °C on CYT agar (1 g pancreatic digest of casein, 0.5 g CaCl₂·2H₂O, 0.5 g MgSO₄·7H₂O, 0.5 g yeast extract and 15 g purified agar, per litre distilled water; pH 7.2; Holmes, 1992). The strain was subsequently purified on Plate Count agar (PCA; Oxoid) and stored at –80 °C in liquid medium supplemented with 20% (v/v) glycerol. Strain RW262^T was selected for further investigation based on the results of 16S rRNA gene-targeted oligonucleotide hybridizations described in a previous study (O'Sullivan *et al.*, 2004).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Fluviicola taffensis* RW262^T is AF493694.

Micrographs of cells of strain RW262^T and a table showing complete whole-cell fatty acid profiles of RW262^T and the type species of other members of the family *Cryomorphaceae* are available as supplementary material in IJSEM Online.

The following phenotypic tests were performed on strain RW262^T and *Crocinitomix catalasitica* NCIMB 1418^T as a reference. Unless otherwise stated, the strains were revived from freezer stocks at 20 °C on either nutrient agar (NA; Oxoid) or PCA prior to investigation. The agar used to grow *Crocinitomix catalasitica* was supplemented with artificial sea water (24.7 g NaCl, 0.7 g KCl, 6.3 g MgSO₄·7H₂O, 4.6 g MgCl₂·6H₂O, 1 g anhydrous CaCl₂ and 0.2 g NaHCO₃, per litre distilled water). Gram-testing was by the KOH method (Gregerson, 1978) and flexirubin pigments were determined according to Fautz & Reichenbach (1980). Growth on Tryptone Soya agar (TSA; Oxoid), Marine agar 2216 (MA2216; Difco), MacConkey agar and Cetrimide agar (Oxoid) was assessed after 1 week. Cell size and gliding motility of strain RW262^T was established by phase-contrast microscopy of colonies after cultivation (3 days) under high humidity on agar. For scanning electron microscopy, RW262^T cells were fixed (1 h, 3 % glutaraldehyde), washed (0.1 M cacodylate buffer, pH 7.5), post-fixed (1 % OsO₄), dehydrated in ethanol, critical-point dried and coated with gold. Temperature and salinity requirements were determined by incubation on NA at 4, 16, 30 and 37 °C and with 0, 2.5, 5 and 10 % NaCl, respectively. Individual colony formation indicated positive results and growth without colony formation indicated weakly positive results. Standard methods (Smibert & Krieg, 1994) were used for oxygen requirements (thioglycollate medium), oxidase and catalase activity, DNA hydrolysis (DNase agar), starch hydrolysis (NA plus 1 g starch l⁻¹) and xylanase activity (NA plus 4 g birch wood xylan l⁻¹). Nitrate and nitrite reduction, indole production, urease activity, and hydrolysis of Tween 80, gelatin and arginine were analysed according to Cowan & Steel (1975). Strains were also characterized using an API 20NE kit (bioMérieux), according to the manufacturer's instructions, except that the incubation was performed at 20 °C and *Crocinitomix catalasitica* was inoculated in artificial sea water containing 1 g Casamino acids l⁻¹ and 1 ml trace elements l⁻¹ (2.85 g H₃BO₃ l⁻¹, 1.8 g MnCl₂·4H₂O l⁻¹, 1.36 g FeSO₄ l⁻¹, 26.9 mg CuCl₂·2H₂O l⁻¹, 20.8 mg ZnCl₂ l⁻¹, 40.4 mg CoCl₂·6H₂O l⁻¹, 25.2 mg Na₂MoO₄·2H₂O l⁻¹ and 1.77 g sodium tartrate l⁻¹). Antimicrobial susceptibility was assessed with antibiotic diffusion discs (Mast Diagnostics) with ampicillin (10 µg), kanamycin (30 µg), penicillin G (10 units), chloramphenicol (10 µg), streptomycin (10 µg), tetracycline (10 µg) and rifampicin (5 µg).

Fatty acid composition was determined with *Crocinitomix catalasitica* cultivated to confluence on MA2216, and strain RW262^T cultivated on the equivalent of MA2216 but without NaCl (g l⁻¹: 5 peptone, 1 yeast extract, 0.5 CaCl₂·2H₂O and 0.5 MgSO₄·7H₂O). Strains were grown at 20 °C for 72 h, harvested in PBS (Sigma) and freeze-dried. Fatty acid analyses followed the procedure described in the Microbial Identification System protocol (MIDI; <http://www.midi-inc.com>), and therefore represent the whole fatty acid composition of the cell hydrolysate. More-detailed analyses with other methods (Serrano-Carreón *et al.*, 1992)

showed no significant difference between hydrolysate fatty acid and phospholipid-derived fatty acid (PLFA) compositions, indicating that most fatty acids were derived from PLFAs.

The DNA base composition of strain RW262^T was determined at the German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and purified (Cashion *et al.*, 1977). The G + C content was determined by HPLC (Mesbah *et al.*, 1989). The 16S rRNA gene of strain RW262^T was PCR amplified (O'Sullivan *et al.*, 2004) from a small amount of purified colony biomass by using two sets of primers (27F, 1392R and 27F, 1492R; Lane, 1991). The PCR product obtained using primers 27F and 1392R was purified and sequenced (O'Sullivan *et al.*, 2004), and the product obtained with primers 27F and 1492R was cloned by using the TOPO TA system (Invitrogen) and sequenced with primers M13F and M13R (O'Sullivan *et al.*, 2002). A consensus from six overlapping sequences was compared with those most similar to RW262^T in the GenBank database, including representative type strains from four families of the phylum 'Bacteroidetes'. The CLUSTAL W alignment (Thompson *et al.*, 1994) was trimmed to 1300 bp, evolutionary distances were calculated and a phylogenetic tree was constructed (O'Sullivan *et al.*, 2004).

Strain RW262^T was aerobic, Gram-negative, motile by gliding and flexirubin-pigmented. Cells were long flexible rods with rounded ends, 0.4–0.5 µm in diameter and 1.5–5.7 µm in length. Rarer longer filaments when observed were of up to 51 µm in length (see the supplementary figure in IJSEM Online). After 7 days on PCA at 20 °C, flat, transparent, shiny colonies, 1–5 mm in diameter, with a yellow–orange colour and creamy consistency, were produced. Growth occurred at 4 and 20 °C, but not in the presence of Na⁺ ions. Growth occurred on NA, PCA, TSA and DNase agar, but not on MA2216, MacConkey agar or Cetrimide agar. The strain was catalase-positive, hydrolysed DNA and weakly hydrolysed gelatin. It was negative for nitrate and nitrite reduction; indole production; oxidase, β-galactosidase, urease and xylanase activity; hydrolysis of agar, arginine, aesculin and starch; and acid production from carbohydrates. The strain could not utilize glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenyl acetate. It was resistant to chloramphenicol, streptomycin and kanamycin, but was susceptible to penicillin G, ampicillin, rifampicin and tetracycline.

The whole-cell fatty acid composition of strain RW262^T is shown in Table 1 (taken from the supplementary table in IJSEM Online). The predominant cellular fatty acid was i15:0 (44.2 %). The G + C content of the genomic DNA of strain RW262^T was 37.2 mol%. The phylogenetic position of strain RW262^T was determined by analysis of the almost-complete 16S rRNA gene sequence. The greatest sequence similarity observed was with members of the families

Table 1. Major whole-cell fatty acids that differentiate *F. taffensis* RW262^T from type species of the genera *Brumimicrobium*, *Cryomorpha*, *Crocinitomix* and *Owenweeksia* of the family *Cryomorphaceae*

Strains: 1, RW262^T (data from this study); 2a, *Crocinitomix catalasitica* NCIMB 1418^T (this study); 2b, *Crocinitomix catalasitica* NCIMB 1418^T (Bowman *et al.*, 2003); 3, *B. glaciale* IC156^T (Bowman *et al.*, 2003); 4, *Cryomorpha ignava* 1-22^T (Bowman *et al.*, 2003); 5, *O. hongkongensis* UST20020801^T (Lau *et al.*, 2005). In this study, strain RW262^T and *Crocinitomix catalasitica* were grown on MA2216 at 20 °C for 3 days (without NaCl for RW262^T), whereas in the study by Bowman *et al.* (2003) *Crocinitomix catalasitica*, *B. glaciale* and *Cryomorpha ignava* were grown on MA2216 at 10–12 °C for 3–5 days. *O. hongkongensis* was grown on MA2216 at 10 °C for 5 days. —, <0.01 % detected.

Fatty acid	Fatty acid content (% of total)					
	1	2a	2b	3	4	5
Saturated fatty acids						
15:0	7.5	19.2	29.3	2.9	0.8	—
Sum	13.9	21.5	31.4	7.1	1.7	0.3
Saturated branched-chain fatty acids						
i14:0	—	—	—	0.8	13.8	—
i15:0	44.2	24.5	36.3	36.4	7.8	18.7
i15:1	—	—	—	—	—	28.0
Sum	45.3	24.7	36.7	43.4	35.2	47.2
Unsaturated branched-chain fatty acids						
i15:1 ω 10c	11.8	17.6	10.9	—	7.1	—
a15:1 ω 10c	—	—	—	45.0	8.2	—
i17:1 ω 9c	—	—	—	—	—	7.3
Sum	13.6	20.4	12.8	45.9	25.0	8.6
Monounsaturated fatty acids						
15:1 ω 11c	1.2	19.2	8.3	—	—	—
16:1 ω 12c	4.9	6.3	4.4	—	—	—
Sum	6.8	29.0	14.8	3.6	0.0	0.3
Hydroxy fatty acids						
2-OH i14:0	—	—	—	—	6.7	—
2-OH i15:0	3.5	—	—	—	1.6	—
2-OH a15:0	—	—	—	—	18.4	—
3-OH 16:0	—	—	—	—	2.9	—
3-OH i17:0	12.3	4.9	3.3	—	—	18.0
Sum	20.4	5.4	4.3	0.0	36.4	28.3

Cryomorphaceae and *Flavobacteriaceae* of the phylum ‘*Bacteroidetes*’. The 16S rRNA gene sequence of strain RW262^T was 90 % similar to that of *Ornithobacterium rhinotracheale* LMG 9086^T (GenBank accession no. ORU87101) and 89 % similar to that of *Crocinitomix catalasitica* NCIMB 1418^T (GenBank accession no. AB078042) of the families *Flavobacteriaceae* and *Cryomorphaceae*, respectively. However, construction of a neighbour-joining phylogenetic tree clearly illustrated that strain RW262^T formed a monophyletic lineage with the family *Cryomorphaceae*, positioned between the families *Flavobacteriaceae* and *Bacteroidaceae* (Fig. 1). The phylogenetic analysis indicated that strain RW262^T represents a distinct genus within the *Cryomorphaceae*.

Strain RW262^T was similar to the members of the family *Cryomorphaceae*, sharing their apparent lack of nutritional versatility, inability to utilize carbohydrates and their

existence as rod-shaped cells of variable length (Bowman *et al.*, 2003). All members were also Gram-negative, catalase-positive, negative for indole production, and did not catalyse the hydrolysis of arginine, aesculin or starch. The 37.2 mol% G + C content of strain RW262^T also fell within the 35–39 mol% known range for the family *Cryomorphaceae* (Bowman *et al.*, 2003). The differential characteristics of strain RW262^T and the type species of the four other *Cryomorphaceae* genera are shown in Table 2. Strain RW262^T was only capable of weak growth at 4 °C, and was therefore not psychrotolerant as are other members of the family (Bowman *et al.*, 2003). Strain RW262^T was also capable of DNA hydrolysis, whereas other members were not. In addition, strain RW262^T could be distinguished from *Crocinitomix catalasitica* NCIMB 1418^T by its susceptibility to tetracycline and lack of growth at 30 °C, from *Brumimicrobium glaciale* IC156^T by its aerobic metabolism and inability to reduce nitrate, from

Fig. 1. A 16S rRNA gene phylogenetic tree illustrating the relationship between strain RW262^T and other members of the phylum 'Bacteroidetes'. Sequences were retrieved from GenBank at the National Centre for Biotechnology Information (NCBI) web site (<http://www.ncbi.nlm.nih.gov>). Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and the alignment was trimmed to 1300 bp. The tree was reconstructed with the neighbour-joining and Jukes-Cantor algorithms in TREECON (Van de Peer & De Wachter, 1994), and was rooted with *Cytophaga hutchinsonii* ATCC 33406^T (GenBank accession no. M58768). Bootstrap values from 100 replicate trees are shown at the nodes, and the bar represents 10% difference in nucleotide sequence.

Table 2. Differential phenotypic characteristics of *F. taffensis* RW262^T and type species of the genera *Brumimicrobium*, *Cryomorpha*, *Crocinitomix* and *Owenweeksia* of the family *Cryomorphaceae*

Strains: 1, *F. taffensis* RW262^T; 2, *Crocinitomix catalasitica* NCIMB 1418^T (data from this study and Bowman *et al.*, 2003); 3, *B. glaciale* IC156^T (Bowman *et al.*, 2003); 4, *Cryomorpha ignava* 1-22^T (Bowman *et al.*, 2003); 5, *O. hongkongensis* UST20020801^T (Lau *et al.*, 2005). Abbreviations: +, positive; -, negative; ND, no data available; w, weakly positive; A, aerobic; F, facultatively anaerobic; S, sensitive; R, resistant. All strains grow at 20 °C and are catalase-positive. All strains are negative for the Gram-test; indole production; growth on 10% NaCl; hydrolysis of arginine, aesculin and starch; acid production from glucose; and utilization of arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, malate and citrate.

Characteristic	1	2	3	4	5
Habitat	Freshwater	Marine	Marine	Marine	Marine
Filaments	+	+	-	-	+
Metabolism	A	A	F	A	A
Gliding motility	+	+	+	-	+
Growth at/on:					
4 °C	w	+	+	+	+
30 °C	-	+	-	-	+
37 °C	-	-	-	-	+
0% NaCl	+	-	-	-	-
2.5% NaCl	-	+	+	-	+
5% NaCl	-	+	+	-	+
TSA	+	-	-	-	ND
NA	+	-	-	-	ND
MA2216	-	+	+	+	ND
DNA hydrolysis	+	-	-	w	-
Gelatin hydrolysis	w	-	-	-	+
Glucose utilization	-	-	+	-	-
Nitrate reduction	-	-	+	-	-
Oxidase activity	-	-	-	-	+
Tetracycline susceptibility	S	R	ND	ND	S
DNA G+C content (mol%)	37.2	35	38-40	36-37	39-40

Cryomorpha ignava 1-22^T by its gliding motility, and from *Owenweeksia hongkongensis* UST20020801^T by its inability to grow at 37 °C or on 5 % NaCl and its lack of oxidase activity.

The main distinguishing feature was that strain RW262^T originated from a freshwater habitat and could not grow in the presence of Na⁺ ions, whereas all other genera originated from marine habitats and had an absolute requirement for Na⁺ ions or natural sea water. Consequently, RW262^T could grow on TSA and NA but not on MA2216, whereas the opposite was true for *Crocinitomix catalasitica*, *B. glaciale* and *Cryomorpha ignava*. Strain RW262^T was previously shown to form a monophyletic lineage with uncultivated bacteria represented in freshwater 16S rRNA gene libraries (O'Sullivan *et al.*, 2004). This suggests that RW262^T may be a member of a freshwater clade within the family *Cryomorphaceae* which is distinct from the other marine members. Similar phylogenetic differentiation between freshwater and marine bacteria is commonplace within the closely related family *Flavobacteriaceae*, and was originally noted by Reichenbach *et al.* (1981). Specifically, the monophyletic branch representing the genus *Flavobacterium* contains mostly freshwater bacteria, whereas the monophyletic branch that represents many genera including *Tenacibaculum*, *Polaribacter*, *Zobellia* and *Cellulophaga* contains mostly marine bacteria.

The fatty acid profiles of strain RW262^T and the type species of the four other *Cryomorphaceae* genera were examined (Table 1). Cultivation conditions are known to influence fatty acid profiles (Männistö & Puhakka, 2001) and therefore conditions were standardized as much as possible to allow comparison with the results of Bowman *et al.* (2003). In this study, strain RW262^T and *Crocinitomix catalasitica* were grown on MA2216 at 20 °C for 3 days (minus NaCl for RW262^T), whereas in the study by Bowman *et al.* (2003) *Crocinitomix catalasitica*, *B. glaciale* and *Cryomorpha ignava* were grown on MA2216 at 10–12 °C for 3–5 days. Overall, the fatty acids identified for *Crocinitomix catalasitica* were very similar between the two studies; however, there were some differences in the proportions of individual fatty acids (Table 1). Like many flavobacteria and cryomorpha, strain RW262^T contained high levels of C13–C17 branched-chain fatty acids (58.9%), particularly saturated i15:0 (44.2%) and unsaturated i15:1ω10c (11.8%) fatty acids. There was also a large proportion of hydroxy fatty acids (20.4%), mainly composed of 3-OH i17:0 (12.3%). The whole-cell fatty acid profiles shown in Table 1 clearly distinguish RW262^T from the other members of the *Cryomorphaceae*.

The results of the polyphasic taxonomic analysis indicated that strain RW262^T did not belong to any of the other genera currently described within the phylum 'Bacteroidetes'. We therefore propose that strain RW262^T should be classified as *Fluviicola taffensis* gen. nov., sp. nov.

Description of *Fluviicola* gen. nov.

Fluviicola [Flu.vi.i.co'la. L. masc. n. *fluvius* -ii river; L. suff. -cola (from L. masc. n. *incola*) inhabitant, dweller; N.L. masc. n. *Fluviicola* river dweller].

Non-flagellated, rod-shaped cells including rarer long filaments. Gram-negative. Strictly aerobic. Motile by gliding. Cell mass is pigmented yellow-orange and flexirubins are synthesized. Utilize carbohydrates for growth. Fatty acids contain a high proportion of branched-chain fatty acids (mainly i15:0 and i15:1ω10c), as well as significant amounts of hydroxy fatty acids (mainly 3-OH i17:0). Isolated from freshwater environments, and do not grow in the presence of Na⁺ ions. 16S rRNA gene sequence analysis indicates that the genus is a member of the family *Cryomorphaceae* in the phylum 'Bacteroidetes'. The type species is *Fluviicola taffensis*.

Description of *Fluviicola taffensis* sp. nov.

Fluviicola taffensis (taf.fen'sis. N.L. masc. adj. *taffensis* pertaining to the River Taff, a river in Wales).

Exhibits the following characteristics in addition to those properties described for the genus. Cells are 0.4–0.5 µm wide and 1.5–5.7 µm long, with rarer longer filaments of up to 51 µm in length. Colonies are 1–5 mm in diameter, circular, flat, transparent, shiny, yellow-orange and creamy on high nutrient solid media. Catalase-positive. Oxidase-negative. Growth occurs at 4–25 °C, with an optimum at about 20 °C. Growth occurs on NA, PCA, TSA and DNASE agar, but not on MA2216, MacConkey agar or Cetrimide agar. Negative for nitrate and nitrite reduction. Indole is not produced. Negative for β-galactosidase, urease and xylanase activity. Capable of DNA hydrolysis and weak gelatin hydrolysis, but not hydrolysis of agar, arginine, aesculin or starch. Acid is not produced from glucose. Does not utilize glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenyl acetate. Resistant to chloramphenicol, streptomycin and kanamycin, but susceptible to penicillin G, ampicillin, rifampicin and tetracycline. The G+C content of the DNA is 37.2 mol%.

The type strain is RW262^T (=NCIMB 13979^T=DSM 16823^T), which was isolated from water of the River Taff, Cardiff, UK.

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References

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended

classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatidis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.

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.

Brettar, I., Christen, R. & Höfle, M. G. (2004). *Belliella baltica* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga–Flavobacterium–Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 65–70.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Cottrell, M. T. & Kirchman, D. L. (2000). Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Appl Environ Microbiol* **66**, 5116–5122.

Cowan, S. T. & Steel, K. J. (1975). *Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.

DeLong, E. F., Franks, D. G. & Alldredge, A. L. (1993). Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol Oceanogr* **38**, 924–934.

Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.

Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microbiol* **65**, 3721–3726.

Gregerson, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.

Holmes, B. (1992). The genera *Flavobacterium*, *Sphingobacterium*, and *Weeksella*. In *The Prokaryotes*, 2nd edn, vol. 4, pp. 3620–3629. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.

Ivanova, E. P., Alexeeva, Y. V., Flavier, S., Wright, J. P., Zhukova, N. V., Gorshkova, N. M., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2004). *Formosa algae* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **54**, 705–711.

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: Wiley.

Lau, K. W. K., Ng, C. Y. M., Ren, J., Lau, S. C. L., Qian, P.-Y., Wong, P.-K., Lau, T. C. & Wu, M. (2005). *Owenweeksia hongkongensis* gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes'. *Int J Syst Evol Microbiol* **55**, 1051–1057.

Männistö, M. K. & Puhakka, J. A. (2001). Temperature- and growth-phase-regulated changes in lipid fatty acid structures of psychrotolerant groundwater *Proteobacteria*. *Arch Microbiol* **177**, 41–46.

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.

Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003a). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga–Flavobacterium–Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.

Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003b). *Vitellibacter vladivostokensis* gen. nov., sp. nov., a new member of the phylum *Cytophaga–Flavobacterium–Bacteroides*. *Int J Syst Evol Microbiol* **53**, 1281–1286.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 7 other authors (2003c). *Mesonía algae* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz.) Kornm. *Int J Syst Evol Microbiol* **53**, 1967–1971.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M. S., Lysenko, A. M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2004). *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 119–123.

Nikitin, D. I., Strömpl, C., Oranskaya, M. S. & Abraham, W.-R. (2004). Phylogeny of the ring-forming bacterium *Arcicella aquatica* gen. nov., sp. nov. (ex Nikitin *et al.* 1994), from a freshwater neuston biofilm. *Int J Syst Evol Microbiol* **54**, 681–684.

O'Sullivan, L. A., Weightman, A. J. & Fry, J. C. (2002). New degenerate *Cytophaga–Flexibacter–Bacteroides*-specific 16S ribosomal DNA-targeted oligonucleotide probes reveal high bacterial diversity in River Taff epilithon. *Appl Environ Microbiol* **68**, 201–210.

O'Sullivan, L. A., Fuller, K. E., Thomas, E. M., Turley, C. M., Fry, J. C. & Weightman, A. J. (2004). Distribution and culturability of the uncultivated 'AGG58 cluster' of the *Bacteroidetes* phylum in aquatic environments. *FEMS Microbiol Ecol* **47**, 359–370.

Reichenbach, H., Kohl, W. & Achenbach, H. (1981). The flexirubin-type pigments, chemosystematically useful compounds. In *The Flavobacterium–Cytophaga Group (Proceedings of the International Symposium on Yellow-Pigmented Gram-Negative Bacteria of the Flavobacterium–Cytophaga Group)*, pp. 101–108. Edited by H. Reichenbach & O. B. Weeks. Weinheim: Verlag Chemie.

Serrano-Carreón, L., Hathout, Y., Bensoussan, M. & Belin, J. M. (1992). Quantitative separation of *Trichoderma* lipid classes on a bonded phase column. *J Chromatogr* **584**, 129–133.

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.

Sohn, J. H., Lee, J.-H., Yi, H., Chun, J., Bae, K. S., Ahn, T.-Y. & Kim, S.-J. (2004). *Kordia algicida* gen. nov., sp. nov., an algicidal bacterium isolated from red tide. *Int J Syst Evol Microbiol* **54**, 675–680.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

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.

Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. (2004). *Gillisia limnaea* gen. nov., sp. nov., a new member of the family *Flavobacteriaceae* isolated from a microbial mat in Lake Fryxell, Antarctica. *Int J Syst Evol Microbiol* **54**, 445–448.