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CHAPTER 6.4

The Genus *Flavobacterium*

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Introduction

The genus *Flavobacterium* Bergey, Harrison, Breed, Hammer, and Huntoon 1923, emend. Bernardet, Segers, Vancanneyt, Berthe, Kersters, and Vandamme 1996 (Bergey et al., 1923; Bernardet et al., 1996) is probably one of the best examples of the revolution brought to the classification of a bacterial taxon by the use of the phylogenetic techniques based on the comparison of 16S rRNA sequences. Of the seven species considered in the previous edition of *Bergey's Manual of Systematic Bacteriology* (Holmes et al., 1984), only the type species *F. aquatile* was finally retained while several other taxa, previously misclassified in other genera, were added to form an extensively emended genus *Flavobacterium* (Bernardet et al., 1996). Since then, the genus has considerably expanded owing to the description of many new species mostly originating from polar habitats. The organisms currently included in the genus *Flavobacterium* were distributed among different chapters in previous editions of *Bergey's Manual* and of *The Prokaryotes*. In *Bergey's Manual*, *Flavobacterium aquatile* was dealt with in the chapter "Genus *Flavobacterium*" (Holmes et al., 1984), while several other *Flavobacterium* species were considered (under other generic epithets) in the chapter "Order I. *Cytophagales*" (Reichenbach, 1989) in the second edition of *The Prokaryotes*. The latter species were also included in the chapter The Order Cytophagales in the second edition, while *F. aquatile* was excluded from the chapter The Genera *Flavobacterium*, *Sphingobacterium*, and *Weeksella* also from this Volume and not considered anywhere else! These chapters contain a wealth of information and are still well worth consulting.

The history and structure of the family Flavobacteriaceae, of which the genus *Flavobacterium* is the type genus, are presented in the chapter "An Introduction to the Family Flavobacteriaceae" in this Volume. The taxonomic and nomenclatural issues concerning the genus *Flavobacterium* are dealt with by the subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes. This subcommittee has issued minimal standards for the description of new taxa in the genus *Flavobacterium* and other genera in the family Flavobacteriaceae (Bernardet et al., 2002).

Few species in the genus *Flavobacterium* have been extensively studied and most of them are represented by one strain (i.e., the type species *F. antarcticum* as well as *F. aquatile*, *F. flevense*, *F. frigidarium*, *F. gillisiae*, *F. granuli*, *F. hydatis*, *F. omnivorum*, *F. pectinovorum*, *F. saccharophilum*, *F. xanthum* and *F. xinjiangense*) or a limited number of strains in culture collections. Exceptions are the fish-pathogenic species, of which many strains are usually available; owing to their economic significance worldwide, they have been the subject of numerous studies (see the sections "Habitat and Ecology" and "Pathogenicity and Epidemiology" in this Chapter) and will provide many examples in this chapter.

Phylogeny

Sequences of 16S rRNA and DNA gyrase large subunit (*gyrB*) genes (Weisburg et al., 1985; Woese et al., 1990; Nakagawa and Yamasato, 1993; Suzuki et al., 2001) locate the genus *Flavobacterium* within the phylum "Bacteroidetes," clustering appropriately in a central position in the family Flavobacteriaceae (Bernardet et al., 1996) where it represents the type genus. Within the family Flavobacteriaceae, the genus *Flavobacterium* branches between two primary lineages, one consisting of mostly marine genera and the other of non-marine genera (see the chapters An Introduction to the Family Flavobacteriaceae in this Volume and The Marine Clade of the Family Flavobacteriaceae: The Genera *Aequorivita*, *Arenibacter*, *Cellulophaga*, *Croceibacter*, *Formosa*, *Gelidibacter*, *Gillisia*, *Maribacter*, *Mesonia*, *Muricauda*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Robiginitalea*, *Salegentibacter*, *Tenacibaculum*, *Ulvibacter*, *Vitellibacter* and *Zobellia* in this Volume). This

intermediate position corresponds well with the ecophysiology of the genus, which is relatively diverse (including species from freshwater and from marine ecosystems; see the section Habitat and Ecology in this Chapter). The deepest known branching members of the genus (*F. gelidilacus*, *F. columnare* and *F. aquatile*) are from freshwater ecosystems, suggesting that more halotolerant and marine-derived members of the genus (such as *F. flevense*, *F. gillisiae*, *F. frigidarium*, etc.) may have been distributed into and adapted to seawater/estuarine type environments in the past. Since practically no member of the genus is known to require Na^+ ions for growth, the adaptation to marine environments appears so far to be incomplete (see the section Physiology in this Chapter). Figure 1 presents the phylogenetic tree of the genus *Flavobacterium* based on 16S rRNA gene sequences.

Sequencing of the 16S rRNA gene has over time helped clarify the taxonomy of the genus *Flavobacterium* and is the means to definitively assign new strains within the genus. Phylogenetic data however indicate that several nomenclatural anomalies remain unresolved. Though many *Flavobacterium* species originally included in the older descriptions of the genus (Holmes et al., 1984) have been reclassified (Bernardet et al., 1996; see the section Taxonomy), the taxonomy of several validly described *Flavobacterium* species (J. P. Euzéby, List of Bacterial Names with Standing in Nomenclature) is yet to be officially corrected. For example, the species [*F.*] *devorans* and [*F.*] *yabuuchiae* are believed to be synonyms of *Sphingomonas paucimobilis* (Yabuuchi et al., 1979; Bauwens and De Ley, 1981) and *Sphingobacterium spiritovorum* (Takeuchi and Yokota, 1992), respectively. The species [*F.*] *mizutaii* and [*F.*] *ferrugineum* are clearly generically misclassified and are most closely related to the genera *Sphingobacterium* and *Chitinophaga*, respectively (Gherna and Woese, 1992; Nakagawa and Yamasato, 1993). No phylogenetic data are available for several species including [*F.*] *acidificum*, [*F.*] *acidurans*, [*F.*] *oceanosedimentum*, [*F.*] *resinovorum* or [*F.*] *thermophilum*. On the basis of DNA base composition data, all of these rather obscure species are misclassified and constitute species in completely separate bacterial phyla. For example, Holmes et al. (1984) considered [*F.*] *acidificum* to be a strain of "*Erwinia herbicola* subsp. *Ananas*" (now *Pantoea ananatis*), a member of the Gammaproteobacteria, while others such as [*F.*] *oceanosedimentum* are Gram-positives belonging to the *Bacillus* branch of the Firmicutes.

There are also discrepancies even with better-studied species. For example, sequences provided for the type strain of *F. psychrophilum* are quite different (Fig. 1). Substantial heterogeneity amongst strains classified as *F. columnare* and *F. johnsoniae* is also notable. However, in the case of *F. columnare*, this apparent heterogeneity is caused by a misidentification of the strain ATCC 43622, which more likely belongs to *F. johnsoniae*. The bona fide *F. columnare* strains are indeed grouped within a single cluster in the phylogenetic tree; the division of this cluster in three subclusters was already reported by Triyanto and Wakabayashi (1999), who based their description of three *F. columnare* genomovars on differences in 16S rRNA sequences, restriction fragment length polymorphism (RFLP), and DNA-DNA hybridization. A misidentification is not the explanation for the distance between the *F. johnsoniae* strain DSM 425 and the *F. johnsoniae* type strain, however, since their whole-cell protein and fatty acid profiles are closely related (J.-F. Bernardet, unpublished data). Some of these limitations have already been reported as early as 1984 by Reichenbach (1989) and this genetic diversity may also suggest the potential presence of additional *Flavobacterium* species amongst culture collection strains.

Taxonomy

The long and complex history of the genus *Flavobacterium* up to its emendation in 1996 has been presented in several publications (Holmes et al., 1984; Holmes et al., 1992; Bernardet et al., 1996; the chapter on The Genera *Flavobacterium*, *Sphingobacterium*, and *Weeksella* in this Volume) and will not be repeated here. As a result of an extensive polyphasic study (Bernardet et al., 1996), the genus *Flavobacterium* was shown to comprise the ten following valid species: *F. aquatile* (Holmes et al., 1984), *F. branchiophilum* (Wakabayashi et al., 1989), *F. columnare* (Bernardet and Grimont, 1989), *F. flevense* (Van der Meulen et al., 1974), *F. hydatis* (basonym, [*Cytophaga*] *aquatilis*; Strohl and Tait, 1978), *F. johnsoniae* (Reichenbach, 1989), *F. pectinovorum* (Reichenbach, 1989), *F. psychrophilum* (Bernardet and Grimont, 1989), *F. saccharophilum* (Agbo and Moss, 1979), and *F. succinicans* (Anderson and Ordal, 1961). (The references given here are the most relevant, although not necessarily the original descriptions.) This study also demonstrated that the invalid taxa "[*Cytophaga*] allerginae" (see the section Pathogenicity and Epidemiology in this Chapter), "[*Cytophaga*] *xantha*," "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*," "[*Promyxobacterium*] *flavum*," and "[*Sporocytophaga*] *cauliformis*" belong to the genus (Bernardet et

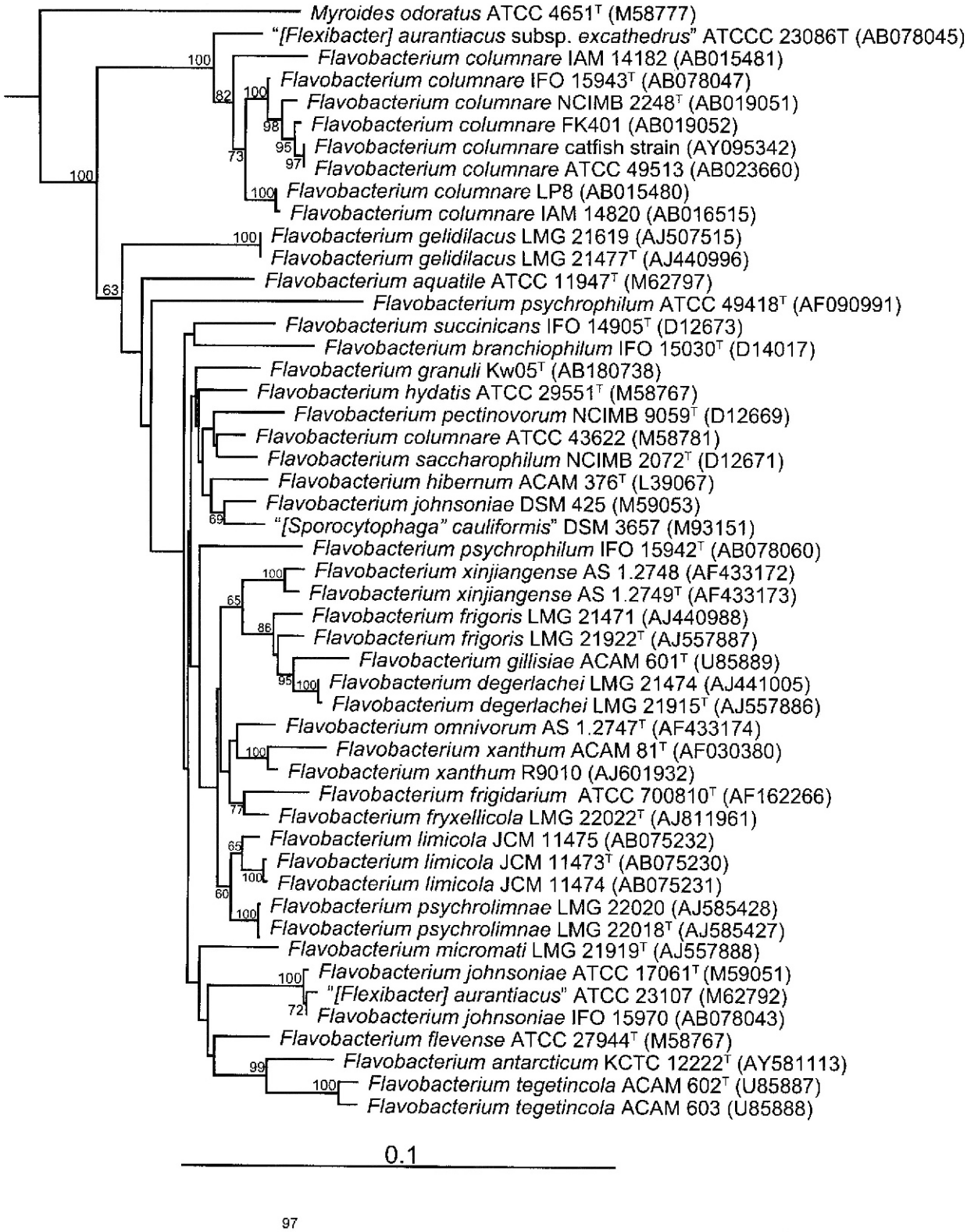

Fig. 1. A maximum likelihood distance phylogenetic tree created by the neighbor-joining procedure (Saitou and Nei, 1987) based on near complete 16S rRNA gene sequences of the genus *Flavobacterium*. The outgroup for the tree was *Myroides odoratus*. The bar indicates maximum likelihood distance.

al., 1996). Except the latter, these invalid and poorly studied taxa are each represented by a single strain. Since then, "[*Cytophaga*] *xantha*" has been validly published under the name *Flavobacterium xanthum* as a result of an extensive study of Antarctic organisms (McCammon and Bowman, 2000). The scant information available on the four remaining invalid taxa may be found in Reichenbach (1989). The determination of the 16S rRNA sequence of "[*Sporocytophaga*] *cauliformis*" and "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*" has confirmed their attribution to the genus *Flavobacterium* (Fig. 1).

Since the emendation of the genus *Flavobacterium*, members of the family Flavobacteriaceae have been shown to represent an important bacterial group in Antarctic environments (Bowman et al., 1997; Van Trappen et al., 2002; see the section Habitat and Ecology in this Chapter); however, the presence of gliding "cytophagas" (which would likely be classified as *Flavobacterium* nowadays) in polar lakes had been noticed long ago (Christensen, 1974). Subsequently, extensive studies of bacterial strains retrieved from various regions of Antarctica have resulted in the description of several new *Flavobacterium* species: *F. antarcticum* (Yi et al., 2005) and *F. fryxellicola* and *F. psychrolimnae* (Van Trappen et al., 2005), *F. hibernum* (McCammon et al., 1998), *F. gillisiae* and *F. tegetincola* (McCammon and Bowman, 2000), *F. frigidarium* (Humphry et al., 2001), *F. gelidilacus* (Van Trappen et al., 2003a), and *F. degerlachei*, *F. frigoris* and *F. micromati* (Van Trappen et al., 2003a). Not from Antarctica but still from cold environments came *F. limicola* (Tamaki et al., 2003), *F. omnivorum* and *F. xinjiangense* (Zhu et al., 2003). Hence, taking into consideration the previously described *F. psychrophilum*, sixteen among the twenty-six current valid *Flavobacterium* species are distinctly psychrophilic or psychrotolerant. While *F. flevense* was the only halophilic species of the ten known in 1996, nine of those published since then may be considered halophilic or halotolerant (see the sections Habitat and Ecology, Identification, and Physiology in this Chapter). Features of the valid *Flavobacterium* species and invalid taxa are listed in Table 1.

Many other organisms, validly published or not, were included in the genus *Flavobacterium* since it was created in 1923 (Bergey et al., 1923). Most of them were subsequently attributed to other or new genera (e.g., the genera *Cellulophaga*, *Chryseobacterium*, *Empedobacter*, *Myroides*, *Pedobacter*, *Psychroflexus*, *Salegentibacter*, *Sphingobacterium* and *Zobellia*; see the corresponding chapters The Genera *Flavobacterium*, *Sphingobacterium*, and *Weeksella* in the second edition; The Genus *Chryseobacterium* in this Volume; The Marine Clade of the Family Flavobacteriaceae: The Genera *Aequorivita*, *Arenibacter*, *Cellulophaga*, *Croceibacter*, *Formosa*, *Gelidibacter*, *Gillisia*, *Maribacter*, *Mesonia*, *Muricauda*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Robiginitalea*, *Salegentibacter*, *Tenacibaculum*, *Ulvibacter*, *Vitellibacter* and *Zobellia*; An Introduction to the Family Flavobacteriaceae; The Genera *Bergeyella* and *Weeksella*; and The Genera *Myroides* and *Empedobacter* all in this Volume) while others (for which no 16S rRNA sequences are available) still appear in the lists of *Flavobacterium* species pending further investigation. Complete lists of organisms currently belonging to the genus *Flavobacterium* or that were attributed to it for some time may be found in the chapter "Taxonomic Outlines of the Prokaryotes" of the second edition of Bergey's Manual (Garrity and Holt, 2001) and in the *List of Bacterial Names with Standing in Nomenclature* (Euzéby, 1997; List of Bacterial Names with Standing in Nomenclature). These lists also provide the current taxonomic status and standing in nomenclature of the taxa that were subsequently excluded from the genus *Flavobacterium*.

Habitat and Ecology

Habitats known to harbor *Flavobacterium* strains are listed in Table 2.

Members of the genus *Flavobacterium* are widely distributed in nature, occurring mostly in aquatic ecosystems ranging in salinity from freshwater to seawater. *Flavobacterium* species have never been isolated from hypersaline waters: the extremely halotolerant Antarctic species formally referred to as "[*F.*] *salegens*" and "[*F.*] *gondwanense*" are now *Salegentibacter* and *Psychroflexus*, respectively (Bowman et al., 1998; McCammon and Bowman, 2000), genera which are core members of the marine clade of the family Flavobacteriaceae. By comparison, *Flavobacterium* species have a distinct predilection to low salinity, cool to cold environments and are commonly isolated from polar lakes (Van Trappen et al., 2002) and from streams, rivers, lakes and muddy soils in other cold environments. Unidentified members of the genus were also reported from marine algae (Maeda et al., 1998; Adachi et al., 2002) and mangrove environments (Nakagawa et al., 2001), although none of them actually required seawater for growth. Most *Flavobacterium* species are psychrotolerant (rather than psychrophilic, as defined by Morita 1975) and grow well at 4°C and optimally at 20–30°C. Currently, *Flavobacterium* species have been successfully isolated from temperate to polar low salinity ecosystems which on a biogeographical level correlates well with their

cold-oriented ecophysiology. Various *Flavobacterium* spp. thus have biotechnological potential and ecological interest owing to their ability to produce cold-active enzymes. Some of these enzymes may even be implicated in pathogenicity such as in coldwater disease in fish caused by *F. psychrophilum*.

As a matter of fact, several freshwater species are (or potentially are) the etiological agents of fish diseases (see the section Pathogenicity and Epidemiology in this Chapter), including *F. columnare*, *F. branchiophilum*, *F. hydatis*, *F. johnsoniae*, *F. psychrophilum* and *F. succinicans*. Strains of these species have been isolated from external lesions and internal organs of salmon and other fish, but in some cases also from the surrounding water. The growth requirements of species such as *F. branchiophilum* and *F. psychrophilum* are rather fastidious (see the section Isolation and Cultivation in this Chapter), suggesting their primary habitat could be limited to the fish tissue environment where they exist as a parasite or saprophyte depending on the immunological state of the fish and the virulence of individual strains. Recent data suggest pathogens such as *F. psychrophilum* can survive for long periods in water, with survival enhanced by sediment-derived nutrients; a salinity of 6 however drastically reduces the number of culturable *F. psychrophilum* cells (Madetoja et al., 2003). Other species such as *F. succinicans* and *F. hydatis* are less exacting and thus may be simple

Table 1. Currently recognized species classified in the genus *Flavobacterium*.[a]

Species[b]	Type strain	G+C (mol%)	Source	Reference(s)	Number of strains
Flavobacterium antarcticum	JCM 12383	38	Soil, King George Island, Antarctica	Yi et al., 2005	1
Flavobacterium aquatile [T, AL, c]	ATCC 11947	33	Deep well, United Kingdom	Holmes et al., 1984; Bernardet et al., 1996	1
Flavobacterium branchiophilum [d]	ATCC 35035	34	Gills of salmon, Japan, 1977	Wakabayashi et al., 1989; Bernardet et al., 1996	16, 2, 30
Flavobacterium columnare [e]	NCIMB 2248	32	Kidney of salmon, United States, 1955	Bernardet and Grimont, 1989c; Bernardet et al., 1996	7, 5, 20
Flavobacterium degerlachei	LMG 21915	34	Microbial mats, Lake Ace, Vestfold Hills, Antarctica	Van Trappen et al., 2003b	14, 5, 14
Flavobacterium flevense [AL, f]	ATCC 27944	35	Freshwater lake, The Netherlands	Van der Meulen et al., 1974; Bernardet et al., 1996	1
Flavobacterium frigidarium [g]	ATCC 700810	35	Marine sediment, Adelaide Island, Antarctica	Humphry et al., 2001	1
Flavobacterium frigoris	LMG 21922	34	Microbial mats, Watts Lake, Vestfold Hills, Antarctica	Van Trappen et al., 2003b	19, 5, 19
Flavobacterium fryxellicola	LMG22022	35	Microbial mats, Lake Fryxell, Dry Valley, Antarctica	Van Trappen et al., 2005	3, 3, 3
Flavobacterium gelidilacus	LMG 21477	30	Microbial mats, freshwater and saline lakes, Antarctica	Van Trappen et al., 2003a	22, 5, 22
Flavobacterium gillisiae	ACAM 601	32	Sea ice, Prydz Bay, Antarctica	McCammon and Bowman, 2000	1
Flavobacterium granuli	IAM15099	36	Wastewater treatment plant, beer brewery, South Korea	Askan et al., 2005	1
Flavobacterium hibernum [h]	ACAM 376	34	Freshwater lake, Antarctica	McCammon et al., 1998	2, 2, 2
Flavobacterium hydatis [AL, j]	ATCC 29551	34	Gills of salmon, United States, 1974	Strohl and Tait, 1978; Bernardet et al., 1996	5, 1, 5
Flavobacterium johnsoniae [AL, j]	ATCC 17061	35	Soil or mud, United Kingdom	Reichenbach, 1989; Bernardet et al., 1996	5, 11, 30
Flavobacterium limicola	JCM 11473	34	River sediment, Ibaraki Prefecture, Japan	Tamaki et al., 2003	3, 3, 3
Flavobacterium micromati	LMG 21919	33	Microbial mats, Grace Lake, Vestfold Hills, Antarctica	Van Trappen et al., 2003b	3, 3, 3
Flavobacterium omnivorum	AS 1.2747	35	Frozen soil, China No. 1 glacier, Xinjiang, China, 1999	Zhu et al., 2003	1
Flavobacterium pectinovorum [k]	NCIMB 9059	35	Soil, United Kingdom	Reichenbach, 1989; Bernardet et al., 1996	8, 1, 8
Flavobacterium psychrolimnae	LMG22018	35	Microbial mats, Lake Fryxell, Dry Valleys, Antarctica	Van Trappen et al., 2005	4, 4, 4

Table 1. *Continued*

Species[b]	Type strain	G+C (mol%)	Source	Reference(s)	Number of strains
Flavobacterium psychrophilum [l]	NCIMB 1947	33	Kidney of salmon, United States, 1955	Bernardet and Grimont, 1989c; Bernardet et al., 1996	7, 6, 200
Flavobacterium saccharophilum [m]	NCIMB 2072	36	River Wey, United Kingdom, 1976	Agbo and Moss, 1979; Reichenbach, 1989; Bernardet et al., 1996	4, 1, 4
Flavobacterium succinicans [n]	DSM 4002	37	Fin of salmon, United States, 1954	Anderson and Ordal, 1961; Reichenbach, 1989; Bernardet et al., 1996	3, 3, 3
Flavobacterium tegetincola	ACAM 602	34	Cyanobacterial mat, marine salinity lake, Antarctica	McCammon and Bowman, 2000	2, 1, 2
Flavobacterium xinjiangense	AS 1.2749	34	Frozen soil, China No. 1 glacier, Xinjiang, China, 1999	Zhu et al., 2003	1
Flavobacterium xanthum [o]	IAM 12026	36	Soil, Showa station, Antarctica, 1967	Inoue and Komagata, 1976; Reichenbach, 1989; McCammon and Bowman, 2000	1
"[*Cytophaga*] *allerginae*"	ATCC 35408	34	Water in air-cooling unit, Florida	Liebert et al., 1984; Reichenbach, 1989; Bernardet et al., 1996	1
"[*Flexibacter*] *aurantiacus* subsp. *excathedrus*"	ATCC 23086	33	Pool in cathedral, Cartago, Costa Rica	Lewin, 1969; Reichenbach, 1989; Bernardet et al., 1996	1
"[*Promyxobacterium*] *flavum*"	DSM 3577	35	Rhizosphere of tomato plant, Russia	Reichenbach, 1989; Bernardet et al., 1996	1
"[*Sporocytophaga*] *cauliformis*"	NCIMB 9488	36	Water, Lake Constance, Germany	Gräf, 1962 Reichenbach, 1989; Bernardet et al., 1996	2, 2, 2

Abbreviations: [T], type species; [AL], the species is cited on the Approved Lists of Bacterial Names (Skerman et al., 1980; Moore et al., 1985); ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Tasmania, Australia; AS, culture collection of the Institute of Microbiology, Academia Sinica, Beijing, China; ATCC, American Type Culture Collection, Manassas, VA, United States; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; IAM, culture collection of the institute of Applied Microbiology, University of Tokyo, Japan; JCM, Japanese Collection of Microorganisms, Tokyo, Japan; LMG, BCCM/LMG bacteria collection, Laboratorium voor Microbiologie, University of Gent, Gent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

[a] Names in quotation marks have not been validly published. Brackets indicate generically misclassified bacteria. Accession number is that in the recognized culture collection in which the type strain was first deposited. The origin and G + C content are those of the type strain. When more than one strain are known, three numbers are given, i.e., the number of strains on which the original description was based, the number of strains currently available in the largest culture collections (ATCC, CIP [Collection de I'Institut Pasteur, Paris, France], DSM, LMG, and NCIMB), and the approximate number of strains which have been included in further studies and which are or may still be maintained in specialized culture collections, respectively.

[b] Previous names and corrected epithets of species (taken from Euzéby [1997]; List of Bacterial Names with Standing in Nomenclature) are as stated in footnotes c to o.

[c] "[*Bacillus*] *aquatillis*" Frankland and Frandlnad 1889, "*Bacterium aquatillis*" Chester 1897, "[*Flavobacterium*] *aquatilis*" Bergey et al. 1923, "[*Chromobacterium*] *aquatilis*" Topley and Wilson 1929, and "[*Empedobacter*] *aquatile*" Brisou et al. 1960.

[d] *Flavobacterium branchiophila* Wakabayashi et al. 1989.

[e] "[*Bacillus*] *columnaris*" Davis 1922, "[*Chondrococcus*] *columnaris*" Ordal and Rucker 1944, [*Cytophaga*] *columnaris* Garnjobst 1945, Reichenbach 1989, and [*Flexibacter*] *columnaris* Leadbetter 1974, Bernardet and Grimont 1989c.

[f] [*Cytophaga*] *flevensis* van der Meulen et al. 1974, Reichenbach 1989.

[g] "*Flavobacterium xylanivorum*," name as deposited in the 16S rRNA sequence databases and in the ATCC.

[h] "*Flavobacterium ameridies*," name as deposited in the 16S rRNA sequence databases.

[i] [*Cytophaga*] *aquatilis* Strohl and Tait 1978.

[j] [*Cytophaga*] *johnsonae* Stanier 1947, Reichenbach 1989, and "[*Cytophaga*] *johnsonii*" Stanier 1957.

[k] "*Flavobacterium pectinovorum*" Dorey 1959, "[*Empedobacter*] *pectinovorum*" Kaiser 1961, and [*Cytophaga*] *pectinovora* Reichenbach 1989.

[l] [*Cytophaga*] *psychrophila* Borg 1960, Reichenbach 1989, and [*Flexibacter*] *psychrophilus* Bernardet and Grimont 1989c.

[m] [*Cytophaga*] *saccharophila* Agbo and Moss 1979.

[n] [*Cytophaga*] *succinicans* Anderson and Ordal 1961, Reichenbach 1989, and "[*Flexibacter*] *succinicans*" Leadbetter 1974.

[o] "[*Cytophaga*] *xantha*" Inoue and Komagata 1976.

Table 2. Habitats which are known to harbor described *Flavobacterium* species.

Species	Known habitat(s)
	Freshwater fish
F. branchiophilum	Diseased gills of various fish species; United States, Canada, Hungary, Japan, and Korea
F. columnare	External lesions and internal organs of many fish species; worldwide
F. hydatis	Diseased gills of salmon; United States
F. psychrophilum	External lesions and internal organs of (mostly) salmonid fish; United States, Canada, Chile, Europe, Japan, Korea, Tasmania
F. succinicans	External lesions of salmon and aquarium water (containing salmon); United States
	Temperate freshwater and soil
F. aquatile	Well water; United Kingdom
F. flevense	Freshwater and muddy soils, e.g., from Ijsselmeer, the Netherlands
F. granuli	Wastewater treatment plant, beer brewery; South Korea
F. johnsoniae	Soil (as for *F. pectinovorum*), compost, freshwater and freshwater sediment, also external lesions of various fish species; worldwide
F. limicola	Freshwater river sediment, e.g., from Japan
F. pectinovorum	Temperate, moist, organic-rich soils, e.g., from the United Kingdom
F. saccharophilum	Freshwater sediment; United Kingdom
"[*Cytophaga*] *allerginae"*	Water in an air-cooling unit; United States
"[*Flexibacter*] *aurantiaus* subsp. *Excathedrus"*	Freshwater pool; Costa Rica
"[*Promyxobacterium*] *flavum"*	Rhizosphere of tomato plant; Russia
"[*Sporocytophaga*] *cauliformis"*	Freshwater lake (Lake Constance)
	Cold environments
F. antarcticum	Organic-rich soil (penguin habitat); Antarctica
F. degerlachei	Microbial mats, freshwater lakes; Antarctica
F. frigidarium	Marine sediment, sea-ice; Antarctica
F. frigoris	Microbial mats, freshwater lakes; Antarctica
F. fryxellicola	Microbial mats, fresh/brackish water lakes; Antarctica
F. gelidilacus	Microbial mats, freshwater and saline lakes; Antarctica
F. gillisiae	Rich basal algal assemblages of sea-ice; Antarctica
F. hibernum	Freshwater lakes fed by glacial run-off; Antarctica
F. micromati	Microbial mats, freshwater lakes; Antarctica
F. omnivorum	Frozen soil, glacier; China
F. tegetincola	Microbial mats, freshwater to marine salinity lakes; Antarctica
F. xanthum	Muddy soil, pond mud, algal mats, freshwater lakes in Antarctica; freshwater eutrophic lakes in Germany
F. xinjiangense	Frozen soil, glacier; China

freshwater commensals but may possess opportunistic pathogenic responses. Strains of *F. columnare* also can kill cysts of the protistan pathogen *Giardia lamblia* in the presence of elevated Ca^{2+} ions (Rodgers et al., 2003), suggesting that the biological interactions of pathogenic and other *Flavobacterium* species could be quite diverse (see the section Applications in this Chapter).

Other *Flavobacterium* species appear to be harmless, chemoheterotrophic species that play a role in mineralizing various types of organic matter (carbohydrates, amino acids, proteins, and polysaccharides) in aquatic ecosystems (see the sections Identification and Physiology in this Chapter), although many early reports of bacteria, fungi, dead insects, and nematods being degraded by so-called "*Flavobacterium*" in soil are questionable. A member of the genus *Flavobacterium* was recently retrieved from paper mill slime, but its role in the process was not determined (Oppong et al., 2003). The 16S rRNA gene sequence was the only clue to the attribution to the genus *Flavobacterium* of several bacterial strains retrieved from the guts of mosquitoes (Campbell et al., 2004). Some species appear to be cosmopolitan (e.g., *F. johnsoniae* and *F. xanthum*), isolated from different habitats across several continents; however, the knowledge of the distribution of most *Flavobacterium* species is severely limited and data for most species are based only on one survey. In addition, specific ecological knowledge for most species is absent, including their specific functional roles and interactions with microorganisms and metazoa. The fish pathogens are exceptions, though even for these the data are still relatively limited.

Temperate freshwater and freshwater sediments (groundwater, lakes, rivers and ponds) are known to be habitats for the species *F. johnsoniae*, *F. saccharophilum*, *F. flevense*, *F. limicola*

and *F. xanthum* (Tamaki et al., 2003), while microbial mats and water of Antarctic oligotrophic lakes harbor *F. tegetincola*, *F. xanthum*, *F. hibernum*, *F. xinjiangense*, *F. gelidilacus*, *F. degerlachei*, *F. micromati*, *F. frigoris*, *F. fryxellicola* and *F. psychrolimnae* (Jaspers et al., 2001; Van Trappen et al., 2002; Van Trappen et al., 2003a; Van Trappen et al., 2003b; Pearce, 2003; Van Trappen et al., 2005; Table 2). The latter set of species tends to be more cold-adapted than the temperate species with growth temperature limits only slightly over 25°C. Soil is also a habitat for *Flavobacterium* species including *F. pectinovorum*, *F. johnsoniae* and *F. antarcticum* (Yi et al., 2005). The soils in which they have been found were moist and organic rich; arid desert or infertile soils were not known to harbor *Flavobacterium* species until a *Flavobacterium* sp. was recently isolated from samples of arid soil (Bodour et al., 2003). Two species, *F. omnivorum* and *F. xinjiangense*, were isolated from entrained frozen soil recovered from a glacier in western China (Xinjiang Province; Zhu et al., 2003). The soil was frozen into the glacier long in the past with the freeze-resistant species probably surviving there ever since. Most bacteria found in glacial deposits are usually hardy soil-type bacteria that are probably distributed into glacial environments either by wind-dispersion or by being trapped during one of the past ice ages. The species *F. gillisiae* and *F. frigidarium* were isolated from polar sea-ice, Antarctic marine salinity lakes, and marine sediment (e.g., Bowman et al., 1997; McCammon and Bowman, 2000; Humphry et al., 2001; Brinkmeyer et al., 2003) and could have a wider distribution in cold ecosystems. Culture-independent studies using polymerase chain reaction (PCR)-amplification and sequencing of cloned 16S rRNA genes so far indicate *Flavobacterium* species are more or less limited to the environments already indicated above (see the section Identification in this Chapter). Using phenotypic characteristics and 16S rRNA gene sequences, Zdanowski et al. (2004) identified several *Flavobacterium* sp., *Chryseobacterium* sp. and *Gelidibacter* sp. strains among the extremely diverse bacterial community in penguin guano in Antarctica. Indeed, the new species *F. antarcticum* was isolated from a soil sample collected from a penguin habitat (Yi et al., 2005).

Such artificial, moist environments as air-cooling units are also colonized by members of the genus *Flavobacterium*; for instance, a *Flavobacterium* sp. strain was involved in several human cases of respiratory disease which occurred during the eighties in a United States textile facility (Flaherty et al., 1984; Liebert et al., 1984; see the section Pathogenicity and Epidemiology in this Chapter). Another, very unusual environment for *Flavobacterium* strains was discovered when amoebae were shown to harbor intracellular members of the genus (Müller et al., 1999; Horn et al., 2001; see the section Pathogenicity and Epidemiology in this Chapter). One of the most recently described *Flavobacterium* species, *F. granuli*, was isolated from anaerobic granules in the wastewater treatment plant of a beer brewing factory in South Korea (Aslam et al., 2005). These granules, composed of microorganisms, inorganic nuclei and extracellular polymers, result from the flocculation of sludge in the reactor.

The ecology and habitats of bacterial genera belonging to the family Flavobacteriaceae were reviewed by Jooste and Hugo (1999). They were also extensively described for some of the bacterial species now encompassed in the genus *Flavobacterium* by Reichenbach (1989) and in the chapter The Order Cytophagales in the second edition. Many early publications reported the degradation of food products by poorly identified "*Flavobacterium*" strains; however, these food-related members of the family Flavobacteriaceae have subsequently been moved to the genera *Bergeyella*, *Chryseobacterium*, *Empedobacter*, *Myroides* and *Weeksella* (Engelbrecht et al., 1996; Jooste and Hugo, 1999; see the three corresponding chapters in this Volume—The Genera *Bergeyella* and *Weeksella*, The Genera *Myroides* and *Empedobacter* and The Genus *Chryseobacterium*). *Flavobacterium* spp. were isolated or identified from their 16S rRNA gene sequence from poultry and the air of poultry processing establishments (Georgnaras et al., 1996; Ellerbroek, 1997), dairy products (Cousin, 1982), and traditional alcoholic beverages in Nigeria (Sanni et al., 1999) and Mexico (Escalante et al., 2004), but the identification procedure was insufficient to determine whether these isolates were true *Flavobacterium* strains or belonged to other genera in the family Flavobacteriaceae. Bacterial strains, identified as members of the genus *Flavobacterium* based on phenotypical characteristics, were isolated from the surface and muscle of fresh or ice-stored sardines, but they were not considered to play an important role in the spoilage of the fish (Gennari and Cozzolino, 1989; Elotmani et al., 2004). This was also the opinion of González et al. (2000) about the *F. aquatile* strain they isolated from muscle samples of ice-stored rainbow trout. The isolation of *Flavobacterium* strains from fish products is not surprising since they have long been known to belong to the normal bacterial flora of freshwater fish and fish eggs. From their phenotypic traits, the *Cytophaga*-like strains reported from the surface of salmonid eggs (Bell et al., 1971; Barker et al., 1989) and gills (Trust, 1975; Nieto et al., 1984) could actually well have been *Flavobacterium* isolates. Unidentified *Flavobacterium* strains were also reported from

egg, skin, gill, and intestine samples (Trust, 1975; Barker et al., 1989; Nedoluha and Westhoff, 1997; Al-Harbi and Uddin, 2003; Míguez and Combarro, 2003; Mahmoud et al., 2004), as well as from the rearing pond water and sediment (Nedoluha and Westhoff, 1997; Al-Harbi and Uddin, 2003) of various fish species, but not from the fish diet (Nedoluha and Westhoff, 1997). Members of the *Flavobacterium-Cytophaga* group represented 9% of all bacteria identified by fluorescent in situ hybridization (FISH) in the tank water of an eel intensive culture system, and even higher numbers of the same organisms occurred in the eel slime (Moreno et al., 1999).

Isolation and Cultivation

Being classic aerobic chemoheterotrophs, members of *Flavobacterium* species can be readily cultivated on several commercially available organic media. The presence of NaCl and the concentration of organic extracts strongly affect growth. This is the major reason growth of freshwater species, including the fish pathogens, is so poor on media such as marine and nutrient agars that contain substantial amounts of NaCl. The procedures available for the enrichment, isolation, and cultivation of some of the bacterial species now encompassed in the genus *Flavobacterium* were extensively described by Reichenbach (Reichenbach, 1989; Reichenbach, 1992); the corresponding information for the *Flavobacterium* species published since then was included in their individual descriptions. The cultivation of bacterial genera belonging to the family Flavobacteriaceae was reviewed by Jooste and Hugo (1999). Media and optimal incubation temperatures for known *Flavobacterium* spp. are indicated in Table 3. Although they do allow limited bacterial growth, some incubation tem-

Table 3. Media and incubation temperature for routine cultivation of *Flavobacterium* species.

Species	Anacker and Ordal's agar	Nutrient agar	Trypticase soy agar	Marine 2216 agar	Incubation temperature (°C)
Na^+-sensitive fish pathogens[a]					
F. branchiophilum	+[b]	–	–	–	20–25
F. columnare	+	–	–	–	25
F. psychrophilum	+[c]	+	–	–	15–20
Freshwater and soil species					
F. aquatile	+	–	w	–	25
F. fryxellicola	+	+	+	–	20
F. granuli	NT	+	+	NT	25–30
F. hibernum	+	+	+	–	20–25
F. hydatis	+	+	+	–	25
F. johnsoniae	+	+	+	–	25
F. limicola	+	+	+	–	20
F. omnivorum	+	+	+	–	10–15
F. pectinovorum	+	+	+	–	25
F. psychrolimnae	+	+	+	–	20
F. saccharophilum	+	+	+	–	25
F. succinicans	+	+	+	–	25
F. xinjiangense[d]	+	w	–	–	10–25
"[*Cytophaga*] *allerginae*"	+	+	NT	NT	25
"[*Flexibacter*] *aurantiacus* subsp. *excathedrus*"	+	+	NT	NT	25
"[*Promyxobacterium*] *flavum*"	+	+	NT	NT	30
"[*Sporocytophaga*] *cauliformis*"	+	+	NT	NT	25
Salt tolerant (and most polar) species					
F. antarcticum	+	+	+	+	20
F. degerlachei	+	+	+	+	20
F. flevense	+	+	+	+	25
F. frigidarium	+	+	+	+	15–20
F. frigoris	+	–	+	+	20
F. gelidilacus	+	+	+	+	20
F. gillisiae	+	+	+	+	15–20
F. micromati	+	+	w	w	20
F. tegetincola	+	+	+	+	15–20
F. xanthum	+	+	+	+	15–20

Abbreviations: +, good growth; w, weak or reduced growth; –, no or scant growth; and NT, not tested.

[a]Fish-pathogenic species will also grow well on Modified Shieh's medium (Song et al., 1988a).

[c]*Flavobacterum psychrophilum* strains grow better on Anacker and Ordal's agar containing an enriched amount of tryptone (e.g., 5 g·liter^{-1}) or on Modified Shieh's agar.

[d]For the routine cultivation of *F. xinjiangense*, PYG agar or broth can also be used (Zhu et al., 2003).

peratures and media in this table are not recommended for routine cultivation of some species.

All species can be grown conveniently on agar media although *F. columnare* strains tend to adhere strongly to the agar surface (see the section Identification in this Chapter). Many species can grow on rich media such as nutrient broth and agar and trypticase soy broth and agar (TSA); most of them can also be readily grown on media containing a lower concentration of nutrients such as R2A and other media typically used for growing freshwater bacterial chemoheterotrophic species. *Flavobacterium omnivorum* and *F. xinjiangense* grow well on rich glucose-containing media such as peptone-yeast extract-glucose (PYG; Zhu et al., 2003); this is presumably also true for other *Flavobacterium* species, although glucose does not improve the growth of *F. columnare* (Song et al., 1988b). Modified Shiehs (Song et al., 1988b) or Anacker and Ordal's media (Anacker and Ordal, 1955), with incubation at 20°C, can be used for general cultivation, as almost all *Flavobacterium* species should grow well under these conditions. Nutrient, trypticase soy, Marine 2216, and R2A broth and agar media are commercially available. The formulae for some of the other media mentioned are detailed at the end of this section.

Flavobacterium are best isolated directly from source material (water, mud, soil, fish tissue, etc.) onto agar media. Selective culturing techniques generally have not been developed for the majority of *Flavobacterium* species, except for the fish pathogens (see below). The distinctive yellow pigmentation and shiny, slightly mucoid appearance of the colonies of most *Flavobacterium* species provide a direct means for selection of putative *Flavobacterium* strains. Further identification relies then on phenotypic characteristics and 16S rRNA gene sequencing (see the section Identification in this Chapter).

The isolation procedure used can take advantage of the fact that most *Flavobacterium* species are adapted to cool to cold ecosystems: incubation temperature thus can be set to 4–20°C. Indeed, the isolation of *Flavobacterium* species from permanently cold ecosystems such as sea-ice, glacial seawater, and polar lake samples can be enhanced by a pre-enrichment step at a low temperature, typically 2–4°C for 1–2 days in a suitable medium. This boosts the populations of heterotrophic psychrophilic species, which respond rapidly to high levels of organic nutrients. The pre-enrichment culture is then spread-plated onto the corresponding agar medium. This process was used for the isolation of *F. gillisiae* and *F. tegetincola* from Antarctic ice and water samples using Marine 2216 agar (McCammon and Bowman, 2000), for the isolation of *F. xinjiangense* and *F. omnivorum* from glacial entrained soil using PYG medium (Zhu et al., 2003), and for the isolation of several species from Antarctic lake algal mat material using TSA and R2A (Van Trappen et al., 2002).

Some *Flavobacterium* species can be isolated on media containing a specific substrate, such as chitin or pectin (Reichenbach, 1989). *Flavobacterium frigidarium* can be enriched from marine sediments in a liquid xylan-containing mineral salts medium; after several weeks enrichment at 4°C, the species can be isolated and purified using the same medium solidified with agar (Humphry et al., 2001).

Understandably, the fish-pathogenic *Flavobacterium* species (see the sections Habitat and Ecology and Pathogenicity and Epidemiology in this Chapter) have received considerable attention to develop appropriate isolation media (Holt et al., 1993; Turnbull, 1993; Wakabayashi, 1993; Austin and Austin, 1999). After the Anacker and Ordal's medium (frequently called "cytophaga medium" in the fish pathology literature) was first proposed for the cultivation of *F. columnare* (Anacker and Ordal, 1955), several other media were evaluated for improved bacterial growth, including the Shieh's medium, both in its original formula (Shieh, 1980) and in a modified, more simple formula (Song et al., 1988b), and the tryptone-yeast extract-salts medium (TYES; Holt, 1988; Holt et al., 1993). *Flavobacterium columnare* is usually isolated from superficial lesions in diseased fish (see the section Pathogenicity and Epidemiology in this Chapter) in which many other bacteria also proliferate (Hawke and Thune, 1992; Tiirola et al., 2002). To prevent the overgrowth of the pathogen by saprophytic bacteria and their antagonistic effect (Chowdhury and Wakabayashi, 1989; Tiirola et al., 2002), selective media were devised on the basis of the resistance of *F. columnare* to neomycin, polymyxin B and tobramycin (Fijan, 1969; Bullock et al., 1986; Griffin, 1992; Hawke and Thune, 1992; Decostere et al., 1997) and on its ability to degrade gelatin (Bullock et al., 1986; Griffin, 1992). One such formula (Selective Flexibacter Medium; Bullock et al., 1986) is given below. Serial dilution of infected samples has also been advocated to favor the isolation of fish-pathogenic *Flavobacterium* species (Tiirola et al., 2002). No growth of *F. columnare* was reported on TSA, nutrient agar or Marine 2216 media. All *F. columnare* strains are able to grow at 18, 25 and 30°C; some of them may also grow at 15 or 37°C, depending on the temperature of the environment they were isolated from (Decostere et al., 1998; Triyanto and Wakabayashi, 1999).

Many formulae have also been proposed for the growth of *F. psychrophilum* (Holt et al., 1993; Madetoja and Wiklund, 2002; Nematollahi et al., 2003b; Cepeda et al., 2004). Anacker and Ordal's

medium has been widely used. To improve the growth of *F. psychrophilum*, several modifications of the original formula have been proposed, such as increasing the tryptone content to 2 g·liter^{-1} (Wakabayashi and Egusa, 1974) or 5 g·liter^{-1} (Bernardet and Kerouault, 1989; Crump et al., 2001) or adding 10% fetal bovine serum (Obach and Baudin-Laurencin, 1991; Brown et al., 1997) or 3% fish blood (Crump et al., 2001). Daskalov et al. (1999) also reported improved growth, gliding motility, and pigmentation of *F. psychrophilum* when grown in Anacker and Ordal's agar and broth supplemented with skimmed milk and 0.5 g·liter^{-1} each of D(+) galactose, D(+) glusose, and L-rhamnose. The Shieh's medium (Shieh, 1980; Holt et al., 1993), the tryptone-yeast extract-salts medium (TYES; Holt et al., 1993; Crump et al., 2001), and the nutrient agar and broth (Secades et al., 2001; Secades et al., 2003) also give good growth. Although the growth of *F. psychrophilum* on TSA was sometimes reported, most authors agree that no or only negligible growth occurs on this medium. The isolation of *F. psychrophilum* from blisters or internal organs usually results in the growth of pure cultures; but a tobramycin-containing medium has been proposed to prevent the overgrowth of *F. psychrophilum* by other bacteria when external lesions are sampled (Kumagai et al., 2004). This organism is not particularly fastidious, although its growth is inevitably slower than that of other fish-pathogenic *Flavobacterium* species owing to its lower range of temperature tolerance. *Flavobacterium psychrophilum* grows at temperatures between 4 and 23°C; most strains do not grow or grow very weakly at 25°C (Bernardet and Kerouault, 1989; Holt et al., 1989; Brown et al., 1997; Uddin and Wakabayashi, 1997), although growth at 25°C was reported for some strains (Ostland et al., 1997; Madetoja et al., 2001). Optimum growth occurs at 15 (Holt et al., 1989) to 20°C (Uddin and Wakabayashi, 1997). One of the main reasons for trying to improve the yield of *F. psychrophilum* in culture was to obtain large masses of bacteria that could be used for preparing a vaccine against the severe salmonid diseases it is responsible for worldwide (see the section Pathogenicity and Epidemiology in this Chapter). Recently, Crump et al. (2001) achieved large-scale production of *F. psychrophilum* in a 35-liter fermenter containing 28 liters of TYES broth supplemented with 1% maltose and 0.02% sodium acetate with stirring and aeration. Supplementation of TYES with 0.5 g·l^{-1} glucose also enhanced the growth of *F. psychrophilum*, resulting in improved recovery of the bacterium from stored cultures and infected fish and in increased production of biomass in liquid culture (Cepeda et al., 2004). Standard procedures for such investigations of *F. psychrophilum* as experimental infection, antimicrobial susceptibility testing, and virulence studies require the use of accurate numbers of viable bacteria. Improvements in the growth media and careful handling of the bacteria in isotonic suspension media resulted in predictable production of viable *F. psychrophilum* cells and allowed an absorbance/colony-forming-units relation curve to be established (Michel et al., 1999). However, as stated by Faruk (2000): "It should be noted that the growth of each *F. psychrophilum* isolate differed considerably. Ideally, a growth curve should have been prepared for each isolate to examine individual life cycles of the isolates." *F. psychrophilum* may enter a "viable but non-cultivable" state (Michel et al., 1999; Madetoja and Wiklund, 2002; Vatsos et al., 2002).

Strains of *F. johnsoniae* (originally isolated from soil samples on chitin-containing media; Stanier, 1947; Reichenbach, 1989) and *F. hydatis* grow well on media with rather low-nutrient content such as Anacker and Ordal's and modified Shieh's, as well as on more nutrient-rich nutrient media and TSA, on which they produce flat, translucent, spreading, pale yellow colonies with filamentous to rhizoid margins or raised to convex, round to undulate, opaque, bright yellow colonies with entire to irregular margins, respectively (Strohl and Tait, 1978; Reichenbach, 1989; J.-F. Bernardet, unpublished data). Hence, these media are currently used for the isolation of *F. johnsoniae* strains from fish (Carson et al., 1993), as well as for the growth of the numerous *F. johnsoniae*-like and *F. hydatis*-like strains also isolated from fish (J.-F. Bernardet, unpublished data; see the section Pathogenicity and Epidemiology in this Chapter). However, as for *F. columnare*, these organisms occur in external lesions and their recovery may be hampered by the presence of numerous saprophytic bacteria. Given the very limited impact of these organisms in fish pathology, no selective medium was devised. Incubation temperatures of 20–30°C are convenient for routine cultivation.

Flavobacterium branchiophilum is the most fastidious of all fish-pathogenic *Flavobacterium* species (Heo et al., 1990; Turnbull, 1993; Ko and Heo, 1997; see the section Pathogenicity and Epidemiology in this Chapter). Growth on Anacker and Ordal's agar is slow, with colonies appearing only after 2–5 days of incubation at 18°C. Colonies are light yellow, translucent, non-spreading, circular with regular edges; the latter characteristic is due the absence of gliding motility in *F. branchiophilum* (Wakabayashi et al., 1989; Ostland et al., 1994). Growth periods of up to seven days were used for some studies, and the enrichment of the medium with serum, gelatin or starch was suggested (Ko and Heo, 1997). For

primary isolation from gill samples, Ostland et al. (1994) plated serial 10-fold dilutions of gill tissue on Anacker and Ordal's agar and incubated the plates at 12 or 18°C for 6–12 days. The growth of several *F. branchiophilum* strains was compared on different media: no growth occurred on TSA, nutrient agar, and Mueller Hinton; only limited growth occurred on Anacker and Ordal's agar enriched to 5 g·liter^{-1} of tryptone; growth was distinctly better on original Anacker and Ordal's agar, modified Shieh's agar, *Microcyclus-Spirosoma* agar (medium no. 81 of the National Collections of Industrial, Food and Marine Bacteria [NCIMB] catalogue of strains), and Medium for Freshwater Flexibacteria (NCIMB medium no. 218; Lewin, 1969); and the best growth was achieved on casitone yeast extract agar (NCIMB medium no. 101; J.-F. Bernardet, unpublished data). Different temperatures were also compared, and the 20–25°C range was found convenient (J.-F. Bernardet, unpublished data).

The four invalid taxa which belong to the genus *Flavobacterium*, i.e., "[*Cytophaga*] *allerginae*," "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*," "[*Promyxobacterium*] *flavum*" and "[*Sporocytophaga*] *cauliformis*," all grow well on nutrient, Anacker and Ordal's, and modified Shieh's media at 25°C; growth on TSA was not tested (Reichenbach, 1989; J.-F. Bernardet, unpublished data).

Anacker and Ordal's Medium (Anacker and Ordal, 1955)

Tryptone (or pancreatic digest of casein)	0.5 g
Yeast extract	0.5 g
Beef extract	0.2 g
Sodium acetate	0.2 g
Agar (optional)	10 g
Distilled water	1000 ml

Adjust pH to 7.2–7.4 and autoclave 15–20 min at 121°C.

The original recipe, used to grow *F. columnare* in agar deeps, included 0.4% agar only; in their 1959 publication, the same authors raised the agar concentration to 0.9% for streaking material from lesions of fish (Anacker and Ordal, 1959). However, an agar concentration of 1–1.5% is recommended for more comfortable streaking (Wakabayashi and Egusa, 1974; Bernardet, 1989a; Michel et al., 1999). A selective medium for the isolation of *F. columnare* was obtained by adding neomycin (5 μg·ml^{-1}) and polymyxin B (200 IU·ml^{-1}) to this medium (Hawke and Thune, 1992), while tobramycin (5 μg·ml^{-1}) was added to modified Anacker and Ordal's medium for the selective isolation of *F. psychrophilum* (Kumagai et al., 2004). The brand of beef extract used was shown to influence the growth of *F. psychrophilum* (Lorenzen, 1993).

Modified Shieh's Medium (Song et al., 1988b)

Yeast extract	0.5 g
Peptone	5 g
Sodium acetate	10 mg
$BaCl_2 \cdot H_2O$	10 mg
K_2HPO_4	0.1 g
KH_2PO_4	50 mg
$MgSO_4 \cdot 7H_2O$	0.3 g
$CaCl_2 \cdot 2H_2O$	6.7 mg
$FeSO_4 \cdot 7H_2O$	1.0 mg
$NaHCO_3$	50 mg
Distilled water	1000 ml
Agar (optional)	15 g

Autoclave 15–20 min at 121°C.

The original Shieh's recipe (Shieh, 1980) was modified by omitting the glucose, pyruvate and citrate following the demonstration that these components did not improve the growth of *F. columnare*. Decostere et al. (1997) devised a selective medium for the isolation of *F. columnare* by adding 1 μg·ml^{-1} of tobramycin to this medium.

Peptone-Yeast Extract-Glucose (PYG; Zhu et al., 2003)

Polypeptone	5 g
Tryptone	5 g
Yeast extract	10 g
Salt solution	40 ml
Agar (optional)	15–20 g
Distilled water	910 ml
Salt Solution	
$CaCl_2$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.4 g
K_2HPO_4	1.0 g
KH_2PO_4	1.0 g
$NaHCO_3$	10 g
NaCl	2.0 g
Distilled water	1000 ml

Adjust pH to 7.2 and autoclave 15–20 min at 121°C. Add 50 ml of 20% (w/v) D-glucose filter-sterilized stock solution to each 1 liter of medium following autoclaving.

Selective Flexibacter Medium (Bullock et al., 1986)

Tryptone	2.0 g
Yeast extract	0.5 g
Gelatin	3.0 g
Agar	15 g
Distilled water	1000 ml

Autoclave 15–20 min at 121°C; when cooled to 45°C, add filter-sterilized neomycin sulfate (0.004 g).

Tryptone Yeast Extract Salts (TYES; Holt, 1988)

Tryptone	4.0 g
Yeast extract	0.4 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2 \cdot 2H_2O$	0.5 g
Distilled water	1000 ml

Adjust pH to 7.2 and autoclave 15–20 min at 121°C.

Identification

Characteristics which differentiate members of the genus *Flavobacterium* from members of other genera in the family Flavobacteriaceae are listed in Table 2 of the chapter An Introduction to the Family Flavobacteriaceae in this Volume; those characteristics that differ among species in the genus *Flavobacterium* are listed in Table 4 below; see also the section Physiology.

The following description of the genus *Flavobacterium* is cited from Bernardet et al., 1996:

Emended description of the genus *Flavobacterium* Bergey, Harrison, Breed, Hammer, and Huntoon 1923. Cells are rods with parallel or slightly irregular sides and rounded or slightly tapered ends and usually are 2 to 5 μm long and 0.3 to 0.5 μm wide. Under certain growth conditions, some species may also produce shorter (1-μm) or longer (10- to 40-μm) filamentous cells. The longer rods are flexible. Motile by gliding (this characteristic has not been observed in *Flavobacterium branchiophilum*). Flagella are absent. Gram negative. Resting stages are not known. Intracellular granules of poly-β-hydroxybutyrate are absent. Colonies are circular, convex or low convex, and shiny with entire or wavy edges (sometimes sunken into the agar) on solid media containing high levels of nutrients. On solid media containing low levels of nutrients most species also produce flat or very thin, spreading, sometimes very adherent swarms with uneven, rhizoid, or filamentous margins. Colonies are typically yellow (they vary from cream to bright orange) because of nondiffusible carotenoid or flexirubin types of pigments or both, but nonpigmented strains do occur. Most species do not grow on seawater-containing media; an exception to this is *Flavobacterium flevense*. Most species are able to grow on nutrient agar and on Trypticase soy agar. Chemoorganotrophic. Aerobic with a respiratory type of metabolism. When certain growth factors are provided, *Flavobacterium hydatis* and *Flavobacterium succinicans* also grow anaerobically (Anderson and Ordal, 1961; Chase, 1965; Reichenbach, 1989; Strohl and Tait, 1978). Peptones are used as nitrogen sources, and NH_3 is released from peptones; growth occurs on peptone alone. Acid is produced from carbohydrates by all species except *Flavobacterium columnare* and *F. psychrophilum*. All species except *Flavobacterium pectinovorum* decompose gelatin and casein, and several species also hydrolyze various polysaccharides, including starch, chitin, pectin, and carboxymethyl cellulose. *Flavobacterium pectinovorum* and *Flavobacterium saccharophilum* are also agarolytic. Cellulose is never decomposed. Tributyrin and Tween compounds are decomposed. Indole is not produced. Catalase is produced. Cytochrome oxidase is produced by all species except *Flavobacterium saccharophilum*.

Menaquinone 6 is the only respiratory quinone. The predominant fatty acids are 15:0, 15:0 iso, 15:1 iso G, 15:0 iso 3OH, summed feature 4 (15:0 iso 2OH, 16:1 ω7c, or 16:1 ω7t or any combination of these fatty acids), 16:0 iso 3OH, 17:1 iso ω9c, and 17:0 iso 3OH. Sphingophospholipids are absent. Homospermidine is the major polyamine in all 10 *Flavobacterium* species; all species except *Flavobacterium branchiophilum* and *Flavobacterium saccharophilum* also contain putrescine as a minor component (Hamana and Matsuzaki, 1990; Hamana and Matsuzaki, 1991; Hamana et al., 1995). Spermidine and spermine are also minor components in *Flavobacterium branchiophilum*, while *Flavobacterium johnsoniae* is the only member of the genus that contains minor amounts of agmatine and 2-hydroxyputrescine (Hamana et al., 1995). The optimum temperature range for most species is 20 to 30°C; the optimum temperature range for *F. psychrophilum* is 15 to 18°C.

These organisms are widely distributed in soil and freshwater habitats, where they decompose organic matter. Several species are pathogenic for freshwater fish (*Flavobacterium branchiophilum*, *Flavobacterium columnare*, *F. psychrophilum*) or occasionally are isolated from diseased freshwater fish (*Flavobacterium hydatis*, *Flavobacterium johnsoniae*, *Flavobacterium succinicans*). The G+C contents of the DNAs are 32 to 37 mol%. The type species is *Flavobacterium aquatile* (Frankland and Frankland, 1889) Bergey, Harrison, Breed, Hammer, and Huntoon 1923.

The publication of sixteen additional *Flavobacterium* species since 1996 did not challenge the core of this emended description. However, additional comments are necessary on some of the characteristics included; these comments are cited below in the order of the above comments.

In some *Flavobacterium* species, spherical degenerative forms usually considered nonviable, often referred to as "spheroplasts," appear in aging liquid cultures (Reichenbach, 1989); they were long mistaken for microcysts (Ordal and Rucker, 1944; Pacha and Ordal, 1970; Bullock et al., 1971; Farkas, 1985; Fig. 2). They have been noticed in *F. columnare* (Garnjobst, 1945; Song et al., 1988a, 1988b; Tiirola et al., 2002; J.-F. Bernardet, unpublished data), *F. succinicans* (Anderson and Ordal, 1961), *F. psychrophilum* (J.-F. Bernardet, unpublished data), *F. johnsoniae* (Stanier, 1947; Liao and Wells, 1986) and *F. branchiophilum* (Ostland et al., 1994).

Few electron microscopy studies have been performed on members of the genus *Flavobacterium*. Reichenbach (1989) reviewed the early

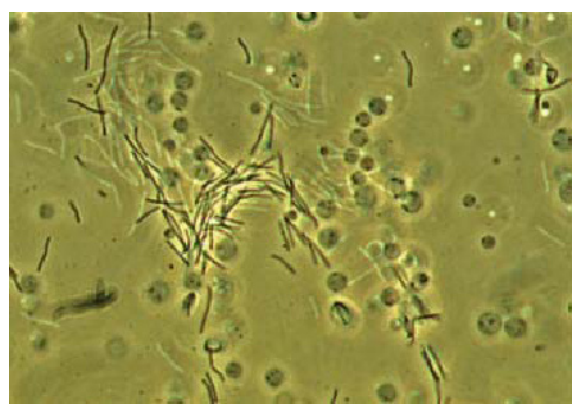

Fig. 2. Spheroplasts of *F. columnare*. Spherical degenerative forms called "spheroplasts" appeared as soon as 24 h in this shaken Anacker and Ordal's broth culture incubated at room temperature (phase-contrast microscopy, ×1000).

Table 4. Differentiating characteristics among the valid species in the genus *Flavobacterium*.

	F. antarcticum	*F. aquatile*	*F. branchiophilum*	*F. columnare*	*F. degerlachei*	*F. flevense*	*F. frigidarium*	*F. frigoris*	*F. fryxellicola*	*F. gelidilacus*	*F. gillisiae*
Habitat	Polar soil	Temperate freshwater	Temperate freshwater	Temperate freshwater	Polar saline lakes	Temperate freshwater	Polar marine sediment	Polar freshwater and saline lakes	Polar freshwater lakes	Polar freshwater and saline lakes	Sea ice
Morphology of the colonies on Anacker and Ordal's agar	Convex, round with entire margins	Low convex, round, with entrie margins	Low convex, round, with entire margins	Flat, adherent to the agar, with rhizoid margins	Flat, round, with entrie margins	Low convex, round, sunken into the agar	Flat, round, with entire margins	Flat, round, with entire margins	Flat, round, with entire margins	Flat, round, with entire margins	ND
Gliding motility	–	+	–	+	–	+	–	–	–	v	–
Congo red absorption	–	–	–	+	–	–	+[a]	–	–	–	ND
Flexirubin type pigment	–	–	–	+	–	–	–	–	–	–	–
Growth on											
Marine agar	+	–	–	–	+	+	+	+	–	+	+
Nutrient agar	+	+	–	–	+	+	+	–	+	+	+
Trypticase-soy agar	+	(+)	–	–	+	+	+	+	(+)	+	+
Growth at:											
20°C	+	+	+	+	+	+	+	+	+	+	+
25°C	(+)	+	+	+	+	+	–	(+)	(+)	+	(+)
Glucose utilization	–	ND	ND	–	+	+	+	+	+	–	+
Acid produced from carbohydrates	(+)	+	+	–	–	+	–	–	–	–	+
Degradation of											
Gelatin	+	+	+	+	–	–	+	–	–	v	–
Casein	+	+	+	+	–	–	+	+	–	+	+
Starch	–	+	+	–	+	+	–	+	–	+	+
Carboxymethyl cellulose	–	–	–	–	–	–	–	–	–	–	–
Agar	–	–	–	–	–	+	–	–	–	–	–
Alginate	–	ND	ND	ND	–	–	ND	–	–	–	ND
Pectin	–	ND	ND	ND	–	+	–	–	–	–	–
Chitin	–	–	–	–	–	–	–	–	–	–	+
Esculin	–	+	–	–	+	+	+	+	+	–	+
DNA	+	–	–	+	–	–	–	–	–	–	–
Urea	–	–	–	–	–	–	–	–	–	–	–
Tween compounds	+	+	+	+	ND	+	ND	ND	ND	ND	+
Tyrosine	–	+	+	–	–	–	–	+	–	–	–
Brown diffusible pigment on tyrosine agar	(+)	–	–	v	–	–	–	–	–	–	–
Precipitate on egg rolk agar	–	+	+	+	–	–	–	–	–	–	–
Hydrolysis of o-nitrophenyl-b-D-galactopyranoside	–	+	+	–	–	+	–	–	–	–	–
Production of											
Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+	–
Hydrogen sulfide	–	–	–	+	–	–	–	–	–	–	–
Nitrate reduction	–	+	–	+	–	–	–	v	–	–	–
Susceptibility to the vibriostatic compound O/129[b]	ND	–	+	+	ND	+	ND	ND	ND	ND	–
Range of G+C content (mol%)	38	32 (33)[c]	29–31 (33–34)	30 (32–33)	34	33–35	35	34	35	30	32

Symbols: +, all strains positive; –, all strains negative, (+) weak or delayed positive response; v, variable among strains; V, variable among references; and ND, no data available.

[a]"There seemed to be absorption of Congo red by the colonies" (Humphry et al., 2001).

[b]Diffusion method, 500-mg disks.

[c]The values cited in parentheses are those determined by Bernardet et al. (1996) when different from previously published values.

From Anderson and Ordal (1961); van der Meulen et al. (1974); Christensen (1977); Strohl and Tait (1978); Agbo and Moss (1979); Oyaizu and Komagata (1981); Holmes et al. (1984); Bernardet (1989); Bernardet and Grimont (1989); Reichenbach (1989); Wakabayashi et al. (1989); Carson et al. (1993); Bernardet et al. (1996); McCammon et al. (1998); McCammon and Bowman (2000); Humphry et al. (2001); Tamaki et al. (2003); Van Trappen et al. (2003a,b); Zhu et al. (2003); Aslam et al., 2005; Van Trappen et al., 2005; Yi et al., 2005 and J.-F. Bernardet, unpublished data.

F. granuli	*F. hibernum*	*F. hydatis*	*F. johnsoniae*	*F. limicola*	*F. micromati*	*F. omnivorum*	*F. pectinovorum*	*F. psychrolimnae*	*F. psychrophilum*	*F. saccharophilum*	*F. succinicans*	*F. tegetincola*	*F. xanthum*	*F. xinjiangense*
Wastewater treatment plant	Polar freshwater lake	Temperate freshwater	Temperate soil and freshwater	Cold freshwater	Polar freshwater and saline lakes	Frozen soil	Temperate soil	Polar freshwater lakes	Temperate freshwater	Temperate freshwater	Temperate freshwater lake	Polar saline	Polar soil	Frozen soil
ND	ND	Flat, spreading, with filamentous margins	Flat, spreading, with filamentous margins	ND	Flat, round, with entire margins	ND	Low convex, round, with entire margins	Convex, round, with entire margins	Low convex, round, with entire or uneven margins	Flat, spreading, sunken into the agar	Flat, spreading, with filamentous margins	ND	ND	ND
−	+	+	+	−	−	−	+	−	(+)	+	+	+	−	−
−	−	−	−	+	−	ND	−	−	−	−	−	ND	ND	ND
−	+	+	+	−	−	−	+	−	+	+	−	−	−	−
ND	−	−	−	−	(+)	−	−	−	−	−	−	+	+	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)
+	+	+	+	+	(+)	+	+	+	−	+	+	+	+	−
+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
+	+	+	+	+	(+)	−	+	+	v	+	+	(+)	(+)	−
+	+	+	+	+	−	+	+	+	−	+	+	+	+	+
−	+	+	+	−	−	−	+	−	−	ND	+	+	+	−
−	+	+	+	+	−	−	+	−	+	+	(+)	−	+	+
−	+	+	+	+	−	+	+	+	+	+	+	−	+	+
−	+	+	+	+	−	+	+	+	−	+	+	−	+	−
−	−	−	+	−	−	+	+	−	−	(+)	(+)	−	−	−
−	−	−	−	+	−	−	−	−	−	+	−	−	−	−
ND	−	−	+	−	−	+	+	−	−	ND	ND	ND	ND	−
ND	−	+	+	−	−	+	+	−	−	+	ND	−	−	−
−	−	(+)	+	−	−	+	+	−	−	−	−	−	−	+
ND	+	+	+	+	+	+	+	+	−	+	+	−	+	+
−	+	+	+	−	−	−	+	−	(+)	−	+	−	−	−
+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
ND	+	+	+	−	ND	−	+	ND	+	+	+	+	+	−
−	+	+	+	+	−	−	+	−	v	+	−	−	−	−
ND	−	−	v	+	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
+	+	+	+	−	−	+	+	+	−	+	+	−	−	−
+	v	+	+	+	+	+	V	+	(+)	−	V	−	+	+
−	−	−	−	−	−	−	−	−	−	+	−	−	+	+
−	+	+	v	−	−	+	+	−	−	+	v	−	V	−
ND	ND	−	−	ND	ND	ND	+	ND	+	+	+	−	−	ND
36	34	32–34	33 (35)	34–35	33	35	34 (35)	34	32 (33–34)	32–36 (36)	38 (35–37)	34	36	34

studies, and some of the recently described species, namely *F. branchiophilum* (Wakabayashi et al., 1989; Ostland et al., 1994; Ko and Heo, 1997), *F. frigidarium* (Humphry et al., 2001), *F. limicola* (Tamaki et al., 2003) and *F. xinjiangense* and *F. omnivorum* (Zhu et al., 2003), were also investigated using transmission electron microscopy. These studies revealed a structure of the cell wall typical of Gram-negative bacteria. *Flavobacterium branchiophilum* and *F. frigidarium* exhibit fimbria-like structures (Heo et al., 1990; Humphry et al., 2001). Nonflagellar appendages were also found in *F. aquatile*; nonfunctional pseudoflagellar structures were noticed in the same species, but this was not confirmed by further investigations (see Holmes et al. [1984] and references therein). A capsule was evidenced in *F. columnare* and *F. frigidarium* using electron microscopy and biochemical studies (Decostere et al., 1999; Humphry et al., 2001; MacLean et al., 2003); conventional microscopy also showed *F. hibernum* cells have a thick capsule when grown at 4°C (McCammon et al., 1998). Most of the newly described *Flavobacterium* species do not exhibit gliding motility; in *F. gelidilacus*, gliding motility is a strain-dependent characteristic (Van Trappen et al., 2003a). In this species and in *F. hibernum*, gliding only occurs on nutrient-poor media (McCammon et al., 1998; Van Trappen et al., 2003), which is consistent with previous reports on other *Flavobacterium* species (Reichenbach, 1989; Bernardet et al., 1996; Bernardet et al., 2002). *F. psychrophilum* is considered a poor glider, although gliding motility was indeed observed on some strains (Schmidtke and Carson, 1995; Lumsden et al., 1996; Ostland et al., 1997; Lee and Heo, 1998; Madetoja et al., 2001); however, *F. psychrophilum* usually exhibits only a slow and weak gliding motility compared to other gliding *Flavobacterium* species (Bernardet and Kerouault, 1989; Cipriano et al., 1995; Iida and Mizokami, 1996). *Flavobacterium pectinovorum* was the only species able to grow on marine media among the ten *Flavobacterium* species known in 1996. Except *F. antarcticum*, the *Flavobacterium* species which were isolated from soil or freshwater, i.e. *F. hibernum*, *F. limicola*, *F. omnivorum*, *F. xinjiangense*, *F. granuli*, *F. fryxellicola* and *F. psychrolimnae*, cannot grow on marine media. All other newly described *Flavobacterium* species (i.e., *F. degerlachei*, *F. frigidarium*, *F. frigoris*, *F. gelidilacus*, *F. gillisiae*, *F. micromati*, *F. tegetincola* and *F. xanthum*) were retrieved from marine or saline environments and are consequently able to grow on marine media (McCammon et al., 1998; McCammon and Bowman, 2000; Humphry et al., 2001; Tamaki et al., 2003; Van Trappen et al., 2003a; Van Trappen et al., 2003b; Zhu et al., 2003; Aslam et al., 2005). However, since most of them do not require NaCl for growth, they were described as merely halotolerant, not halophilic organisms. The range of NaCl concentrations they tolerate varies widely among species; in the case of *F. frigidarium*, it also varies with the composition of the growth medium used (Humphry et al., 2001). NaCl inhibits the growth of *F. limicola*, although it may tolerate up to 1.5% NaCl (Tamaki et al., 2003). Although different terms are sometimes used (e.g., chemoheterotroph [McCammon and Bowman, 2000; Tamaki et al., 2003] and heteroorganotrophic [Zhu et al., 2003]), all *Flavobacterium* species described since 1996 are chemoorganotrophic and strictly aerobic. *Flavobacterium branchiophilum* was confirmed as an obligate aerobe (Ostland et al., 1994) rather than a facultative anaerobe as previously reported (Ferguson et al., 1991). No growth factors are necessary; however, growth may be stimulated by various mineral or biological compounds, e.g., vitamins for *F. gillisiae* (McCammon and Bowman, 2000), yeast extract for *F. limicola* (Tamaki et al., 2003), and fetal bovine serum and trace mineral elements for *F. psychrophilum* (Michel et al., 1999).

In the emended description of the genus *Flavobacterium* (Bernardet et al., 1996), indole production was negative and catalase production positive for all ten species; this is still the case for the sixteen *Flavobacterium* species described since then. However, the production of catalase is weak in some *Flavobacterium* species (e.g., *F. psychrophilum*; Bernardet and Kerouault, 1989); in such cases, the production of oxygen bubbles by bacteria flooded with oxygen peroxide should be tested on fresh colonies and checked under a stereomicroscope (×20). Characteristics that are variable between *Flavobacterium* species (i.e., degradation of various polysaccharides, casein and gelatin, production of acid from carbohydrates, and production of cytochrome oxidase) are listed in Table 4. Surprisingly, even though several among the newly described *Flavobacterium* species originate from marine environments, the only new agarolytic species is *F. limicola*, isolated from freshwater (Tamaki et al., 2003). Tween 80 was decomposed by all ten *Flavobacterium* species in 1996. Although this characteristic was not tested for all newly described species, *F. limicola*, *F. omnivorum* and *F. xinjiangense* do not decompose this compound (Tamaki et al., 2003; Zhu et al., 2003), contrary to *F. antarcticum* (Yi et al., 2005). The decomposition of tributyrin, a trait for which all ten *Flavobacterium* species gave a positive reaction, was not tested in the sixteen new species. *Flavobacterium limicola*, *F. johnsoniae* and *F. granuli* were also shown to be the only urea-hydrolyzing *Flavobacterium* species among those tested (Reichenbach, 1989; Carson et al., 1993; Tamaki et al., 2003; Aslam et al., 2005). The overall fatty

acid profiles of the *Flavobacterium* species described since 1996 resemble those of the ten previously known species, with only slight discrepancies in the major and minor constituents (McCammon et al., 1998; McCammon and Bowman, 2000; Humphry et al., 2001; Tamaki et al., 2003; Van Trappen et al., 2003a; Van Trappen et al., 2003b; Zhu et al., 2003). Only *F. hibernum* and *F. xanthum* have been tested for their polyamine composition; homospermidine is still the only or major polyamine in these two species (Hamana and Nakagawa, 2001). The low optimum temperature range for *F. psychrophilum*, which was an exception in the 1996 genus description, is now shared by all new species; this is not surprising since they were all retrieved from cold environments. (*Flavobacterium granuli*, isolated from a wastewater treatment plant, is an exception; it grows at 37°C and maybe more (Aslam et al., 2005). Their optimum temperature varies from 11°C for *F. omnivorum* and *F. xinjiangense* (Zhu et al., 2003) to 26°C for *F. hibernum* (McCammon et al., 1998) and is around 15–20°C for all other species. The lowest temperature for growth is 0°C for *F. gillisiae*, *F. tegetincola*, and *F. limicola* (McCammon and Bowman, 2000; Tamaki et al., 2003), lower than 0°C for *F. frigidarium* and *F. xanthum* (McCammon and Bowman, 2000; Humphry et al., 2001), and as low as –7°C for *F. hibernum* (McCammon et al., 1998). All *F. columnare* strains tested are able to grow at 18 and 30°C, but only some of them grow at 15 or 37°C (Triyanto and Wakabayashi, 1999); strains of *F. psychrophilum* usually grow at 4–23°C (Holt et al., 1989; see the sections Isolation and Cultivation and Pathogenicity and Epidemiology in this Chapter). Cold and polar (as well as marine or saline) environments must be added to the temperate soil and freshwater habitats listed in the 1996 description, but no other fish-pathogenic *Flavobacterium* species was described. Since the G+C content of *F. gelidilacus* is around 30 mol% (Van Trappen et al., 2003b), the range of G+C content for species in the genus *Flavobacterium* is now 30–37 mol%. Although slightly lower (29 mol% for some *F. branchiophilum* strains; Wakabayashi et al., 1989) and higher (38 mol% for *F. succinicans*; Anderson and Ordal, 1961) values were reported, they have subsequently been re-evaluated (Reichenbach, 1989; Bernardet et al., 1996).

In the minimal standards proposed for describing new genera and species in the family Flavobacteriaceae (Bernardet et al., 2002), particular methods were suggested to determine some of these phenotypic properties since the conditions in which the tests are performed are critical. These procedures (updated from Bernardet et al., 2002) are listed below in the order shown in Table 4.

Colony Morphology and Gliding Motility

Provided identical growth conditions are used, colony morphology may help in differentiating between *Flavobacterium* species (Reichenbach, 1989; J.-F. Bernardet, unpublished results). Characteristics such as iridescent waves (Fig. 3A) and spreading (Figs. 3B and 3C) to rhizoidal

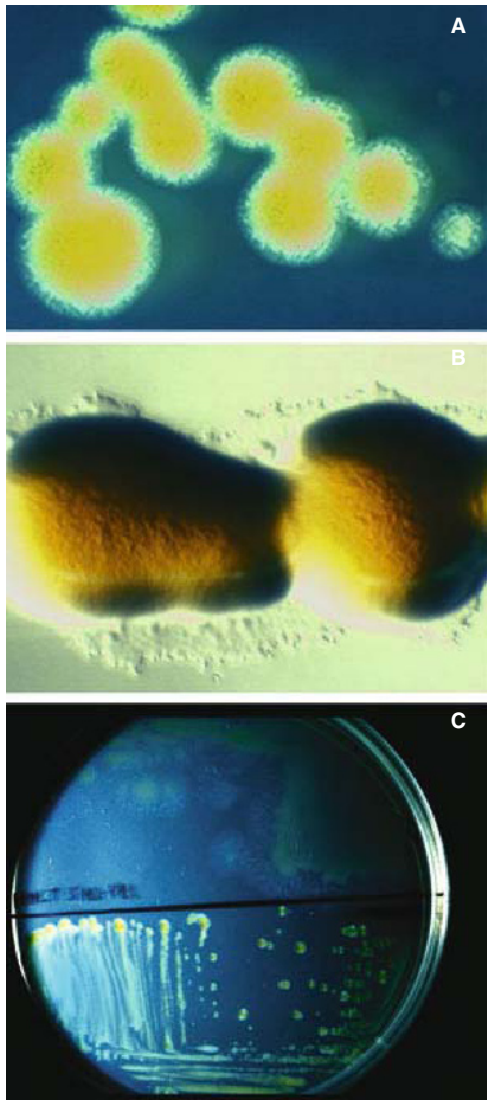

Fig. 3. Colonies of *F. psychrophilum*. Typical yellow, circular, convex, nonadherent colonies of *F. psychrophilum* after a five-day incubation on Anacker and Ordal's agar (containing 0.5% tryptone instead of 0.05%) at 18°C; under the stereomicroscope and oblique transillumination, shiny, iridescent, kaleidoscopic waves are clearly visible (A; size of the colonies is ~1–3 mm). Colonies of another strain, observed under the same conditions, exhibit zones of thin bacterial growth spreading from otherwise entire edges (B; size of the colonies ~0.5–1.5 mm). On the same Anacker and Ordal's agar plate (5 days, 18°C), two *F. psychrophilum* strains exhibit very different colony types: at the bottom of the photograph, the type strain NCIMB 1947^T produced typical circular colonies, whereas at the top another strain produced flat spreading colonies (C).

edges (Figs. 4A to 4F) are best revealed under stereomicroscopic examination (×20) through oblique transmitted light (Anderson and Ordal, 1961; Bernardet, 1989b; Bernardet and Kerouault, 1989). Different strains of *F. columnare*, *F. psychrophilum*, *F. pectinovorum* and *F. succinicans* may produce different colony types, i.e., compact with regular margins or spreading with uneven to rhizoid margins (Fig. 3C); some strains may even exhibit different colony types on the same agar plate (Anderson and Ordal, 1961; Bernardet, 1989a; Bernardet, 1989b; Bernardet and Kerouault, 1989; Schmidtke and Carson, 1995; Lee and Heo, 1998).

Anacker and Ordal's medium (see the section Isolation and Cultivation in this Chapter) is recommended to observe the typical spreading or swarming colonies exhibited by most gliding *Flavobacterium* species (Holmes et al., 1984; Bernardet, 1989a; Bernardet, 1989b; Bernardet and Kerouault, 1989; see the section Isolation and Cultivation in this Chapter). Under direct microscopic examination, their rhizoid or filamentous margin is composed of numerous bacterial cells, alone or in small groups, slowly gliding on the wet surface of the agar and progressing towards the periphery (Fig. 4F). Gliding may be readily observed; in other cases, the slime tracks left on agar by gliding cells are clearly visible (Burchard, 1981; Reichenbach, 1992; J.-F. Bernardet, unpublished data; Fig. 4G). The cells are able to glide either as single cells or as groups of cells. In this sense, their behavior is strikingly similar to the "A" (adventurous) and "S" (social) motility of *Myxoccoccus xanthus*, referred to in the chapter on The Myxobacteria in this Volume.

Information on gliding motility, methods available to observe it in liquid and solid cultures, and the relationship among gliding, production of slime, and adherence are given in Bernardet et al. (2002) and in the chapter An Introduction to the Family Flavobacteriaceae in this Volume.

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. Colonies of *F. pectinovorum* merely exhibit a sticky or mucoid consistency, while separating colonies from agar can be nearly impossible in some *Flavobacterium columnare* strains (Garnjobst, 1945); adherence of this pathogen to fish tissue is considered an important virulence factor (see the section Pathogenicity and Epidemiology in this Chapter). However, adherence may be lost after several subcultures: the National Collections of Industrial, Food, and Marine Bacteria (NCIMB, Aberdeen, Scotland) replaced its culture of the *F. columnare* type strain (NCMB 1038^T) by an adherent subculture (NCIMB 2248^T) after the original one lost its typical adherence. Adherence is also exhibited by *F. columnare* cells in still or slowly agitated liquid cultures: yellow filamentous clumps or tufts of bacterial cells adhere at the surface of the glass flask, forming a thick ring at the upper level of the liquid medium (Fig. 5). When the liquid culture is agitated using a magnet, the latter may be completely covered by these adherent clumps (Garnjobst, 1945; Shamsudin and Plumb, 1996; Newton et al., 1997; Decostere et al., 1998; J.-F. Bernardet, unpublished results).

Such clumps (as well as the warty center of some colonies and the "columns" of bacterial cells that appear on infected tissue; see the section Pathogenicity and Epidemiology in this Chapter) were already noticed in early studies and mistaken for fruiting bodies. This confusion, together with that of the spheroplasts for microcysts, led early authors to assign *F. columnare* to the myxobacteria, and hence the long used epithet "[*Chondrococcus*] *columnaris*" (Ordal and Rucker, 1944; Pacha and Ordal, 1970; Bullock et al., 1971). Conversely, Garnjobst correctly identified the spheroplasts and, since she did not observe fruiting bodies, she ascribed this organism to the genus *Cytophaga*, which was indeed much closer to the currently accepted taxonomic affiliation (Garnjobst, 1945).

Adsorption of Congo Red

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 two min, the dye is gently rinsed with water and the color of these colonies compared to that of control colonies not covered with the dye (Fig. 6). In the case of *Flavobacterium columnare*, the Congo red-staining material has been shown to be an extracellular galactosamine glycan in the slime (Johnson and Chilton, 1966).

Congo red may also be included in the agar; *F. columnare* colonies growing on this medium are red (P. Koski, personal communication). However, when *F. psychrophilum* was tested on a Congo red-containing agar, bacterial growth was strongly inhibited by the dye (Crump et al., 2001). Among the tested *Flavobacterium* species described since 1996, only colonies of *F. limicola* and *F. frigidarium* were shown to adsorb Congo red (Humphry et al., 2001; Tamaki et al., 2003). For the latter species, this trait was actually not definitely demonstrated since the authors carefully stated that "There seemed to be absorption of Congo red by colonies . . ."; in the same study, Congo red was also used to reveal the xylanase activity of the isolate (Humphry et al., 2001).

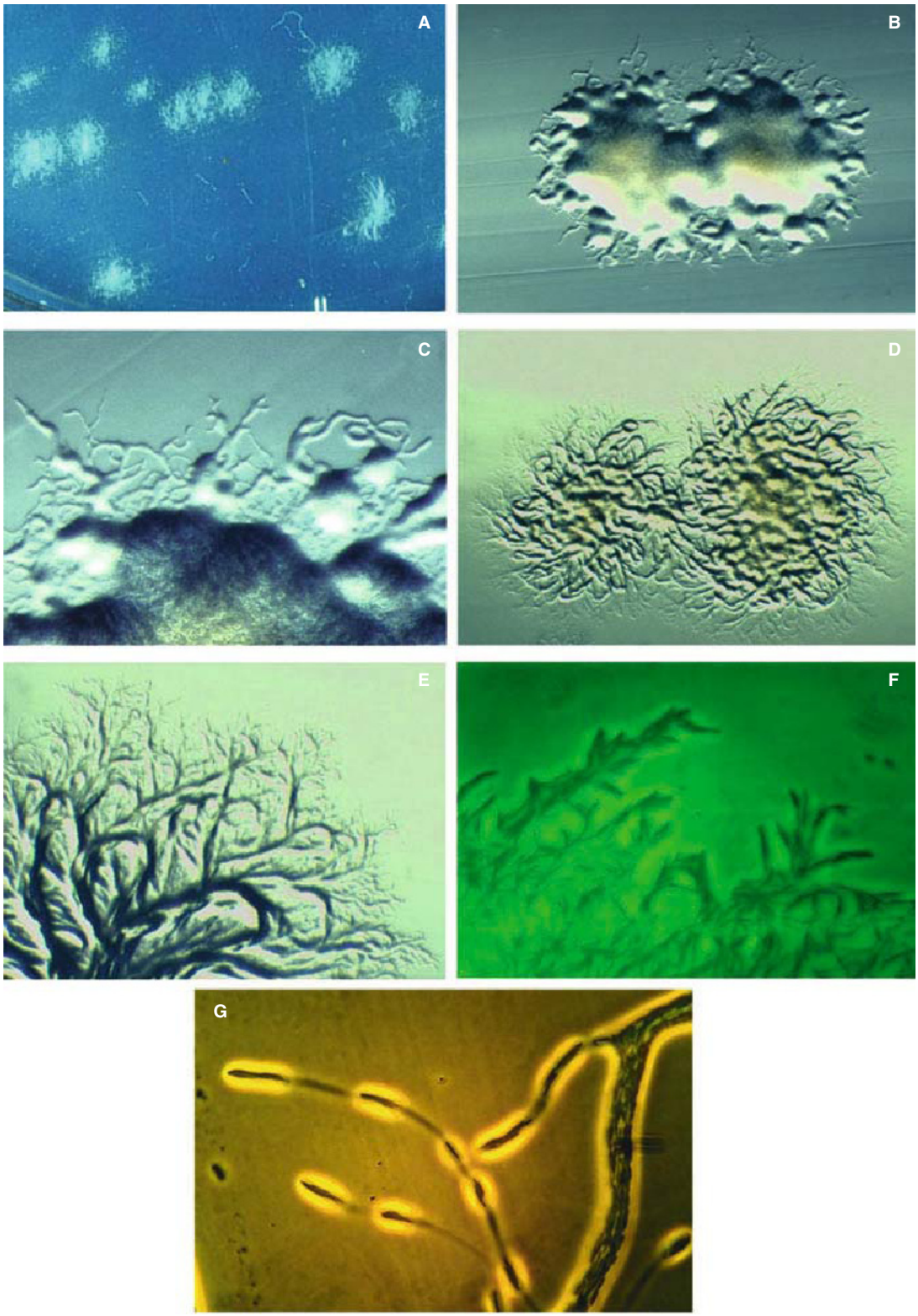

Fig. 4. Colonies of *F. columnare*. Grown on Anacker and Ordal's agar at 22°C for three days, a *F. columnare* strain produced typical pale yellow, flat, dry, rhizoid and adherent colonies (A; field of the view: approx. 5 × 3 cm). The rhizoid aspect of the edges is better seen under the stereomicroscope and oblique transmitted illumination: B and C, moderately rhizoid colonies, and D and E, very rhizoid colonies (size of the colonies: approx. 5 mm). Phase-contrast microscopy reveals groups of parallel bacterial cells spreading from the edge of a colony of the *F. columnare* type strain, NCIMB 2248^{T} (F; field of the view: approx. 0.2 × 0.14 mm). When growth and optical conditions are favorable, the observation of the edge of a *F. columnare* colony on Anacker and Ordal's agar under phase-contrast microscopy (×40) may reveal the slime tracks left by groups of bacterial cells gliding at the surface of the agar (G).

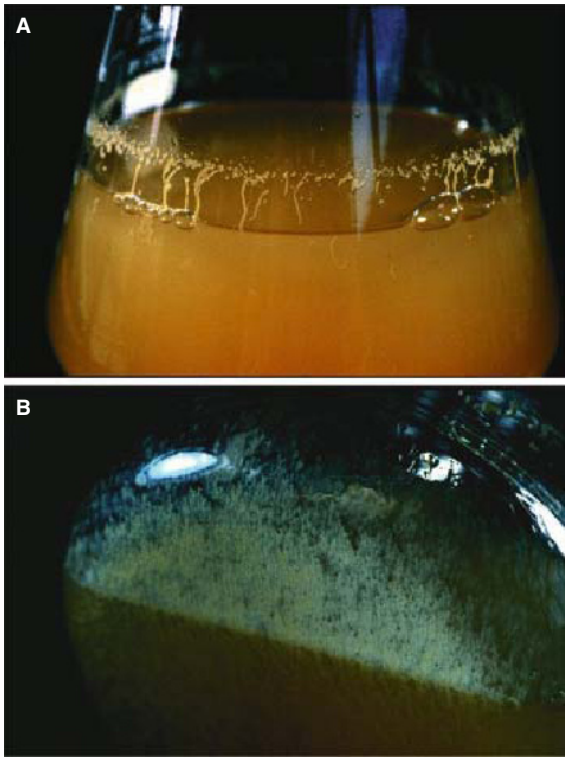

Fig. 5. Adherence of *F. columnare*. A *F. columnare* strain was cultivated for three days at 25°C in 1 liter of Anacker and Ordal's broth (enriched to 0.5% tryptone instead of 0.05%) with gentle orbital shaking. Numerous filamentous clumps of bacteria adhered to the glass, forming a ring at the top edge of the broth surface (A) and carpeting the walls of the flask (B shows the bottom of the tilted flask).

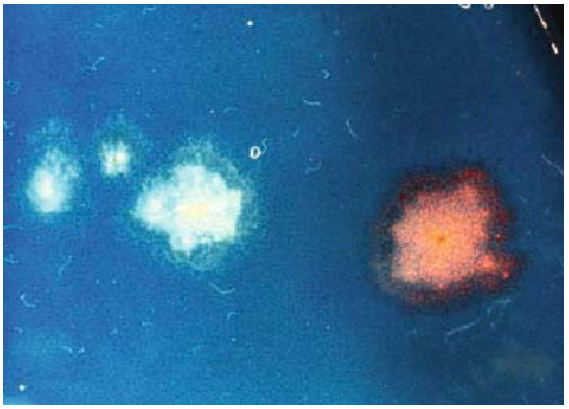

Fig. 6. Congo red test. The color of rhizoid *F. columnare* colonies was checked after flooding the colony on the right hand side with an aqueous 0.01% Congo red solution and gentle rinsing using distilled water: the colony has adsorbed the red dye, demonstrating the presence of a galactosamine glycan component in the bacterial slime (field of the view: approx. 5 × 3 cm).

Determination of Pigment

Information on the different types of pigment produced by members of the family Flavobacteriaceae and on methods to identify these pigments is given in Bernardet et al. (2002). Briefly, an easy although not absolutely specific method to demonstrate the production of flexirubin type pigments is to flood a mass of bacterial cells collected on agar with 20% KOH and to compare the resulting color with that of a control mass that has not been flooded with KOH. When the yellow color of the strain is caused by flexirubin type of pigments, the mass immediately turns dark red, purple or brown (Reichenbach, 1989; Fig. 7). When the yellow color is presumably caused by carotenoid type of pigments, no color change develops.

Several other KOH techniques were proposed. For example, KOH may be directly poured over colonies on an agar plate (e.g., Crump et al., 2001). However, the resulting color-shift may pass unnoticed on a thin layer of bacteria (Cipriano et al., 1996); this was probably why *F. psychrophilum* was first considered devoid of flexirubin type pigments by some authors (e.g., Holt, 1988). Alternatively, a loopful of bacterial cells may be streaked onto filter paper soaked in 1N NaOH (Brown et al., 1997). A "string test" was also proposed (Cipriano et al., 1996). Finally, the KOH test may be performed after methanol/chloroform extraction of the pigments from the pellet of a liquid culture (Maeda et al., 1998). The genus *Flavobacterium* includes carotenoid-producing and flexirubin-producing species, as well as species that produce both types of pigments (Reichenbach, 1989; Bernardet et al., 1996). The more frequent association of carotenoid pigments with marine species and flexirubin pigments with freshwater or soil organisms (Reichenbach, 1989) is not especially clear for members of the genus *Flavobacterium*: the yellow color of *F. frigidarium* and *F. gillisiae*, the only species originating from marine environments, is indeed caused by carotenoid pigments, but several other *Flavobacterium* species produce this type of pigment even though they occur in nonmarine environments (Table 4).

Degradation of Cellulose and Cellulose Derivatives

Some species in the family Flavobacteriaceae degrade soluble cellulose derivatives such as carboxymethylcellulose or hydroxyethylcellulose but, since enzymes other than cellulases can degrade these compounds, this does not demonstrate that these species are cellulolytic. The decomposition of crystalline cellulose (i.e., filter paper) requires the production of a specific cel-

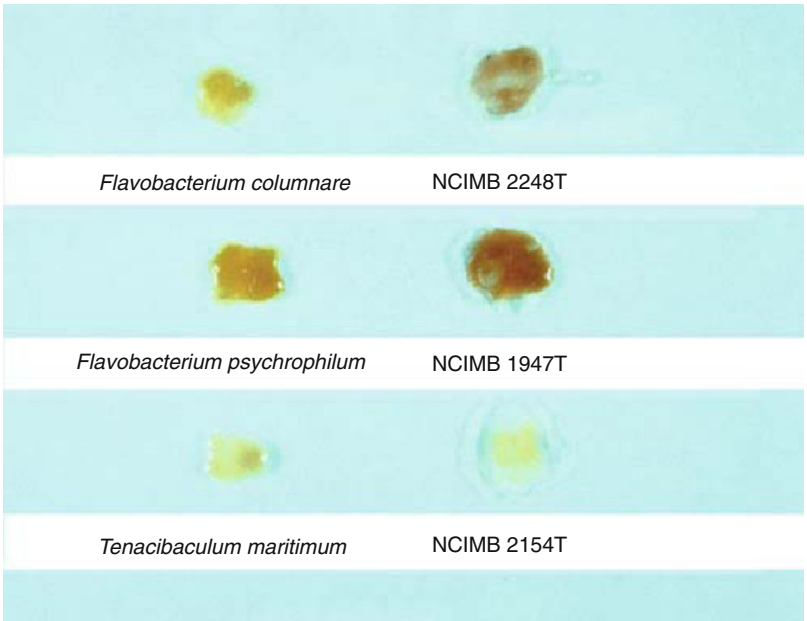

Fig. 7. KOH test for the detection of flexirubin type pigments. After two masses of bacterial cells of *Flavobacterium columnare*, *F. psychrophilum* and *Tenacibaculum maritimum* were deposited on a glass slide, those on the right hand side were flooded with a drop of 20% KOH. The immediate color shift from yellow to brownish pink of the two *Flavobacterium* strains revealed the presence of flexirubin type pigments, whereas the unchanged cream color of the *Tenacibaculum* strain suggested that it is rather due to carotenoid type pigments.

lulase, and hence only strains able to degrade filter paper should be regarded as cellulose degraders (Reichenbach, 1989). The ten *Flavobacterium* species known in 1996 were considered unable to degrade crystalline cellulose and this inability has subsequently been confirmed in members of the recently described *Flavobacterium* species (J. P. Bowman, unpublished results). Hence, this characteristic distinguishes members of the genus *Flavobacterium* from those of the genus *Cytophaga*, now restricted to cellulolytic organisms (Nakagawa and Yamasato, 1996). Two recent publications may challenge this clear-cut distinction. The degradation of cellulosic plant fibers by a number of soil organisms was investigated, and nine cellulolytic isolates were assigned to *F. johnsoniae* (Lednicka et al., 2002). However, these strains were exclusively identified through fatty acid analysis, a technique that is considered to provide taxonomic information mainly at the generic level in the family Flavobacteriaceae (Bernardet et al., 2002). A cellulose-degrading bacterial strain isolated from freshwater proved capable of killing the cyst form of the protozoan parasite *Giardia lamblia*; the determination of the sequence of its 16S rRNA showed a close relationship with those of several *F. columnare* strains, and the isolate shares a number of phenotypic traits with this species (Rodgers et al., 2003). Although some other traits as well as the G+C content were at variance with *F. columnare*, and although a high 16S rRNA homology does not guarantee conspecificity (Bernardet et al., 2002), it is still likely this isolate indeed belongs to the genus *Flavobacterium* or at least to the family Flavobacteriaceae.

Several *Flavobacterium* species are able to degrade various cellulose derivatives, carboxymethylcellulose (CMC) being the most frequently tested (Table 4), for instance, on a 0.5% CMC overlay agar (McCammon et al., 1998). Alternatively, 3% of high viscosity CMC sodium salt may be added to any liquid medium with a rather low nutrient content (e.g., Anacker and Ordal's medium; see the section Isolation and Cultivation in this Chapter); the medium is homogenized by vigorous shaking, distributed in deep glass tubes, and autoclaved. After the gelified medium was inoculated and incubated for several days, its consistency is compared to that of a control, uninoculated, tube. Depending on the ability of the strain to degrade CMC, the medium may be partially or totally liquefied (Bernardet, 1989a; Van Trappen et al., 2003a; Van Trappen et al., 2003b; Fig. 8).

Degradation of Other Biomacromolecules

The degradation of biomacromolecules other than those mentioned above, particularly polysaccharides and proteins, by bacterial species now included in the genus *Flavobacterium* was extensively reviewed by Reichenbach (1989). The degradation of various macromolecules has also been reported in recently described *Flavobacterium* species (Table 4). Most *Flavobacterium* species are able to degrade a variety of polysaccharidic components of algae, plants, fungi, or insects such as agar (Fig. 9), alginate, chitin, laminarin, pectin, xylan, etc. (Stanier, 1947; Van der Meulen et al., 1974; Liao and Wells, 1986; Reichenbach, 1989; Humphry et al., 2001).

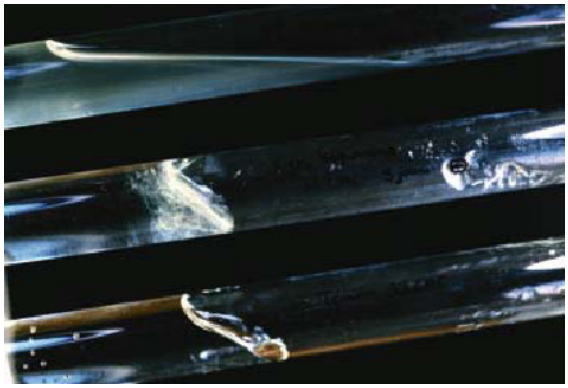

Fig. 8. Hydrolysis of carboxymethylcellulose. Each tube contained 10 ml of Anacker and Ordal's broth gelified with 3% high-viscosity CMC. The tube at the bottom of the figure was an uninoculated control, whereas the two other tubes were inoculated and incubated at 22°C for three days. The tubes were then tilted to reveal the consistency of the medium. Inoculated with *Pedobacter heparinus*, which did not hydrolyze CMC, the medium in the middle tube had the same consistency as that in the control tube. Conversely, the *F. johnsoniae* strain inoculated in the upper tube completely hydrolyzed CMC, turning the medium to liquid.

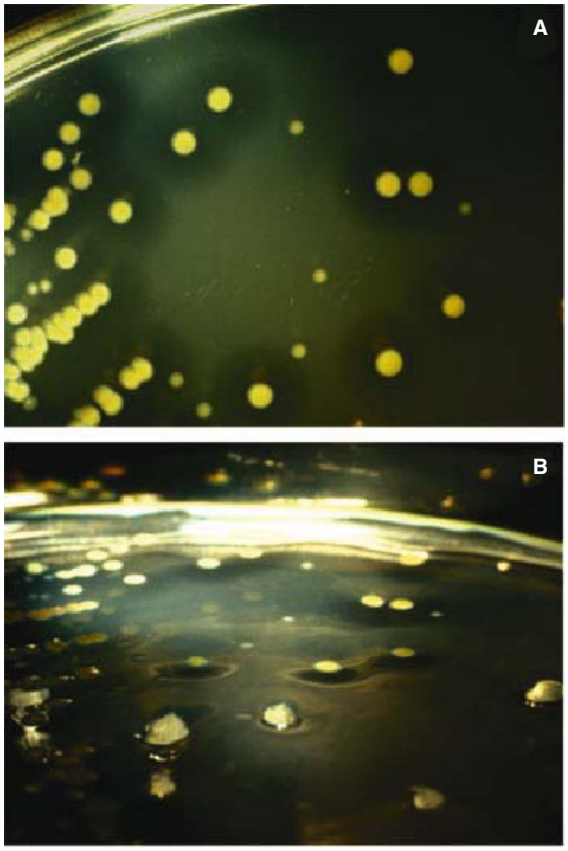

Fig. 9. Hydrolysis of agar. After a four-day incubation at 22°C on Anacker and Ordal's medium with 1.2% (w/v) agar, *F. flevense* has hydrolyzed the surrounding agar: colonies are situated in the center of shallow craters (Fig. 9B), under and around which the agar is softened and more transparent; hence the clear halos surrounding colonies (Fig. 9A).

Interestingly, the fish pathogens *F. columnare* and *F. psychrophilum* have long been considered unable to degrade any polysaccharide (Bernardet and Grimont, 1989). However, it was later discovered that they are actually able to degrade some particular complex acidic polysaccharides of connective tissue such as chondroitin sulfate and hyaluronic acid (Otis, 1984; Griffin, 1991; Teska, 1993; Stringer-Roth et al., 2002). Degradation of chondroitin sulfate was even included in an identification scheme for *F. columnare* (Griffin, 1992). Hence, these polysaccharide-degrading enzymes likely participate in the severe skin and muscular necrotic lesions that frequently occur in infected fish (see the section Pathogenicity and Epidemiology in this Chapter) in combination with the various extracellular proteases also produced by *F. columnare* and *F. psychrophilum*. These proteases, considered important virulence factors, are able to degrade components of muscle, cartilage, and connective tissue such as elastin, type IV collagen, fibrinogen, gelatin, laminin, fibronectin, actin and myosin (Otis, 1984; Holt, 1988; Bertolini and Rohovec, 1992; Bertolini et al., 1994; Nomura and Ohara, 1994; Newton et al., 1997; Ostland et al., 2000; Secades et al., 2001; Secades et al., 2003). The two metalloproteases purified from *F. psychrophilum*, produced under different physiological conditions, exhibit a broad range of hydrolytic activity and are adapted to low temperatures, being optimally produced at 12°C (Secades et al., 2001; Secades et al., 2003). Ostland et al. (2000) demonstrated that in vitro casein-, gelatin- and collagen-degrading activities of *F. psychrophilum* strains were associated with their ability to produce muscle necrosis when injected in rainbow trout. Interestingly, the optimum temperature for protease production was consistently 4–9°C below the optimum temperature for growth for all strains tested (Uddin and Wakabayashi, 1997). The low temperature of protease production may be related to the range of temperature at which outbreaks of the diseases caused by *F. psychrophilum* in salmonid fish occur (see the section Pathogenicity and Epidemiology in this Chapter). In *F. branchiophilum*, the extracellular products contain a protease, a phosphatase, and a phosphoamidase but no hemolysin or endotoxin; when juvenile rainbow trout were immersed in the extracellular products, gill lesions similar to those which occur during the natural infection were observed (Ototake and Wakabayashi, 1985; see the section Pathogenicity and Epidemiology in this Chapter). As in many microorganisms adapted to cold

environments, the production of the extracellular protease found in *F. limicola* is enhanced at low temperatures (Tamaki et al., 2003).

Production of Cytochrome Oxidase

Although the different techniques available to detect the production of cytochrome oxidase have not been compared on all *Flavobacterium* species, it has been demonstrated that the presence of cytochrome oxidase was more readily evidenced in *F. columnare* and *F. psychrophilum* using discs impregnated with dimethyl-*p*-phenylene diamine oxalate than using liquid tetramethyl-*p*-phenylene diamine dihydrochloride reagents (Koski et al., 1993; J.-F. Bernardet, unpublished results). This characteristic should be tested on fresh colonies since it becomes difficult to detect after more than 3–4 days cultivation, especially on species that yield a weak positive reaction such as *F. psychrophilum* (Bernardet and Kerouault, 1989).

Commercial Identification Galleries

In addition to (or instead of) the standard biochemical tests, commercially available kits, strips, and galleries have been extensively used over the last decade to help identify *Flavobacterium* strains. However, since most of these systems are aimed at the identification of human pathogens, the temperature and time of incubation must be adapted to test bacteria that do not grow at 37°C. API galleries (bioMérieux) have frequently been used. While API 20E galleries are not well adapted to most *Flavobacterium* strains (J.-F. Bernardet, unpublished results), API 20NE, API ZYM, API ID 32E, API 32GN and API 50CH have yielded interesting results when used at temperatures that were close to the optimum of the *Flavobacterium* species tested. As for API ZYM galleries, the time of incubation usually varied from the 4 h recommended by the manufacturer (Van Trappen et al., 2003a; Van Trappen et al., 2003b; Aslam et al., 2005) to overnight (Bernardet, 1989b; Bernardet and Grimont, 1989; Bernardet and Kerouault, 1989; Madetoja et al., 2001), although incubation time up to two or three days was sometimes used (Ostland et al., 1994; Humphry et al., 2001). API ZYM galleries were used to differentiate the most common fish pathogenic bacteria, including *F. columnare* (Sakai et al., 1993). API galleries in which bacterial growth is necessary were also incubated for two to three days (McCammon et al., 1998; Humphry et al., 2001; Van Trappen et al., 2003a; Van Trappen et al., 2003b) and up to ten days for API 50CH galleries (Bernardet, 1989b; Bernardet and Grimont, 1989; Bernardet and Kerouault, 1989). Except *F. columnare* (Bernardet, 1989b) and *F. psychrophilum* (Bernardet and Kerouault, 1989), all *Flavobacterium* species tested in API 50CH galleries were able to utilize a variety of substrates. Preliminary studies also demonstrated that *F. johnsoniae*-like fish isolates utilize a number of substrates in Biotype 100 galleries (bioMérieux; J.-F. Bernardet and C. Bizet, unpublished data). Various substrates were also utilized by *F. hibernum* (after incubation times of 4 and 24 h) and *F. limicola* in GN MicroPlates (Biolog; McCammon et al., 1998; Tamaki et al., 2003).

Besides the above-mentioned identification methods, other usually more sophisticated techniques are available to help identify *Flavobacterium* strains and species. The application of most of these techniques to the differentiation of members of the family Flavobacteriaceae was already considered by Bernardet et al. (2002) and in the chapter An Introduction to the Family Flavobacteriaceae in this Volume; some additional information primarily on the genus *Flavobacterium* is provided below.

Composition of the Cell Wall and Membrane

Reichenbach (1989) reviewed data available on the structure and composition of the cell wall and membrane in several bacterial species now attributed to the genus *Flavobacterium*. Since then, fish-pathogenic species were investigated in this regard, mostly to characterize antigens that could be targets for potential vaccines and diagnostic tests (see the section Pathogenicity and Epidemiology in this Chapter). Low- and high-molecular-mass lipopolysaccharides were identified in *F. psychrophilum*, the latter containing the O-antigen (Crump et al., 2001; MacLean et al., 2001); a very unusual sugar in the O-chain may constitute a specific diagnostic target (Crump et al., 2001). Also in *F. psychrophilum*, the outer membrane protein was tested as a possible vaccine (Rahman et al., 2002), a major glycoprotein antigen was purified and characterized (Merle et al., 2003) and a surface antigenic protein was isolated and partially characterized (Massias et al., 2004). Outer membrane proteins and the lipopolysaccharide of *F. columnare* were also studied (Shamsudin, 1994); the structure of the lipopolysaccharide O-antigen was only recently characterized and shown to differ from that of *F. psychrophilum* (MacLean et al., 2003). The structure of the glycopeptides resulting, together with the lipopolysaccharide (LPS) and the capsular polysaccharide from the phenol-water extraction of *F. columnare* cells, was determined; its role in pathogenesis and its antigenic

properties are not known yet (Vinogradov et al., 2003).

As do other members of the family Flavobacteriaceae, *Flavobacterium* species contain menaquinone 6 as their only respiratory quinone and do not contain sphingophospholipids (Bernardet et al., 1996).

The kind of chemotaxonomic information resulting from the analysis of whole-cell fatty acid methyl esters (FAMEs) is discussed in the chapter An Introduction to the Family Flavobacteriaceae in this Volume. Recently, fatty acid analysis has been used extensively for rapidly clustering large numbers of polar bacterial isolates; representative strains in each cluster were then submitted to phylogenetic analysis (see Van Trappen et al. [2002] and references therein). Those isolates that were subsequently shown to belong to the genus *Flavobacterium* mostly contained branched fatty acids (saturated, unsaturated, or saturated and hydroxylated; Van Trappen et al., 2002). These fatty acids, which contribute to the membrane fluidity at low temperature, were abundant in all psychrophilic *Flavobacterium* species (Zhu et al., 2003) and no evidence of polyunsaturated fatty acids was found (Humphry et al., 2001). The overall fatty acid compositions of newly described *Flavobacterium* species were similar to that cited in the genus description (see above), except for some discrepancies in relative proportions (e.g., Van Trappen et al., 2003, 2005; Aslam et al., 2005; Yi et al., 2005) and failure to detect 15:1 *iso* G. Among those fatty acids already listed in the genus description, 15:0, 15:0 *iso*, 15:0 *iso* 3OH, 16:0 *iso* 3OH, 17:1 *iso* 30H and particularly 16:1 ω7c were the most abundant components in most new species (McCammon et al., 1998; McCammon and Bowman, 2000; Humphry et al., 2001; Tamaki et al., 2003; Van Trappen et al., 2003a, Van Trappen et al., 2003b, 2005; Zhu et al., 2003; Aslam et al., 2005; Yi et al., 2005). Additional components which may account for a significant fraction of the total fatty acid composition of the recently described *Flavobacterium* species were 15:0 *anteiso*, 15:1, 15:1 *iso*, 15:1 ω6c, 15:1 *iso* ω10c, 16:0 *iso*, 16:1 *iso*, 17:1 ω6c, and summed feature 3. The decisive influence of growth temperature and medium on the relative amounts of the various fatty acids, although already recognized (McGrath et al., 1990), was again noticed in *F. limicola* (Tamaki et al., 2003). Therefore, although there are no species-specific fatty acid profiles in the genus *Flavobacterium*, it is still possible to define a rather typical profile for the genus itself. Given the necessary technical skill and equipment, the determination of the fatty acid profile helps assignment of new isolates to the genus.

Whole-Cell Protein Analysis

Again, provided the skill and equipment for highly standardized sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), numerical analysis of the resulting electrophoregram, and database comparison are available, the determination of whole-cell protein profiles may also help assign new isolates to published *Flavobacterium* species or delineate new species (Bernardet et al., 1996; Bernardet et al., 2002). A good correlation between high DNA homology and high similarity of whole-cell protein patterns was noticed among several members of the family Flavobacteriaceae (Vandamme et al., 1996; Bernardet et al., 2002; the chapter An Introduction to the Family Flavobacteriaceae in this Volume). Some *Flavobacterium* spp. (*F. columnare* and *F. psychrophilum*) could indeed be readily identified by their typical protein profile, but others (*F. branchiophilum*, *F. johnsoniae* and *F. succinicans*) because of intraspecific heterogeneity could not (Bertolini and Rohovec, 1992; Bernardet et al., 1996; Cipriano et al., 1996). For yet other species, no conclusion could be drawn owing to the limited number of strains tested/available. In *F. columnare* and *F. psychrophilum*, whole-cell protein patterns remained consistent regardless of the growth medium used (Cipriano and Teska, 1994). Unfortunately, none of the sixteen recently described *Flavobacterium* species was investigated for their protein profiles.

Multilocus Enzyme Electrophoresis

Multilocus Enzyme Electrophoresis (MEE) was used as an attempt to classify 106 strains of yellow, Gram-negative, nonflagellated organisms isolated from fish or freshwater environments; one of the four groups delineated by this technique was entirely composed of *F. columnare* strains (Starliper et al., 1988).

DNA-DNA Hybridization

DNA-DNA similarity, as determined using quantitative DNA-DNA hybridization, is still the acknowledged standard for species delineation, together with ΔT_m when available (Wayne et al., 1987; Stackebrandt et al., 2002). The different techniques available and a review of their application to members of the family Flavobacteriaceae were discussed extensively by Bernardet et al. (2002), and only additional information especially focused on the genus *Flavobacterium* will be given here. DNA-DNA hybridization

experiments performed on members of the species classified in the genus have revealed that the 70% cut-off value proposed by Wayne et al. (1987) does apply to *Flavobacterium* species. Strains belonging to the same species share DNA relatedness well above 70% (i.e., 75–100%), whereas DNA relatedness is distinctly below this value (i.e., 4–50%) when strains belonging to different *Flavobacterium* species are hybridized (Bernardet and Grimont, 1989; McCammon et al., 1998; McCammon and Bowman, 2000; Humphry et al., 2001; Tamaki et al., 2003; Van Trappen et al., 2003a; Van Trappen et al., 2003b; Zhu et al., 2003; Van Trappen et al., 2005). The range of DNA relatedness between different strains of the same species may be rather wide (from approximately 75% to close to 100%); this is the case for *F. columnare* (Bernardet and Grimont, 1989), *F. branchiophilum* (Wakabayashi et al., 1989), *F. limicola* (Tamaki et al., 2003) and *F. fryxellicola* (Van Trappen et al., 2005). In the particular case of *F. branchiophilum* and *F. johnsoniae*, DNA relatedness values among some strains were as low as 67% (Wakabayashi et al., 1989) and 60% (Bernardet et al., 1996), respectively; however, ΔT_m values well below 5°C have shown that such strains nevertheless belong to the same species (Bernardet et al., 1996). Three distinct hybridization groups were delineated within a collection of *F. columnare* strains; each was characterized by a particular 16S rDNA restriction pattern and a particular nucleotide signature in its 16S rRNA sequence (Triyanto and Wakabayashi, 1999). However, since no phenotypic characteristics were available to differentiate them, these groups could only be considered genospecies or genomovars. Intraspecific DNA relatedness cannot be assessed in *Flavobacterium* species for which only one bona fide strain is available, namely *F. antarcticum*, *F. aquatile*, *F. flevense*, *F. frigidarium*, *F. gillisiae*, *F. granuli*, *F. hydatis*, *F. omnivorum*, *F. pectinovorum*, *F. saccharophilum*, *F. xanthum* and *F. xinjiangense*.

Sequence Analysis of Small Subunit rRNA and *gyrB* genes

Sequences of the 16S rRNA gene of all valid *Flavobacterium* species are now available; their comparison, combined with earlier data resulting from DNA-rRNA hybridization experiments (Bauwens and De Ley, 1981; Bernardet et al., 1996), provides a distinct view of the phylogenetic relationships within the genus *Flavobacterium* (Fig. 1) and between the genus and other members of the family Flavobacteriaceae (see Figs. 1 and 2 of the chapter An Introduction to the Family Flavobacteriaceae in this Volume). Although 16S rRNA delivers valuable information to delineate taxa at the genus and family level, it should not be used as the only genomic method to delineate bacterial species, with the exception of noncultivable organisms. As shown by several studies, some of them dealing with members of the very family Flavobacteriaceae, the arbitrary cut-off value of 97% 16S rRNA sequence homology is frequently not correlated with DNA-DNA homology (see Bernardet et al. [2002] and references therein). For instance, values of 16S rRNA sequence similarity of 97.2–98.7% were recently found between several *Flavobacterium* species (Van Trappen et al., 2003b, 2005). The sequence of *gyrB* genes, encoding the subunit B protein of DNA gyrase, may also be used for phylogenetic studies. Until now, only the *gyrB* sequences of *F. aquatile*, *F. johnsoniae*, *F. psychrophilum* and "[*Sporocytophaga*] *cauliformis*" are available (Izumi and Wakabayashi, 2000; Suzuki et al., 2001); hence, an evaluation of the respective phylogenetic interest of *gyrB* and 16S rRNA is not possible yet. In the genus *Tenacibaculum*, another member of the family Flavobacteriaceae, interspecific DNA relatedness values have been shown to be more distinctly correlated to the sequence similarity of *gyrB* than to that of 16S rRNA (Suzuki et al., 2001).

A combination of two or more of the above techniques will provide a polyphasic approach to the identification of new *Flavobacterium* isolates (Vandamme et al., 1996). Indeed, it is this kind of approach that has allowed the successful identification and description of all recently published *Flavobacterium* species. Below, we list some other possible techniques for detecting *Flavobacterium* strains in the environment and in clinical samples without requiring previous culture, as well as the methods currently available to type collections of *Flavobacterium* strains.

Molecular Detection and Identification

Several culture-independent molecular methods have been used extensively over the last decade to investigate the phylogenetic composition of bacterial communities in various environments (see the section Habitat and Ecology in this Chapter and the chapter An Introduction to the Family Flavobacteriaceae in this Volume). However, although part of the panel of species used to design the rRNA-targeted oligonucleotide probes for fluorescent in situ hybridization (FISH) was *Flavobacterium* species, no probe specifically targeting members of the genus

Flavobacterium is available yet. Published probes had a broad specificity, usually including the whole *Cytophaga-Flavobacterium-Bacteroides* (CFB) group or parts of it (Manz et al., 1996; Maeda et al., 1998; Weller et al., 2000; Adachi et al., 2002; O'Sullivan et al., 2002). Hence, it is not possible to determine the proportion of *Flavobacterium* species among other members of the CFB group in the environments investigated. Other surveys of bacterial communities used the sequencing of cloned 16S rRNA gene fragments; this technique allowed the detection of several organisms distinctly belonging to the genus *Flavobacterium* in the Columbia and Delaware estuaries (Crump et al., 1999; Kirchman et al., 2003). Denaturing gradient gel electrophoresis (DGGE) of 16S rDNA fragments has also become a popular method to assess the diversity of bacterial flora. It was recently found, however, that *Flavobacterium* strains were the only members of the aquatic bacterioplankton studied that possessed multiple melting domains in their 16S rDNA; these domains were responsible for fuzzy bands on the gel and consequently for poor resolution of DGGE at the species level when commonly applied universal primers were used (Kisand and Wikner, 2003). This problem could lead to misinterpretation of DGGE data and thereby to incorrect estimates of the number of *Flavobacterium* species in natural bacterial communities.

A variety of PCR tests were published for the economically significant fish-pathogenic *Flavobacterium* species, i.e., *F. branchiophilum*, *F. columnare* and *F. psychrophilum* (see the section Pathogenicity and Epidemiology in this Chapter). Classical PCR assays using either 16S rRNA- or *gyrB*-targeted primers (Toyama et al., 1994; Toyama et al., 1996; Urdaci et al., 1998; Izumi and Wakabayashi, 2000) were devised, and procedures for nested PCR (Baliarda et al., 2002; Taylor and Winton, 2002), multiplex PCR (del Cerro et al., 2002), and TaqMan PCR (del Cerro et al., 2002) were also published. These PCR tests were used either to confirm the identification of cultured isolates or to specifically detect the pathogens in the tissue of diseased or apparently healthy fish (Wiklund et al., 2000; Baliarda et al., 2002), in ovarian fluid and eggs (Izumi and Wakabayashi, 1997; Kumagai and Takahashi, 1997; Baliarda et al., 2002), and in water samples (Wiklund et al., 2000; Madetoja and Wiklund, 2002). *F. psychrophilum* was also detected in water samples (Vatsos et al., 2002) and fish organs (Liu et al., 2001) by in situ hybridization using fluorescein-labeled PCR primers. Attempts were made to identify fish-pathogenic *Flavobacterium* species from samples of skin lesion by direct broad-range PCR amplification using universal primers followed by sequencing of the partial 16S rDNA PCR products; sequences similar to those of *F. columnare*, *F. psychrophilum* and *F. hibernum* were found (Tiirola et al., 2002). Presumptive identification at the generic and specific level of different fish pathogens including *F. columnare* and *F. psychrophilum* was also obtained directly from fish tissue using terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes (Nilsson and Strom, 2002).

Typing of Collections of *Flavobacterium* Strains

MOLECULAR TYPING. Several different molecular methods have been used to type collections of *Flavobacterium* strains. Understandably, because of the economic significance of the fish pathogenic species, and because collections of strains of widely different geographic origins and host species were available, typing methods have preferentially been tested on these species. Depending on the primers used, random amplified polymorphic DNA (RAPD) was shown to provide both a typing of *F. psychrophilum* and *F. columnare* strains and a clear differentiation between all *Flavobacterium* species tested (Chakroun et al., 1997); from a practical point of view, this technique was able to distinguish *F. psychrophilum* from other fish-pathogenic *Flavobacterium* species which may occur in the same environment (Crump et al., 2001). Ribotyping (or rRNA gene restriction pattern analysis) was also performed on *F. psychrophilum* strains using several different restriction endonucleases. All four strains tested by Ciprian et al. (1996) exhibited the same ribotype; however, different ribotypes were found when larger collections of strains were studied. Similar to what was obtained using RAPD, some of the ribotypes identified among 85 *F. psychrophilum* strains were distinctly correlated with the fish species from which the strains were isolated, whereas no correlation with the geographic origin was found (Chakroun et al., 1998). One ribotype was highly dominant among 299 Danish strains, and a relationship was found among this ribotype, serotype, and virulence (Madsen and Dalsgaard, 2000). Madetoja et al. (2001) also noted this relationship, but again no correlation with the geographic origin was found; interestingly, this study showed that different clones of *F. psychrophilum* could be isolated during the same outbreak. Three different restriction fragment length polymorphism (RFLP) patterns of PCR-amplified 16S rDNA were found in a collection of *F. columnare* strains, each corresponding to one of the three DNA-DNA hybridization groups identified in this species (Triyanto and

Wakabayashi, 1999; see above). In *F. psychrophilum*, RFLP of the amplified *gyrB* gene yielded different results depending on the kind of primers (i.e., universal versus *F. psychrophilum*-specific) used to amplify the gene; a certain correlation between RFLP patterns and the species of fish host was noticed (Izumi et al., 2003b). Five different plasmid profiles were found when a collection of mostly Danish *F. psychrophilum* isolates was investigated; a ~3.2-kb plasmid was present in most strains (Lorenzen et al., 1997). Chakroun et al. (1998) found that most of the 85 *F. psychrophilum* strains they tested harbored at least one plasmid and that eleven different plasmid profiles occurred; even strains belonging to the same ribotype could exhibit different plasmid profiles, and the approximate size of the plasmids varied from 2.1 to 20 kb. Thirty-seven strains originating from widely different geographic areas (i.e., the United States, Chile, Japan, and several European countries), most of them retrieved from rainbow trout, shared the same 3.5-kb plasmid (Chakroun et al., 1998). A 3.3-kb plasmid was found in 284 among 299 Danish *F. psychrophilum* strains studied, but no clear correlation was noted between the presence of this plasmid and the virulence of strains (Madsen and Dalsgaard, 2000). Among the four different plasmids found in a collection of *F. psychrophilum* isolates from various fish species and geographic areas, the 3.4 kb plasmid occurred mostly in rainbow trout isolates while two other plasmids seemed restricted to Japanese isolates; no plasmid was associated with antibiotic resistance (Izumi and Aranishi, 2004a). Given the rough method used in these studies to evaluate the plasmid size, it is most likely that the 3.2-, 3.3-, 3.4- and 3.5-kb plasmids they reported are actually identical. This plasmid, called "pCP1," was sequenced and used to develop cloning vectors for *F. johnsoniae* (McBride and Kempf, 1996; see the section Genetics in this Chapter).

Hence, the above-mentioned fingerprinting techniques may be powerful molecular tools for the epidemiological tracing of the infections caused by *Flavobacterium* species in fish, provided a sufficient number of strains representing a variety in origins is tested.

Recently, repetitive extragenic palindromic DNA-PCR (rep-PCR) fingerprinting was used to investigate the genomic diversity of clusters previously delineated by fatty acid analysis among a collection of polar isolates (Van Trappen et al., 2003, 2005). The results confirmed that strains sharing the same rep-PCR profile are closely related. Among the six new *Flavobacterium* species described by these authors, *F. degerlachei*, *F. micromati*, *F. fryxellicola* and *F. psychrolimnae* were each comprised of strains that exhibited the same rep-PCR profile, whereas *F. frigoris* and *F. gelidilacus* included strains representing two or three different rep-PCR profiles, respectively (Van Trappen et al., 2003a, Van Trappen et al., 2003b, 2005). Hence, this technique may either differentiate *Flavobacterium* species from each other or be used for intraspecific typing.

SEROLOGICAL TYPING. Fish-pathogenic *Flavobacterium* species have been extensively investigated for possible serotyping with applications to epidemiological studies and diagnostic tests in view; however, only a few studies included strains originating from various fish species and geographic areas. Early studies were reviewed by Reichenbach (1989). Many serotyping schemes were published, but it is only recently that investigators began identifying and characterizing bacterial antigens presumably responsible for the different serotypes (see the section Composition of the Cell Wall and Membrane in this Chapter). As for *F. psychrophilum*, three to seven serotypes were recognized by different authors depending on the origin and number of strains studied and on the technique used (i.e., rabbit or trout mono- or polyclonal antibodies; microtiter or slide agglutination with or without reciprocal absorption of antisera; double immunodiffusion; or enzyme linked immunosorbent assay [ELISA]; Holt, 1988; Wakabayashi et al., 1994; Lorenzen and Olesen, 1997; Izumi and Wakabayashi, 1999; Dalsgaard and Madsen, 2000; Madetoja et al., 2001; Mata et al., 2002). Recently, a new serotype was described and the different serotyping systems were compared and harmonized (Izumi et al., 2003a). No association was found between the serotypes of *F. psychrophilum* strains, their geographical origin, and the host fish species of origin (Faruk, 2000). Serological studies on *F. columnare* are scarce and early (Pacha and Ordal, 1970; Bullock, 1972; Sanders et al., 1976); again, serological relationships did not reveal a pattern based on fish host, tissue, or geographic origin (Shamsudin, 1994). Only limited numbers of *F. branchiophilum* strains have been studied; although common antigens were shared by Japanese, Canadian, and Hungarian strains, antigenic diversity was also found, even among isolates originating from the same region (Huh and Wakabayashi, 1989; Ostland et al., 1994; Ko and Heo, 1997).

BACTERIOCIN TYPING. Bacteriocins produced by some *F. columnare* strains are lethal to other strains of the same species; nine *F. columnare* types were defined according to their susceptibility to seven different bacteriocins, and a partial correlation was noted between these types and serotypes (Anacker and Ordal, 1959). Chase (1965) observed growth medium-dependent differences in the production of

these "columnaricins"; the highest production occurred during the late exponential growth phase (Needleman and Pacha, 1974).

MISCELLANEOUS. Early studies, reviewed by Reichenbach (1989), have reported the presence of phages in *F. columnare* and *F. johnsoniae*, but no practical utilization (e.g., for the typing of strains or as a control method during outbreaks of fish disease) was suggested. Recently, a *Flavobacterium* sp. was identified as a phage host in Arctic sea ice, but its highest 16S rRNA sequence homology (with *F. hibernum*) was only 94% (Borriss et al., 2003).

The only *Flavobacterium* species for which a particular odor, called "sickening," "distinct, fruity," or "pronounced and somewhat characteristic," was noted is *F. columnare* (Garnjobst, 1945; McCarthy, 1975; Shamsudin and Plumb, 1996).

The susceptibility to a range of antibiotics has been tested in only some members of the genus *Flavobacterium*. On the whole, and contrary to other members of the family Flavobacteriaceae such as the genus *Chryseobacterium* (see the corresponding chapter The Genus *Chryseobacterium* in this Volume), *Flavobacterium* strains are not considered to be highly resistant to antimicrobial drugs. Obviously, the fish pathogens have been particularly studied in this regard to determine which drugs might be used to treat infections. The drug susceptibility of *F. columnare* and *F. psychrophilum* were determined (Fijan and Voorhees, 1969; Amin et al., 1988; Hawke and Thune, 1992; Soltani et al., 1995; Shamsudin and Plumb, 1996) and their intrinsic resistance to certain antibiotics was used to propose selective media (Fijan, 1969; Decostere et al., 1997; Kumagai et al., 2004; see the section Isolation and Cultivation in this Chapter). A particular medium was proposed to test the antibiotic susceptibility of *F. columnare* (Hawke and Thune, 1992). Antimicrobial resistance patterns of bacterial strains and minimum inhibitory concentrations of commonly used antibiotics were determined for *F. columnare*, *F. johnsoniae* and *F. psychrophilum* to validate their clinical efficacy when fish were administered bath or oral treatment (Bernardet, 1989b; Bernardet and Grimont, 1989; Bernardet and Kerouault, 1989; Soltani et al., 1995; Lorenzen et al., 1997; Rangdale et al., 1997; Bruun et al., 2000, 2003; Izumi and Aranishi, 2004). Media were compared and standards were recommended to assess the minimal inhibitory concentration of chloramphenicol and florfenicol in various bacterial fish pathogens, including *F. psychrophilum* (Michel et al., 2003). In *F. psychrophilum* as in other Gram-negative bacteria, the co-occurrence of resistance to quinolones and specific mutations of the A subunit of DNA gyrase (GyrA) demonstrated that GyrA is an important target for quinolones (Izumi and Aranishi, 2004b). An extreme susceptibility to penicillin G was noted in *F. branchiophilum* (Ostland et al., 1994). Antibiotics for treatment of fish were selected from among those antibiotics that are toxic to fish-pathogenic *Flavobacterium* species but are not toxic to fish or banned from use on fish farms. Further information on the practical use of antibiotics in the treatment of fish is given in the section Pathogenicity and Epidemiology. Among the recently described *Flavobacterium* species, only *F. frigidarium* (Humphry et al., 2001) and *F. granuli* (Aslam et al., 2005) were tested for their antimicrobial resistance pattern, which proved similar to those of *F. columnare* and *F. psychrophilum*; *F. frigidarium* was found to be highly resistant to kanamycin (Humphry et al., 2001). The carbapenem-hydrolyzing β-lactamase from the *F. johnsoniae* type strain has recently been purified and characterized; the analysis of its sequence revealed that it is closely related to the β-lactamase produced by other members of the family Flavobacteriaceae such as *Chryseobacterium meningosepticum* and *C. indologenes* (Naas et al., 2003; see the chapter The Genus *Chryseobacterium* in this Volume).

Preservation

Flavobacterium species can be preserved for multiple-year periods by simple cryopreservation at –70°C or less, using 10–30% (v/v) glycerol as a cryoprotectant within a routine growth medium such as Anacker and Ordal's broth (see media formulae in the section Isolation and Cultivation). This procedure has been proposed for *F. branchiophilum* (Wakabayashi et al., 1989), *F. columnare* (Shamsudin and Plumb, 1996), *F. psychrophilum* (Madsen and Dalsgaard, 1998), and *F. antarcticum* (Yi et al., 2005) and used successfully by J.-F. Bernardet (unpublished data) for all *Flavobacterium* species using the CAS broth (i.e., 1% casitone [Difco], 0.1% $MgSO_4 \cdot 7H_2O$, pH 6.8 unadjusted) recommended by Reichenbach (1989) to which 10% sterile glycerol was added. Commercial cryopreservative media are also convenient (Cepeda et al., 2004). Storage in liquid nitrogen may also be used, the CAS medium being supplemented with 5% dimethylsulfoxide (DMSO; Reichenbach, 1989).

A study by Desolme and Bernardet (1996) indicated that lyophilization (freeze-drying) of *F. columnare* and *F. psychrophilum* (and thus of most other generally more robust *Flavobacterium* species) was effective using a suspension medium consisting of 2/3 Difco Bacto Brucella broth and 1/3 filtered and heat-inactivated (1 h at 56°C) horse serum. Lyophilization was also

used to preserve *F. branchiophilum* (Ostland et al., 1994). The frozen cells can be revived using Anacker and Ordal's agar. Better results for some of the more sensitive fish pathogenic strains, such as *F. psychrophilum*, can be obtained by reviving on Anacker and Ordal's medium enriched to 0.5% tryptone and supplemented with 5% defibrinated horse serum (Difco) and a trace element solution as described by Michel et al. (1999).

Most of the cold-adapted species can also be stored as live cultures on agar slants at low temperature. The best temperature is 2°C, which reduces the incidence of fungal contamination. However this procedure needs to be verified on a case-by-case basis, as some species may show poor survival in this manner of storage after more than 3 months. Many of the cold-adapted Antarctic species such as *F. tegetincola* can survive at least 18 months this way. Most fish-pathogenic *Flavobacterium* strains will survive for several months or even years when heavily inoculated in deep tubes containing Anacker and Ordal's medium gelified with 0.4% (w/v) agar maintained at 4°C (Anacker and Ordal, 1955; J.-F. Bernardet, unpublished data).

The influence of the preservation procedure on the virulence stability of a *F. psychrophilum* strain was evaluated using experimental infection of rainbow trout fingerlings and determination of the lethal dose 50%. Cultures were revived after 1, 8 and 23 months of storage by lyophilization, freezing at –80°C, and maintenance at 4°C in Anacker and Ordal's agar deeps: whatever the preservation procedure, virulence was maintained after 1 and 8 months of storage; after 23 months, the bacteria kept in agar deeps at 4°C had lost some of their virulence, while the lyophilized and frozen ones were only slightly attenuated (Michel and Garcia, 2003).

Physiology

Though specific aspects of the biochemical physiology of members of the genus *Flavobacterium* remain to be discovered, almost all *Flavobacterium* species are strictly aerobic and appear to lack fundamental idiosyncrasies in their carbon and energy pathways. Most species use glucose as a carbon source and probably employ the Embden-Meyerhof-Parnas pathway for their catabolism (Reichardt and Morita, 1982). Exceptions to this generalization are the species *F. succinicans* and *F. hydatis*, which can grow anaerobically when provided peptone or yeast extract, e.g., modified Shiehs medium (Anderson and Ordal, 1961; Strohl and Tait, 1978; Reichenbach, 1989; see the section Isolation and Cultivation in this Chapter). Some components of these media presumably contain so far undetermined growth factors required to drive anaerobic growth. *Flavobacterium succinicans* also requires CO_2 (which can be provided by adding 15–25 mM $NaHCO_3$ to growth media) and glucose for anaerobic growth (Chase, 1965). *Flavobacterium antarcticum* grows weakly under microaerobic conditions and poorly under anaerobic conditions (Yi et al., 2005). Capnophilic requirements, as exhibited by the related genus *Capnocytophaga* and other members of the family Flavobacteriaceae (see the chapters The Genus Capnocytophaga in this Volume and An Introduction to the Family Flavobacteriaceae in this Volume), are otherwise absent or negligible for known *Flavobacterium* spp. Anaerobic growth can occur fermentatively on carbohydrates or yeast extract or by respiration using nitrate as the electron acceptor. *Flavobacterium succinicans* can also grow by fermentation of pyruvate (converting it to mostly acetate); otherwise organic acids or amino acids are not utilized. Alternative anaerobic electron acceptors to nitrate (sulfur, ferric compounds, etc.) have yet to be tested. Fermentation products formed during growth are mostly succinate, acetate and formate. Anderson and Ordal (1961) proposed the CO_2 requirement of *F. succinicans* stems from a lack of lactate dehydrogenase; instead this species condenses CO_2 with phosphoenolpyruvate (using phosphoenolpyruvate carboxylase and guanosine 5′-diphosphate [GDP]), resulting in oxaloacetate. Oxaloacetate is then reduced further via the reduced form of nicotinamide adenine dinucleotide (NADH), forming succinate as an end product. Interesting physiological comparisons may be made between the capnophilic members of the family Flavobacteriaceae, i.e., the genera *Capnocytophaga* (see the chapter The Genus Capnocytophaga in this Volume), *Coenonia*, *Ornithobacterium* and *Riemerella* (see the chapter Capnophilic Bird Pathogens in the Family Flavobacteriaceae: Riemerella, Ornithobacterium and Coenonia in this Volume). Interestingly, *F. granuli* appears to be strictly aerobic although it was isolated from granules that had been kept under anaerobic conditions for two years (Aslam et al., 2005).

Energy metabolism can be supplemented by reduction of nitrate to nitrite by many *Flavobacterium* species, particularly freshwater species. Some strains (but not the type strains) of *F. johnsoniae* and *F. columnare* have been reported to grow by anaerobic denitrification (Stanier, 1947; Christensen, 1977; Callies and Mannheim, 1978; Nogales et al., 2002). It is possible, however, that these denitrifying strains actually belong to separate species or even genera (Reichenbach, 1989).

Carbon and nitrogen metabolism pathways are poorly understood in the genus; however, on the basis of phenotypic data, they appear quite diverse between species. This can be best seen in the capacity for carbohydrate catabolism, which varies strongly at the species level. Some species utilize a wide range of carbohydrates and polysaccharides including *F. pectinovorum*, the aptly named *F. saccharophilum*, *F. johnsoniae*, *F. hydatis* and *F. omnivorum*. On the other hand, *F. branchiophilum*, *F. columnare*, *F. psychrophilum*, *F. tegetincola* and *F. micromati* are essentially asaccharolytic or poorly saccharolytic, preferring nitrogen-containing substrates, primarily amino acids and proteins. Asaccharolytic species also lack common glycolytic enzymes (e.g., as revealed by API ZYM galleries; see Table 6 in Bernardet et al., 1996), which can be useful for their rapid discrimination from saccharolytic species. All *Flavobacterium* species except *F. branchiophilum* (which requires yeast extract) can grow on single amino acids, such as L-glutamate or L-asparagine, as sole nitrogen sources. In general, *Flavobacterium* species have little propensity to utilize other types of substrates including sugar alcohols, alcohols, organic acids, hydrocarbons and aromatics. The ability to utilize xenobiotics occasionally ascribed to *Flavobacterium* sp. (e.g., Topp et al., 1988) is almost certainly erroneous and the strains concerned are likely instead *Sphingomonas* spp., which can be confused with *Flavobacterium* owing to superficial morphological similarities.

Several other aspects pertaining to the physiology of members of the genus *Flavobacterium* are dealt with in other sections in this Chapter, particularly the section Identification.

Genetics

Besides investigations targeting the whole genome (DNA-DNA hybridization and determination of the G+C content) or particular genes (sequencing of 16S rRNA and *gyrB* genes) for taxonomic and phylogenetic purposes or for the molecular typing of bacterial strains (see the section Identification in this Chapter), genetics of members of the genus *Flavobacterium* has been poorly investigated to date. However, a group of scientists studied extensively the genetics of gliding motility in *F. johnsoniae*. Tools for the genetic manipulation of this organism were first developed: a *Bacteroides* transposon was shown to function in *F. johnsoniae* and cloning vectors based on a cryptic plasmid of *F. psychrophilum* were devised (McBride and Kempf, 1996). The system of gene transfer, the selectable marker, the suicide vector, and the transposon developed to genetically manipulate *F. johnsoniae* were also used successfully on *F. succinicans*, on other members of the family Flavobacteriaceae (i.e., *Chryseobacterium meningosepticum*), and on other members of the *Cytophaga-Flavobacterium-Bacteroides* phylum (i.e., [*Flexibacter*] *canadensis* and *Cytophaga hutchinsonii*; McBride and Baker, 1996). Recently, methods for the genetic manipulation of *F. psychrophilum* were also developed; selectable markers, plasmid cloning vectors, a *lacZY* reporter construct, and a transposon are now available and should allow for the analysis of virulence mechanisms and the development of vaccine strains (Alvarez et al., 2004). The most common plasmid in *F. psychrophilum* strains, pCP1 (see the section Molecular Typing in this Chapter), was entirely sequenced (M. J. McBride, seq. no. NC004811). Mutagenesis was used to address the genetic mechanisms of gliding motility in *F. johnsoniae*; at least eight different genes were shown to be required (Kempf and McBride, 2000; Hunnicutt and McBride, 2001; Hunnicutt et al., 2002; McBride et al., 2003, McBride and Braun, 2004). The most recently discovered genes, *gldH* and *gldI*, are required for gliding motility, chitin utilization, and infection by bacteriophages; these properties are lost in *gldH* or *gldI* mutants and restored when *gldH* or *gldI* is reintroduced in the genome via a plasmid (McBride et al., 2003). However, mechanisms responsible for gliding motility are complex and probably differ among the organisms that exhibit this kind of motility (McBride, 2001). Recently, the nucleotide sequence of the *F. aquatile* operon coding for the *Fau*I restriction-modification system was determined (Abdurashitov et al., 2003).

Pathogenicity and Epidemiology

Plant Pathogens

The decay of various fresh plants and vegetables called "soft rot" has been attributed to *F. johnsoniae*, among other bacterial species (Liao and Wells, 1986; Lelliott and Stead, 1987). The pathogenic strains were shown to produce more pectate lyase in broth medium than the non-pathogenic ones; however, *F. johnsoniae* was mostly considered an opportunistic pathogen (Liao and Wells, 1986).

Human Medicine

After several human cases of a lung disease similar to hypersensitivity pneumonitis occurred in a United States textile facility, a bacterial endotoxin was isolated from the biomass growing in

the air humidification system and shown serologically to be the cause of the clinical disease; the main representatives of the bacterial flora were isolated from the system and a *Cytophaga* strain was identified as the source of the endotoxin (Flaherty et al., 1984). Further studies showed that the bacterium was related to several *Cytophaga*, *Flexibacter*, and *Flavobacterium* reference strains; although DNA relatedness with *F. hydatis* was 78%, several biochemical discrepancies with this species led the authors to propose the name "[*Cytophaga*] *allerginae*" (Liebert et al., 1984). Fatty acid analysis, whole-cell protein profile, and DNA-rRNA hybridization (Bernardet et al., 1996), as well as a rather high DNA relatedness with *F. johnsoniae* (J.-F. Bernardet, unpublished data), confirmed the relationships of "[*C.*] *allerginae*" with members of the genus *Flavobacterium* (see the section Taxonomy in this Chapter). To our knowledge, no other case of lung disease in humans related to such organisms was reported since then. However, since bacterial strains closely affiliated to *F. johnsoniae* and *F. succinicans* were shown to occur intracellularly in amoebae retrieved from drinking water in hospitals and from a human corneal sample (Müller et al., 1999; Horn et al., 2001), other human pathology may possibly result from the close contact with such endocytobionts.

Veterinary Medicine

Although some members of the genus *Flavobacterium* are part of the normal bacterial flora in the mucus at the surface of fish and fish eggs (see the section Habitat and Ecology in this Chapter), several *Flavobacterium* species exhibit various degrees of pathogenicity for fish. To date, freshwater fish are the only animals known to be extensively affected by flavobacterial diseases, although infections in newt (Brown et al., 1997) and frog tadpole (Bullock et al., 1971) were exceptionally reported. Wild fish may be infected (Lehmann et al., 1991; Wiklund et al., 1994; Iida and Mizokami, 1996), but farmed fish are particularly exposed to these diseases owing to the high density of fish in farms and to the extensive national and international trade in fish and fish eggs. The fish-pathogenic *Flavobacterium* species are listed below in the order of decreasing economic significance. Data on cultural, phenotypical, serological, and molecular characteristics of these pathogens, as well as on their antibiotic susceptibility, are provided in other sections in this chapter and in Tables 2, 3 and 4; additional information may also be found in recent reviews (Austin and Austin, 1999; Shotts and Starliper, 1999). The terms used by fish farmers and fish pathologists to designate these bacteria reflect the great taxonomic and nomenclatural confusion that long characterized this bacterial group. Owing to the fact that spheroplasts were mistaken for microcysts and bacterial clumps for fruiting bodies (see the section Identification in this Chapter), fish pathogens were wrongly attributed to the fruiting gliding bacteria and the global term "myxobacteria" was consequently used (and still frequently is). Such generic epithets as *Chondrococcus* or *Cytophaga* were also commonly used for the most important fish pathogens until they were classified in the genus *Flexibacter* on the basis of phenotypic characteristics (Bernardet and Grimont, 1989) and finally in the emended genus *Flavobacterium* after extensive genomic and phylogenetic studies (Bernardet et al., 1996; Bernardet et al., 2002).

FLAVOBACTERIUM PSYCHROPHYLUM. Following the description of "peduncle disease" (see below) by Davis (1946), the responsible bacterium was first isolated by Borg (1948), who named it [*Cytophaga*] *psychrophila* and studied it extensively (Borg, 1960). For the following decades, the disease seemed restricted to the United States and Canada (Borg, 1960; Holt, 1988). *F. psychrophilum* was first recognized in Europe during the mid-eighties (Von Weis, 1987; Bernardet et al., 1988) and subsequently appeared in all other major areas of salmonid aquaculture (Japan, Tasmania, and Chile) during the nineties; its distribution is now considered worldwide. It is suspected that the pathogen extended its geographical range through the international trade in fish and fish eggs (Wakabayashi et al., 1994; Kumagai and Takahashi, 1997). Nevertheless, *F. psychrophilum* may also have occurred in some areas long ago, passing unnoticed because it was not responsible for serious outbreaks before intensive farming was put into practice and because media and temperature appropriate to its isolation were not commonly used. In contrast to *F. columnare* (see below), *F. psychrophilum* mostly infects salmonid fish (as well as the related species called "ayu," *Plecoglossus altivelis*, reared in Japan and Korea), although it may occasionally be isolated from eel, various cyprinids, and other fish species (Lehmann et al., 1991; Iida and Mizokami, 1996; Amita et al., 2000; Izumi et al., 2003b) in which it usually causes less severe infections. Hence, resident non-salmonid fish species as well as salmonid fish having survived an outbreak (Dalsgaard and Madsen, 2000; Madetoja et al., 2000) likely act as carriers or reservoirs of *F. psychrophilum* for salmonids in the same river. The pathogen can survive starvation outside fish hosts: although its culturability and viability declined when maintained in stream water, growth was still possible after 36 weeks provided

a resuscitation step was performed (Vatsos et al., 2003). Similarly, Madetoja et al. (2003) reported a survival capacity of more than 300 days in sterilized fresh water at 15°C even though the virulence declined much more rapidly. Consequently, *F. psychrophilum* was detected in fish-farm water, river water, and algae growing at the surface of stones on riverbeds (Amita et al., 2000; Madetoja and Wiklund, 2002; Vatsos et al., 2002). However, the main sources of infection are the high numbers of bacterial cells that are released into the water by infected and dead fish; disruption of the skin is likely a major invasion route into the fish, whereas oral contamination trials using live feed as a vector did not succeed (Madetoja et al., 2000). *F. psychrophilum* is currently one of the main bacterial pathogens in reared and wild salmonids in temperate to cold water and is responsible for considerable economic losses in salmonid culture worldwide (Izumi et al., 2003b). Besides the above-mentioned general reviews of fish-pathogenic *Flavobacterium* species, much information on the diseases caused by *F. psychrophilum* may be found in reviews focused on this pathogen (Dalsgaard, 1993; Holt et al., 1993; Nematollahi et al., 2003b).

Several different pathological entities have been described depending on the fish species and size (Holt et al., 1993; Nematollahi et al., 2003b). In the classical form observed in Northern America since the forties in the two most susceptible species, coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*), the area around the adipose fin becomes dark and eroded; pathological changes progressively extend to the whole caudal peduncle and to the tail, usually resulting in a deep ulcerative lesion exposing the spinal cord (Davis, 1946; Pacha and Ordal, 1970; Holt et al., 1993), and hence the name "peduncle disease" (Fig. 10). Similar lesions may occur around the dorsal fin; this particular location won the condition the name "saddleback disease."

The terms "bacterial cold-water disease" and "low temperature disease" are also frequently used since outbreaks usually occur when water temperature is below 10°C. When groups of experimentally infected salmonids were kept at 3–23°C, the shortest time from infection to death was reported in the group kept at 12–15°C (Holt et al., 1989). In contrast to *F. columnare* infections (see below), those caused by *F. psychrophilum* rapidly become septicemic and the bacterium may be isolated from internal organs such as the spleen and kidney. Mortality may reach up to 50% in coho salmon fingerlings and 20% in larger fish (Holt, 1988). Gill damage due to *F. psychrophilum* (which is less severe than that due to *F. columnare* or *F. branchiophilum*

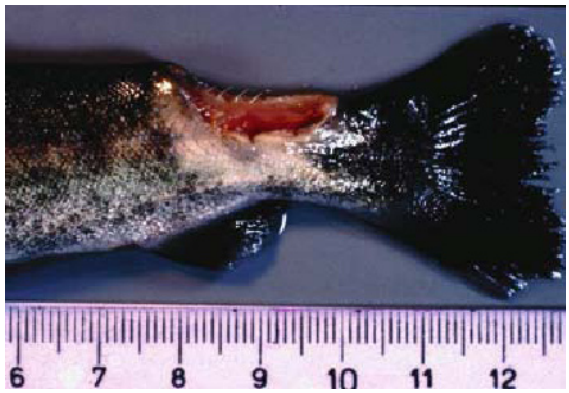

Fig. 10. "Peduncle disease" caused by *F. psychrophilum*. Extensive necrotic lesions may appear in various locations on the body of infected fish, including the caudal peduncle. On this young rainbow trout, extracellular enzymes of the pathogen have destroyed the skin, connective tissue and muscle, revealing the spinal column.

infections [see below]) has been reported in several salmonid species in Ontario (Ostland et al., 1999). Nervous forms of this disease are also known in the United States: young salmon and trout having recovered from acute infection displayed ataxia and spiral swimming behavior stemming from compression of nervous tissue by cranial lesions and spinal deformities; in such cases, osteitis, osteochondritis, meningitis and myeloencephalitis were noted (Kent et al., 1989; Meyers, 1989; Ostland et al., 1997). Such clinical signs as anemia, exophthalmia, necrotic scleritis, and skin bullae or blisters evolving into cavernous ulcers due to necrotic myositis were also reported (Lumsden et al., 1996; Ostland et al., 1997; Ostland et al., 2000). In Europe, although cases of typical cold-water disease were occasionally reported in table-size rainbow trout (Dalsgaard, 1993; Wiklund et al., 1994; Madetoja et al., 2001), *F. psychrophilum* infection is mostly a concern in rainbow trout fry and fingerlings; hence the terms "rainbow trout fry syndrome" (RTFS) and "fry mortality syndrome" (FMS; see Nematollahi et al. [2003b] and references therein). These conditions may result in high to very high mortality (i.e., 20–70%). Infection is septicemic and fish usually display only limited and nonspecific clinical signs such as anemia, lethargy, loss of appetite, dark color, and exophthalmia (Lorenzen, 1994), although fin erosion, blisters (from which *F. psychrophilum* may be isolated in pure culture; J.-F. Bernardet, unpublished data), ulcers, as well as mouth and eye lesions may also occur in fingerlings (Von Weis, 1987; Bernardet and Kerouault, 1989; Lehmann et al., 1991; Dalsgaard, 1993; Lorenzen, 1994; Martínez et al., 2004). The most consistent

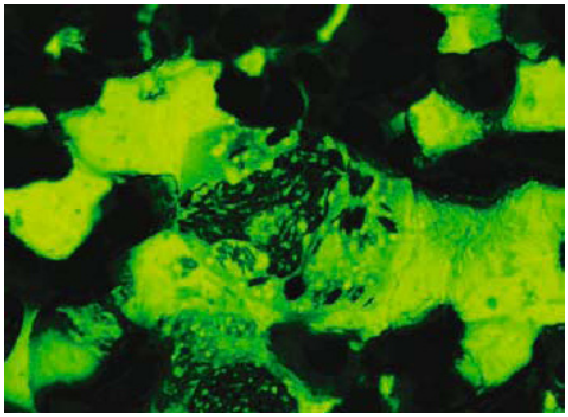

Fig. 11. *Flavobacterium psychrophilum* in a fish spleen macrophage. A spleen imprint from a rainbow trout with hemorrhagic septicemia caused by *F. psychrophilum* reveals numerous bacterial cells in the vacuoles of a macrophage (May-Grunwald-Giemsa, ×450).

internal sign is a severe splenic hypertrophy; in spleen imprints or histological preparations, numerous bacterial rods are dispersed in the parenchyma throughout the tissue (Rangdale et al., 1999; Ekman and Norrgren, 2003; Ekman et al., 2003) as well as located within phagocytes and endothelial cells in the spleen, liver, heart, and kidney (Lehmann et al., 1991; Ekman and Norrgren, 2003; Ekman et al., 2003; Fig. 11).

The presence of bacteria in the spleen tissue is considered pathognomonic for RTFS together with the replacement of the border of the spleen by a loose eosinophilic layer, fibrinous inflammation, and intercellular edema (Rangdale et al., 1999). Clinical signs and pathological findings were similar in the three salmonid species experimentally infected (Ekman and Norrgren, 2003). Following intraperitoneal injection of *F. psychrophilum* cells to rainbow trout, epifluorescence microscopy revealed that the intracellularly located bacteria in the spleen of fry were still viable and increased in number with time, suggesting that the ability of bacteria to escape from the bactericidal mechanisms of phagocytes and to be protected against the humoral defense mechanisms of the host could explain, at least partly, why fry display clinical signs and suffer mortality. Conversely, older fish displayed no sign of infection and no bacteria were detected in their spleen phagocytes (Decostere et al., 2001). Wiklund and Dalsgaard (2003) showed that 1) the *F. psychrophilum* cells associated with kidney phagocytes of large rainbow trout in vitro were rapidly destroyed, 2) the differences in association with the phagocytes were not clearly related to the serotype or virulence of the strains, and 3) a phagocyte toxin was not necessary for the virulence.

Besides the resistance to the defense mechanisms of the fish, several other factors have been suggested to explain the virulence of *F. psychrophilum* (see Dalsgaard [1993] and Nematollahi et al. [2003a] and references therein) and its wide variation among strains (Holt, 1988; Faruk, 2000). Adherence is likely the initial step of infection. The adherence of *F. psychrophilum*, studied on a gill perfusion model, was influenced by water temperature and chemical composition; also, a high virulence strain attached more readily than a low virulence one (Nematollahi et al., 2003a). The adhesion of *F. psychrophilum* to the eggs of rainbow trout (Vatsos et al., 2001) and to the body surface of ayu (Kondo et al., 2002) was also studied; initial attachment of the bacterium was shown to occur preferentially on the jaw and caudal peduncle, which are precisely the locations of some of the most typical lesions. However, the role of the slime layer of *F. psychrophilum* in the adhesion process (unlike that of *F. columnare* [see below]) has not yet been demonstrated (Nematollahi et al., 2003b). Surprisingly, when the adhesion of *F. psychrophilum* to mucus preparations from rainbow trout esophagus, stomach, intestine, gills and skin was compared to that of other fish-pathogenic bacteria, it proved to exhibit the lowest adhesion ability, a detectable adhesion being only reported to mucus from stomach and intestine (Nikoskelainen et al., 2001). The ability of *F. psychrophilum* to agglutinate and hemolyze rainbow trout erythrocytes (Lorenzen, 1994; Lorenzen et al., 1997) may explain, at least partially, the severe anemia that occurs in infected fish. Recently, a sialic acid-binding lectin was shown to be involved in the hemagglutinating properties exhibited by *F. psychrophilum* strains belonging to some serotypes (Møller et al., 2003). The extracellular proteases (Otis, 1984; Holt, 1988; Bertolini and Rohovec, 1992; Bertolini et al., 1994; Nomura and Ohara, 1994; Newton et al., 1997; Faruk, 2000; Ostland et al., 2000; Secades et al., 2001; Secades et al., 2003) and chondroitin lyase (Otis, 1984) produced by *F. psychrophilum* are certainly important virulence factors which may explain to some degree the extensive necrotic lesions displayed by diseased fish (see the section Identification in this Chapter). Scanning electron microscopy investigations of infected fin rays of Atlantic salmon revealed that erosion of the ray axis resulted from the formation of grooves and tubular boreholes likely caused by *F. psychrophilum* extracellular proteases (Martínez et al., 2004). Among the various proteolytic capacities of *F. psychrophilum*, the elastin-degrading capacity in particular has been suggested as a virulence factor, since the mortality rates in groups of rainbow trout experimentally infected

with an elastin-positive strain were significantly higher compared to those of groups infected with an elastin-negative strain; a certain correlation was also found between the elastin-degrading capacity and the serotype of the strains studied (Madsen and Dalsgaard, 1998). However, the opposite result was observed when rainbow trout eggs were injected with *F. psychrophilum* strains with or without elastin-degrading capacity, suggesting that other virulence factors are involved in the virulence of the pathogen (Ekman et al., 2003). It has also been suggested that the lytic activity displayed by *F. psychrophilum* strains against other bacterial species may constitute an advantage, indirectly contributing to its virulence (Nematollahi et al., 2003a). Wiklund and Dalsgaard (2002) showed that virulent as well as nonvirulent strains of *F. psychrophilum* were resistant to the action of the complement present in the serum of rainbow trout. Although the structure of the lipopolysaccharide of *F. psychrophilum* was recently determined (Crump et al., 2001; MacLean et al., 2001; see the section Identification in this Chapter), its role in pathogenesis has not been evaluated. A certain relationship between serotype, ribotype, and virulence was found in *F. psychrophilum* isolates; however, virulence was not clearly correlated to the plasmid content (Madsen and Dalsgaard, 2000; see the section Identification in this Chapter).

The diagnosis of *F. psychrophilum* infections usually relies on the isolation of the pathogen, preferably from internal organs (see the section Isolation and Cultivation in this Chapter), and on its identification using phenotypical and serological characteristics (see the section Identification in this Chapter). The molecular techniques available to specifically detect the bacterium in infected tissue and to identify isolates are listed in the section Identification. Serological methods such as ELISA (Rangdale and Way, 1995; Faruk, 2000), immunohistochemistry (Evensen and Lorenzen, 1996; Ekman and Norrgren, 2003), and immunofluorescence (Lorenzen and Karas, 1992; Amita et al., 2000; Faruk, 2000) have been devised to detect *F. psychrophilum* in fish tissue and to reveal antigenic differences between isolates.

F. psychrophilum has long been suspected to be vertically transmitted from brood fish to progeny, i.e., within fish eggs. If confirmed, vertical transmission associated with the international trade in fish eggs would help explain the dramatic worldwide spread of rainbow trout fry syndrome within only a few years despite disinfection procedures routinely applied to eggs (Izumi and Wakabayashi, 1997; Kumagai and Takahashi, 1997). The detection using cultivation and/or molecular methods of *F. psychrophilum* in the coelomic fluid and at the surface of the ova of infected female salmonids, as well as in the milt of certain males, is well documented (Holt, 1988; Lorenzen, 1994; Rangdale et al., 1996; Brown et al., 1997; Izumi and Wakabayashi, 1997; Kumagai and Takahashi, 1997; Ekman et al., 1999; Amita et al., 2000; Baliarda et al., 2002; Taylor, 2004). However, since *F. psychrophilum* is embedded in the mucus at the surface of the egg, a complete disinfection is difficult to achieve (Brown et al., 1997; Kumagai and Takahashi, 1997) and thus transmission within disinfected eggs rather than on their still-infected or re-infected surfaces is difficult to demonstrate. The strongest evidence for in ovo transmission of *F. psychrophilum* came from Brown et al. (1997), who reported the isolation of the pathogen from crushed steelhead trout eggs after they were disinfected using iodine compounds and after the sterility of their surface was verified by a 72-h incubation in an appropriate broth medium. The same authors also demonstrated that *F. psychrophilum* is resistant to concentrations of lysozyme greater than those found within salmonid eggs and that the pathogen is able to survive within the content of the eggs. Injection of infected females with erythromycin or tetracycline prior to fecundation did not significantly reduce the proportion of infected eggs (Brown et al., 1997; Roberts, 1998). Recent investigations by Kumagai et al. (2000) using immunofluorescence on frozen sections of experimentally infected coho salmon eggs and by Taylor (2004) using a nested PCR assay on yolk material from four different naturally infected Pacific salmonid species also succeeded in revealing *F. psychrophilum* cells within the eggs. Nanoinjection of *F. psychrophilum* in rainbow trout eggs was recently used to mimic vertical transmission and to compare the virulence of two strains (Ekman et al., 2003).

The study of the pathogenesis of *F. psychrophilum* infections and the evaluation of candidate vaccines (see below) were made possible by various experimental infection and challenge models reviewed by Nematollahi et al. (2003b). Clinical disease was successfully reproduced using subcutaneous, intramuscular, or intraperitoneal injection of bacterial suspensions, but these routes were criticized for not being natural and for bypassing the surface defense mechanisms of the fish. To better approximate the natural route of infection, other infection models were devised, using cohabitation with diseased fish or immersion in bacterial suspensions. These procedures usually failed to induce infection, except when fish were previously stressed somehow (see Madetoja et al. [2000], Busch et al. [2003], and Nematollahi et al. [2003b] and references therein); yet, skin injury or osmotic stress

did not significantly increase the mortality of rainbow trout submitted to a bath infection (Garcia et al., 2000). Presence of ectoparasites was expected to favor the penetration of *F. psychrophilum* in fish through the microlesions they cause on the skin; however, no distinct enhancement of infection could be demonstrated in rainbow trout fry bathed in a bacterial suspension and exposed to a monogenean ectoparasite compared to fry only exposed to the bacterium (Busch et al., 2003).

Although the mucus and serum of infected rainbow trout were shown to contain specific antibodies against *F. psychrophilum*, the number of bacterial cells incubated in the serum decreased only slightly, suggesting that high levels of antibodies alone do not provide protection against the pathogen and that stimulation of nonspecific immune factors is necessary to achieve nearly complete protection (LaFrentz et al., 2002; Wiklund and Dalsgaard, 2002). The inability of antibodies to provide complete protection was also demonstrated by the partial success of passive immunization experiments (LaFrentz et al., 2003). Among the vaccination attempts to prevent *F. psychrophilum* infections (see the reviews by Bernardet [1997] and Nematollahi et al. [2003b]), injection of or immersion in formalin-killed bacterial cells has met with varying degrees of success. Recently, Kondo et al. (2003) have claimed to induce protection of juvenile ayu using an oral vaccination procedure. Mortality of ayu injected intraperitoneally with formalin-killed bacterin mixed with water-soluble adjuvants was significantly lower than that of un-vaccinated fish (Nagai et al., 2003; Rahman et al., 2003). Improved vaccines could result from the identification of several immunogenic cell surface molecules (Crump et al., 2001) and from the characterization of the O-antigen of the *F. psychrophilum* lipopolysaccharide (MacLean et al., 2001). Recently, a good protection was obtained using the outer membrane protein of *F. psychrophilum* as a vaccine (Rahman et al., 2002). The major glycoprotein antigen purified from the bacterial cell membrane is another candidate for a subunit vaccine against *F. psychrophilum* (Merle et al., 2003). Following the identification of several immunogenic fractions of the bacterium using western-blot analysis of rainbow trout immune sera, immunization with the 70–100 kDa fraction resulted in a very good protection of fish (LaFrentz et al., 2004). Since antibodies in the serum of protected fish recognized high molecular weight proteins and the O-polysaccharide component of the lipopolysaccharide, these antigens may be responsible for the protective immune response and could serve as vaccine candidates. Methods for the genetic manipulation of *F. psychrophilum* are now available (Alvarez et al., 2004; see the section Genetics in this Chapter); they should facilitate the rational design of vaccine strains.

Holt et al. (1993), Austin and Austin (1999), and Nematollahi et al. (2003b) have reviewed the different antibacterial drugs (e.g., sulfonamides, furans, oxytetracycline, and florfenicol) that may be used in the control of *F. psychrophilum* infections. Although antibacterial therapy remains the most effective control method, the progressive development of resistance to oxytetracycline, oxolinic acid and amoxicillin, as well as the importance of performing in vitro antimicrobial susceptibility testing, are well documented (Soltani et al., 1995; Bruun et al., 2000; Bruun et al., 2003; Dalsgaard and Madsen, 2000; Schmidt et al., 2000; see the section Identification in this Chapter). However, a recent survey of the susceptibility of fish-pathogenic bacteria isolated in France to chloramphenicol and florfenicol found no resistant strain of *F. psychrophilum* (Michel et al., 2003). Manipulation of water temperature or salinity has also been suggested to reduce the morbidity due to *F. psychrophilum* infections in farmed fish (Soltani and Burke, 1994).

FLAVOBACTERIUM COLUMNARE. Although different generic epithets were successively used to designate it (see above and the comments to Table 1 in the chapter An Introduction to the Family Flavobacteriaceae in this Volume), *F. columnare* has long been recognized as an important fish pathogen in relatively warm freshwater worldwide, causing dramatic losses in a considerable variety of farmed and wild, food and ornamental fish. The list of fish species which may be infected and the history of "columnaris disease," as it is called by fish farmers and fish pathologists since its discovery by Davis (1922), may be found in the extensive literature devoted to this organism, particularly in recent reviews (Wakabayashi, 1993; Austin and Austin, 1999; Shotts and Starliper, 1999). Early reports of *F. columnare* from seawater or marine fish (see Bullock et al. [1971] and references therein) were subsequently invalidated following the demonstration that the bacterium was unable to survive in seawater (Chowdhury and Wakabayashi, 1988); in fact, the organism responsible for "marine columnaris" was likely *Tenacibaculum maritimum* (see the chapter The Marine Clade of the Family Flavobacteriaceae: The Genera Aequorivita, Arenibacter, Cellulophaga, Croceibacter, Formosa, Gelidibacter, Gillisia, Maribacter, Mesonia, Muricauda, Polaribacter, Psychroflexus, Psychroserpens, Robiginitalea, Salegentibacter, Tenacibaculum, Ulvibacter, Vitellibacter and Zobellia in this Volume). Even though the overall economic losses due to *F. psychrophilum* worldwide are now far higher

than those caused by *F. columnare*, the latter remains one of the most serious bacterial pathogens in commercially reared channel catfish (*Ictalurus punctatus*) in the United States (Hawke and Thune, 1992; Newton et al., 1997) and in several tropical and/or ornamental fish (Decostere et al., 1998; Michel et al., 2002; Tripathi et al., 2005).

Many environmental and host-related factors (such as water temperature and composition, skin or gill damage, stress due to handling and high stocking density, and feed or oxygen deprivation) may influence the impact of columnaris disease on fish populations (Pacha and Ordal, 1970; Becker and Fujihara, 1978; Austin and Austin, 1999). Columnaris disease is usually problematic only in the warmer periods of the year: the optimum temperature for an outbreak is 20–30°C and mortality usually does not occur below 15°C, although highly virulent strains may still be pathogenic at lower temperatures (see Wakabayashi [1991] and references therein). Field studies demonstrated that various species of feral fish serve as reservoir of infection (Pacha and Ordal, 1970; Becker and Fujihara, 1978; Wakabayashi, 1991). The survival of *F. columnare* in water depends on the concentration of various ions, on the presence of organic matter (such as particulate fish feeds on which the bacterium grows well), and on the temperature (Wakabayashi, 1991).

The pathology of columnaris disease has been extensively described (e.g., Pacha and Ordal, 1970; Amin et al., 1988; Shotts and Starliper, 1999; Decostere et al., 2002) and gross clinical signs may help achieve diagnosis (Austin and Austin, 1999). The gross pathology in young fish infected by highly virulent *F. columnare* strains is usually very limited; the gill is the major site of damage, but the body, mouth (Fig. 12), fins and tail are also frequently damaged. In adult fish, yellowish areas of necrotic tissue appear in the gills, eventually destroying completely the gill filament (Fig. 13).

Lesions on the body begin as small eroded and hyperemic areas which progressively extend to large ulcerative and hemorrhagic necrotic lesions exposing the underlying muscle tissue. A particular aspect of columnaris disease in salmonids is named "saddleback" because the lesion is typically located around the dorsal fin (Cone et al., 1980; Morrison et al., 1981). In severe cases of *F. columnare* infection, a septicemia may also occur. In tropical ornamental fish, columnaris disease is frequently called "cotton-wool" disease or "mouth fungus" since the filaments composed of numerous bacterial cells emerge from the lesions; however, recent descriptions of columnaris disease in three different tropical fish species only mentioned local skin discoloration, with or without ulceration, and degeneration of muscle fibers (Decostere et al., 1998; Michel et al., 2002). When fragments of infected fish tissue are examined under the microscope in wet mounts, great numbers of bacterial cells are

Fig. 12. Mouth lesion in fish infected by *F. columnare*. Bacteria have destroyed the floor of the oral cavity in a young rainbow trout; such necrotic lesions ("mouth rot") are common and may also affect other parts of the body ("fin rot," "tail rot," skin ulcers, etc.).

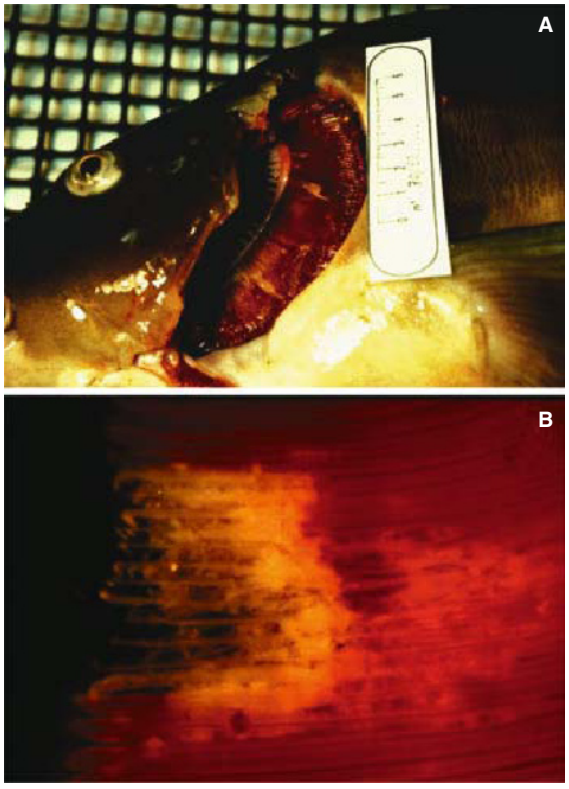

Fig. 13. Gill necrosis in carp caused by *F. columnare*. Yellowish necrotic lesions in the gills of a heavily infected common carp (A). Under the stereomicroscope (×10), gill tissue appears completely destroyed by the extracellular enzymes of *F. columnare*, and yellow bacterial growth progressively invades the gills (B).

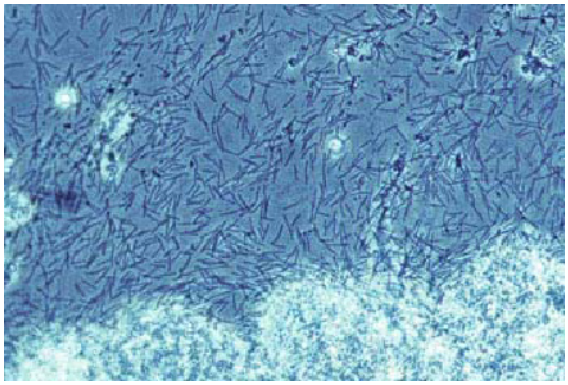

Fig. 14. Direct microscopic examination of a fragment of *F. columnare*-infected tissue. Under phase-contrast microscopy (×1000), a fragment of infected skin tissue from neon tetra (*Paracheirodon innesi*) shows numerous actively gliding bacterial cells; the dense, column-like masses of gliding bacterial cells frequently found on the surface of infected tissue are at the origin of the specific epithet "*columnare*."

observed, slowly gliding and aggregating in columnar masses, from which the specific epithet of *F. columnare* is derived (Fig. 14). Histopathology of columnaris disease was described in several fish species (Pacha and Ordal, 1967; Cone et al., 1980; Morrison et al., 1981; Amin et al., 1988; Shamsudin, 1994; Decostere et al., 1999; Tripathi et al., 2005).

Flavobacterium columnare may usually be readily isolated from external lesions, provided samples are taken from the edge of recent lesions and streaked on convenient media (see the section Isolation and Cultivation in this Chapter). When columnaris disease reaches a septicemic phase, the bacterium may also be isolated from internal organs, preferably spleen or kidney (Morrison et al., 1981; Hawke and Thune, 1992; Decostere et al., 1998; Tripathi et al., 2005). Although different colony types may occur (Song et al., 1988a; Shamsudin and Plumb, 1996), most *F. columnare* strains may be readily identified from their typical colonies on Anacker and Ordal's agar (Anacker and Ordal, 1955). These are pale greenish yellow, flat, spreading, with more or less rhizoid margins, are adherent to the agar, and may appear warty or elevated at the center (Pacha and Ordal, 1970). The gliding motility in liquid culture is also easy to observe and those who know this organism well recognize its odor. Of course, additional tests are necessary for an accurate identification (see other sections in this chapter and Table 4). Various PCR techniques have been proposed to detect *F. columnare* in the tissue of diseased fish or to identify isolates (Toyama et al., 1996; Nilsson and Strom, 2002; Tiirola et al., 2002; Darwish et al., 2004; Tripathi et al., 2005; see also the section Isolation and Cultivation in this Chapter). The typing of *F. columnare* isolates may be achieved using biochemical, serological and molecular techniques; early descriptions of bacteriocin and phage typing were also published (see the section Identification in this Chapter).

Columnaris disease was experimentally reproduced using different routes. These experiments showed *F. columnare* strains exhibit various degrees of virulence: highly virulent strains killed all experimental fish within 24 h while strains exhibiting a low virulence required over 96 h to reach 100% mortality; some of the least virulent *F. columnare* strains failed to infect the fish unless their skin was previously scarified or did not kill the fish at all (Pacha and Ordal, 1970; Amin et al., 1988; Shamsudin, 1994; Chowdhury, 1995; Decostere et al., 1999; Tripathi et al., 2005). Extensive external lesions only appear when the disease lasts several days, i.e., when it is caused by strains with low or moderate virulence. Although the virulence mechanisms of *F. columnare* are far from completely elucidated, several putative virulence factors have been studied. No correlation was found between the degree of virulence and such epidemiological markers as serotypes (Pacha and Ordal, 1970), plasmid or RAPD profiles, and ribotypes (see the section Identification in this Chapter). The various enzymes, such as extracellular proteases (Newton et al., 1997) and chondroitin AC lyase (Stringer-Roth et al., 2002), produced by *F. columnare* are presumably responsible for the extensive necrotic lesions (see the section Identification in this Chapter), and a correlation was indeed found between the degree of virulence and that of chondroitin AC lyase activity (Stringer-Roth et al., 2002). The adherence of *F. columnare* was extensively studied in vivo (Decostere et al., 1999) and ex vivo using a model of isolated gill arches (Decostere et al., 1999; Decostere et al., 1999); these studies demonstrated that 1) the adhesion of bacterial cells to the gill tissue was an important step in pathogenesis, 2) adhesion was favored by the presence of nitrite or organic matter in the water and by high water temperatures, and 3) a high virulence strain adhered more readily than a low virulence one. The role of adherence in pathogenesis was further substantiated when a decrease in virulence was observed in fish challenged with an adhesion-defective mutant of *F. columnare* obtained by serial passages on an ampicillin-enriched medium (Bader et al., 2005). Adherence ability of *F. columnare* was correlated to its hemagglutination capacity, and a lectin-like component of the capsule was responsible for the attachment of the bacterium to gill tissue; moreover, the capsule was thicker and denser in highly virulent strains than is low virulence ones (Decostere et al., 1999). Nevertheless, according

to MacLean et al. (2003), the adhesins involved have not been definitely identified; possible candidates are the lipopolysaccharide, the capsule, the fimbriae, or other appendages of the bacterium.

The control of columnaris disease is difficult to achieve and outbreaks frequently occur in the same river, lake or fish farm when environmental conditions are adequate; healthy fish may be infected by resident carrier fish, by bacteria surviving in the environment, or by recently introduced infected fish. The facts that rainbow trout surviving a columnaris disease outbreak were resistant to a re-exposure the following year (Becker and Fujihara, 1978) and that channel catfish, rainbow trout and tilapia injected with suspensions of heat- or formalin-killed virulent strains produced specific antibodies in plasma and skin mucus (Fujihara and Nakatani, 1971; Schachte and Mora, 1973, Grabowski et al., 2004) have led to various vaccination trials. Although bath or parenteral vaccination of fish against columnaris disease with killed whole-cell bacterins have shown some promise (Fujihara and Nakatani, 1971; Moore et al., 1990; Bernardet, 1997), the protection is neither strong nor long lasting, and better vaccines are needed. As suggested by Newton et al. (1997), detoxified proteases of *F. columnare* could possibly be more effective antigens. Seven outer membrane proteins, some of them common to all *F. columnare* isolates, were strongly recognized by antibodies in the serum of convalescent channel catfish; they may also prove useful in developing an improved vaccine (Davidson, 1996). Rabbit antisera raised against the outer membrane proteins of *F. columnare* were used to screen an expression library of the bacterium; two genes, encoding a zinc metalloprotease (possibly representing a new family of zincins) and a prolyl oligopetidase (Pop), both membrane-associated, were identified (Xie et al., 2004). Comparison of immunodominant antigens in whole-cell lysates of *F. columnare* showed that formalin treatment (but not pressure treatment) inactivates one of the protein antigens (Bader et al., 1997). The recently characterized lipopolysaccharide *O*-polysaccharide antigen and the glycopeptides of *F. columnare* may also provide target molecules for vaccines (MacLean et al., 2003; Vinogradov et al., 2003). The modification of water temperature and salinity has been proposed as a control method during outbreaks of columnaris disease, when possible (Holt et al., 1993; Soltani and Burke, 1994). Chemotherapy remains the most widely used control method in fish farms, but very few antibiotics are at the same time authorized for use in fish, nontoxic to fish, and actually effective in vivo despite demonstration of in vitro activity against *F. columnare* (Soltani et al., 1995; see the section Identification in this Chapter). Oxytetracycline is commonly used in feed or as a bath for external infections; oral administration of oxolinic acid, sulfonamides or florfenicol is also recommended (Wakabayashi, 1993; Austin and Austin, 1999; Shotts and Starliper, 1999). Bath, flush or dip treatments with various disinfectants (sodium chloride, copper sulfate, potassium permanganate, hydrogen peroxide, chloramine-T, quaternary ammonium compounds, Diquat, etc.) have met with varying degrees of success (Altinok, 2004; Thomas-Jinu and Goodwin, 2004). After having been advocated as a strategy to control infectious diseases of channel catfish, withholding feed has been shown to actually reduce the resistance of fish to *F. columnare* (Shoemaker et al., 2003).

FLAVOBACTERIUM BRANCHIOPHILUM. Although *F. columnare*, *F. psychrophilum* and presumably *F. hydatis* are able to cause bacterial gill disease in various fish species (see the corresponding paragraphs), a different, nongliding, yellow filamentous organism first recognized from gill disease in salmonids in Japan (Kimura et al., 1978) is actually the main causative agent. Following the identification of other, serologically different strains from similar cases in Oregon (Wakabayashi et al., 1980) and from gill disease in rainbow trout, sheatfish, and silver carp in Hungary (Farkas, 1985), comparative studies of representative isolates originating from these three geographic areas resulted in the description of the new species *Flavobacterium branchiophila* (Wakabayashi et al., 1989), which was rapidly corrected to *branchiophilum* (Von Graevenitz, 1990). The new pathogen was also recognized in Ontario, Canada, as the causative agent of gill disease, one of the most important conditions affecting the salmonid industry in this region (Ferguson et al., 1991; Turnbull, 1993; Ostland et al., 1994). More recently, cases were also identified in Korea (Ko and Heo, 1997). As for *F. psychrophilum*, it is difficult to know whether what appears to be a fast, worldwide diffusion of a new pathogen could actually be only related to improvements in the isolation and identification of a long-established bacterial species after fish pathologists became aware of the disease in other parts of the world. Bacterial gill disease was reproduced experimentally in various fish species (Wakabayashi et al., 1980; Ferguson et al., 1991; Ostland et al., 1995). Histopathological changes in infected gills were evaluated and the ability of all *F. branchiophilum* strains to attach to the gills was demonstrated, although only virulent strains were able to further colonize the gills and cause mortality (Turnbull, 1993; Ostland et al., 1995). Contrary to the two above-mentioned fish pathogens, *F. branchiophilum* is considered noninvasive and is

usually not isolated from internal organs; it is a relatively fastidious organism, easily overgrown by other bacteria (Turnbull, 1993). The virulence for gills was connected to the various enzymatic and hemagglutinating activities detected in the extracellular products (Ototake and Wakabayashi, 1985) which affect respiratory functions of rainbow trout (Wakabayashi and Iwado, 1985). Serological studies (see the section Identification in this Chapter) made possible the use of immunofluorescence and ELISA to specifically detect and quantify *F. branchiophilum* at the gill surface (Heo et al., 1990; MacPhee et al., 1995). Indirect enzyme immunoassay showed that infected fish produce serum and gill-surface antibodies (Lumsden et al., 1993); however, intermittent occurrence of gill disease outbreaks in the same fish population suggested that surviving fish are not protected against the pathogen (Heo et al., 1990). Nevertheless, immunization trials (reviewed by Bernardet, 1997) showed that the local gill immune response was probably responsible for the significant reduction in gill-associated *F. branchiophilum* and for the partial protection seen in bath-vaccinated fish challenged with relatively low numbers of bacteria (Lumsden et al., 1994). The use of NaCl (Heo et al., 1990), H_2O_2 (Derksen et al., 1999), formalin and chloramine-T (Ostland et al., 1995), and nifurpirinol (now banned; Ostland et al., 1989) was evaluated for the bath treatment of bacterial gill disease and has met with varying degrees of success (Turnbull, 1993; Shotts and Starliper, 1999).

FLAVOBACTERIUM JOHNSONIAE. [*Cytophaga*] *johnsonae* was long known as a common soil organism involved in the degradation of various biomacromolecules (Stanier, 1947; Reichenbach, 1989). It was later transferred to the genus *Flavobacterium* and its specific epithet was corrected (Bernardet et al., 1996). After strains isolated from diseased fish were first mentioned by Christensen (1977), similar strains were frequently recognized in external lesions of different fish species worldwide (Rintamäki-Kinnunen et al., 1997; J.-F. Bernardet, unpublished data). The best description of a fish disease caused by *F. johnsoniae* was published following its isolation in pure culture from extensive skin lesions in juvenile farmed barramundi (*Lates calcarifer*) in Australia (Carson et al., 1993). However, the fact that this outbreak occurred in intensive farming conditions after an increase in suspended solids in the water and a sudden drop in water temperature indicated that special conditions had to be met for *F. johnsoniae* to become pathogenic. This was confirmed when experimental infection trials in various fish species were shown to be effective only in barramundi and then only when fish were challenged after being submitted to a drop in water temperature (Soltani et al., 1994). Hence, these studies confirmed *F. johnsoniae* as an opportunistic pathogen. Although phenotypic and chemotaxonomic (i.e., whole-cell protein profiles and fatty acid analysis) investigations of a collection of fish isolates showed they were indeed highly related to *F. johnsoniae*, only rather low DNA relatedness was found between the isolates tested and the type strain (J-F. Bernardet, unpublished data). Hence, pending further research, such strains should preferably be referred to as "*F. johnsoniae*-like." Since a certain degree of heterogeneity was noticed between the strains available in culture collections (Bernardet et al., 1996), the splitting of *F. johnsoniae* into two or several species may be necessary in the future.

OTHER *FLAVOBACTERIUM* SPECIES. Several other so-called *Flavobacterium* species have been either retrieved from fish or described as fish pathogens; however, most of them have subsequently been moved to other or new genera, and they have not been involved in any outbreak since their original description.

Several *Flavobacterium*-like organisms were described from diseased marine fish; for instance, "[*Flavobacterium*] *piscicida*," isolated from various fish species in Florida, was subsequently reclassified as "[*Pseudomonas*] *piscicida*" and finally as *Pseudoalteromonas piscicida* (see Bernardet [1998] and references therein; the chapter The Genus *Alteromonas* and Related Proteobacteria in Volume 6).

[*Flavobacterium*] *balustinum* was originally isolated from the surface of dead fish; the current opinion is that this organism was a fish spoilage agent rather than a pathogen (see Austin and Austin [1999] and references therein). It was moved to the genus *Chryseobacterium* as *C. balustinum* after further taxonomic investigations (Vandamme et al., 1994).

A new bacterial species isolated from turbot (*Scophthalmus maximus*) and seawater in Scotland was first named "[*Flavobacterium*] *scophthalmum*" (Mudarris et al., 1994); this bacterium, responsible for gill disease and hemorrhagic septicemia, was also transferred to the genus *Chryseobacterium* as *C. scophthalmum* (Vandamme et al., 1994).

A related bacterium, although not isolated from fish, should be mentioned: an organism responsible for hemorrhagic septicemia in farmed bullfrog (*Rana catesbeiana*) in Taiwan was characterized and named "[*Flavobacterium*] *ranacida*" (see Faung et al. [1996] and references therein); recently, this organism was identified as *Chryseobacterium meningosepticum* (J.-F. Bernardet, unpublished data; for the three above-mentioned organisms, see

the chapter The Genus *Chryseobacterium* in this Volume).

Three strains of a facultatively anaerobic gliding bacterium were isolated from skin lesions of salmon and from a water sample in a Washington state hatchery, characterized, and named "[*Cytophaga*] *succinicans*" (Anderson and Ordal, 1961) in spite of phenotypic differences between the three strains (Reichenbach, 1989). Whole-cell protein profile and fatty acid content confirmed that one strain differs from the two others (Bernardet et al., 1996). No other strain of this bacterial species was ever described, and it was subsequently transferred to the genus *Flavobacterium* as *F. succinicans* (Bernardet et al., 1996). Also isolated from external (i.e., gill) lesions in salmon in a Michigan hatchery, another facultatively anaerobic gliding bacterium was described as the new species [*Cytophaga*] *aquatilis* (Strohl and Tait, 1978). This species was later transferred to the genus *Flavobacterium* under the new epithet *F. hydatis* (Bernardet et al., 1996). Similar strains have frequently been isolated from external lesions (i.e., gill or fin necrosis, skin ulcers, etc.) of fish since then (e.g., Austin and Austin, 1999; J.-F. Bernardet, unpublished data). As in the case of *F. johnsoniae*, the fish isolates were phenotypically and chemotaxonomically similar to *F. hydatis* in spite of rather low DNA relatedness between those that were tested and the type and only bona fide *F. hydatis* strain available in culture collections (J.-F. Bernardet, unpublished data); consequently, these strains should be referred to as "*F. hydatis*-like." The pathogenicity of *F. succinicans* and *F. hydatis* for fish was never actually demonstrated (Austin and Austin, 1999); these organisms likely act as opportunistic pathogens, invading fish already weakened by other pathogens, poor farming conditions, or environmental disorders.

Bacterial strains whose 16S rRNA gene sequence matched that of *F. frigidarium* were recently identified among the bacterial community in amoebae-infested gill tissue of salmon, but the relative implication of bacteria and parasites in the disease is not known (Bowman and Nowak, 2004).

Applications

Flavobacterium species produce a variety of enzymes (see the section Identification in this Chapter) that have potential biotechnological applications in processes involving the degradation of such biomacromolecules as agar, alginate, chitin, laminarin, pectin, xylan, etc. For instance, a keratinase-producing *Flavobacterium* sp. was recently isolated from a poultry industry (Riffel and Brandelli, 2002). This organism was able to completely degrade raw feathers and could be used to hydrolyze keratin; however, its affiliation to the genus *Flavobacterium* was only based on a scant phenotypic study. Some bona fide members of the genus have particular biotechnological potential and ecological interest because they can produce cold-active enzymes (see the section Habitat and Ecology in this Chapter).

Over the last few years, a number of algicidal bacteria were identified, especially in Japan; they have attracted much attention as possible tools to regulate the population of phytoplankton responsible for the "red tide" blooms, which frequently cause serious damage to the aquaculture industry in coastal seawaters. Some of these bacteria were attributed to the genus *Flavobacterium* thanks to their 16S rRNA sequence; though isolated from seawater, these isolates did not require NaCl for growth (Maeda et al., 1998). A whole-cell hybridization method using fluorescently labeled probes was developed for the specific detection and enumeration of another algicidal *Flavobacterium* sp. (Adachi et al., 2002).

A *F. columnare*-like organism capable of killing the cyst form of the protozoan parasite *Giardia lamblia* was recently isolated from freshwater (Rodgers et al., 2003). Provided the concentration of Ca^{2+} in the medium was high and the bacteria and cyst were in direct contact, up to 80% of the cysts were destroyed within 48 h. Hence, such strains could be used as biological control agents against *Giardia* cysts in drinking water treatment plants.

A biosurfactant-producing *Flavobacterium* sp. was isolated from samples of arid soil (Bodour et al., 2003). It produces a mixture of at least 37 compounds representing a new class of biosurfactants, named "flavolipids," exhibiting a unique polar moiety and strong and stable emulsifying and solubilizing activities; they have a variety of potential biotechnological and industrial applications, for instance, in the remediation of contaminated soils (Bodour et al., 2004).

Studying the dynamics of bacterial community in a soil contaminated with petroleum hydrocarbons, Kaplan and Kitts (2004) noticed that organisms attributed to the genera *Flavobacterium* and *Pseudomonas* from their 16S rRNA gene sequence were most abundant during the fast degradation phase. This observation supported the importance of *Flavobacterium* spp. in the bioremediation of hydrocarbon-contaminated soils already reported by previous publications (Kaplan and Kitts, 2004 and references therein), although the identification of bacterial strains may not have been based on firm grounds in all these studies.

The degradation potential of a *Flavobacterium* sp. strain on paper mill effluents was investi-

gated; degradation appeared more effective after the bacterium was pre-treated with sinusoidal magnetic fields (Aarthi et al., 2004).

A *Flavobacterium* sp. strain producing antifungal compounds may have some potential for the biocontrol of fungi responsible for the banana crown rot (Gunasinghe et al., 2004).

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