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Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family

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In this paper minimal standards for the description of new genera and cultivable species in the family *Flavobacteriaceae* are proposed in accordance with Recommendation 30b of the *Bacteriological Code* (1990 Revision). In addition to specified phenotypic characteristics, the description of new species should be based on DNA–DNA hybridization data, and the placement of new taxa should be consistent with phylogenetic data derived from 16S rRNA sequencing. An emended description of the family is also proposed as several new taxa have been described since 1996. These proposals have been endorsed by the members of the Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes.

Keywords: minimal standards, *Flavobacteriaceae*

INTRODUCTION

Recommendation 30b of the *Bacteriological Code* (1990 Revision) (Lapage *et al*., 1992) calls for the development of minimal standards for describing new bacterial taxa. The aim of this paper is to propose minimal standards for descriptions of new genera and species of the family *Flavobacteriaceae*.

Current taxonomy of the *Flavobacteriaceae*

The family *Flavobacteriaceae* constitutes one of the main phyletic lines within the domain *Bacteria* together with the families *Bacteroidaceae*, *Cytophagaceae*, *Sphingobacteriaceae* and *Spirosomaceae*, as well as several taxa unaffiliated to any family (Woese *et al*., 1985; Bernardet *et al*., 1996). This line has been given several names, such as the 'flavobacter–bacteroides' phylum (Gherna & Woese, 1992), the *Flavobacterium–Cytophaga* complex (Nakagawa & Yamasato, 1993), rRNA superfamily V (Segers *et al*., 1993b), and the *Cytophaga–Flavobacterium–Bacteroides* group (Hirsch *et al*., 1998). Reichenbach (1992a) proposed that this phyletic line be equated with the order *Cytophagales*, although the position of the family *Bacteroidaceae*, which contains several genera of anaerobic bacteria (Holdeman *et al*., 1984), was not clear at that time. Since then, the family *Bacteroidaceae* has been unequivocally allocated to the phylum by several phylogenetic studies (Paster *et al*., 1994; Nakagawa & Yamasato, 1996; Vandamme *et al*., 1996b; Hirsch *et al*., 1998), though it falls within the competence of the Subcommittee on the taxonomy of Gram-negative anaerobic rods. The family *Sphingobacteriaceae*, which is defined on firm genomic and phenotypic grounds (Steyn *et al*., 1998), encompasses closely related soil and clinical organisms. In contrast, phylogenetic studies (Bernardet *et al*., 1996; Nakagawa & Yamasato, 1996) demonstrated that the distance between most of the organisms once included in the family *Cytophagaceae* solely on the basis of phenotypic characteristics (Reichenbach, 1989, 1992a) is actually considerable, hence a thorough emendation of this family is necessary. Similarly, the family *Spirosomaceae* (Larkin & Borrall, 1984; Raj & Maloy, 1990a) should probably be emended since the four genera it contains are only distantly related (Manz *et al*., 1996; Sly *et al*., 1998),

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Table 1. Currently recognized genera and species classified in the family *Flavobacteriaceae*

Names of type species are underlined. AL indicates that the species is cited on the Approved Lists of Bacterial Names (Skerman *et al*., 1980; Moore *et al*., 1985). Names in quotation marks have not been validly published. Previous names and corrected epithets are taken from Euzéby (1997). Accession number is that in the recognized culture collection in which the type strain was first deposited. ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; ATCC, American Type Culture Collection, Manassas, VA, USA; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; IAM, Institute of Applied Microbiology, University of Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japanese Collection of Microorganisms, Tokyo, Japan; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCTC, National Collection of Type Cultures, London, UK; NIBHT, Culture Collection of the National Institute of Bioscience and Human Technology, Tsukuba, Japan.

Genus and species*	Type strain	G+C (mol%)	Source	Reference(s)
Genus *Bergeyella*				Vandamme *et al*. (1994a)
Bergeyella zoohelcum[a]	NCTC 11660	35	Human sputum, USA	Holmes *et al*. (1986b)
Genus *Capnocytophaga*				Leadbetter *et al*. (1979); Holt & Kinder (1989)
Capnocytophaga canimorsus	ATCC 35979	37	Human blood after dog bite, USA	Brenner *et al*. (1989); Vandamme *et al*. (1996b)
Capnocytophaga cynodegmi	ATCC 49044	36	Dog's mouth, USA, 1979	Brenner *et al*. (1989); Vandamme *et al*. (1996b)
Capnocytophaga gingivalis[AL]	ATCC 33624	40	Periodontitis in human, USA, 1978	Leadbetter *et al*. (1979); Vandamme *et al*. (1996b)
Capnocytophaga granulosa	JCM 8566	42	Human dental plaque, Japan	Yamamoto *et al*. (1994); Vandamme *et al*. (1996b)
Capnocytophaga haemolytica	JCM 8565	44	Human dental plaque, Japan	Yamamoto *et al*. (1994); Vandamme *et al*. (1996b)
Capnocytophaga ochracea[AL]	ATCC 27872	39	Human oral cavity	Leadbetter *et al*. (1979); Vandamme *et al*. (1996b)
Capnocytophaga sputigena[AL]	ATCC 33612	38	Periodontitis in human, USA, 1978	Leadbetter *et al*. (1979); Vandamme *et al*. (1996b)
Genus *Cellulophaga*				Johansen *et al*. (1999)
Cellulophaga algicola	ACAM 630	37	Surface of marine alga, Antarctica	Bowman (2000)
Cellulophaga baltica	LMG 18535	33	Surface of marine alga, Svaneke, Denmark	Johansen *et al*. (1999)
Cellulophaga fucicola	LMG 18536	32	Surface of marine alga, Hirsholm, Denmark	Johansen *et al*. (1999)
Cellulophaga lytica[AL,b]	ATCC 23178	33	Beach mud, Limon, Costa Rica	Lewin (1969); Reichenbach (1989); Johansen *et al*. (1999)
Cellulophaga uliginosa[c]	ATCC 14397	42	Marine sediment	ZoBell & Upham (1944); Reichenbach (1989); Bowman (2000)
Genus Chryseobacterium				Holmes *et al*. (1984a); Vandamme *et al*. (1994a)
Chryseobacterium balustinum[AL,d]	NCTC 11212	33	Blood of freshwater fish, France, 1959	Holmes *et al*. (1984a)
Chryseobacterium gleum[AL,e]	ATCC 35910	37	Human vaginal swab, UK, 1979	Holmes *et al*. (1984b)
Chryseobacterium indologenes[AL,f]	NCTC 10796	38	Human trachea at autopsy, 1958	Yabuuchi *et al*. (1983)
Chryseobacterium indoltheticum[AL,g]	ATCC 27950	34	Marine mud	Campbell & Williams (1951)
'*Chryseobacterium joostei*'	LMG 18212	37	Raw cow's milk, South Africa, 1981	Hugo (1997)
Chryseobacterium meningosepticum[AL,h]	ATCC 13253	37	Human cerebrospinal fluid, USA, 1949	King (1959); Holmes *et al*. (1984a)
'*Chryseobacterium proteolyticum*'	NIBHT P17664	37	Soil, rice field, Tsukuba, Japan	Yamaguchi & Yokoe (2000)
Chryseobacterium scophthalmum[i]	CCM 4109	34	Gills of marine fish, UK, 1987	Mudarris *et al*. (1994)
Genus *Coenonia*				Vandamme *et al*. (1999)
Coenonia anatina	LMG 14382	35	Peking duck, Germany, 1991	Vandamme *et al*. (1999)
Genus *Empedobacter*				Vandamme *et al*. (1994a)
Empedobacter brevis[AL,j]	NCTC 11099	33	Human bronchial secretion, Switzerland, 1976	Holmes *et al*. (1978); Holmes *et al*. (1984a)
Genus *Flavobacterium*				Bernardet *et al*. (1996)
Flavobacterium aquatile[AL,k]	ATCC 11947	33	Deep well, UK	Holmes *et al*. (1984a); Bernardet *et al*. (1996)
Flavobacterium branchiophilum[l]	ATCC 35035	34	Gills of salmon, Japan, 1977	Wakabayashi *et al*. (1989); Bernardet *et al*. (1996)
Flavobacterium columnare[m]	NCIMB 2248	32	Kidney of salmon, USA, 1955	Bernardet & Grimont (1989); Bernardet *et al*. (1996)
Flavobacterium flevense[AL,n]	ATCC 27944	35	Freshwater lake, The Netherlands	van der Meulen *et al*. (1974); Bernardet *et al*. (1996)
Flavobacterium gillisiae	ACAM 601	32	Sea ice, Prydz Bay, Antarctica	McCammon & Bowman (2000)
Flavobacterium hibernum[o]	ACAM 376	34	Freshwater lake, Antarctica	McCammon *et al*. (1998)
Flavobacterium hydatis[AL,p]	ATCC 29551	34	Gills of salmon, USA, 1974	Strohl & Tait (1978); Bernardet *et al*. (1996)
Flavobacterium johnsoniae[AL,q]	ATCC 17061	35	Soil or mud, UK	Reichenbach (1989); Bernardet *et al*. (1996)
Flavobacterium pectinovorum[r]	NCIMB 9059	35	Soil, UK	Reichenbach (1989); Bernardet *et al*. (1996)
Flavobacterium psychrophilum[s]	NCIMB 1947	33	Kidney of salmon, USA	Bernardet & Grimont (1989); Bernardet *et al*. (1996)
Flavobacterium saccharophilum[t]	NCIMB 2072	36	River Wey, UK, 1976	Agbo & Moss (1979); Reichenbach (1989); Bernardet *et al*. (1996)
Flavobacterium succinicans[u]	DSM 4002	37	Fin of salmon, USA, 1954	Anderson & Ordal (1961); Reichenbach (1989); Bernardet *et al*. (1996)
Flavobacterium tegetincola	ACAM 602	34	Cyanobacterial mat, marine salinity lake, Antarctica	McCammon & Bowman (2000)
Flavobacterium xanthum[v]	IAM 12026	36	Soil, Showa station, Antarctica, 1967	Inoue & Komagata (1976); Reichenbach (1989); McCammon & Bowman (2000)
Genus *Gelidibacter*				Bowman *et al*. (1997)
Gelidibacter algens	ACAM 536	36	Sea ice, Antarctica	Bowman *et al*. (1997)

Table 1 (*cont.*)

Genus and species*	Type strain	G+C (mol%)	Source	Reference(s)
Genus *Myroides*				Vancanneyt *et al.* (1996)
Myroides odoratus[AL,w]	ATCC 4651	36	Unknown	Holmes *et al.* (1977, 1984a); Vancanneyt *et al.* (1996)
Myroides odoratimimus	NCTC 11180	32	Human wound, UK	Vancanneyt *et al.* (1996)
Genus *Ornithobacterium*				Vandamme *et al.* (1994b)
Ornithobacterium rhinotracheale	CCUG 23171	38	Respiratory tract of turkey, UK	Vandamme *et al.* (1994b)
Genus *Polaribacter*				Gosink *et al.* (1998)
Polaribacter filamentus	ATCC 700397	32	Surface sea water, Alaska, 1992	Gosink *et al.* (1998)
Polaribacter franzmannii	ATCC 700399	32	Sea ice, Antarctica, 1992	Gosink *et al.* (1998)
Polaribacter glomeratus[x]	ACAM 171	33	Marine salinity lake, Antarctica, 1984	McGuire *et al.* (1987); Gosink *et al.* (1998)
Polaribacter irgensii[y]	ATCC 700398	31	Sea water, Antarctica, 1986	Gosink *et al.* (1998)
Genus *Psychroflexus*				Bowman *et al.* (1998)
Psychroflexus gondwanensis[z]	ACAM 44	39	Hypersaline lake, Antarctica, 1986	Dobson *et al.* (1993); Bowman *et al.* (1998)
Psychroflexus torquis	ACAM 623	33	Sea ice, Antarctica	Bowman *et al.* (1998)
Genus *Psychroserpens*				Bowman *et al.* (1997)
Psychroserpens burtonensis	ACAM 188	28	Marine salinity lake, Antarctica	Bowman *et al.* (1997)
Genus *Riemerella*				Segers *et al.* (1993a)
Riemerella anatipestifer[AL,aa]	ATCC 11845	35	Duck's blood, USA	Segers *et al.* (1993a)
Riemerella columbina	LMG 11607	36	Pigeon palatine cleft, Germany, 1989	Vancanneyt *et al.* (1999)
Genus *Salegentibacter*				McCammon & Bowman (2000)
Salegentibacter salegens[bb]	ACAM 48	37	Water, Organic Lake, Antarctica, 1986	Dobson *et al.* (1993); McCammon & Bowman (2000)
Genus *Tenacibaculum*				Suzuki *et al.* (2001)
Tenacibaculum amylolyticum	IFO 16310	31	Marine alga, Palau, Philippines	Suzuki *et al.* (2001)
Tenacibaculum maritimum[cc]	ATCC 43398	32	Diseased marine fish, Japan, 1977	Wakabayashi *et al.* (1986); Bernardet & Grimont (1989); Suzuki *et al.* (2001)
Tenacibaculum mesophilum	IFO 16307	32	Marine sponge, Numazu, Japan	Suzuki *et al.* (2001)
Tenacibaculum ovolyticum[dd]	ATCC 51887	30	Marine fish egg, Norway, 1989	Hansen *et al.* (1992); Suzuki *et al.* (2001)
Genus *Weeksella*				Holmes *et al.* (1986a)
Weeksella virosa	NCTC 11634	37	Human urine, USA	Holmes *et al.* (1986a)
Genus *Zobellia*				Barbeyron *et al.* (2001)
Zobellia galactanivorans[ee]	DSM 12802	43	Red marine alga, Brittany, France	Barbeyron *et al.* (2001)
Zobellia uliginosa[c]	ATCC 14397	42	Marine sediment	ZoBell & Upham (1944); Reichenbach (1989); Barbeyron *et al.* (2001)
Unaffiliated taxa				Bernardet *et al.* (1996); Hanzawa *et al.* (1995)
[*Cytophaga*] *latercula*	ATCC 23177	32	Seawater aquarium outflow, La Jolla, USA	Lewin (1969); Reichenbach (1989)
[*Cytophaga*] *marinoflava*	NCIMB 397	37	Sea water, UK	Colwell *et al.* (1966); Reichenbach (1989)

* Previous names: *a*, [*Weeksella*] *zoohelcum* Holmes *et al.* 1986b; *b*, [*Cytophaga*] *lytica* Lewin 1969; *c*, [*Flavobacterium*] *uliginosum* ZoBell and Upham 1944, Weeks 1974, '*Agarbacterium uliginosum*' Breed 1957a, [*Cytophaga*] *uliginosa* Reichenbach 1989 [this taxon has successively been reclassified in the genus *Cellulophaga* (Bowman, 2000) and in the genus *Zobellia* (Barbeyron *et al.*, 2001); hence, it is provisionally listed within both genera in this table]; *d*, [*Flavobacterium*] *balustinum* Harrison 1929; *e*, [*Flavobacterium*] *gleum* Holmes *et al.* 1984b; *f*, [*Flavobacterium*] *indologenes* Yabuuchi *et al.* 1983; *g*, [*Flavobacterium*] *indoltheticum* Campbell and Williams 1951, '*Beneckea indolthetica*' Campbell 1957; *h* [*Flavobacterium*] *meningosepticum* King 1959; *i*, '*Cytophaga scophthalmis*', name as listed in 1989 in the catalogue of strains of the Czech Collection of Microorganisms, [*Flavobacterium*] *scophthalmum* Mudarris *et al.* 1994; *j*, '[*Bacillus*] *brevis*' Lustig 1890, '*Bacterium breve*' Chester 1901, '[*Flavobacterium*] *brevis*' Bergey *et al.* 1923, '*Pseudobacterium brevis*' Krasil'nikov 1949, '*Empedobacter breve*' Prévot 1961, [*Flavobacterium*] *breve* Holmes and Owen 1982; *k*, '[*Bacillus*] *aquatilis*' Frankland and Frankland 1889, '*Bacterium aquatilis*' Chester 1897, '[*Flavobacterium*] *aquatilis*' Bergey *et al.* 1923, '[*Chromobacterium*] *aquatilis*' Topley and Wilson 1929, '[*Empedobacter*] *aquatile*' Brisou *et al.* 1960; *l*, *Flavobacterium branchiophila* Wakabayashi *et al.* 1989; *m*, '[*Bacillus*] *columnaris*' Davis 1922, '[*Chondrococcus*] *columnaris*' Ordal and Rucker 1944, [*Cytophaga*] *columnaris* Garnjobst 1945, Reichenbach 1989, [*Flexibacter*] *columnaris* Leadbetter 1974, Bernardet and Grimont 1989; *n*, [*Cytophaga*] *flevensis* van der Meulen *et al.* 1974, Reichenbach 1989; *o*, '*Flavobacterium ameridies*', name as deposited in the 16S rRNA sequence databases; *p*, [*Cytophaga*] *aquatilis* Strohl and Tait 1978; *q*, [*Cytophaga*] *johnsonae* Stanier 1947, Reichenbach 1989, '[*Cytophaga*] *johnsonii*' Stanier 1957; *r*, '*Flavobacterium pectinovorum*' Dorey 1959, '[*Empedobacter*] *pectinovorum*' Kaiser 1961, [*Cytophaga*] *pectinovora* Reichenbach 1989; *s*, [*Cytophaga*] *psychrophila* Borg 1960, Reichenbach 1989, [*Flexibacter*] *psychrophilus* Bernardet and Grimont 1989; *t*, [*Cytophaga*] *saccharophila* Agbo and Moss 1979; *u*, [*Cytophaga*] *succinicans* Anderson and Ordal 1961, Reichenbach 1989, '[*Flexibacter*] *succinicans*' Leadbetter 1974; *v*, '[*Cytophaga*] *xantha*' Inoue and Komagata 1976; *w*, '[*Flavobacterium*] *odoratum*' Stutzer *in* Stutzer and Kwaschnina 1929; *x*, [*Flectobacillus*] *glomeratus* McGuirre *et al.* 1987; *y*, '*Antarcticum vesiculatum*', '*Vesiculatum antarctica*', names as deposited in the databases of 16S rRNA sequences; *z*, [*Flavobacterium*] *gondwanense* Dobson *et al.* 1993, *Psychroflexus gondwanense* Bowman *et al.* 1998; the original spelling of the specific epithet was corrected on validation (Bowman *et al.*, 1999); *aa*, '*Pfeifferella anatipestifer*' Hendrickson and Hilbert 1932, [*Moraxella*] *anatipestifer* Bruner and Fabricant 1954, [*Pasteurella*] *anatipestifer* Breed 1957b; *bb*, [*Flavobacterium*] *salegens* Dobson *et al.* 1993; *cc*, '[*Flexibacter*] *marinus*' Hikida *et al.* 1979, [*Flexibacter*] *maritimus* Wakabayashi *et al.* 1986, [*Cytophaga*] *marina* Reichenbach 1989; *dd*, [*Flexibacter*] *ovolyticus* Hansen *et al.* 1992; *ee*, '[*Cytophaga*] *drobachiensis*' Potin *et al.* 1991, *Zobellia galactanovorans* Barbeyron *et al.* 2001; the original spelling of the specific epithet was corrected on notification (*International Journal of Systematic Bacteriology*, 2001).

although they do share some 16S rRNA sequence signatures (Woese *et al*., 1990a). The *Cytophaga–Flavobacterium–Bacteroides* phylum also comprises several other genera and species which are phylogenetically distant (Manz *et al*., 1996; Nakagawa & Yamasato, 1996); difficulties will probably arise in delineating new families for some of these taxa, most of which are poorly described and only represented by single strains.

The family *Flavobacteriaceae* was proposed by Jooste (1985) and included in the first edition of the *Bergey's Manual of Systematic Bacteriology* (see Reichenbach, 1989), but the taxon was not formally described (Holmes, 1997). The name of the family was subsequently validated (Reichenbach, 1992b) and an emended description was published (Bernardet *et al*., 1996). The family included *Flavobacterium* (Bernardet *et al*., 1996), the type genus, and the genera *Bergeyella* (Holmes *et al*., 1986b; Vandamme *et al*., 1994a), *Capnocytophaga* (Holt & Kinder, 1989; Vandamme *et al*., 1996b), *Chryseobacterium* (Holmes *et al*., 1984a; Vandamme *et al*., 1994a), *Empedobacter* (Holmes *et al*., 1978; Vandamme *et al*., 1994a), *Ornithobacterium* (Vandamme *et al*., 1994b), *Riemerella* (Segers *et al*., 1993a; Vancanneyt *et al*., 1999) and *Weeksella* (Holmes *et al*., 1986a). Another taxon included in the family, [*Flavobacterium*] *odoratum* (brackets indicate generically misclassified bacteria) (Holmes *et al*., 1977), was subsequently transferred to the new genus *Myroides* (Vancanneyt *et al*., 1996); a second *Myroides* species was also described. Several new species have been added to the family *Flavobacteriaceae* since 1996, namely *Flavobacterium hibernum* (McCammon *et al*., 1998); *Flavobacterium gillisiae*, *Flavobacterium tegetincola* and *Flavobacterium xanthum* (previously '[*Cytophaga*] *xantha*') (McCammon & Bowman, 2000); *Riemerella columbina* (Vancanneyt *et al*., 1999); '*Chryseobacterium joostei*' (Hugo, 1997); and '*Chryseobacterium proteolyticum*' (Yamaguchi & Yokoe, 2000). The new genus *Coenonia* has also been assigned to the taxon (Vandamme *et al*., 1999).

The family *Flavobacteriaceae* also includes a rather complex group of halophilic organisms, many of which are psychrophilic. The structure of this group has been progressively unravelled following the emended description of the family (Bernardet *et al*., 1996). Five new genera of polar organisms have been described, namely the monospecific genera *Gelidibacter* and *Psychroserpens* (Bowman *et al*., 1997), *Polaribacter* (with four species, one of which was previously called [*Flectobacillus*] *glomeratus*) (Gosink *et al*., 1998), *Psychroflexus* (with two species, one of which was previously called [*Flavobacterium*] *gondwanense*) (Bowman *et al*., 1998) and *Salegentibacter* (comprising the taxon previously called [*Flavobacterium*] *salegens*) (McCammon & Bowman, 2000). The new genus *Cellulophaga* has been proposed for [*Cytophaga*] *lytica* and two new marine species (Johansen *et al*., 1999) (this genus was erroneously included in the family *Cytophagaceae*); a new *Cellulophaga* species has subsequently been described and [*Cytophaga*] *uliginosa* has also been reclassified in this genus (Bowman, 2000). More recently, it has been proposed to reclassify [*Cytophaga*] *uliginosa* in the new genus *Zobellia*, together with a new species (Barbeyron *et al*., 2001). [*Flexibacter*] *maritimus* and [*Flexibacter*] *ovolyticus* are two phylogenetically close and well-defined species that are represented by several strains (Bernardet *et al*., 1996); the new genus *Tenacibaculum* has recently been proposed to classify these organisms and two new species (Suzuki *et al*., 2001). In contrast, [*Cytophaga*] *latercula* and [*Cytophaga*] *marinoflava* are phylogenetically distant from the other halophilic taxa and cannot be assigned to a single genus; they remain generically misclassified and probably constitute the core of new genera (Hanzawa *et al*., 1995; Bowman *et al*., 1997, 1998). These two organisms will not be considered in this paper, as minimal standards cannot accommodate such phylogenetically isolated and poorly described species represented by single strains (Colwell *et al*., 1966; Lewin, 1969; Reichenbach, 1989). Several poorly described algicidal and/or algal-lytic gliding bacteria have been allocated to the group of the halophilic *Flavobacteriaceae* (Hanzawa *et al*., 1995; Maeda *et al*., 1998; Kondo *et al*., 1999). This group is also well represented in mangrove environments (Nakagawa *et al*., 2001).

A list of the currently recognized taxa classified in the family *Flavobacteriaceae*, namely the 18 well-defined genera and the two unaffiliated organisms, is shown in Table 1. The position of the family *Flavobacteriaceae* in the *Cytophaga–Flavobacterium–Bacteroides* phylum is shown in Fig. 1. The phylogenetic relationships of the taxa classified in the family *Flavobacteriaceae* are shown in Fig. 2. Although not included in the Tables and not further considered in this paper, invalid taxa belonging to the family *Flavobacteriaceae* for which 16S rRNA sequence is available have been included in Fig. 2 for information; their names are given in quotation marks.

A rather surprising result from several phylogenetic investigations was the allocation to the family *Flavobacteriaceae* of several intracellular symbionts of insects. In addition to the long established genus *Blattabacterium* (Dasch *et al*., 1984), which encompasses organisms that live in the tissues of several cockroach species (Bandi *et al*., 1994), these organisms include various termite symbionts and ladybird beetle male-killing agents (Hurst *et al*., 1997, 1999). The sequences of the 16S rRNA of these organisms are the only clue to their phylogenetic affiliation as they have yet to be isolated, cultivated and described. The symbiont group is unequivocally included in the family *Flavobacteriaceae* in some phylogenetic analyses (Manz *et al*., 1996; Bowman *et al*., 1998; Hirsch *et al*., 1998) while in others it branches slightly below the other members of the family (Bandi *et al*., 1994; Hurst *et al*., 1997, 1999; and the present study; see Fig. 1). Although the close relationship of these organisms with the family *Flavobacteriaceae* is established, little

more can be said about them here as minimal standards cannot be applied to unculturable bacteria. The provisional category *Candidatus* (Murray & Stackebrandt, 1995) has been included as an Appendix in the *Bacteriological Code* (1990 Revision) for such microorganisms (Labeda, 1997). Several intracellular bacteria isolated from amoebae have also been allocated to the family *Flavobacteriaceae* based on phenotypic characteristics and fatty acid profiles, but these potentially novel organisms have not been fully described (Müller *et al*., 1999, and references therein).

General principles

The primary aim of this paper is to provide bacteriologists involved in the taxonomy of the family *Flavobacteriaceae* with a framework for describing new taxa. The list of characters whose determination is strongly recommended in the following paragraphs may seem long and demanding but this should not discourage scientists from publishing the description of new species since minimal standards are only designed to avoid the publication of poorly characterized 'new' taxa. It is well known that such taxa are difficult to retract and can confuse bacterial nomenclature for decades. The general principles outlined here have already been mentioned in corresponding contributions dealing with other bacterial taxa (Graham *et al*., 1991; Vincent Lévy-Frébault & Portaels, 1992; Ursing *et al*., 1994; International Committee on Systematic Bacteriology Subcommittee on the taxonomy of Mollicutes, 1995; Oren *et al*., 1997; Freney *et al*., 1999; Dewhirst *et al*., 2000).

A polyphasic approach to bacterial systematics has been progressively adopted by most bacteriologists over the last decade (Murray *et al*., 1990; Vandamme *et al*., 1996a). This approach integrates phenotypic and chemotaxonomic characterization with genomic and phylogenetic data. The description of new taxa in the family *Flavobacteriaceae* should thus rely on a wide variety of phenotypic and molecular properties in order to consider the greatest percentage of the bacterial genome possible. It is not possible to recommend a single medium that supports the growth of all members of the *Flavobacteriaceae*, hence it is suggested that the best growth medium and the optimal growth conditions be determined before strains are further investigated. When primary isolation is performed on environmental samples or external lesions of animals, bacterial cultures are frequently mixed; consequently, the purity of cultures should be checked by an accurate examination of agar plates under a stereomicroscope ($\times 20$). All studies should be performed on actively growing cultures. As many isolates as possible of a candidate new taxon should be included, preferably representing a wide variety of independent sources (i.e. different animal hosts, geographical locations, environmental samples, years of isolation); these data should be specified in the article.

The clustering of strains by numerical taxonomic methods (Sneath & Sokal, 1973) is highly recommended when a sufficient number of phenotypic properties is examined.

When a potentially new species is studied, the type strains of all related species should be included for comparison. In order to restrict the number of these species (and consequently the amount of technically demanding investigations, such as DNA–DNA hybridizations), the new species can first be located within the 16S rRNA tree; this is straightforward given the extensive database now available for the family *Flavobacteriaceae* (Woese *et al*., 1990b; Gherna & Woese, 1992; Nakagawa & Yamasato, 1993, 1996; Dobson *et al*., 1993; Bandi *et al*., 1994; Vandamme *et al*., 1996b; Hurst *et al*., 1997; Bowman *et al*., 1997, 1998; Gosink *et al*., 1998; Maidak *et al*., 1999; Bowman, 2000; Suzuki *et al*., 2001). Such comparative studies should include as many related organisms as possible in order to improve the significance of the tree. Differences in 16S rRNA sequences of up to 5% have been found among strains of some species classified in the family (Clayton *et al*., 1995; Triyanto & Wakabayashi, 1999); hence comparison of sequences of several strains is desirable since this will improve the soundness of the phylogenetic hypotheses and provide an estimation of the genomic diversity of the new taxon.

It is very important that the methods of alignment and the treeing algorithms used in phylogenetic studies are stated. Phylogenetic trees should be constructed using more than two methods, e.g. choice from maximum-likelihood, maximum-parsimony, unweighted pair-group method using arithmetic averages (UPGMA) or neighbour-joining (Sneath & Sokal, 1973; Nei, 1987; Saitou & Nei, 1987). In addition, the reliability of branchings should be statistically evaluated using a criterion of goodness such as a bootstrap analysis (Felsenstein, 1985). It is imperative that new 16S rRNA sequences are deposited in a recognized database and that the accession numbers are included in the species description. In most databases, bacterial nomenclature is not always properly updated; hence new 16S rRNA sequences should preferably be deposited under the laboratory code or culture collection number of the isolate or, for instance, under such provisional denominations as '*Flavobacterium* sp. no. X', 'strain no. Y' or 'fish isolate no. Z'. This practice will ensure that databases, publications and phylogenetic trees do not contain prematurely attributed invalid Latin names.

The sequence of molecules other than 16S rRNA may also provide interesting phylogenetic informations: Yamamoto & Harayama (1996) have shown that a phylogenetic analysis based on the DNA gyrase B subunit gene (*gyrB*) may have a greater degree of resolution than one based on the 16S rRNA sequence because protein-encoding genes evolve faster than rRNA genes. However, the two techniques have

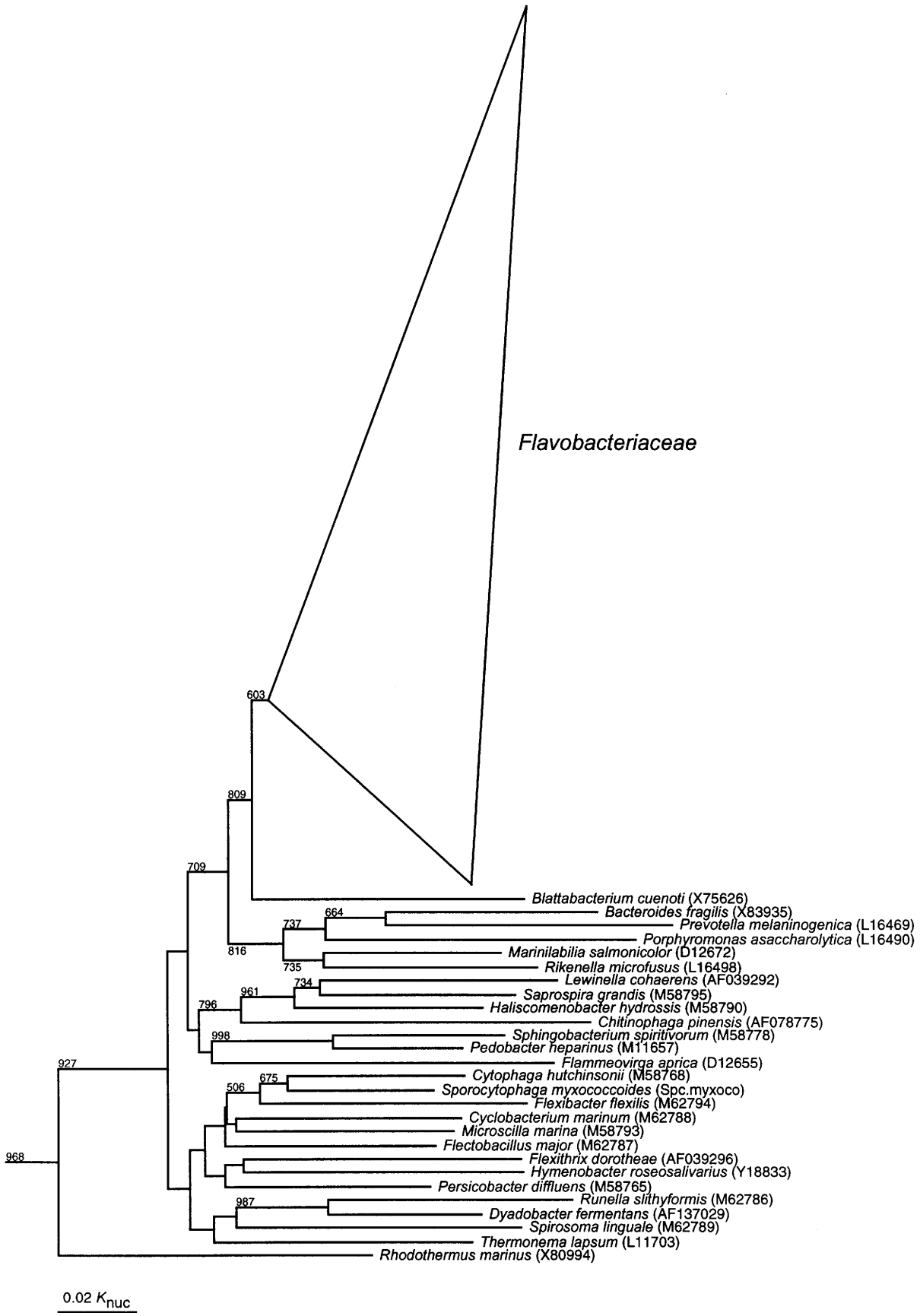

Fig. 1. For legend see facing page.

recently been shown to generate almost equivalent phylogenetic structures for the *Cytophaga–Flavobacterium–Bacteroides* phylum (Suzuki *et al*., 2001).

New isolates, or at least a selection of representative strains, should be preserved in the laboratory by freeze-drying, liquid drying, or by storage in liquid nitrogen or at −80 °C; two methods should preferably be used. Several procedures for the preservation of members of the family *Flavobacteriaceae* have been described (Holmes *et al*., 1984a; Reichenbach, 1989, 1992a; Holmes, 1992; Ostland *et al*., 1994; Desolme & Bernardet, 1996).

A type species must be designated when a new genus is described, and a type strain must be selected for every novel species. According to Recommendation 30a of the *Bacteriological Code* (1990 Revision) (Lapage *et al*., 1992), all type strains should be deposited in a recognized culture collection; this Recommendation has been revised (i) to *require* that this deposition occurs; (ii) and that it occurs *before* the validation of the new taxa (Labeda, 1997). It is now required that type strains be deposited in at least two different recognized culture collections, preferably not in the same country (Labeda, 2000). It is also good practice to deposit a few additional representative strains of the new taxon and to store duplicate sets of preserved strains in two separate locations. Additional changes have been proposed for the Rule governing the use of 'patent strains' as type strains (Tindall, 1999).

All taxonomic methods included in the description of new taxa should be given in detail or references to the appropriate publications in English should be given. It has been suggested that 'phenotypic descriptions of strains of existing species used for comparisons to a proposed new species should be based on tests performed on those strains in the authors' own laboratories, rather than on published data, to assure comparability of results' (International Committee on Systematic Bacteriology, 1997). Discrepancies in the phenotypic properties of some species classified in the family *Flavobacteriaceae* have indeed been noticed when the same strains have been examined in different studies (denoted by '*v*' in Table 2), probably because different methods were used. Commercially available galleries such as API ZYM (Yabuuchi *et al*., 1983; Holmes *et al*., 1984b; Bernardet & Grimont, 1989; Hansen *et al*., 1992; Mudarris *et al*., 1994; Vandamme *et al*., 1994b, 1999; Bernardet *et al*., 1996; Vancanneyt *et al*., 1996, 1999), API 50CH (Bernardet, 1989b; Bernardet & Grimont, 1989); API 20E (McCammon *et al*., 1998), API 20NE (McCammon *et al*., 1998; Vancanneyt *et al*., 1996; Vancanneyt *et al*., 1999; Vandamme *et al*., 1999; J.-F. Bernardet, unpublished results), API ID 32E (Vancanneyt *et al*., 1999; Vandamme *et al*., 1999), Biotype 100 (Vancanneyt *et al*., 1996; J.-F. Bernardet, unpublished results) or Biolog GN MicroPlate (Vancanneyt *et al*., 1996; McCammon *et al*., 1998; Johansen *et al*., 1999) may be used. For instance, some *Chryseobacterium* species are included in the analytical profile index of API 20NE galleries and most strains are actually able to grow at the temperature recommended by the manufacturer (i.e. 30 °C). However, some other galleries have been devised for clinically significant organisms grown at 37 °C; hence results obtained with environmental, polar or fish isolates grown at lower temperatures must be carefully interpreted. Moreover, discrepancies may occur between the results of conventional tests and those of the corresponding tests included in galleries (see below).

The descriptions of new taxa should preferably be published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM); when published in another journal, a reprint should be submitted to the IJSEM so that the new taxon can be rapidly included in one of the Validation Lists that appear periodically in that journal as required by the *Bacteriological Code* (1990 Revision) (Lapage *et al*., 1992; *International Journal of Systematic Bacteriology*, 1992). If the scientists proposing a new taxon are not familiar with bacterial nomenclature or with the use of Latin for naming scientific taxa, they are strongly advised to read the specialized literature (Bousfield, 1993; MacAdoo, 1993; Buchanan, 1994; Trüper, 1996) or refer to a Latin scholar.

Some of the taxa that have been assigned to the family *Flavobacteriaceae* contain organisms that are rather fastidious (e.g. some animal pathogens and some capnophilic organisms) or occur in low numbers in the environment (e.g. some aquatic organisms). The description of such bacteria may thus require some flexibility in the application of the general principles stated above, as well as in the recommended minimal

Fig. 1. Phylogenetic position of the family *Flavobacteriaceae* in the *Cytophaga–Flavobacterium–Bacteroides* phylum based on 16S rRNA sequence comparisons using the neighbour-joining method (Saitou & Nei, 1987). Sequences are taken from the DDBJ and GenBank nucleotide databases, apart from the sequence of *Sporocytophaga myxococcoides* which comes from the Ribosomal Database Project (Maidak *et al*., 1999). The type species of all validly described genera in the phylum are represented by the sequence of their type strain. *Mitsuokella multacida* could not be included because the comparison of the available 16S rRNA sequence did not result in its allocation to the phylum. Accession numbers for the sequences are given in parentheses. Scale bar, 0·02 K_{nuc} (Kimura, 1980). The numbers on the branches represent the confidence limits (expressed as percentages rounded up to whole numbers) estimated by a bootstrap analysis (Felsenstein, 1985) of 1000 replicates; confidence limits less than 50% are not shown. Sequences were aligned using the CLUSTAL W version 1.8 software package (Thompson *et al*., 1994). The alignments were modified manually against the 16S rRNA secondary structure of *Escherichia coli* (Brosius *et al*., 1978). Positions at which the secondary structures varied in the strains (positions 66–104, 143–220, 447–487, 841–845, 991–1045, 1134–1140 and 1446–1456) and all sites which were not determined in any sequence were excluded from the analysis. The number of nucleotides compared was 831 bp. *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Escherichia coli* were used as outgroups.

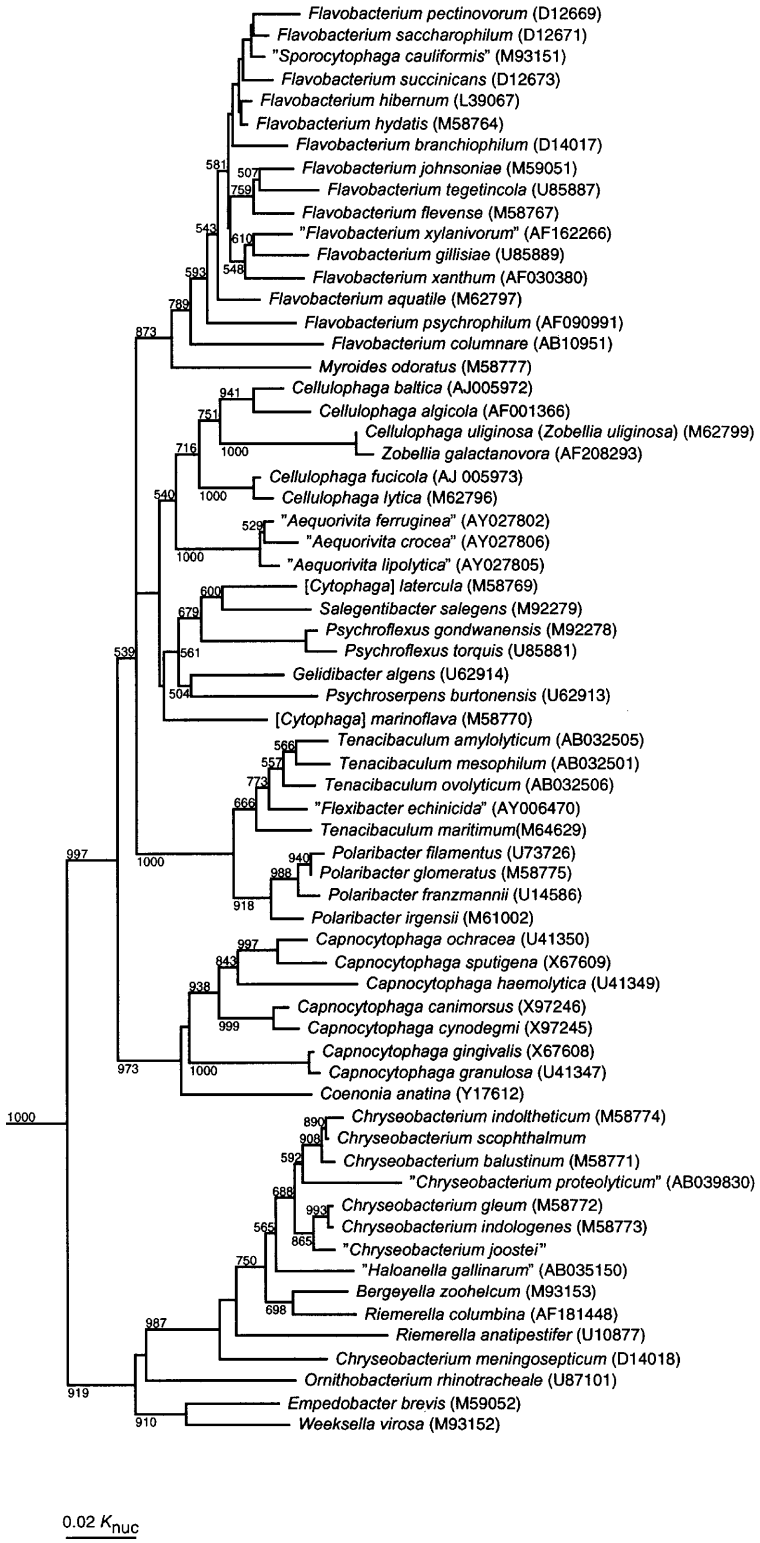

Fig. 2. Phylogenetic relationships among representatives of the family *Flavobacteriaceae* based on comparisons of 16S rRNA sequence. All species of *Flavobacteriaceae* are included (except *Myroides odoratimimus* for which no data are available) and represented by the sequence of their type strain (except *Empedobacter brevis*). Invalid taxa which 16S rRNA sequence is available have also been included for information; their names are in quotation marks. The 16S rRNA sequences of *Tenacibaculum* species were obtained from Makoto Suzuki, and those of *Chryseobacterium scophthalmum* and '*Chryseobacterium joostei*' from Paul Segers (personal communications). The number of nucleotides compared was 899 bp. *Agrobacterium tumefaciens*, *Bacillus subtilis*, and *Escherichia coli* were used as outgroups. Other details are given in the legend to Fig. 1.

features that should be included in descriptions of new taxa. The description of new taxa based on single strains is not encouraged as such taxa cannot take biological diversity into account; this in turn makes it difficult to decide whether some features are relevant to the description of the species or the genus. It is preferable to study at least two strains. However, when a single strain has been retrieved from an important source or has important biological properties, it does make sense to describe it as a new species though in such cases an extensive phenotypic, genomic and phylogenetic study should be undertaken to ensure

that the organism differs significantly from members of all related species.

General features of species classified in the family *Flavobacteriaceae*

An emended description of the family *Flavobacteriaceae* is needed due to the changes outlined above. The modifications and additions resulting from nomenclatural changes and from the descriptions of several additional taxa since the first description of the family (Bernardet *et al*., 1996) are in bold.

Emended description of the family *Flavobacteriaceae* Reichenbach 1989

Cells are short to moderately long rods with parallel or slightly irregular sides and rounded or slightly tapered ends. They are usually 0·3–0·6 μm wide and 1–10 μm long, though members of some species may form filamentous flexible cells **(e.g. *Flavobacterium* and *Tenacibaculum*) or coiled and helical cells (*Polaribacter*, *Psychroflexus* and *Psychroserpens* strains)** under certain growth conditions; **ring-shaped cells are not formed**. Cells in old cultures may form spherical or coccoid bodies **(e.g. *Flavobacterium*, *Gelidibacter*, *Psychroserpens* and *Tenacibaculum*)**. Gram-negative. Non-spore-forming. **Gas vesicles are produced in members of some *Polaribacter* species.** Flagellae **are usually** absent; **the only *Polaribacter irgensii* strain available is flagellated, but motility has not been observed in wet mounts.** Non-motile (*Bergeyella*, *Chryseobacterium*, ***Coenonia***, *Empedobacter*, ***Myroides***, *Ornithobacterium*, ***Polaribacter*, *Psychroserpens***, *Riemerella*, ***Salegentibacter*** and *Weeksella* strains, and ***Psychroflexus gondwanensis*** strains) or motile by gliding (*Capnocytophaga*, ***Cellulophaga***, ***Gelidibacter***, *Flavobacterium*, ***Tenacibaculum*** **and *Zobellia* strains**, and ***Psychroflexus torquis* strains**).

Growth is aerobic (*Bergeyella*, ***Cellulophaga***, *Chryseobacterium*, *Empedobacter*, *Flavobacterium*, ***Gelidibacter***, ***Myroides***, ***Polaribacter***, ***Psychroflexus***, ***Psychroserpens***, ***Salegentibacter***, ***Tenacibaculum***, *Weeksella* **and *Zobellia*** strains) or microaerobic to anaerobic (*Capnocytophaga*, ***Coenonia***, *Ornithobacterium* and *Riemerella* strains). The optimum temperature is usually in the range 25–35 °C, **but members of some species or genera are psychrophilic or psychrotolerant (*Flavobacterium psychrophilum* and the Antarctic *Flavobacterium* species, as well as *Gelidibacter*, *Polaribacter*, *Psychroflexus*, *Psychroserpens* and *Salegentibacter* strains). Members of some taxa are halophilic to varying degrees (*Cellulophaga*, *Gelidibacter*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Salegentibacter*, *Tenacibaculum*** and ***Zobellia* strains).**

Colonies are non-pigmented (*Bergeyella*, ***Coenonia***, *Ornithobacterium* and *Weeksella* strains) or pigmented by carotenoid or flexirubin pigments or both (*Capnocytophaga*, ***Cellulophaga***, *Chryseobacterium*, *Empedobacter*, *Flavobacterium*, ***Gelidibacter***, ***Myroides***, ***Polaribacter***, ***Psychroflexus***, ***Psychroserpens***, *Riemerella*, ***Salegentibacter***, ***Tenacibaculum*** and ***Zobellia*** strains).

Menaquinone 6 is either the only respiratory quinone or the major respiratory quinone. Chemo-organotrophic. Intracellular granules of poly-β-hydroxybutyrate are absent. Sphingophospholipids are absent. Homospermidine is the major polyamine though agmatine, cadaverine and putrescine are frequently present as minor components. **Crystalline** cellulose **(i.e. filter paper)** is not decomposed. The DNA base composition ranges from **27 to 44** mol% G+C.

Mostly saprophytic in terrestrial and aquatic habitats. Several members of the family are commonly isolated from diseased humans or animals, some species are considered true pathogens. The type genus is *Flavobacterium* Bergey, Harrison, Breed, Hammer and Huntoon 1923, as emended in 1996 (Bernardet *et al*., 1996).

Other taxa included in the family *Flavobacteriaceae* are the genera *Bergeyella*, *Capnocytophaga*, ***Cellulophaga***, *Chryseobacterium*, ***Coenonia***, *Empedobacter*, ***Gelidibacter***, ***Myroides***, *Ornithobacterium*, ***Polaribacter***, ***Psychroflexus***, ***Psychroserpens***, *Riemerella*, ***Salegentibacter***, ***Tenacibaculum***, *Weeksella* **and *Zobellia*. Several species unaffiliated to any genus also belong to the family. Several intracellular symbionts of insects and intracellular parasites of amoebae are closely related to the family.**

Among the properties listed above, some may be considered particularly important because they allow a clear differentiation between members of the family *Flavobacteriaceae* and those of other families in the *Cytophaga–Flavobacterium–Bacteroides* phylum. These properties should be investigated if the necessary equipment and knowledge are available (Suzuki *et al*., 1993). The presence of sphingophospholipids, for instance, characterizes members of the family *Sphingobacteriaceae*. In addition, all current members of the family *Flavobacteriaceae* exhibit menaquinone 6 as their only or major respiratory quinone (Bernardet *et al*., 1996), whereas menaquinone 7 is present in members of all related families and taxa that have been tested (Hanzawa *et al*., 1995).

Some species in the family *Flavobacteriaceae* degrade soluble cellulose derivatives such as carboxymethylcellulose or hydroxyethylcellulose but, since these compounds may be degraded by enzymes other than cellulases, this does not demonstrate that these species are cellulolytic. The decomposition of crystalline cellulose (i.e. filter paper) requires the production of a specific cellulase, hence only strains able to degrade filter paper should be regarded as cellulose degraders (Reichenbach, 1989). The inability to degrade crystalline cellulose has been confirmed in members of most taxa included in the family *Flavobacteriaceae* (Bernardet, 1989a; Reichenbach, 1989, 1992a; J.-F. Bernardet, unpublished) including members of the

Table 2. Differential characteristics of taxa classified in the family *Flavobacteriaceae*

Bergeyella zoohelcum; 2, *Capnocytophaga*; 3, *Cellulophaga*; 4, *Chryseobacterium*; 5, *Coenonia anatina*; 6, *Empedobacter brevis*; 7, *Flavobacterium*; 8, *Gelidibacter algens*; 9, *Myroides*; 10, *Ornithobacterium rhinotracheale*; 11, *Polaribacter*; 12, *Psychroflexus*; 13, *Psychroserpens burtonensis*; 14, *Riemerella*; 15, *Salegentibacter*; 16, *Tenacibaculum*; 17, *Weeksella virosa*; 18, *Zobellia*. Habitat: P, parasitic; S, saprophytic; FL, free-living; (me), marine environment. Data taken from Barbeyron *et al.* (2001), Bernardet (1989a), Bernardet & Grimont (1989), J.-F. Bernardet (unpublished results), Bowman *et al.* (1997, 1998), Bowman (2000), J. P. Bowman (personal communication), Bruun & Ursing (1987), Dees *et al.* (1986), Dobson *et al.* (1993), Gosink *et al.* (1998), Hansen *et al.* (1992), Holmes (1992), Holmes *et al.* (1977, 1978, 1984a, 1986a, 1986b), Hugo (1997), Johansen *et al.* (1999), Lewin & Lounsbery (1969), London *et al.* (1985), McCammon & Bowman (2000), McGuire *et al.* (1987), Ostland *et al.* (1994), Oyaizu & Komagata (1981), Reichenbach (1989), Segers *et al.* (1993a), Suzuki *et al.* (2001), Ursing & Bruun (1991), Vancanneyt *et al.* (1999), Vandamme *et al.* (1994a, 1994b, 1999), Yabuuchi *et al.* (1983), Yamaguchi & Yokoe (2000) and Yamamoto *et al.* (1994). Additional phenotypic characteristics that differentiate (a) the *Capnocytophaga* species, *Coenonia anatina*, *Ornithobacterium rhinotracheale* and *Riemerella anatipestifer* are described in Vandamme *et al.* (1994b, 1999), (b) the genera *Cellulophaga*, *Gelidibacter*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Salegentibacter*, *Tenacibaculum*, *Zobellia*, and other related halophilic organisms are described in Bowman *et al.* (1997, 1998), Gosink *et al.* (1998), Johansen *et al.* (1999), McCammon & Bowman (2000), Barbeyron *et al.* (2001) and Suzuki *et al.* (2001), (c) the genera *Bergeyella*, *Chryseobacterium*, *Empedobacter*, *Flavobacterium*, *Myroides* and *Weeksella* are described in Bernardet *et al.* (1996). [*Cytophaga*] *latercula* and [*Cytophaga*] *marinoflava* are not included in this table; their phenotypic characteristics are described by Lewin (1969) and Colwell *et al.* (1966), respectively, as well as by Reichenbach (1989), Dobson *et al.* (1993) and Bernardet *et al.* (1996). +, Positive reaction; –, negative reaction; (+), weak positive reaction; v, varies within and/or between species; v, varies between references; ND, not determined or determined for some species only. The type of yellow pigment (when determined) is indicated by a F (flexirubin-type pigment) or a C (carotenoid type pigment). A greyish-white to beige pigment is produced by *Riemerella anatina* strains on some solid media.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Habitat	P or S	P or S	FL (me) or S	FL, S or P	P	FL or S	FL or S	FL (me) or S	FL or S	P	FL (me) or S	FL (me) or S	FL (me) or S	P	FL (me) or S	P or S	P or S	FL (me) or S
Pigment production	–	+ F[a]	+ F and/or C	+ F[b]	–	(+) F	+ F and/or C	+ C	+ F	–	+ C[c]	+ C	+ C	–/(+)	+ C	+/(+) C	–	+ F
Gliding motility	–	+	+	–	–	–	11[d]	+	–	–	–	v	–	–	–	+	–	+
Sea water requirement	–	–	v	–	–	–	–	+	–	–	+[e]	v	+	–	–	v	–	+
Capnophilic metabolism	–	+	–	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–
Growth at:																		
25 °C	+	ND	+	+	(+)	+	13[d]	v	+	–	–	v	–	v	+	+	+	+
37 °C	+	+	v	v	+	+[e]	–	–	+	+	–	–	–	+	–	v	+	+
42 °C	–[f]	ND	–	v	–	–	–	–	–	+	–	–	–	+	–	–	+	+
Growth on:																		
MacConkey agar	–	–	ND	v[g]	–	+	ND	–	+	–	ND	–[h]	–	–	ND	ND	+	ND
β-Hydroxybutyrate	–	ND	ND	+[i]	ND	+	ND	–	+	ND	ND	–[h]	–	ND	ND	ND	+	ND
Acid production from:																		
Glucose	–	+	v	+[j]	+	+[e]	v	+	–	v	+	v	–	+	v	ND	–	+
Sucrose	–	+[k]	v	v	–	–	v	–	–	–	v	–[h]	–	–	v	ND	–	+
Production of:																		
Dnase	–	ND	v	+	ND	+	v	+	+	–	ND	+	–	ND	+	+	–	+
Urease	+	v	v	v	–	v	v	–	+	+	–	v	–	v	v	ND	–	–
Catalase	+	v	+	+	+	+	+/(+)	+	+	–	+/(+)	+	+	+	+	+	+	+
Indole	+	–	ND	+[j]	–	+	–	–	–	–	–	–[h]	–	v	ND	ND	+	+
β-Galactosidase	–	v	ND	v	+	–	v	–	–	+	v	–	v	–	+	ND	–	+
Nitrate reduction	–	v	v	v	–	–	v	–	–	–	–	–	–	–	+	v	–	+
Carbohydrate utilization	–	v	ND	+	+	v	v	+	–	+	+	+	–	+	+	ND	–	+

Table 2 (*cont.*)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Degradation of:																		
Agar	–	–	+	–	–	–	v	–	–	–	–	–	–	–	–	–	–	+
Starch	–	v	+	v	ND	v	11[d]	+	–	ND	+/(+)	+	–	ND	+	v	–	v
Aesculin	–	v	ND	+	+	–	10[d]	+	–	–	v	v	–	v	+	–	–	+
Gelatin	+	v	v	+	–	+	11[d]	v	+	–	v	v	v	+	+	+	+	+
Resistance to penicillin G	–	–	ND	+[i]	ND	+	v	ND	ND	v	ND	ND	ND	–	ND	–[l]	–	ND
G+C content (mol%)	35–37	36–44	32–42	33–38	35–36	31–33	32–37	36–38	30–38	37–39	31–34	32–39	27–29	29–37	37–38	30–32	37–38	42–43

a, Type of pigment in *Capnocytophaga gingivalis*. Not determined in the six other *Capnocytophaga* species.

b, *Chryseobacterium meningosepticum* strains are either not pigmented or produce a weak yellow pigment (e.g. the type strain). Members of all other *Chryseobacterium* species produce a bright yellow to orange pigment.

c, Type of pigment in *Polaribacter glomeratus*. Not determined in the three other *Polaribacter* species.

d, Number of species positive for this characteristic among the 14 valid *Flavobacterium* species. Only specified when most (i.e. 10 or more) species are positive.

e, Most strains are positive for this characteristic.

f, Most strains are negative for this characteristic.

g, Strain dependent for *Chryseobacterium indologenes*. Positive for all other *Chryseobacterium* species except *Chryseobacterium scophthalmum* and '*Chryseobacterium proteolyticum*'.

h, Negative for *Psychroflexus torquis* (Bowman, personal communication), not determined for *Psychroflexus gondwanensis*.

i, Not determined for '*Chryseobacterium proteolyticum*', positive for all other *Chryseobacterium* species.

j, Positive for all *Chryseobacterium* species, except *Chryseobacterium scophthalmum*.

k, Positive for all *Capnocytophaga* species, except *Capnocytophaga canimorsus*.

l, Negative for *Tenacibaculum maritimum* (Burchard, 1999). Not determined in the three other *Tenacibaculum* species.

recently described genera *Cellulophaga*, *Gelidibacter*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Salegentibacter* and *Zobellia* (Gosink *et al*., 1998; Barbeyron *et al*., 2001; J. P. Bowman, personal communication; P. Nielsen, personal communication) and members of the recently described *Flavobacterium* species (J. P. Bowman, personal communication). This characteristic distinguishes members of the family from those of the genus *Cytophaga*, now restricted to cellulolytic organisms (Nakagawa & Yamasato, 1996). It is essential (i) that members of new taxa in the family *Flavobacteriaceae* be tested for their ability to degrade filter paper on both a nutrient-containing agar (presence of a cellulase) and on a mineral agar (ability to use cellulose as only carbon source) (Reichenbach, 1992a) and (ii) that the cellulose derivatives that are used be specified.

Minimal standards for the description of genera classified in the family *Flavobacteriaceae*

The main differential characteristics for separating the genera classified in the family *Flavobacteriaceae* are listed in Table 2, apart from the phylogenetic data (see above) and the properties included in the description of the family. The tests used to determine these characteristics should be considered as the minimal standards for delineation of new genera. The conditions in which the tests are performed are critical for the reliability and reproducibility of the results, hence the recommended procedures described below should be followed.

Determination of pigments. Members of most genera classified in the family *Flavobacteriaceae* produce light to bright yellow or orange pigments though non-pigmented taxa (*Bergeyella zoohelcum*, *Coenonia anatina*, *Ornithobacterium rhinotracheale* and *Weeksella virosa*) and strains (e.g. some *Chryseobacterium meningosepticum* strains, including the type strain) do occur. These pigments may belong to the carotenoid or to the flexirubin types depending on the genus. However, the genus *Flavobacterium* includes carotenoid-producing and flexirubin-producing species, as well as species that produce both types of pigment (Reichenbach, 1989; Bernardet *et al*., 1996). Carotenoid pigments are usually produced by members of marine species while flexirubin pigments are more frequently associated with clinical, freshwater or soil organisms (Reichenbach, 1989). Pigments are usually non-diffusible, except the beige pigment produced by members of *Riemerella* species on some solid media (e.g. trypcase-soy agar) (J.-F. Bernardet, unpublished results).

Chromatography and spectrophotometry are the most accurate techniques for determining the type of pigment produced by a bacterial strain (Weeks, 1981) but, when it is not possible to use these approaches, a very simple test may be performed: colonies having a flexirubin type of pigment exhibit an immediate colour shift from yellow or orange to red, purple or brown when flooded with 20% KOH, and revert to their initial colour when flooded by an acidic solution once the excess of KOH has been removed (Reichenbach, 1989). It is strongly recommended that the test be performed on a small mass of bacterial cells collected with a loop and deposited on a glass slide placed on a white background as the colour-shift may pass unnoticed when the KOH solution is poured directly over a thin colony on an agar plate (J.-F. Bernardet, unpublished results). If possible, a second similar mass of bacteria should be deposited on the slide so that one of the preparations can be flooded with KOH; the resulting colour may be then compared with the initial colour of the other mass. This colour change is not absolutely specific for the flexirubin type of pigment (Fautz & Reichenbach, 1980), but it is still helpful when combined with the results of other tests.

Gliding motility. This type of bacterial motility is not restricted to the *Cytophaga–Flavobacterium–Bacteroides* phylum (Burchard, 1981; Reichenbach, 1989). However, this property is important for differentiating between genera classified in the family *Flavobacteriaceae*, though some precautions need to be taken as its unequivocal recognition is not always easy. Gliding motility is highly dependent on growth conditions such as the temperature (McGrath *et al*., 1990) and the concentration of nutrients in the growth medium; the latter should preferably be low (Reichenbach & Dworkin, 1981). When gliding is strongly suspected but is not readily observed by microscopic examination of a drop of liquid culture with a conventionally used cover slip, it should be tested using the hanging drop technique: the cover slip on which the drop has been deposited should be turned upside down and placed on tiny stands on a glass slide; bacteria are then observed through the cover slip. Gliding must be checked on the edge of the hanging drop and at the bottom surface of the cover slip as this phenomenon is exclusively exhibited by bacteria in contact with a solid surface. The movements involved in gliding motility have been well described elsewhere (Reichenbach & Dworkin, 1981; Reichenbach, 1989). When gliding motility is very slow and hardly noticeable, it may be detected by comparing the position of bacteria in the same area at an interval of several minutes. Gliding can usually be suspected from the more or less rhizoidal aspect of the edge of the colonies (provided the agar is not too dry) but it cannot usually be detected in bacteria collected on agar and suspended in saline. Direct microscopic examination of the edge of a young colony on an open agar plate (at the highest magnification possible without using immersion oil) may reveal either gliding itself or the slime tracks left on agar by gliding bacteria (Burchard, 1981; Reichenbach, 1992a; J.-F. Bernardet, unpublished results). Chamber culture may also be used (Reichenbach & Dworkin, 1981). The use of phase-contrast microscopy is always preferable to visualize gliding motility, but this technique may be difficult to apply on agar plates and on hanging drops.

Salinity requirement. The salinity requirement of members of a potentially new taxon is an important property to be investigated since several genera included in the family *Flavobacteriaceae* are composed of strains retrieved from sea water, ice or sediments, marine fish, beach mud, marine algae, hypersaline lakes or lakes with salinity similar to that of sea water (see Table 1) (Bowman *et al*., 1997, 1998; Gosink *et al*., 1998; Barbeyron *et al*., 2001). It must be clearly shown whether isolates merely tolerate sea water (e.g. members of all *Chryseobacterium* species are able to grow on marine agar although only members of two of them were actually isolated from marine environments; J.-F. Bernardet, unpublished results) or if they really require a high salinity. In the latter case, it is necessary to determine whether they have a requirement for artificial or natural sea water (pure or diluted) or if the mere addition of NaCl to standard media facilitates bacterial growth. The salinity range and the optimal salinity should be determined when NaCl is sufficient for growth.

Capnophilic metabolism. Members of most genera assigned to the family *Flavobacteriaceae* are composed of aerobic organisms. However, *Capnocytophaga*, *Coenonia*, *Ornithobacterium* and *Riemerella* strains exhibit various levels of capnophilic metabolism. Primary isolation and initial *in vitro* growth should be performed on blood agar plates incubated in microaerobic conditions [i.e. a CO_2-enriched (5–10 %) and O_2-depleted (5–10 %) atmosphere or a moisture-saturated atmosphere of 5 % O_2, 10 % CO_2 and 85 % N_2 in a commercial gas-generation system] as these conditions provide the highest isolation rates and optimal growth (K.-H. Hinz, personal communication). After several subcultivations, some strains may be adapted to grow under aerobic conditions, although growth is always significantly better under microaerobic conditions (Segers *et al*., 1993a; Vandamme *et al*., 1994b, 1996b, 1999; Vancanneyt *et al*., 1999). Growth should be tested under both conditions. Growth is usually very poor or absent under strict anaerobic conditions.

Determination of fatty acid methyl esters. When the necessary equipment [i.e. gas-liquid chromatography (Vauterin *et al*., 1991; Suzuki *et al*., 1993)] and experience are available, this technique provides high quality taxonomic information, mainly at the generic and specific levels (Vandamme *et al*., 1996a). The predominant fatty acids found in members of the family *Flavobacteriaceae* are usually characteristic of genera though some fatty acid profiles help to differentiate species (Vandamme *et al*., 1994b, 1996b; Bernardet *et al*., 1996; Vancanneyt *et al*., 1996; Bowman *et al*., 1997, 1998; Gosink *et al*., 1998; Barbeyron *et al*., 2001). Comparison between fatty acid profiles yielded by different experiments is only possible when culture conditions are standardized as these conditions may markedly affect fatty acid composition (McGrath *et al*., 1990).

Determination of DNA base composition. Determination of the base composition of bacterial DNA is important for the description of new species since the range of G+C content of genera classified in the family *Flavobacteriaceae* is relatively wide. Members of most genera can be assigned to one of three groups according to their G+C content: very low (approx. 27–32 mol %), intermediate (approx. 33–38 mol %) and medium (approx. 39–44 mol %) (see Table 2). The procedures required for determinating G+C content have been described in detail in minimal standards recommended for other bacterial groups (Vincent Lévy-Frébault & Portaels, 1992). The DNA of a reference strain, such as the type strain of *Escherichia coli* (51 mol % G+C) should be included so that the G+C content of the tested strains can be expressed relative to the reference strain (Ursing *et al*., 1994). Species descriptions should include information on the G+C content of the proposed type strain, the range of G+C content for all strains tested, and the procedure used to acquire these results.

Minimal standards for the description of species classified in the family *Flavobacteriaceae*

Phenotypic characteristics. The description of a new species within the different genera classified in the family *Flavobacteriaceae* should be based on characteristics necessary for assigning the new taxon to the corresponding genus and on characteristics that serve to differentiate the new taxon from existing taxa of the genus. The main phenotypic properties used for differentiating between species are listed below. The recommended tests used to acquire these data form the minimal standards for descriptions of new species within the different genera and taxa. However, when different methods are available to test phenotypic characteristics, they have only rarely been compared on a given species and usually not on all members of a genus. For instance, it has been demonstrated that the presence of cytochrome oxidase in some *Flavobacterium* species was more readily evidenced using discs impregnated with dimethyl-*p*-phenylenediamine oxalate than using liquid tetramethyl-*p*-phenylenediamine dihydrochloride reagents (Koski *et al*., 1993; J.-F. Bernardet, unpublished results). However, this comparison has not been performed on all *Flavobacterium* species nor in other genera; consequently, the recommendation of the disc method cannot be extended to the whole genus, even less to other genera. A similar situation occurred when the production of *β*-galactosidase was tested in *Chryseobacterium* (Holmes *et al*., 1984b; Bruun & Ursing, 1987; J.-F. Bernardet, unpublished results) and *Coenonia* (Vandamme *et al*., 1999) species using different substrates (i.e. *o*-nitrophenyl-*β*-D-galactopyranoside on filter paper discs and in API 20E galleries, *p*-nitrophenyl-*β*-D-galactopyranoside in API 20NE and API ID 32E galleries, or 2-naphthyl-*β*-D-galactopyranoside in API ZYM galleries). Moreover, the members of the different genera have very different growth requirements (i.e. ionic content, temperature, composition of the media and atmosphere). For these reasons, the tests recom-

mended below for the different genera are mostly those successfully used by authors who have studied them extensively; the corresponding references are given in parentheses after the name of each taxon. When the only publication available is the original description of the genus, the methods used to determine its characteristics should preferably be followed to describe new taxa in this genus. When no particular method was specified, standardized, well-described tests and methods (e.g. West & Colwell, 1984; Barrow & Feltham, 1993; Gerhardt *et al*., 1994; Smibert & Krieg, 1994; Richard & Kiredjian, 1995) should preferably be followed. When G+C determinations clearly help to differentiate between species, this property should be added to the list of phenotypic characteristics.

Bergeyella zoohelcum (Holmes *et al*., 1986a, 1986b; Holmes, 1992). Members of this species were originally classified in the genus *Weeksella*, hence its differentiation from *W. virosa* relied on its inability to grow at 42 °C, on MacConkey agar, and on β-hydroxybutyrate, as well as on its urease activity on Christensen's medium (Christensen, 1946). These characteristics now differentiate the genera *Bergeyella* and *Weeksella* (see Table 2).

Capnocytophaga species (Socransky *et al*., 1979; Brenner *et al*., 1989; Holt & Kinder, 1989; Yamamoto *et al*., 1994). Hydrolysis of aesculin (e.g. Holdeman *et al*., 1977), dextran and gelatin; hydrolysis of starch (for example using the method of Barrow & Feltham, 1993) and urea (preferably on Christensen's medium; Christensen, 1946); β-galactosidase activity; acid production from lactose, melibiose and raffinose (e.g. Holdeman *et al*., 1977); production of catalase (with percentage of H_2O_2 solution and time of observation given) and cytochrome oxidase (e.g. Barrow & Feltham, 1993); β-haemolysis; ability to grow aerobically; presence of granular inclusions; nitrate reduction (e.g. Holdeman *et al*., 1977); and G+C content of DNA.

Cellulophaga species (Johansen *et al*., 1999; Bowman, 2000). Type of pigment and sea water requirement (see above); production of cytochrome oxidase; acid production from glucose and sucrose; DNase and urease activities; nitrate reduction; utilization of carbohydrates (for example using the Biolog GN MicroPlate method); degradation of alginic acid (2%, w/v, in a mineral medium; e.g. van der Meulen *et al*., 1974) and autoclaved yeast cells (tested on VY/2 substrate containing 20‰ sea salts, according to Reichenbach, 1989); degradation of casein, elastin, fibrinogen and gelatin (0·5, 0·1, 0·1 and 0·6%, w/v, respectively, added to Difco marine agar 2216 or to trypticase-soy agar containing 20‰ sea salts); and G+C content of DNA.

Chryseobacterium species (Yabuuchi *et al*., 1983, 1990; Holmes, 1992; Mudarris *et al*., 1994; Hugo, 1997; Yamaguchi & Yokoe, 2000). Growth on cetrimide and MacConkey agars and at 5, 37 and 42 °C; acid production from different sugars (preferably tested in ammonium salt medium; Barrow & Feltham, 1993); nitrate and nitrite reduction (for example using the method described by West & Colwell, 1984); production of L-phenylalanine deaminase (preferably by the technique of Richard & Kiredjian, 1995), urease and indole (preferably using a very dense suspension of bacteria in a urea-indole medium and Kovács' reagent; Richard & Kiredjian, 1995; Hugo, 1997), and H_2S (on commercial Kliger iron agar, according to Smibert & Krieg, 1984); formation of a precipitate on 10% egg yolk nutrient (Barrow & Feltham, 1993) or trypcase-soy agar; hydrolysis of starch and Tween 80 (for example using the methods of West & Colwell, 1984); hydrolysis of L-tyrosine on 0·5% L-tyrosine nutrient (Barrow & Feltham, 1993) or trypcase-soy agar; and β-galactosidase activity (preferably using commercial ONPG discs or API 20NE galleries). *Chryseobacterium indologenes* and *Chryseobacterium gleum* differ in their ability to degrade aesculin after 4 h (Yabuuchi *et al*., 1990).

Coenonia anatina. The properties considered characteristic of this taxon and the recommended tests used to acquire them are listed in the species description (Vandamme *et al*., 1999).

Empedobacter brevis. An extensive list of the characteristics of this taxon and of the recommended tests used to acquire them were given by Holmes *et al*. (1978).

Flavobacterium species (Holmes *et al*., 1984a; Reichenbach, 1989, 1992a; Bernardet & Grimont, 1989; Bernardet *et al*., 1996 and references therein; McCammon *et al*., 1998; McCammon & Bowman, 2000). Morphology of colonies on Anacker and Ordal's agar (Anacker & Ordal, 1955); adherence of colonies to the agar and Congo red adsorption (see below); growth on marine, nutrient and trypcase-soy agars and at 25 °C; presence of gliding motility and production of flexirubin type of pigments (see above); utilization of glucose as a sole carbon and energy source (for example using API 20NE galleries; McCammon *et al*., 1998); production of acid from carbohydrates aerobically, preferably tested in ammonium salt medium (e.g. Barrow & Feltham, 1993); degradation of aesculin (using a commercial aesculin agar), agar, alginate (e.g. West & Colwell, 1984), carboxymethylcellulose (for example using a 0·5% CMC overlay agar; McCammon *et al*., 1998), casein, chitin (for example using a 20% chitin overlay agar; Reichenbach & Dworkin, 1981), DNA (on any commercial DNA agar; Bernardet & Kerouault, 1989), gelatin, pectin (preferably according to Hildebrand, 1971), starch, L-tyrosine and urea; production of a brown diffusible pigment on L-tyrosine agar; formation of a precipitate on egg yolk agar (e.g. Barrow & Feltham, 1993); β-galactosidase activity (preferably using commercial ONPG filter paper discs); susceptibility to vibriostatic compound O/129; production of H_2S and cytochrome oxidase; and reduction of nitrate.

Gelidibacter algens. The properties considered characteristic of this taxon and the recommended tests used to acquire them were given in the species description (Bowman *et al*., 1997).

Myroides species (Vancanneyt *et al*., 1996). The two *Myroides* species are mostly differentiated by their profiles in Biotype 100 galleries and Biolog GN MicroPlate assays, and by the slightly different G+C content of their DNAs.

Ornithobacterium rhinotracheale. The properties considered characteristic of this taxon and the recommended tests used to acquire them were listed in the species description (Vandamme *et al*., 1994b).

Polaribacter species (Gosink *et al*., 1998). Cell morphology, coil formation and presence of gas vesicles; growth at 21 °C; hydrolysis of aesculin and gelatin (e.g. Smibert & Krieg, 1994); sea water requirement; utilization of DL-malate, L-glutamate, glycerol and *N*-acetyl-β-glucosamine as carbon sources; oxidation/fermentation of a variety of sugars; β-galactosidase activity; and absorbance wavelength of ethanolic extracts.

Psychroflexus species (Bowman *et al*., 1998). Production of filamentous cells longer than 100 μm; presence of gliding motility; optimal growth temperature and temperature range; optimal salinity and salinity range; yeast extract requirement; hydrolysis of aesculin, gelatin and urea; production of acid from various sugars and from glycerol; and G+C content of DNA.

Psychroserpens burtonensis. The properties considered characteristic of this taxon and the recommended tests used to acquire them were given in the species description (Bowman *et al*., 1997).

Riemerella species (Vancanneyt *et al*., 1999). The only phenotypic characteristics that clearly differentiate the two *Riemerella* species are pigment production on Columbia blood agar and aesculin hydrolysis.

Salegentibacter salegens. The properties considered characteristic of this taxon and the recommended tests used to acquire them were given in the original description of the species (Dobson *et al*., 1993) and by McCammon & Bowman (2000).

Tenacibaculum species (Wakabayashi *et al*., 1986; Hansen *et al*., 1992; Suzuki *et al*., 2001). Pigmentation of colonies and sea water requirement (see above and Suzuki *et al*., 2001); growth at 4, 30 and 37 °C; Congo red adsorption, adherence of colonies to the agar and production of high-viscosity extracellular polysaccharide in liquid culture (see below); growth in pellicle in liquid culture and utilization of sodium glutamate as a nitrogen source (Hansen *et al*., 1992); nitrate reduction and degradation of starch (preferably according to the methods of Smibert & Krieg, 1994); and production of ammonia (according to Lewin & Lounsbery, 1969).

Weeksella virosa, see *Bergeyella zoohelcum*.

Zobellia species (Barbeyron *et al*., 2001). Morphology and colour of colonies; degradation of starch; intensity of the degradation of carrageenans; and assimilation of various sugars in commercial galleries.

Experience is needed to successfully carry out some of the above-mentioned phenotypic tests. Provided identical growth conditions are used, colony morphology may be used to differentiate some species, for instance, in the genus *Flavobacterium* (Reichenbach, 1989; J.-F. Bernardet, unpublished results). Characteristics such as iridescent waves and rhizoid edges are best revealed under stereomicroscopic examination (×20) through oblique transmitted light (Bernardet, 1989b; Bernardet & Kerouault, 1989). However, some strains may exhibit different colony types on the same agar plate (e.g. *Flavobacterium columnare*, *Flavobacterium psychrophilum* and *Tenacibaculum maritimum* strains) (Bernardet, 1989a, 1989b; Bernardet & Kerouault, 1989; J.-F. Bernardet, unpublished results). Strains should preferably be grown on relatively poor media, such as Anacker and Ordal's (Anacker & Ordal, 1955) to observe the typical swarming colonies exhibited by most gliding bacteria. Adherence of colonies to the agar is also a useful feature; it can be determined by trying to collect colonies on agar plates with a loop. Separating colonies from agar can be nearly impossible in some *Flavobacterium columnare* and *Cellulophaga/Zobellia uliginosa* strains, but adherence may be lost after several subcultures (J.-F. Bernardet, unpublished results). Colonies of members of some other species may exhibit a sticky or mucoid consistency; in such cases, the viscosity of liquid cultures is usually increased due to the production of slime (e.g. *Tenacibaculum maritimum*) (J.-F. Bernardet, unpublished results). Congo red adsorption is tested by directly flooding some colonies on the agar with a few drops of a 0·01% aqueous solution of the dye; after about 2 min, the dye is gently rinsed with water and the colour of these colonies compared to that of colonies which have not been flooded. In the case of *Flavobacterium columnare*, the Congo red-staining material has been shown to be an extracellular galactosamine glycan in the slime (Johnson & Chilton, 1966).

Genomic analyses. Relationships to neighbouring species should be determined by quantitative DNA–DNA hybridization (Owen & Pitcher, 1985). According to Wayne *et al*. (1987), 'The phylogenetic definition of a species generally would include strains with approximately 70% or greater DNA relatedness and with 5 °C or less ΔT_m. Both values must be considered'. With regard to the correlation with phenotypic properties, the authors insisted that they 'should agree with this definition and would be allowed to override the phylogenetic concept of species only in a few exceptional cases' and that 'a distinct genospecies that cannot be differentiated from another genospecies on the basis of any known phenotypic property not be named until they can be

differentiated by some phenotypic property' (Wayne *et al.*, 1987). This is important since DNA homology studies cannot be performed in all laboratories; it is thus necessary that the identification of new species be readily possible from a few phenotypic characteristics that can be tested outside specialized laboratories. Most DNA–DNA hybridization experiments performed on members of the species classified in the family *Flavobacteriaceae* have revealed that the 70% cut-off value proposed by Wayne *et al.* (1987) does make sense with respect to these taxa: strains belonging to the same species (according to phenotypic and chemotaxonomic features) share DNA relatedness well above 70% whereas DNA relatedness is distinctly below this value when strains belonging to different species (even within the same genus) are hybridized (Holmes *et al.*, 1986a; Bernardet & Grimont, 1989; Vandamme *et al.*, 1994b; Bowman *et al.*, 1997, 1998; McCammon *et al.*, 1998; Goris *et al.*, 1998; Vancanneyt *et al.*, 1999; McCammon & Bowman, 2000; Yamaguchi & Yokoe, 2000). The range of DNA relatedness between different strains of the same species may be rather wide, from approximately 70% to close to 100% (Holmes *et al.*, 1986b; Bernardet & Grimont, 1989; Wakabayashi *et al.*, 1989; Bernardet *et al.*, 1994; Vancanneyt *et al.*, 1996; Johanssen *et al.*, 1999). Some strains may even exhibit a DNA relatedness value lower than 70% with most other strains of the same species (Holmes *et al.*, 1986b; Wakabayashi *et al.*, 1989; Goris *et al.*, 1998; Vancanneyt *et al.*, 1996, 1999); in some of these cases, reciprocal hybridization experiments (Yamamoto *et al.*, 1994) or ΔT_m values well below 5 °C (Bernardet *et al.*, 1996) have shown that such strains nevertheless belong to the same species. Distinct hybridization groups have been delineated within some *Chryseobacterium* species and *Empedobacter brevis* (Ursing & Bruun, 1987, 1991) and within *Flavobacterium columnare*; in the latter case, these groups have been confirmed by 16S rDNA restriction patterns and sequence analysis (Triyanto & Wakabayashi, 1999). However, according to the second principle by Wayne *et al.* (1987) quoted above, these groups could only be considered genomic species or genomovars as no phenotypic characteristics were available to differentiate them.

The different quantitative techniques used to determine DNA relatedness are reliable (provided reciprocal values and relevant controls are included) but they yield different relative binding ratios; conversely, ΔT_m (i.e. the percentage divergence, which measures the thermal stability of DNA hybrids) is not influenced by the method used but it cannot be determined by using the spectrophotometric method (Grimont *et al.*, 1980). Microplate techniques are also available for DNA–DNA hybridization experiments (Ezaki *et al.*, 1989), but at best they are semiquantitative and may thus not be able to resolve close genomic relationships. Although several hybridization procedures have been applied specifically to members of the family *Flavobacteriaceae*, a comparison of the results yielded by different methods on the same group of strains is only possible in very few cases. DNA relatedness among strains belonging to several *Chryseobacterium* species has been determined spectrophotometrically by the initial renaturation rate method (De Ley *et al.*, 1970) by Mudarris *et al.* (1994) and Hugo *et al.* (1999); similar DNA relatedness values have been obtained by Bernardet (unpublished results) using the S1-nuclease method with adsorption of S1-resistant DNA onto diethylaminoethyl-cellulose filters (Popoff & Coynault, 1980). The DNA of strains belonging to several *Tenacibaculum* species has been hybridized using three different techniques: the initial renaturation method (Hansen *et al.*, 1992), an S1-nuclease method (Bernardet *et al.*, 1994; J.-F. Bernardet, unpublished results) and a microplate method (Suzuki *et al.*, 2001). All three techniques resulted in intraspecific DNA relatedness values higher than 85%. However, there were considerable discrepancies when the DNA of the type strains of *Tenacibaculum maritimum* and *Tenacibaculum ovolyticum* were hybridized: their DNA relatedness was below 6% by the S1-nuclease method, approximately 12–35% by the microplate method, and 43% by the initial renaturation method. The DNA relatedness between several members of the two *Myroides* species has been determined using a microplate hybridization method and the initial renaturation method; a good correlation between the two methods was found for both intraspecific and interspecific hybridization experiments, although DNA relatedness values from the microplate method were slightly higher than those from the initial renaturation method (Goris *et al.*, 1998). It is strongly recommended that the integrity of bacterial DNAs used in genomic studies be previously checked on an agarose gel as fragmented DNAs may yield aberrant G+C content and DNA relatedness values (Goris *et al.*, 1998).

Hybridization studies should include several strains of the newly proposed species, including the proposed type strain, as well as the type strains of all related species. It has been suggested that DNA homology studies also include 'those organisms not currently assigned specific status' (Graham *et al.*, 1991), because such studies could result in their assignation to a new species. DNA–DNA hybridization experiments are laborious, hence a preliminary polyphasic study (e.g. including 16S rRNA sequencing, classical phenotypic tests, protein and fatty acid profiles …) will help to select the test strains. However, with the exception of non-cultivable organisms (see above), 16S rRNA analysis should not be the only genomic method used for delineating new species. Although organisms that share less than 97% 16S rRNA sequence homology rarely display more than 60% DNA homology (Stackebrandt & Goebel, 1994, and references therein), exceptions exist (Harrington & On, 1999; Suzuki *et al.*, 2001). It is also well known that sequence homology values higher than 97% do not guarantee conspecificity, as shown in *Capnocytophaga* (Vandamme *et al.*, 1996b) and *Cellulophaga* (Bowman, 2000) strains.

Similar precautions should be taken when interpreting *gyrB* sequences, although the DNA relatedness values between *Tenacibaculum* species have been shown to be more distinctly correlated to the sequence similarity of *gyrB* than to that of 16S rRNA (Suzuki *et al*., 2001).

Whole-cell protein analysis. Several studies have revealed a correlation between high DNA homology and high similarity in whole-cell protein patterns obtained by SDS-PAGE (Vauterin *et al*., 1993; Vandamme *et al*., 1996a). Within the family *Flavobacteriaceae*, species of the genera *Capnocytophaga* (Vandamme *et al*., 1996b), *Myroides* (Vancanneyt *et al*., 1996) and *Riemerella* (Vancanneyt *et al*., 1999) can be readily differentiated by their protein profiles. This is particularly important for the genera *Myroides* and *Riemerella*, for which few characteristics are available for the differentiation of these taxa. However, for some other genera some species can be identified by their very typical protein profiles whereas some others exhibit intraspecific heterogeneity; this is, for instance, the case in the genus *Flavobacterium* (Bernardet *et al*., 1996). Consequently, whole-cell protein analysis cannot replace DNA homology in the definition of bacterial species. Moreover, in order to compare protein profiles accurately, highly standardized SDS-PAGE electrophoregrams must be scanned and numerically analysed by computer and compared to large databases.

As already mentioned above, the presence or amount of some fatty acids may also be of value for differentiating the species.

Relationship to the host. Some members of the family *Flavobacteriaceae* are commensal organisms or true or opportunistic pathogens. Members of certain species are aetiologic agents of diseases of humans, birds or fishes. When members of a new species are recovered from clinical or veterinary samples, data concerning their pathogenicity for the host should be provided, if available. In the case of possible animal pathogens, the disease should preferably be experimentally reproduced in order to demonstrate pathogenicity.

Recognition of subspecies

When a group of strains exhibits consistent phenotypic discrepancies with most other strains in the species but still shares a high DNA homology with them, it could be warranted subspecies rank. In order to avoid congesting bacterial nomenclature with unnecessary subspecies names, epithets such as biovar, pathovar or serovar should preferably be used when only limited information is available and pending further data.

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NOTE ADDED IN PROOF

After this manuscript was completed, the following taxa were added to the family *Flavobacteriaceae*: *Flavobacterium frigidarium* sp. nov. (Humphry *et al*., 2001), *Arenibacter* gen. nov., sp. nov. (Ivanova *et al*., 2001), *Muricauda ruestringensis* gen. nov., sp. nov. (Bruns *et al*., 2001), *Aequorivita* gen. nov. (including *Aequorivita antarctica* sp. nov., *Aequorivita lipolytica* sp. nov., *Aequorivita crocea* sp. nov. and *Aequorivita sublithincola* sp. nov.) (Bowman & Nichols, 2002) and *Gelidibacter mesophilus* sp. nov. (Macián *et al*., 2002).

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