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Cutting a Gordian Knot: Emended Classification and Description of the Genus *Flavobacterium*, Emended Description of the Family *Flavobacteriaceae*, and Proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978)

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The phylogenetic positions and G+C contents of most species belonging to the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter* and several related taxa were determined. Most of the strains included in this study belong to rRNA superfamily V, as shown by DNA-rRNA hybridization data, but the three main genera are highly polyphyletic. Several so-called *Cytophaga* and *Flexibacter* species isolated from soil and freshwater cluster with the type species of the genus *Flavobacterium*, *Flavobacterium aquatile*, and with *Flavobacterium branchiophilum*. The fatty acid and protein profiles of members of this group of organisms were determined. We provide an emended description of the genus *Flavobacterium* and propose new combinations for the following 7 of the 10 validly described species included in this genus: *Flavobacterium columnare*, *Flavobacterium flevense*, *Flavobacterium johnsoniae* (we also correct the specific epithet of this taxon), *Flavobacterium pectinovorum*, *Flavobacterium psychrophilum*, *Flavobacterium saccharophilum*, and *Flavobacterium succinicans*. A new name, *Flavobacterium hydatis*, is proposed for [*Cytophaga*] *aquatilis* Strohl and Tait 1978. The emended genus *Flavobacterium* contains bacteria that have the following main characteristics: gram-negative rods that are motile by gliding, produce yellow colonies on agar, are chemoorganotrophs and aerobes, decompose several polysaccharides but not cellulose, and are widely distributed in soil and freshwater habitats. Three *Flavobacterium* species are pathogenic for fish. The G+C contents of *Flavobacterium* DNAs range from 32 to 37 mol%. An emended description of the family *Flavobacteriaceae* is also provided.

The long and complex history of the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter* and the heterogeneity of these genera have been well documented. The most recent reviews of the taxonomy of these organisms were published in *Bergey's Manual of Systematic Bacteriology* (40, 68), in *The Prokaryotes* 2nd ed. (37, 69), and in *Advances in the Taxonomy and Significance of Flavobacterium, Cytophaga and Related Bacteria* (38, 70). Because of the numerous phenotypic similarities of *Flavobacterium*, *Cytophaga*, and *Flexibacter* strains, for a long time differentiation of these genera has been based on the presence (in the genera *Cytophaga* and *Flexibacter*) or absence (in the genus *Flavobacterium*) of gliding motility. This characteristic, whose relevance for genus delineation has been questioned, is probably an ancestral property of this bacterial group that was lost by some organisms in the course of evolution (68, 101).

The genus *Flavobacterium* was created in 1923 (7) to accommodate gram-negative, non-spore-forming, yellow-pigmented rods that produce acid from carbohydrates weakly (40). Because of this limited definition, the genus rapidly acquired many poorly defined species and consequently became very heterogeneous. However, through successive emendations, the genus *Flavobacterium* was restricted to nonmotile and nongliding species and thus achieved what could be considered reasonable homogeneity in *Bergey's Manual of Systematic Bacteriology* (40). The acceptability of *Flavobacterium aquatile* as the type species of the genus *Flavobacterium* has been discussed repeatedly (37, 38, 40). This species is represented by a single strain, which is not the strain that was described originally, and several studies have demonstrated that *Flavobacterium aquatile* is indeed more closely related to certain *Cytophaga* species than to other *Flavobacterium* species; *Flavobacterium aquatile* exhibits swarming and gliding motility under certain conditions, and the structure of its cell wall is similar to that of [*Cytophaga*] *johnsonae* (29) (brackets indicate generically misclassified bacteria). As *Flavobacterium aquatile* was not considered representative of the genus, Holmes and Owen requested that this name be rejected as a nomen dubium and the species be replaced with *Flavobacterium breve* as the type species of the genus (39). Because this request was denied by the Judicial Commission (96), *Flavobacterium aquatile* must be retained as the type species. Several species previously placed in the genus *Flavobacterium* have been reclassified and placed in new or different genera, including the genera *Bergeyella* (85), *Cytophaga* (68), *Empedobacter* (85), *Sphingobacterium* (37, 102), and *Weeksella* (37, 43, 44). Recently, the genus *Chryseobacterium* has been proposed for several species previously included in the genus *Flavobacterium*; some of these species are found in aquatic environments, while others are human or fish pathogens (85). Several new *Flavobacterium* species have also been described recently (24, 54).

The genus *Cytophaga*, which was created in 1929 by Winogradsky for aerobic cellulolytic gliding soil bacteria, was subsequently expanded to include many environmental gliding

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organisms that degrade several polysaccharides (e.g., agar, chitin, pectin, heparin, and carboxymethyl cellulose) but form neither microcysts nor fruiting bodies (68). As a consequence, this genus also became very heterogeneous. The same is true for the genus *Flexibacter*, which was created by Soriano in 1945 for soil and freshwater bacteria that were phenotypically similar to *Cytophaga* strains but were not able to degrade cellulose (77). The description of the genus *Flexibacter* was later emended so that it included organisms that produced long, slender, thread-like cells in young cultures, did not degrade any polysaccharide, and produced yellow, red, or pink pigments (50). Because these criteria were not sound and failed to deal with the obvious heterogeneity of the genera *Cytophaga* and *Flexibacter*, Reichenbach proposed that these two genera should be distinguished on the basis of changes in cell morphology in aging cultures, G+C content ranges, and habitats (68). The bacterial species that were similar to *Flexibacter* species but were isolated from marine environments were placed in the genus *Microscilla*, which was described by Pringsheim in 1951 (66) and was emended by Lewin (50, 68).

Since the mid-1980s, 16S rRNA oligonucleotide catalog (63) and sequence (30, 45, 56, 59, 100, 101) data and DNA-rRNA hybridization data (6, 72, 73, 85) have shown that the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter* belong to one of the 10 main phylogenetic branches of the *Bacteria*. Depending on the authors, this branch is called the *Cytophaga-Flavobacterium-Bacteroides* group, rRNA superfamily V (73), or the "flavobacter-bacteroides" phylum (30). More surprising is the fact that this group, which is composed of aerobic bacteria for the most part, also includes the capnophilic genera *Capnocytophaga*, *Riemerella*, and *Ornithobacterium* (58, 72, 88) and the obligately anaerobic genera *Bacteroides* (63), *Mitsuokella* (36), *Prevotella*, *Porphyromonas*, and *Rikenella* (62). Additional data have revealed that the following other taxa also belong to the *Cytophaga-Flavobacterium-Bacteroides* group: the family *Spirosomaceae* (including the genera *Spirosoma*, *Runella*, *Flectobacillus*, and *Cyclobacterium*) (67, 98), the recently described genus "*Taxeobacter*" (names in quotation marks have not been validly published) (71), and the genera *Saprospira*, *Haliscomenobacter*, *Microscilla*, *Flexithrix*, *Sporocytophaga*, and *Chitinophaga* (68). In addition, phylogenetic analyses have revealed that two thermophilic organisms, *Thermonema lapsum* (64) and *Rhodothermus marinus* (5), are also included in the *Cytophaga-Flavobacterium-Bacteroides* group. Recent data from 23S rRNA sequencing studies have suggested that the closest relatives of the *Flavobacterium-Bacteroides-Cytophaga* group are the green sulfur bacteria (99).

The following branches of rRNA superfamily V have been studied previously in detail by the DNA-rRNA hybridization technique: the *Chryseobacterium-Bergeyella-Riemerella* branch (72, 85); the *Weeksella-Empedobacter* branch (85); and the *Ornithobacterium*, *Capnocytophaga*, and *Sphingobacterium* branches (72, 80, 81, 88). In order to determine the phylogenetic relationships within the *Cytophaga-Flavobacterium-Bacteroides* group more precisely, we performed extensive DNA-rRNA hybridization experiments and determined guanine-plus-cytosine (G+C) contents; in this study we used the type strains and well-characterized isolates of most valid and invalid species belonging to the genera *Cytophaga* and *Flexibacter*, as well as several members of the genera *Flavobacterium* and *Sphingobacterium* and related taxa. One major rRNA cluster, which contained the type species of the genus *Flavobacterium*, *Flavobacterium aquatile*, was also studied by using a polyphasic approach that included fatty acid analysis and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins in order to determine the relationships at the generic level. Because of the phenotypic similarity between [*Cytophaga*] *johnsonae* and [*Flexibacter*] *aurantiacus*, DNA-DNA hybridization experiments were performed with four [*Cytophaga*] *johnsonae* strains and the only two [*Flexibacter*] *aurantiacus* strains available in order to determine the levels of DNA relatedness of these organisms.

Our results showed that the genera *Cytophaga* and *Flexibacter* are highly polyphyletic and that most *Cytophaga* and *Flexibacter* species are only distantly related to their respective type species (i.e., *Cytophaga hutchinsonii* and *Flexibacter flexilis*, respectively). Several of these generically misnamed species, including soil and freshwater isolates, are close relatives of *Flavobacterium aquatile* and have a considerable number of phenotypic and chemotaxonomic characteristics in common. Therefore, we propose that these species should be transferred to an emended genus *Flavobacterium* that has *Flavobacterium aquatile* as its type species. Because of the high levels of DNA relatedness between the [*Cytophaga*] *johnsonae* type strain and the two [*Flexibacter*] *aurantiacus* strains, we also propose that [*Flexibacter*] *aurantiacus* strains should be transferred to the species [*Cytophaga*] *johnsonae*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains included in this study are shown in Table 1 along with their sources and the growth media used for them. Most of the bacteria were grown at 25°C; the exceptions were [*Flexibacter*] *ovolyticus* and [*Flexibacter*] *psychrophilus*, which were grown at 19°C, and [*Flectobacillus*] *glomeratus*, which was grown at 15°C. The following media were used to grow the bacteria: Dubos mineral medium (69) supplemented with 1% (wt/vol) D-cellobiose; marine medium 2216E (Difco Laboratories, Detroit, Mich.); *Microcyclus-Spirosoma* medium (medium 81 [57a]); modified Shieh medium (76); and Trypticase soy medium (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). Most of the bacteria were grown on agar plates; the only exceptions were the [*Flexibacter*] *columnaris* strains, which were grown in liquid medium because their colonies adhered strongly to agar.

Preparation of DNA. Previously described methods (12) were used to extract and purify high-molecular-weight native DNAs.

DNA base composition. The G+C contents of the DNAs were determined by the thermal denaturation method and were calculated by using the equation of Marmur and Doty (51), as modified by De Ley (21).

DNA-DNA hybridization experiments. A previously described procedure was used for in vitro labeling of bacterial DNA (nick translation) with tritium-labeled nucleotides (Amersham International, Amersham, England) (31); the only modification was that a nick translation kit (Amersham International) was used in this study. Previously described methods were used for hybridization experiments (the S1 nuclease-DE81 method) (65) and for determining the temperature (T_m) at which 50% of a reassociated DNA became hydrolyzable by S1 nuclease (17). The difference between the T_m of a homologous reaction and the T_m of a heterologous reaction (ΔT_m) was an estimate of the level of divergence between two DNAs.

Preparation of rRNA. The rRNAs of [*Flexibacter*] *columnaris* NCIMB 2248^T (T = type strain), [*Flexibacter*] *maritimus* NCIMB 2154^T, *Cytophaga hutchinsonii* LMG 10844^T, and *Flexibacter flexilis* NCIMB 12853^T were radioactively labeled in vivo by adding ^{3}H-labeled adenine to early-log-phase broth cultures as described by De Ley and De Smedt (23). The labeled rRNAs were extracted by the method of Aiba et al. (2) (slightly modified as described by Vandamme et al. [88]) and were separated into 23S and 16S rRNA fractions by ultracentrifugation on a sucrose gradient (23). Labeled *Flavobacterium aquatile* LMG 4008^T, *Sphingobacterium heparinum* LMG 10339^T, and *Sphingobacterium spiritivorum* LMG 8347^T rRNAs were prepared by the same method during a previous study (72).

DNA-rRNA hybridization experiments. Previously described methods (91) were used for fixation of single-stranded DNAs on cellulose nitrate filters, determination of the amount of DNA fixed on filters, saturation hybridization with labeled rRNAs, RNase treatment, and determination of the thermostability of DNA-rRNA hybrids.

FAME analysis. We determined fatty acid methyl ester (FAME) profiles of strains belonging to the *Flavobacterium aquatile* rRNA cluster (see below). Most of the bacteria were grown on modified Shieh agar for 48 h at 25°C (the only exception was [*Flexibacter*] *psychrophilus*, which was grown at 19°C), and the cells were harvested and used for FAME extraction. The FAMEs were then separated by gas-liquid chromatography, and the FAME profiles obtained were compared by performing a numerical analysis (93).

PAGE of whole-cell proteins. Protein patterns were determined for the same taxa that were used in the FAME analysis, which were grown under the condi-

TABLE 1. Bacterial strains used in this study

Name as received	Strain designation as received[a]	LMG no.[a]	Medium[b]	Source
Chitinophaga pinensis		LMG 13176^T	S	Pine litter, Brisbane, Australia
Chitinophaga pinensis		LMG 13042	S	Freshwater, Queensland, Australia
Cyclobacterium marinus		LMG 13164^T	M	Coelomic fluid of sand dollar, California
[*Cytophaga*] *agarovorans*	NCIMB 2217^T	LMG 13037^T	M	Marine mud, California
"[*Cytophaga*] *allerginae*"	ATCC 35408		S	Water in air-cooling unit, Florida
[*Cytophaga*] *aprica*	ATCC 23126^T		M	Rocky sand, Kailua, Hawaii
[*Cytophaga*] *aprica*	NCIMB 1411		M	Mud, Dubrovnik, Yugoslavia
[*Cytophaga*] *aquatilis*	DSM 2063^T	LMG 8385^T	S	Gills of diseased salmon, Michigan
[*Cytophaga*] *arvensicola*	JCM 2836^T	LMG 8359^T	S	Soil, Osaka, Japan
Cytophaga aurantiaca	NCIMB 8628^T	LMG 1337^T	C	Swampy soil, Germany
[*Cytophaga*] *diffluens*	NCIMB 1402^T	LMG 13036^T	M	Beach mud, Bombay, India
[*Cytophaga*] *fermentans*	NCIMB 2218^T	LMG 1338^T	M	Marine mud, California
[*Cytophaga*] *flevensis*	DSM 1076^T	LMG 8328^T	S	Lake Ijssel, The Netherlands
Cytophaga hutchinsonii		LMG 10844^T	C	Soil
Cytophaga hutchinsonii	NCIMB 10782	LMG 13160	C	Soil
[*Cytophaga*] *johnsonae*	DSM 2064^T	LMG 1341^T	S	Soil or mud, Rothamsted or Cambridge, England
[*Cytophaga*] *johnsonae*	ATCC 29585	LMG 1342	S	Diseased freshwater fish, Manitoba, Canada
[*Cytophaga*] *johnsonae*	ATCC 29586		S	Diseased freshwater fish, Manitoba, Canada
[*Cytophaga*] *johnsonae*	Cy j1[c]	LMG 13142	S	Root surface of grass, Scotland
[*Cytophaga*] *johnsonae*	NCIMB 11391	LMG 13161	S	Soil
[*Cytophaga*] *johnsonae*	UASM 405[d]		S	Soil, Ottawa, Ontario, Canada
[*Cytophaga*] *johnsonae*	UASM 444[d]		S	Unknown
"[*Cytophaga*] *keratolytica*"		LMG 11610	S	Unknown
[*Cytophaga*] *latercula*	NCIMB 1399^T	LMG 1343^T	M	Outflow of marine aquarium, La Jolla, Calif.
[*Cytophaga*] *lytica*	NCIMB 1423^T	LMG 1344^T	M	Beach mud, Limon, Costa Rica
[*Cytophaga*] *lytica*	DSM 2040[e]	LMG 13155	M	Outflow of marine aquarium, La Jolla, Calif.
[*Cytophaga*] *marinoflava*	NCIMB 397^T	LMG 1345^T	M	Seawater off Aberdeen, Scotland
[*Cytophaga*] *pectinovora*	NCIMB 9059^T	LMG 4031^T	S	Soil, England
[*Cytophaga*] *saccharophila*	NCIMB 2072^T	LMG 8384^T	S	River Wey, Surrey, England
[*Cytophaga*] *salmonicolor*	NCIMB 2216^T	LMG 1346^T	M	Marine mud, California
[*Cytophaga*] *succinicans*	NCIMB 2277^T	LMG 10402^T	S	Eroded fin of salmon, Washington
[*Cytophaga*] *succinicans*	NCIMB 2278		S	Lesion of salmon, Snake River, Idaho
[*Cytophaga*] *succinicans*	NCIMB 2279		S	Water from a fish tank, Washington
[*Cytophaga*] *uliginosa*	NCIMB 1863^T	LMG 3809^T	M	Marine sediment
"[*Cytophaga*] *xantha*"		LMG 8372^T	S	Showa Station, Antarctica
Flavobacterium aquatile		LMG 4008^T	S	Deep well, Kent, England
Flavobacterium branchiophilum	ATCC 35035^T	LMG 13707^T	S	Diseased gills of fish, Gumma, Japan
Flavobacterium branchiophilum	NCIMB 2219		S	Diseased gills of fish, Bonneville Hatchery, Oregon
Flavobacterium branchiophilum	BGD 7736[f]		S	Diseased gills of fish, Gumma, Japan
Flavobacterium branchiophilum	FL-15[f]		S	Diseased gills of sheatfish, Hungary
Flavobacterium branchiophilum	THP-1[f]		S	Diseased gills of fish, Tokushima, Japan
Flavobacterium branchiophilum	FDL-1[f]		S	Diseased gills of fish, South Santiam, Oreg.
Flavobacterium branchiophilum	BV-6[f]		S	Diseased gills of fish, Bonneville Hatchery, Oregon
[*Flavobacterium*] *ferrugineum*		LMG 10403^T	T	Unknown
[*Flavobacterium*] *gondwanense*		LMG 13192^T	M	Water, Organic Lake, Antarctica
[*Flavobacterium*] *gondwanense*	ACAM 46[g]		M	Water, Organic Lake, Antarctica
[*Flavobacterium*] *odoratum*		LMG 1233^T	S	Unknown
[*Flavobacterium*] *odoratum*		LMG 4028	S	Urine, England
[*Flavobacterium*] *odoratum*		LMG 4029	S	Wound swab, England
[*Flavobacterium*] *salegens*		LMG 13193^T	M	Water, Organic Lake, Antarctica
[*Flavobacterium*] *salegens*	ACAM 52[g]		M	Water, Organic Lake, Antarctica
"[*Flavobacterium*] *tirrenicum*"	Fv t1^{Tc}	LMG 4037^T	M	Seawater, Gulf of Naples, Italy
[*Flectobacillus*] *glomeratus*		LMG 13858^T	M	Water, Burton Lake, Antarctica
Flectobacillus major		LMG 13163^T	S	Algal culture, Russia
[*Flexibacter*] *aurantiacus*	NCIMB 1382^T	LMG 3987^T	S	Garden soil, Minneapolis, Minn.
[*Flexibacter*] *aurantiacus*	NCIMB 1455	LMG 10404	S	Unknown
"[*Flexibacter*] *aurantiacus* subsp. *copepodarum*"		LMG 10405^T	M	Offshore copepod, La Jolla, Calif.
"[*Flexibacter*] *aurantiacus* subsp. *excathedrus*"		LMG 3986^T	S	Pool in cathedral, Cartago, Costa Rica
[*Flexibacter*] *canadensis*		LMG 8368^T	T	Soil, Canada
[*Flexibacter*] *columnaris*	NCIMB 2248^T	LMG 13035^T	S	Kidney of diseased salmon, Snake River, Washington
[*Flexibacter*] *columnaris*	DD3-69[h]		S	Gill lesion of salmon, Dexter Dam, Oregon
[*Flexibacter*] *columnaris*	JIP 44/87		S	Skin lesion of brown trout, Basse Normandie, France
[*Flexibacter*] *columnaris*	CR7[i]		S	Jaw erosion of trout, Finland

Continued on following page

TABLE 1—*Continued*

Name as received	Strain designation as received[a]	LMG no.[a]	Medium[b]	Source
[*Flexibacter*] *columnaris*	CR8[i]		S	Tail lesion of salmon, Finland
[*Flexibacter*] *elegans*		LMG 10750^T	T	Hot spring, Rotorua, New Zealand
[*Flexibacter*] *filiformis*	ATCC 29495^T	LMG 10391^T	T	Soil, Upolu, Apia, Samoa
Flexibacter flexilis	NCIMB 12853^T	LMG 3989^T	S	Lily pond, San Jose, Costa Rica
"[*Flexibacter*] *flexilis* subsp. *algavorum*"	DSM 4510Te	LMG 13158^T	S	Pond, Saint Petersburg, Russia
"[*Flexibacter*] *flexilis* subsp. *pelliculosus*"		LMG 3991^T	S	Shore of Birch Lake, Minnesota
[*Flexibacter*] *litoralis*	NCIMB 1366^T	LMG 3992^T	M	Outflow of marine aquarium, La Jolla, Calif.
[*Flexibacter*] *maritimus*	NCIMB 2154^T	LMG 11612^T	M	Kidney of diseased sea bream, Hiroshima, Japan
[*Flexibacter*] *maritimus*	NCIMB 2153		M	Kidney of diseased sea bream, Hiroshima, Japan
[*Flexibacter*] *maritimus*	NCIMB 2158	LMG 13038	M	Skin lesion of Dover sole, Hunterston, Scotland
[*Flexibacter*] *ovolyticus*	EKD 002^{Tj}	LMG 13026^T	M	Adherent epiflora of halibut eggs, Austevoll, Norway
[*Flexibacter*] *ovolyticus*	VKB 004[j]	LMG 13027	M	Water in halibut egg incubator, Austevoll, Norway
[*Flexibacter*] *psychrophilus*	NCIMB 1947^T	LMG 13179^T	S	Kidney of coho salmon, Washington
[*Flexibacter*] *psychrophilus*		LMG 10400	S	Salmon, Minter Creek Hatchery, Washington
[*Flexibacter*] *psychrophilus*	SH3-81[h]		S	Kidney of coho salmon, Oregon
[*Flexibacter*] *psychrophilus*	FPC 830[f]	LMG 13183	S	Coho salmon, Migayi, Japan
[*Flexibacter*] *psychrophilus*	JIP 22/90		S	Skin lesion of brown trout, Nord-Pas-de-Calais, France
[*Flexibacter*] *roseolus*	ATCC 23088^T	LMG 13507^T	S	Hot spring, Agua Caliente, Costa Rica
[*Flexibacter*] *ruber*	ATCC 23103^T	LMG 13508^T	S	Hot spring, Geysir, Iceland
[*Flexibacter*] *sancti*	NCIMB 1379^T	LMG 8377^T	S	Soil, Buenos Aires, Argentina
Flexithrix dorotheae	Ft d1^{Tc}	LMG 8379^T	M	Beach silt, Ernakulum, Kerala, India
Haliscomenobacter hydrossis		LMG 10767^T	N	Activated sludge, Oss, The Netherlands
"*Microscilla aggregans*"	NCIMB 1443^T	LMG 8376^T	M	Sand, Canoe Beach, Tema, Ghana
"*Microscilla aggregans*"		LMG 13137	M	Sand, Ernakulum, Kerala, India
"*Microscilla arenaria*"	NCIMB 1413^T	LMG 13024^T	M	Sand, Norse Beach, Puerto Peñasco, Sonora, Mexico
"*Microscilla furvescens*"	NCIMB 1419^T	LMG 13023^T	M	Sand, Samoa
Microscilla marina	NCIMB 1400^T	LMG 13022^T	M	Outflow of marine aquarium, La Jolla, Calif.
"*Microscilla sericea*"	NCIMB 1403^T	LMG 13021^T	M	Outflow of marine aquarium, La Jolla, Calif.
"*Microscilla tractuosa*"	NCIMB 1408^T	LMG 8378^T	M	Sand, Nhatrang, Vietnam
"[*Promyxobacterium*] *flavum*"		LMG 10389^T	S	Rhizosphere of tomato plant, Russia
Runella slithyformis		LMG 11500^T	N	Freshwater lake near Baton Rouge, La.
Saprospira grandis		LMG 10407^T	M	Rock pool, upper littoral, Woods Hole, Mass.
Sphingobacterium heparinum		LMG 10339^T	T	Dry soil
Sphingobacterium mizutae		LMG 8340^T	T	Ventricular fluid of fetus, Japan
Sphingobacterium multivorum		LMG 8342^T	T	Spleen, Washington
Sphingobacterium spiritivorum		LMG 8347^T	T	Uterus, Kansas
Sphingobacterium thalpophilum		LMG 11520^T	T	Wound swab, New York, N.Y.
Spirosoma linguale	DSM 74Te	LMG 13140^T	N	Laboratory water bath
"[*Sporocytophaga*] *cauliformis*" type 1		LMG 8362	S	Water, Lake Constance, Germany
"[*Sporocytophaga*] *cauliformis*" type 2		LMG 8363^T	S	Water, Lake Constance, Germany
Sporocytophaga myxococcoides	ATCC 10010^T	LMG 8393^T	C	Soil, Quebec, Canada
Sporocytophaga myxococcoides	DSM 1813[e]	LMG 13345	C	Sewage water, Germany
"*Taxeobacter gelupurpurascens*"	Tx g1^{Tc}	LMG 13512^T	S	Soil, Alberta, Canada

[a] ACAM, Australian Collection of Antarctic Microorganisms, University of Tasmania, Hobart, Australia; ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; JCM, Japanese Collection of Microorganisms, Tokyo, Japan; JIP, Culture Collection of the Unité de Virologie et Immunologie Moléculaires, Institut National de la Recherche Agronomique, Jouy-en-Josas, France; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom; UASM, Soil Microbiology Laboratory, University of Alberta, Edmonton, Canada.

[b] Strains were grown on Dubos agar supplemented with 1% cellobiose (C), Difco marine agar 2216E (M), National Collection of Industrial and Marine Bacteria medium 81 (N), modified Shieh agar (S), or Trypticase soy agar (T).

[c] Strain provided by H. Reichenbach, Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany.

[d] Strain provided by R. P. Burchard, Department of Biological Sciences, University of Maryland, Baltimore.

[e] Strain provided by K. A. Malik, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany.

[f] Strain provided by H. Wakabayashi, Laboratory of Aquaculture Biology, Department of Fisheries, University of Tokyo, Tokyo, Japan.

[g] Strain provided by C. A. Mancuso, Department of Agriculture Science, University of Tasmania, Hobart, Tasmania, Australia.

[h] Strain provided by R. A. Holt, Department of Microbiology, Oregon State University, Corvallis.

[i] Strain provided by P. Rintamaki, Department of Zoology, University of Oulu, Oulu, Finland.

[j] Strain provided by G. H. Hansen, Department of Microbiology and Plant Physiology, University of Bergen, Bergen, Norway.

tions described above. Previously described methods were used to prepare whole-cell protein extracts, for SDS-PAGE, and to perform a numerical analysis of the scanned protein gel electropherograms with the GelCompar software package (Applied Maths, Kortrijk, Belgium) (92).

RESULTS

DNA base compositions. The DNA base compositions of the strains which we studied are shown in Table 2. Differences of

TABLE 2. DNA base compositions of strains and $T_{m(e)}$ values of DNA-rRNA hybrids

Taxon	Strain[a]	G+C content (mol%)	$T_{m(e)}$ (°C) of hybrid with rRNA from:							
			Flavobacterium aquatile LMG 4008^T	[*Cytophaga*] *johnsonae* LMG 1341^T	[*Flexibacter*] *columnaris* NCIMB 2248^T	[*Flexibacter*] *maritimus* NCIMB 2154^T	*Sphingobacterium spiritivorum* LMG 8347^T	*Sphingobacterium heparinum* LMG 10339^T	*Flexibacter flexilis* LMG 3989^T	*Cytophaga hutchinsonii* LMG 10844^T
Chitinophaga pinensis	LMG 13176^T	45.2			54.0		58.0		56.8	55.3
Chitinophaga pinensis	LMG 13042	45.0			52.4					
Cyclobacterium marinus	NCIMB 1802^T	38.4				58.6	61.5		60.4	61.5
[*Cytophaga*] *agarovorans*	NCIMB 2217^T	41.8			55.6		59.4		56.0	56.5
"[*Cytophaga*] *allerginae*"	ATCC 35408	34.0	71.8		70.8					
[*Cytophaga*] *aprica*	ATCC 23126^T	33.3			57.2		59.6		57.1	60.1
[*Cytophaga*] *aprica*	NCIMB 1411	34.5								59
[*Cytophaga*] *aquatilis*	DSM 2063^T	ND[b]	75.5		71.6					
[*Cytophaga*] *arvensicola*	JCM 2836^T	46.9			51.4		58.6		54.6	57.1
Cytophaga aurantiaca	NCIMB 8628^T	ND							65.1	78.1
[*Cytophaga*] *diffluens*	NCIMB 1402^T	41.3			55.5		61.1		57.8	61.4
[*Cytophaga*] *fermentans*	NCIMB 2218^T	35.7				55.9	60.6		56.1	57.1
[*Cytophaga*] *flevensis*	DSM 1076^T	ND	71.8		70.5					
Cytophaga hutchinsonii	LMG 10844^T	39.6	62.2					60.4	62.5	78.7
Cytophaga hutchinsonii	NCIMB 10782	39.4							63.4	80.1
[*Cytophaga*] *johnsonae*	DSM 2064^T	35.2	71.4	76.9	71.2		58.5			
[*Cytophaga*] *johnsonae*	ATCC 29585	35.5	73.2	75.1						
[*Cytophaga*] *johnsonae*	ATCC 29586	35.2	73.4		71					
"[*Cytophaga*] *keratolytica*"	LMG 11610	40.3					72.8			
[*Cytophaga*] *latercula*	NCIMB 1399^T	31.8				67.3				
[*Cytophaga*] *lytica*	NCIMB 1423^T	33.0	70.0	70.3	66.1	67.4				
[*Cytophaga*] *lytica*	DSM 2040	33.3	68.3		66.3	67.5				
[*Cytophaga*] *marinoflava*	NCIMB 397^T	37.0	71.0	71.6	65.9	67				
[*Cytophaga*] *pectinovora*	NCIMB 9059^T	35.2	70.9	74.7	69					
[*Cytophaga*] *saccharophila*	NCMB 2072^T	35.7	72.3	71.4	70.3					
[*Cytophaga*] *salmonicolor*	NCIMB 2216^T	40.8		68.0	58.1	58.7	59.9		58.1	
[*Cytophaga*] *succinicans*	NCIMB 2277^T	36.7	74.2		70.6					
[*Cytophaga*] *succinicans*	NCIMB 2278	35.2	72.4		70.9					
[*Cytophaga*] *uliginosa*	NCIMB 1863^T	43.0	67.8	67.8	67.8	66.5				
"[*Cytophaga*] *xantha*"	LMG 8372^T	35.9	71.9		67.9					
Flavobacterium aquatile	LMG 4008^T	33.0	76.3	73.1	71.6	67.2	55.7			
Flavobacterium branchiophilum	ATCC 35035^T	34.0	72.5	71.5	71.1					
Flavobacterium branchiophilum	BGD 7736	33.3	72.5	72.3	70.5					
[*Flavobacterium*] *ferrugineum*	LMG 10403^T	48.9	58.0					55.7	54.6	57.1
[*Flavobacterium*] *gondwanense*	LMG 13192^T	35.7	68.3		66.2	67.7				
[*Flavobacterium*] *gondwanense*	ACAM 46	36.2			65.5	67.8				
[*Flavobacterium*] *odoratum*	LMG 1233^T	36.7	69.6	71.3	70.5	64.1				
[*Flavobacterium*] *odoratum*	LMG 4028	ND		72.0						
[*Flavobacterium*] *odoratum*	LMG 4029	ND		71.3						
[*Flavobacterium*] *salegens*	LMG 13193^T	37.4	68.0		66.6	67.7				
[*Flavobacterium*] *salegens*	ACAM 52	37.7			66.4	68.1				
"[*Flavobacterium*] *tirrenicum*"	Fv t1^T	ND			55.2					
[*Flectobacillus*] *glomeratus*	LMG 13858^T	31.3			65.1	71.2				
Flectobacillus major	NCIMB 11363^T	38.9					59.6		62.3	61.6
[*Flexibacter*] *aurantiacus*	NCIMB 1382^T	35.5		76.2	71					
[*Flexibacter*] *aurantiacus*	NCIMB 1455	ND	72.9	76.2	72					

"[*Flexibacter*] *aurantiacus* subsp. *copepodarum*"	LMG 10405^T	29.6			66.1	72.6				
"[*Flexibacter*] *aurantiacus* subsp. *excathedrus*"	LMG 3986^T	32.5	69.5	67.5	75.3					
[*Flexibacter*] *canadensis*	LMG 8368^T	38.4					67.9			
[*Flexibacter*] *columnaris*	NCIMB 2248^T	32.0	71.0		78.7	66.2	60.0			
[*Flexibacter*] *columnaris*	DD3-69	32.0	71.4		77.7		59.1		59.5	60.0
[*Flexibacter*] *columnaris*	JIP 44/87	32.8			78.6					
[*Flexibacter*] *columnaris*	CR8	31.8			78.9					
[*Flexibacter*] *elegans*	LMG 10750^T	47.4			56.7		59.3		61.4	61.1
[*Flexibacter*] *filiformis*	ATCC 29495^T	45.0			55		59.2		57.3	
Flexibacter flexilis	NCIMB 12853^T	43.8			60.5		62.2		78.4	64.5
"[*Flexibacter*] *flexilis* subsp. *algavorum*"	DSM 4510^T	31.1			57.6		60.4		61.1	
"[*Flexibacter*] *flexilis* subsp. *pelliculosus*"	LMG 3991^T	36.9			57.2		60.2		61.2	
[*Flexibacter*] *litoralis*	NCIMB 1366^T	31.1			58.5		59.0			59.1
[*Flexibacter*] *maritimus*	NCIMB 2154^T	31.8	68.4		65.4	76.1	58.8			
[*Flexibacter*] *maritimus*	NCIMB 2153	31.6	67.0			77.0			57.9	59.5
[*Flexibacter*] *maritimus*	NCIMB 2158	31.1				76.8				
[*Flexibacter*] *ovolyticus*	EKD 002^T	29.5				71.8				
[*Flexibacter*] *ovolyticus*	VKB 004	29.8				72.0				
[*Flexibacter*] *psychrophilus*	NCIMB 1947^T	33.0	71.5		70.8		58.7			
[*Flexibacter*] *psychrophilus*	SH3-81	33.5	70.8		71.3					
[*Flexibacter*] *roseolus*	ATCC 23088^T	39.0	60.7	62.2						
[*Flexibacter*] *ruber*	ATCC 23103^T	37.0	58.2	62.3						
[*Flexibacter*] *sancti*	NCIMB 1379^T	43.3			55.2		59.2		57.7	57.6
Flexithrix dorotheae	Ft d1^T	35.9				59.5	62.3		61.1	60.9
Haliscomenobacter hydrossis	LMG 10767^T	ND	56.9				60.6			
"*Microscilla aggregans*"	NCIMB 1443^T	ND			58	60			60.9	
"*Microscilla aggregans*"	LMG 13137	37.9				60.4	61.9			
"*Microscilla arenaria*"	NCIMB 1413^T	30.8				57.3	60.5		57.2	
"*Microscilla furvescens*"	NCIMB 1419^T	45.0				59.9	61.3			61.3
Microscilla marina	NCIMB 1400^T	39.1				57.1	59.7		57.9	61.6
"*Microscilla sericea*"	NCIMB 1403^T	35.7				63.2	62.4			64.3
"*Microscilla tractuosa*"	NCIMB 1408^T	35.5				59.7	61.1		61	
"[*Promyxobacterium*] *flavum*"	LMG 10389^T	34.5	72.6	70	70.6					
Runella slithyformis	LMG 11500^T	47.2			52.6				55.8	60.6
Saprospira grandis	LMG 10407^T	46.4	61.2		58.2			57.1	56.0	54.4
Sphingobacterium heparinum	LMG 10339^T	43.0	63.1				67.1	80.0	59.4	59.9
Sphingobacterium mizutae	LMG 8340^T	39.3					71.2			
Sphingobacterium multivorum	LMG 8342^T	40.5					72.6			
Sphingobacterium spiritivorum	LMG 8347^T	39.8	60.7				77.2	69.5		
Sphingobacterium thalpophilum	LMG 11520^T	ND					69.0			
Spirosoma linguale	DSM 74^T	ND			55.9					56.7
"[*Sporocytophaga*] *cauliformis*" type 1	LMG 8362	33.5			71.4					
"[*Sporocytophaga*] *cauliformis*" type 2	LMG 8363^T	36.2	72.8	74.2	71					
Sporocytophaga myxococcoides	ATCC 10010^T	36.4								65.2
Sporocytophaga myxococcoides	DSM 1813	36.4								68.3
"*Taxeobacter gelupurpurascens*"	Tx g1^T	53.8			57.5		57.9			

[a] See Table 1, footnote *a*.
[b] ND, not determined.

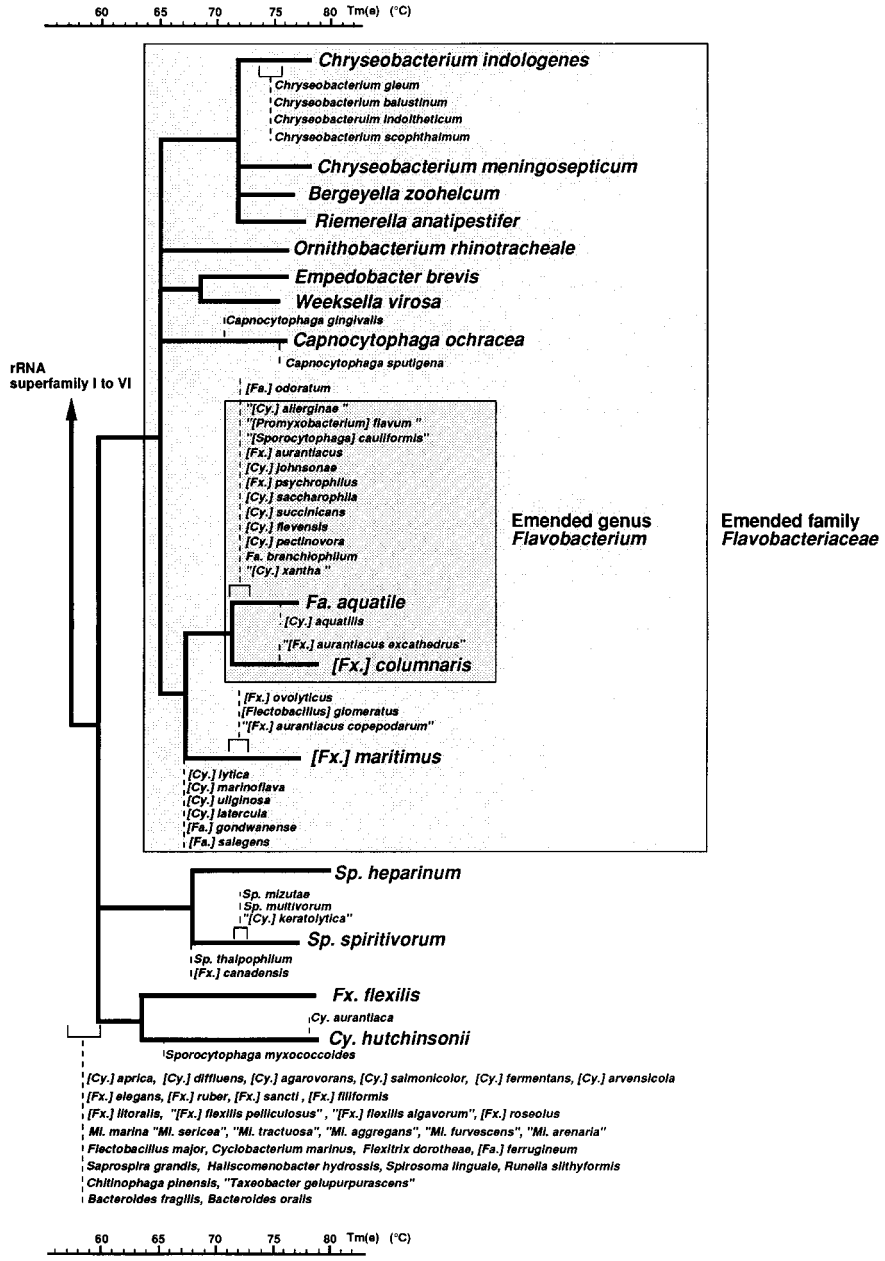

FIG. 1. Simplified rRNA cistron similarity dendrogram for rRNA superfamily V. Data from this study and references 72, 73, 80, 85, and 88. Abbreviations: *Fa*., *Flavobacterium*; *Cy*., *Cytophaga*; *Fx*., *Flexibacter*; *Sp*., *Sphingobacterium*; *Mi*., *Microscilla.*

2 to 4 mol% G+C with previously published data (68, 71) were observed for the following strains: [*Cytophaga*] *fermentans* NCIMB 2218^T, "[*Cytophaga*] *xantha*" LMG 8372^T, [*Flexibacter*] *aurantiacus* NCIMB 1382^T, "*Taxeobacter gelupurpurascens*" Txg1^T, and "[*Sporocytophaga*] *cauliformis*" type 1 strain LMG 8362. The greatest difference was observed with [*Cytophaga*] *salmonicolor* NCIMB 2216^T; the two different DNA batches which we studied had DNA G+C contents of 41 and 42 mol%, respectively, while the previously published value was 37 mol% (68). All of other DNA base ratios determined in this study were similar to the values published previously (35, 40, 48, 53, 68, 71, 95).

DNA-rRNA hybridization experiments. Table 2 shows the results of DNA-rRNA hybridizations between DNAs from strains belonging to the genera *Flavobacterium*, *Cytophaga*, *Flexibacter*, and *Microscilla* and related genera and radioactively labeled rRNAs from several reference strains belonging to rRNA superfamily V. These results are also shown in Fig. 1 as a dendrogram based on melting temperatures of elution [$T_{m(e)}$] ($T_{m(e)}$ is the temperature at which 50% of a DNA-rRNA hybrid is denatured). The $T_{m(e)}$ values obtained from the reciprocal hybridization experiments performed with all of the strains belonging to each rRNA homology cluster were used to calculate the average levels of linkage between pairs of rRNA clusters. The dendrogram in Fig. 1 also includes some previously published DNA-rRNA hybridization data (73, 81, 85, 88).

DNA-DNA hybridization experiments. The results of the

TABLE 3. Levels of DNA relatedness for strains of [*Cytophaga*] *johnsonae* and [*Flexibacter*] *aurantiacus* as determined by the S1 nuclease method at 60°C

Source of unlabeled DNA		% Reassociation with labeled DNA from:		
Species	Strain[a]	[*Cytophaga*] *johnsonae* DSM 2064^T	[*Cytophaga*] *johnsonae* ATCC 29585	[*Flexibacter*] *aurantiacus* NCIMB 1382^T
[*Cytophaga*] *johnsonae*	DSM 2064^T	100[b]	18	60, 63 (0.5)
[*Cytophaga*] *johnsonae*	ATCC 29585	13	100	ND
[*Cytophaga*] *johnsonae*	ATCC 29586	ND[c]	100	ND
[*Cytophaga*] *johnsonae*	NCIMB 11391	13	ND	18
[*Flexibacter*] *aurantiacus*	NCIMB 1382^T	64, 72, 60 (1.2)	20	100
[*Flexibacter*] *aurantiacus*	NCIMB 1455	67, 64	22	85

[a] See Table 1, footnote *a*.
[b] The levels of DNA relatedness are expressed as percentages of relative binding; the results of one, two, or three experiments are shown. The values in parentheses are ΔT_m values (thermal stabilities of heteroduplexes) (in degrees Celsius).
[c] ND, not determined.

DNA-DNA hybridization experiments performed with the [*Cytophaga*] *johnsonae* and [*Flexibacter*] *aurantiacus* strains are shown in Table 3.

FAME analysis. The fatty acid profiles of *Flavobacterium aquatile* and its closest phylogenetic relatives were determined, and the results of the FAME analysis are shown in Table 4. The predominant fatty acids in all of the taxa studied were 15:0, 15:0 iso, 15:0 anteiso, 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 [Table 4]), 16:0 iso 3OH, 17:1 iso ω9c, and 17:0 iso 3OH. The organisms which we studied were differentiated mainly on the basis of quantitative differences in these major fatty acids. However, some minor qualitative differences also occurred, and these differences were used to characterize several taxa. [*Flexibacter*] *columnaris*, [*Flavobacterium*] *odoratum*, and "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*" LMG 3986 did not contain significant amounts of 15:1 ω6c and 17:1 ω6c. In addition, [*Flavobacterium*] *odoratum* also did not contain 15:1 iso G. *Flavobacterium branchiophilum* strains did not contain 16:0 iso. Intraspecific heterogeneity in fatty acid contents was observed in the following four species: [*Cytophaga*] *johnsonae*, [*Cytophaga*] *succinicans*, "[*Sporocytophaga*] *cauliformis*," and [*Flavobacterium*] *odoratum*. In most cases this heterogeneity was based on quantitative differences; however, [*Flavobacterium*] *odoratum* LMG 4029 contained several fatty acids that were not detected in the two other strains of this species that were studied. When we compared our fatty acid data with data for some other taxa belonging to rRNA superfamily V reported previously, we found both significant similarities and diagnostic differences. The members of most taxonomic groups contained high levels of 15:0 iso and 17:0 iso 3OH. The levels of other fatty acids varied in the different genera and species (55, 72, 85, 88, 103). However, it should be noted that the cultivation conditions were different in most of the previous studies, which may have significantly affected the fatty acid compositions.

PAGE of whole-cell proteins. Figure 2 shows the protein profiles of strains belonging to the *Flavobacterium aquatile* rRNA cluster and the corresponding dendrogram obtained after a numerical comparison. For two species, [*Flexibacter*] *psychrophilus* and [*Flexibacter*] *columnaris*, the different strains which we studied produced very similar protein profiles, and thus these taxa could be identified easily on the basis of SDS-PAGE results. In contrast, we observed intraspecific heterogeneity in the protein profiles obtained for several other species, including [*Flavobacterium*] *odoratum*, [*Cytophaga*] *johnsonae*, [*Cytophaga*] *succinicans*, and *Flavobacterium branchiophilum*. The last three species are discussed below. The heterogeneity of the [*Flavobacterium*] *odoratum* strains, which was suspected on the basis of the cellular fatty acid analysis results (Table 4) and the genomic differences among the strains (60), was confirmed by differences between the protein profile of [*Flavobacterium*] *odoratum* LMG 4029 and the protein profiles of the two other strains studied (LMG 1233^T and LMG 4028).

DISCUSSION

Taxonomic structure of rRNA superfamily V. At this time, several major clusters of rRNA branches and a number of solitary taxa that have very distinct positions (e.g., the genera *Ornithobacterium* and *Capnocytophaga*) can be distinguished in rRNA superfamily V on the basis of DNA-rRNA hybridization data (Fig. 1) (73, 85, 88; this study). Similar conclusions were drawn previously on the basis of 16S rRNA sequence data (30, 56, 101).

The genera *Chryseobacterium* (including six species previously considered *Flavobacterium* species [*Chryseobacterium balustinum*, *Chryseobacterium gleum*, *Chryseobacterium indologenes*, *Chryseobacterium indoltheticum*, *Chryseobacterium meningosepticum*, and *Chryseobacterium scophthalmum*]) (85), *Bergeyella* (including only one species, *Bergeyella zoohelcum*, previously considered a *Weeksella* species) (85), and *Riemerella* (including a single species, *Riemerella anatipestifer*, long considered a *Moraxella* species) (72) make up an rRNA cluster that comprises four different rRNA branches. The genera *Empedobacter* (including only *Empedobacter brevis*, formerly [*Flavobacterium*] *breve*) and *Weeksella* (containing only one species, *Weeksella virosa*) (85) belong to two different rRNA branches in a second cluster. A third cluster consists of the *Flavobacterium aquatile* and [*Flexibacter*] *columnaris* rRNA branches, and 13 other taxa (most of which are generically misclassified) are located at the base level between these two rRNA branches. This last rRNA cluster is referred to as the *Flavobacterium aquatile* rRNA cluster and is discussed in detail below. The four members of the [*Flexibacter*] *maritimus* rRNA branch (i.e., the marine organisms "[*Flexibacter*] *aurantiacus* subsp. *copepodarum*," [*Flexibacter*] *maritimus*, [*Flexibacter*] *ovolyticus*, and [*Flectobacillus*] *glomeratus*) and several other marine species located at the base level between [*Flexibacter*] *maritimus* and the *Flavobacterium aquatile* rRNA cluster are the closest relatives of the *Flavobacterium aquatile* rRNA cluster. The average level of linkage between the rRNA clusters mentioned above and the solitary taxa is a $T_{m(e)}$ of 65 ± 1.5°C. A separate rRNA cluster is formed by the genus *Sphingobacterium*, in which *Sphingobacterium heparinum* occupies a rather distinct position (72, 73, 80, 81). Finally, *Flexibacter flexilis* is the only member of

TABLE 4. Fatty acid compositions of the taxa studied[a]

Taxon[b]	% of:							
	13:0 iso	14:0 iso	15:0	15:1 ω6c	15:0 3OH	15:0 iso	15:0 anteiso	15:1 iso G[c]
Flavobacterium aquatile (1)		1.1	12.7	9.1	1.5	21.5	1.6	9.0
Flavobacterium branchiophilum (7)	1.2 ± 0.3	tr	11.0 ± 3.0	7.6 ± 1.6	1.8 ± 2.3	22.2 ± 3.5	2.5 ± 1.3	10.9 ± 2.2
[*Flexibacter*] *columnaris* (5)	1.8 ± 0.5	tr	4.4 ± 0.9		tr	39.0 ± 4.0	1.4 ± 0.8	13.1 ± 1.7
[*Cytophaga*] *flevensis* (1)		2.2	7.6	8.6	1.6	14.5	10.4	5.7
[*Cytophaga*] *aquatilis* (1)		1.2	9.6	5.0	2.0	17.6	tr	3.8
[*Cytophaga*] *johnsonae* (1)[e]	tr	1.5	6.1	1.3	1.2	24.9	3.4	4.9
[*Cytophaga*] *johnsonae* (6)[f]		1.3 ± 0.7	4.5 ± 1.1	3.1 ± 0.8	1.6 ± 0.4	20.1 ± 3.0	3.8 ± 3.6	5.2 ± 1.7
[*Cytophaga*] *pectinovora* (1)		1.2	6.7	6.4	2.1	24.1	2.0	8.0
[*Flexibacter*] *psychrophilus* (5)	1.3 ± 0.2	2.0 ± 0.7	6.0 ± 1.0	5.6 ± 0.6	tr	19.7 ± 1.4	3.7 ± 0.7	11.7 ± 3.5
[*Cytophaga*] *saccharophila* (1)		1.2	8.5	7.3	2.5	9.5	1.3	6.9
[*Cytophaga*] *succinicans* (2)[g]	tr	1.6 ± 0.1	11.8 ± 2.1	10.7 ± 1.6	3.3 ± 1.2	16.8 ± 0.3	1.1 ± 0.7	9.2 ± 1.9
[*Cytophaga*] *succinicans* (1)[h]		tr	7.6	6.9	tr	30.0	1.0	9.0
"[*Cytophaga*] *allerginae*" (1)			7.9	2.0	1.4	27.3	1.2	3.6
"[*Cytophaga*] *xantha*" (1)		3.3	10.9	11.6	1.9	9.0	3.9	3.9
[*Flexibacter*] *aurantiacus* (2)	tr	tr	6.5 ± 2.0	2.4 ± 1.4	1.6 ± 0.3	29.0 ± 3.6	1.3 ± 0.1	6.9 ± 0.6
"[*Flexibacter*] *aurantiacus* subsp. *excathedrus*" (1)	tr	tr	6.4		2.2	25.6	tr	17.1
"[*Promyxobacterium*] *flavum*" (1)		1.2	6.9	4.5	1.5	28.0	1.7	7.3
"[*Sporocytophaga*] *cauliformis*" (1)[i]		2.6	7.8	5.2	1.8	17.0	1.5	6.1
"[*Sporocytophaga*] *cauliformis*" (1)[j]		tr	8.3	3.3	2.1	30.0	tr	4.2
[*Flavobacterium*] *odoratum* (2)[k]			tr			53.5 ± 5.2	1.8 ± 1.2	
[*Flavobacterium*] *odoratum* (1)[l]	3.3	2.7	3.9	tr	1.2	39.0	1.3	

[a] In addition, small amounts (less than 2.5% of the total fatty acids) of one or more of the following fatty acids occur in the taxa studied: unknown 11.541 (fatty acid whose identity is unknown; the number indicates the equivalent chain length), unknown 13.566, unknown 16.580, 14:0, 15:0 2OH, 16:1 ω5c, 16:1 iso G, 17:1 ω8c, 17:0 3OH, 18:1 ω5c, summed feature 3 (14:0 3OH or 16:1 iso I or both), and summed feature 5 (17:1 iso I or 17:1 anteiso B or both).

[b] The numbers in parentheses are the numbers of strains studied. Unless indicated otherwise below, the strains used were the strains shown in Table 1. When two or more strains were used, the mean ± standard deviation is shown.

[c] The double bound position indicated by the capital letter is not known.

[d] The fatty acids 15:0 iso 2OH, 16:1 ω7c, and 16:1 ω7t could not be separated from each other by gas chromatography by using the Microbial Identification System (Microbial ID, Inc., Newark, Del.) software package and together were considered summed feature 4.

[e] [*Cytophaga*] *johnsonae* DSM 2064^T.

[f] All of the [*Cytophaga*] *johnsonae* strains in Table 1 except DSM 2064^T.

[g] [*Cytophaga*] *succinicans* NCIMB 2277^T and NCIMB 2279.

[h] [*Cytophaga*] *succinicans* NCIMB 2278.

[i] "[*Sporocytophaga*] *cauliformis*" LMG 8362.

[j] "[*Sporocytophaga*] *cauliformis*" LMG 8363^T.

[k] [*Flavobacterium*] *odoratum* LMG 1233^T and LMG 4028.

[l] [*Flavobacterium*] *odoratum* LMG 4029.

its rRNA branch, whereas the cellulolytic organisms (*Cytophaga hutchinsonii*, *Cytophaga aurantiaca*, and *Sporocytophaga myxococcoides*) make up the *Cytophaga hutchinsonii* rRNA branch. The *Sphingobacterium* rRNA cluster and the *Cytophaga hutchinsonii* and *Flexibacter flexilis* rRNA branches are linked to each other and to the other rRNA clusters in superfamily V at a $T_{m(e)}$ of 60 ± 2.0°C.

Several organisms, most of which are represented by a single strain, do not belong to any of the rRNA clusters described above. With average $T_{m(e)}$ values less than 60°C, the following organisms occupy peripheral positions on the $T_{m(e)}$ dendrogram: *Bacteroides fragilis* and *Bacteroides oralis* (88); *Chitinophaga pinensis*; *Cyclobacterium marinus* (formerly [*Flectobacillus*] *marinus*) (67); [*Cytophaga*] *agarovorans*; [*Cytophaga*] *aprica*; [*Cytophaga*] *arvensicola*; [*Cytophaga*] *diffluens*; [*Cytophaga*] *fermentans*; [*Cytophaga*] *salmonicolor*; [*Flavobacterium*] *ferrugineum*; *Flectobacillus major*; [*Flexibacter*] *elegans*; [*Flexibacter*] *filiformis*; "[*Flexibacter*] *flexilis* subsp. *algavorum*"; "[*Flexibacter*] *flexilis* subsp. *pelliculosus*"; [*Flexibacter*] *litoralis*; [*Flexibacter*] *roseolus*; [*Flexibacter*] *ruber*; [*Flexibacter*] *sancti*; *Flexithrix dorotheae*; *Haliscomenobacter hydrossis*; "[*Microscilla*] *aggregans*"; "[*Microscilla*] *arenaria*"; "[*Microscilla*] *furvescens*"; [*Microscilla*] *marina*; "[*Microscilla*] *sericea*"; "[*Microscilla*] *tractuosa*"; *Runella slithyformis*; *Saprospira grandis*; *Spirosoma linguale*; and "*Taxeobacter gelupurpurascens*." rRNA sequencing is a better method than DNA-rRNA hybridization for revealing deep phylogenetic relationships (97), and recent data obtained by the former technique have indeed indicated that most of these taxa should be assigned to the *Cytophaga-Flavobacterium-Bacteroides* group (30, 56, 58, 98, 101). Two marine facultatively anaerobic species, [*Cytophaga*] *agarovorans* and [*Cytophaga*] *salmonicolor*, have recently been reclassified in the new genus "*Marinolabilia*" (57). "[*Flavobacterium*] *tirrenicum*" is probably not related to superfamily V, as demonstrated by its very low level of rRNA homology (Table 2) and its quite different polyamine distribution (34), but at this time no rRNA sequence is available to determine the phylogenetic relationships of this organism.

For technical reasons, the following species were not included in this study: [*Cytophaga*] *xylanolytica*, [*Flexibacter*] *polymorphus*, *Sphingobacterium antarcticus*, *Sphingobacterium faecium*, *Sphingobacterium piscium*, "*Taxeobacter ocellatus*," and "*Taxeobacter chitinovorans*." rRNA sequencing data have revealed that [*Cytophaga*] *xylanolytica* and [*Flexibacter*] *polymorphus* belong to the *Cytophaga-Flavobacterium-Bacteroides* group (32, 58). The three new *Sphingobacterium* species mentioned above have been clearly identified as members of this genus on the basis of chemotaxonomic data and DNA-DNA hybridization data (74, 83, 84). No rRNA sequences have been published yet for members of the recently proposed genus

TABLE 4—*Continued*

% of:									
15:0 iso 3OH	16:0	Summed feature 4[d]	16:0 3OH	16:0 iso	16:0 iso 3OH	16:1 iso H	17:1 ω6c	17:1 iso ω9c	17:0 iso 3OH
6.6	tr	4.0	tr	2.4	5.1	2.2	4.1	4.8	7.4
14.3 ± 2.7	1.1 ± 0.3	4.5 ± 1.9	2.3 ± 0.6		1.7 ± 0.6	tr	5.1 ± 1.2	tr	5.7 ± 0.8
8.7 ± 2.5	tr	tr	tr	2.0 ± 1.2	2.6 ± 1.0	tr		7.5 ± 1.6	12.1 ± 1.3
4.9	1.4	18.4	4.1	1.4	3.8		2.7	tr	3.9
8.9	1.3	13.4	4.5	2.0	6.8	1.4	4.1	2.5	8.3
6.6	2.7	12.8	4.7	3.4	4.4		2.2	2.4	9.4
8.8 ± 1.7	1.6 ± 0.5	18.2 ± 1.6	3.7 ± 1.2	1.3 ± 0.5	4.7 ± 2.0	tr	2.8 ± 0.7	2.7 ± 0.7	9.6 ± 1.3
7.9	tr	5.3	tr	1.5	4.7	tr	5.2	5.1	12.1
8.5 ± 2.2	tr	2.3 ± 0.5		2.5 ± 1.0	3.3 ± 1.0	4.2 ± 1.8	2.2 ± 0.6	12.2 ± 1.4	9.0 ± 2.2
5.9	tr	12.3	1.4	3.7	5.3	2.0	7.6	5.6	9.6
8.6 ± 3.7	tr	7.5 ± 6.3	2.2 ± 0.5	1.3 ± 0.5	4.5 ± 1.3	1.8 ± 1.0	4.0 ± 1.0	2.4 ± 1.7	5.8 ± 0.5
10.7	tr	5.8	1.7	1.9	3.3	1.7	3.1	3.3	7.8
6.7	3.0	14.5	4.5	1.5	3.2		2.2	3.1	8.7
5.2	1.0	5.0	1.4	4.1	9.6	2.6	12.5	2.1	6.2
9.2 ± 0.7	1.4 ± 0.3	10.1 ± 0.8	3.4 ± 0.7	1.0 ± 0.6	2.8 ± 1.3	tr	2.2 ± 0.9	3.8 ± 0.5	11.5 ± 1.5
7.5		5.4	tr	1.9	4.3		tr	7.4	12.6
8.7	tr	6.6	tr	2.3	3.1	1.1	3.5	3.7	13.6
7.9	1.1	11.8	3.2	4.7	8.2	2.1	3.2	2.5	7.9
8.7	1.4	12.4	5.1	1.2	4.1		2.1	2.3	7.7
5.7 ± 1.6	tr	1.6 ± 2.3	3.5 ± 1.5	1.4 ± 0.5	tr			13.5 ± 2.9	12.8 ± 2.8
4.0	tr	8.3	5.4	4.4	2.1	1.9	tr	8.3	8.6

"*Taxeobacter*" (71), but an oligonucleotide cataloging study revealed that a "*Taxeobacter ocellatus*" strain is relatively closely related to *Sphingobacterium heparinum* (63). In this study, we observed only low levels of rRNA homology between "*Taxeobacter gelupurpurascens*" and the rRNA probes tested (Table 2).

Genera *Cytophaga* and *Flexibacter*. Our DNA-rRNA hybridization data clearly show that the genera *Cytophaga* and *Flexibacter* (as presently defined) are polyphyletic and thus confirm and extend similar conclusions based on previous DNA-rRNA hybridization results and on 16S rRNA sequence comparisons (6, 30, 45, 46, 56, 58, 72, 73, 85, 101). The genus names *Cytophaga* and *Flexibacter* must be preserved for members of the *Cytophaga hutchinsonii* and *Flexibacter flexilis* rRNA branches, respectively. Other *Cytophaga* and *Flexibacter* species, which belong to several rRNA branches and clusters in rRNA superfamily V or occupy peripheral positions on the $T_{m(e)}$ dendrogram, must be considered generically misclassified (Fig. 1). As the type species of the genus *Flexibacter*, *Flexibacter flexilis*, is the only member of its rRNA branch, the genus *Flexibacter* is restricted to this species. All other *Flexibacter* species should be reclassified. In the case of the genus *Cytophaga*, the type species (*Cytophaga hutchinsonii*) and *Cytophaga aurantiaca* are the only genuine *Cytophaga* species, and the members of the cellulolytic microcyst-forming genus *Sporocytophaga* (*Sporocytophaga myxococcoides* is the type and only valid species) are their closest relatives. The difference in $T_{m(e)}$ between *Sporocytophaga myxococcoides* and *Cytophaga hutchinsonii* (13.5°C) is great enough to justify separate generic status for the former taxon. The cellulolytic *Cytophaga* species can also be clearly differentiated from the other *Cytophaga* and *Flexibacter* species on the basis of their sulfonolipid contents (26). All other *Cytophaga* species (i.e., noncellulolytic *Cytophaga* species) should be reclassified. Such a restriction of the genera *Cytophaga* and *Flexibacter* to the type species and some close phylogenetic relatives was suggested previously on the basis of 16S rRNA sequence data (56, 70).

As shown by DNA-rRNA hybridization data, several generically misclassified *Cytophaga* and *Flexibacter* species are located in the *Sphingobacterium* rRNA cluster (Fig. 1). The generic status of *Sphingobacterium heparinum* has been discussed repeatedly, and transfers of this organism to other or new genera have been proposed (16, 68, 80, 84); however, the specific status of *Sphingobacterium heparinum* has now been established on the basis of genomic data (80). The results of rRNA homology experiments also confirmed that all *Sphingobacterium* species belong to a single rRNA branch (72). These findings corroborate the proposals of Takeuchi and Yokota (83, 84), who included [*Flavobacterium*] *yabuuchiae* in *Sphingobacterium spiritivorum* and [*Cytophaga*] *keratolytica* in *Sphingobacterium multivorum*. [*Flexibacter*] *canadensis* is also a peripheral member of this cluster, a position confirmed by the 16S rRNA sequence of this organism (56). Because *Sphingobacterium thalpophilum* (formerly [*Flavobacterium*] *thalpophilum*) was found to contain sphingophospholipids, transfer of this species to the genus *Sphingobacterium* was proposed (19, 84). As [*Flexibacter*] *canadensis* occurs at the same position on the dendrogram as *Sphingobacterium thalpophilum*, the results of lipid content analyses and the possible presence of sphingophospholipids may be decisive in transferring [*Flexibacter*] *canadensis* to the genus *Sphingobacterium* or in creating a new genus to accommodate this species.

The [*Flexibacter*] *maritimus* rRNA branch includes several species that were isolated from marine environments and are generically misclassified. One of these is [*Flectobacillus*] *glomeratus*, since the type species of the genus *Flectobacillus*, *Flectobacillus major*, is located at a rather low level on the *Cytophaga hutchinsonii* rRNA branch as determined by 16S rRNA sequence data (24). Additional data will be necessary to determine the generic relationships of members of the [*Flexibacter*] *maritimus* rRNA branch, the *Flavobacterium aquatile* rRNA cluster, and the peripherally related taxa [*Cytophaga*] *latercula*, [*Cytophaga*] *lytica*, [*Cytophaga*] *marinoflava*, [*Cytophaga*] *uliginosa*, [*Flavobacterium*] *gondwanense*, and [*Flavobacterium*] *salegens* (Fig. 1). The great genomic heterogene-

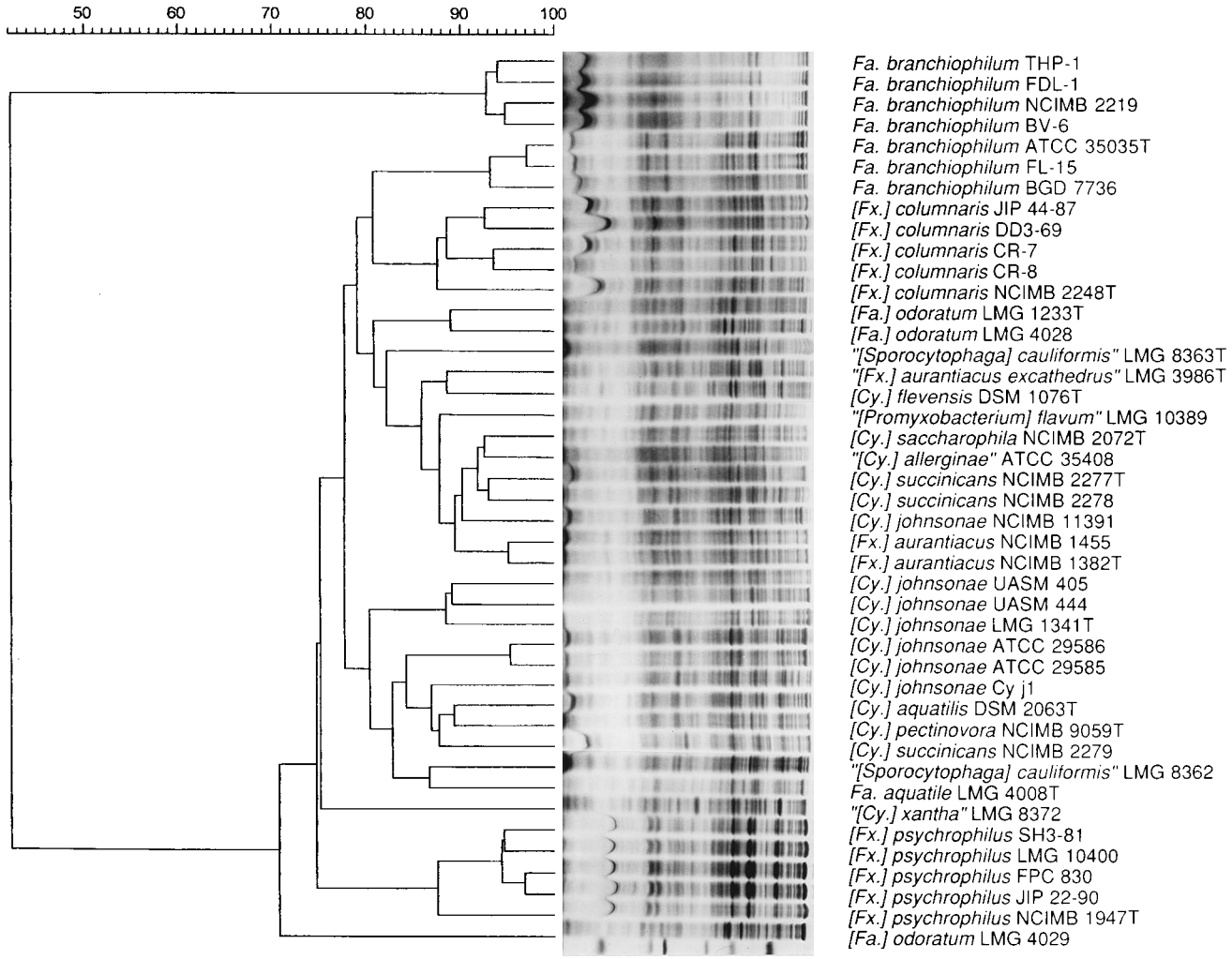

FIG. 2. Electrophoretic protein profiles and corresponding dendrogram for all of the strains studied in the *Flavobacterium aquatile* rRNA cluster. The dendrogram was constructed by using unweighted pair group average linkage of the correlation coefficients for pairs of protein patterns. The molecular weight markers used (indicated at the bottom) were (from left to right) trypsin inhibitor (molecular weight, 20,100), trypsinogen (24,000), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), and bovine albumin (66,000). For abbreviations see the legend to Fig. 1.

ity in the [*Flexibacter*] *maritimus* rRNA branch was also revealed by 16S rRNA sequence data (24, 30, 56, 88).

Genus *Flavobacterium*. Since several species previously included in the genus *Flavobacterium* have been reclassified as members of new or other genera, at this time the only valid *Flavobacterium* species are *Flavobacterium aquatile* (the type species), *Flavobacterium branchiophilum*, [*Flavobacterium*] *ferrugineum*, [*Flavobacterium*] *gondwanense*, [*Flavobacterium*] *odoratum*, and [*Flavobacterium*] *salegens* (24, 40, 85, 95). The large differences in $T_{m(e)}$ between [*Flavobacterium*] ferrugineum, [*Flavobacterium*] *gondwanense*, or [*Flavobacterium*] salegens and *Flavobacterium aquatile* (Table 2 and Fig. 1) demonstrate that [*Flavobacterium*] *ferrugineum*, [*Flavobacterium*] *gondwanense*, and [*Flavobacterium*] *salegens* do not belong to the genus *Flavobacterium* and should be reclassified. In contrast, phenotypic similarities and low differences in $T_{m(e)}$ between *Flavobacterium branchiophilum* and *Flavobacterium aquatile* support inclusion of *Flavobacterium branchiophilum* in the genus *Flavobacterium*. [*Flavobacterium*] *odoratum*, several misclassified *Cytophaga* and *Flexibacter* species, "[*Promyxobacterium*] *flavum*," and "[*Sporocytophaga*] *cauliformis*" all form a single rRNA homology cluster. So far, this cluster includes two rRNA branches; one of these branches includes [*Flexibacter*] *columnaris* and "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*," and the other is composed of *Flavobacterium aquatile* and [*Cytophaga*] *aquatilis*. All other species ([*Flavobacterium*] *odoratum*, "[*Cytophaga*] *allerginae*," [*Cytophaga*] *flevensis*, [*Cytophaga*] *johnsonae*, [*Cytophaga*] *pectinovora*, [*Cytophaga*] *saccharophila*, [*Cytophaga*] *succinicans*, "[*Cytophaga*] *xantha*," *Flavobacterium branchiophilum*, [*Flexibacter*] *aurantiacus*, [*Flexibacter*] *psychrophilus*, "[*Promyxobacterium*] *flavum*," and "[*Sporocytophaga*] *cauliformis*") are located at the base level between the two branches (Table 2 and Fig. 1). The range of levels of genomic divergence in this rRNA cluster is rather wide, corresponding to a difference in $T_{m(e)}$ of about 8°C, although several other genera, such as the genus *Sphingobacterium* (72), have similar levels of genomic divergence. In such cases, all species belonging to the rRNA cluster can be placed in one genus or several genera can be created, depending on the phenotypic characteristics of the species (22, 85).

Besides their similar habitats (soil or freshwater or both) most of the taxa included in this rRNA cluster have a consid-

erable number of characteristics in common. They contain menaquinone 6 as the major respiratory quinone (56, 61); their G+C contents are in the range from 32 to 37 mol% (Table 1) (40, 68); they exhibit clear gliding motility (a possible exception is *Flavobacterium branchiophilum*, but the motility of this organism may have been overlooked, as the motility of *Flavobacterium aquatile* was for a long time); they produce yellow nondiffusible pigments; and they have many classical phenotypic characteristics in common (see below). In addition, the metabolism of most of these organisms is strictly aerobic (the exceptions are [*Cytophaga*] *aquatilis* and [*Cytophaga*] *succinicans*, which can also grow anaerobically when some growth factors are provided [68]). Finally, these organisms have very similar fatty acid profiles (see below) (Table 4). [*Flavobacterium*] *odoratum* can be easily distinguished from the other members of the cluster by its clinical origin, its lack of gliding motility, its good growth at 37°C, its halotolerance (10, 41, 61), and several differences in its fatty acid profile (Table 4).

Therefore, both phenotypic and genotypic criteria justify inclusion of most of the members of the *Flavobacterium aquatile* rRNA cluster in a separate genus; the only exception is [*Flavobacterium*] *odoratum*. This conclusion is supported by 16S rRNA sequence data; the soil and freshwater organisms cluster on a separate branch, while [*Flavobacterium*] *odoratum* occupies a clearly independent position (30, 56, 57). Moreover, researchers have found small-subunit rRNA sequence signatures which clearly differentiate [*Flavobacterium*] *odoratum* from its neighbors (30). Therefore, we propose that *Flavobacterium aquatile* should be the type species of an emended genus *Flavobacterium* and that all valid taxa isolated from soil and freshwater belonging to the *Flavobacterium aquatile* rRNA cluster should be placed in the emended genus *Flavobacterium*. Below, we present an emended description of the genus *Flavobacterium* and propose new combinations for seven of its members.

There is a nomenclatural problem concerning [*Cytophaga*] *aquatilis*. This valid species (82), which at this time is represented by only one available strain, is indeed an independent taxon, as demonstrated by DNA-DNA homology data. The DNA of this organism does not exhibit significant levels of homology with DNAs from *Flavobacterium aquatile* and several other members of the *Flavobacterium aquatile* rRNA cluster (9). The fatty acid and protein profiles of [*Cytophaga*] *aquatilis* are also clearly different from the profiles of the other organisms (see below) (Table 4 and Fig. 2). If the combination [*Cytophaga*] *aquatilis* (Strohl and Tait 1978) was modified in a way that was consistent with its new generic status, it would become a junior homonym of *Flavobacterium aquatile* (Frankland and Frankland 1889). Therefore, below we propose a new name for this taxon, *Flavobacterium hydatis*, on the basis of Rule 12b of the *International Code of Nomenclature of Bacteria* (47a). The new specific epithet was chosen because its meaning is similar to that of the former epithet.

The specific epithet of [*Cytophaga*] *johnsonae* is another problem, as this epithet was incorrectly formed by Stanier in 1947 (78). In 1957 Stanier changed the epithet to *johnsonii*, which is also incorrect (79). As this epithet was created in honor of the American microbiologist Delia E. Johnson, who first isolated this species, it should be a genitive noun with a feminine ending. Therefore, we propose that the new name for this organism should be as *Flavobacterium johnsoniae.*

Other problems concern the taxonomic status of the valid species [*Flexibacter*] *aurantiacus* and the invalid taxa "[*Cytophaga*] *allerginae*," "[*Cytophaga*] *xantha*," "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*," "[*Promyxobacterium*] *flavum*," and "[*Sporocytophaga*] *cauliformis*." We propose that [*Flexibacter*] *aurantiacus* strains should be included in [*Cytophaga*] *johnsonae* and that all of the invalid taxa should be referred to as *Flavobacterium* sp. (see below).

Consequently, the emended genus *Flavobacterium* contains the following species: *Flavobacterium aquatile*, *Flavobacterium branchiophilum*, *Flavobacterium columnare*, *Flavobacterium flevense*, *Flavobacterium hydatis*, *Flavobacterium johnsoniae*, *Flavobacterium pectinovorum*, *Flavobacterium psychrophilum*, *Flavobacterium saccharophilum*, and *Flavobacterium succinicans*. All other *Flavobacterium* species are generically misclassified.

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 nutrient contents. 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 (4, 14, 68, 82). 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 *Flavobacterium psychrophilum*. All species except *Flavobacterium flevense* decompose gelatin and casein, and several species also hydrolyze various polysaccharides, including starch, chitin, pectin, and carboxymethyl cellulose. *Flavobacterium flevense* 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 (33, 34). 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 (34). The optimum temperature range for most species is 20 to 30°C; the optimum temperature range for *Flavobacterium 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*

TABLE 5. Phenotypic characteristics that can be used to differentiate the 10 valid species belonging to the genus *Flavobacterium*[a]

Characteristic	*Flavobacterium aquatile*	*Flavobacterium branchiophilum*	*Flavobacterium columnare*	*Flavobacterium flevense*
Morphology of colonies on AOA[b]	Low convex, round, with entire margins	Low convex, round, with entire margins	Flat, rhizoid, strongly adherent to agar	Low convex, round, sunken into agar
Congo red absorption[c]	−[d]	−	+	−
Growth on seawater media	−	−	−	+
Growth on nutrient agar	−	−	−	+
Growth on Trypticase soy agar	(+)	−	−	+
Gliding motility	+[e]	−	+	+
Flexirubin type of pigments[f]	−	−	+	−
Glucose used as a sole carbon and energy source	ND	ND	−	+
Acid produced aerobically from carbohydrates	+	+	−	+
Degradation of:				
Gelatin	V	+	+	−
Casein	+	+	+	−
Starch	V	+	−	V
Carboxymethyl cellulose	−	−	−	−
Agar	−	−	−	+
Alginate	ND	ND	ND	−
Pectin	ND	ND	ND	+
Chitin	−	−	−	−
Esculin	V	−	−	+
DNA	−	−	+	−
Tyrosine	V	+	−	−
Brown diffusible pigment produced on tyrosine agar	−	−	v	−
Precipitate formed on egg yolk agar	+	+	+	−
β-Galactosidase activity[g]	V	+	−	+
Susceptibility to vibriostatic compound O/129[h]	−	+	+	+
H_2S production	−	−	+	−
Production of cytochrome oxidase	+	+	+	+
Nitrate reduction	V	−	V	V

[a] Data from references 1, 4, 8–10, 13, 15, 16, 40, 61, 68, 82, 90, and 95.
[b] AOA, Anacker-Ordal agar (0.05% tryptone, 0.05% yeast extract, 0.02% beef extract, 0.02% sodium acetate) (3).
[c] Production of an extracellular galactosamin glycan was revealed by flooding the colonies with a 0.01% aqueous solution of Congo red (52).
[d] +, all strains are positive; −, all strains are negative; (+), weakly positive; v, variable among strains; V, variable among references; ND, no data available.
[e] Gliding motility was observed in the type and only strain of *Flavobacterium aquatile* under certain conditions (39).
[f] The presence of the flexirubin type of pigments was revealed by a distinct, reversible color shift of the colonies when they were flooded with a 20% (wt/vol) KOH aqueous solution (28).
[g] Determined by the *o*-nitrophenyl-β-D-galactopyranoside test.
[h] Determined by a diffusion method in which 500-μg disks were used.

branchiophilum, *Flavobacterium columnare*, *Flavobacterium 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.

Description of *Flavobacterium aquatile* (Frankland and Frankland 1889) Bergey, Harrison, Breed, Hammer, and Huntoon 1923. The description of *Flavobacterium aquatile* is the same as that given previously (40), with the following additions and modifications: no flexirubin type of pigment is produced; peptones are used as nitrogen sources, but urea and Casamino Acids are not; gelatin, tyrosine, starch, esculin, tributyrin, and lecithin are degraded, but carboxymethyl cellulose, agar, and chitin are not; nitrate is reduced; and *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed (10, 15, 61).

Description of *Flavobacterium branchiophilum* Wakabayashi, Huh, and Kimura 1989. *Flavobacterium branchiophilum* was originally described as *Flavobacterium branchiophila* (95), but the specific epithet was later corrected (94). The description of this taxon is the same as that given previously (95), with the following additions: tyrosine, tributyrin, lecithin, and Tween compounds are degraded, but carboxymethyl cellulose is not; hydrogen sulfide is not produced; *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed; and no growth occurs at 37°C (9). This species is frequently isolated from diseased gills of fish, and pathogenicity for fish has been demonstrated by experimental infection tests (95).

Description of *Flavobacterium columnare* comb. nov. *Flavobacterium columnare* (basonym, *Flexibacter columnaris* (Davis 1922) Bernardet and Grimont 1989). The following combinations have been used for this species: *Bacillus columnaris* Davis 1922; *Chondrococcus columnaris* Ordal and Rucker 1944; *Cytophaga columnaris* Garnjobst 1945; *Cytophaga columnaris* Reichenbach 1989 (68); *Flexibacter columnaris* Leadbetter 1974 (49); and *Flexibacter columnaris* Bernardet and Grimont 1989 (10). The description of this taxon is the same as that given previously (10), except that tyrosine is not hydrolyzed. This species is frequently isolated from superficial lesions on

TABLE 5—*Continued*

Flavobacterium hydatis	*Flavobacterium johnsoniae*	*Flavobacterium pectinovorum*	*Flavobacterium psychrophilum*	*Flavobacterium saccharophilum*	*Flavobacterium succinicans*
Flat, spreading, with filamentous margins	Flat, spreading, with filamentous margins	Low convex, round, with entire margins	Low convex, round, with entire or uneven margins	Flat, spreading, sunken into agar	Flat, spreading, with filamentous margins
−	V	−	−	−	−
−	−	−	−	−	−
+	+	+	−	+	+
+	+	+	−	+	+
+	+	+	(+)	+	+
+	+	+	+	+	−
+	+	+	−	+	+
+	+	+	−	ND	+
+	+	+	+	+	(+)
+	+	+	+	+	+
+	+	+	−	+	+
V	+	+	−	+	ND
−	−	−	−	+	−
−	+	+	−	ND	ND
+	+	+	−	+	ND
(+)	+	+	−	−	−
+	+	+	−	+	+
+	+	+	(+)	−	+
+	+	+	v	+	−
−	v	−	−	−	−
−	−	−	+	−	−
+	+	+	−	+	+
−	−	+	+	+	+
−	−	V	−	+	−
V	+	+	V	−	+
+	v	+	−	+	v

diseased fish and sometimes from internal organs. Pathogenicity for fish has been demonstrated by experimental infection tests (18).

Description of *Flavobacterium flevense* comb. nov. *Flavobacterium flevense* (basonym, *Cytophaga flevensis* van der Meulen, Harder, and Veldkamp 1974). The description of this taxon is the same as that given previously (90), with the following additions and modifications: starch, esculin, and Tween compounds are degraded; DNA, carboxymethyl cellulose, tyrosine, and lecithin are not degraded; catalase is produced; *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed; and good growth occurs on nutrient agar Trypticase soy agar (10).

Description of *Flavobacterium hydatis* nom. nov. *Flavobacterium hydatis* (basonym, *Cytophaga aquatilis* Strohl and Tait 1978) (hy′ da.tis. Gr. n. *hydor*, water; N. L. gen. n. *hydatis*, from water). The description of this taxon is the same as that given previously for [*Cytophaga*] *aquatilis* (82), with the following additions and modifications: lecithin is not degraded; cytochrome oxidase is produced; *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed; and good growth occurs on Trypticase soy agar (10). The only currently available strain was isolated from the gills of a diseased salmon, but the pathogenicity of this organism has not been tested (82).

Description of *Flavobacterium johnsoniae* comb. nov. corrig. *Flavobacterium johnsoniae* (basonyms, *Cytophaga johnsonae* Stanier 1947, *Cytophaga johnsonii* Stanier 1957) (john.so′ ni.ae. N. L. gen. fem. n. *johnsoniae*, of Johnson, named after D. E. Johnson [see above]). The description of this taxon is the same as that given previously (68), with the following additions: good growth occurs on nutrient agar and Trypticase soy agar; tyrosine, esculin, tributyrin, and Tween compounds are degraded; lecithin is not degraded; and *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed (10, 61). *Flavobacterium johnsoniae* is common in soil and freshwater, and strains are frequently isolated from superficial lesions on diseased fish (9). Thus, for a long time this species was considered opportunistic, but some clues to its pathogenicity have been found recently (13). *Flavobacterium johnsoniae* includes two strains previously known as [*Flexibacter*] *aurantiacus* strains (strains NCIMB 1382^T and NCIMB 1455) (see below).

Description of *Flavobacterium pectinovorum* comb. nov. *Flavobacterium pectinovorum* (basonym, *Cytophaga pectinovora* (Dorey 1959) Reichenbach 1989) was described as *Flavobacterium pectinovorum* by Dorey in 1959 (25), but this name was not included on the Approved Lists of Bacterial Names (75). It was later reclassified as *Cytophaga johnsonae* (15) and then restored as an independent species under the combination *Cytophaga pectinovora* (68). The description of this taxon is the same as that given previously (68), with the following additions: good growth occurs on nutrient agar and Trypticase soy agar; esculin and tyrosine are degraded, but no pigment is produced on tyrosine agar; no precipitate is formed on egg yolk agar; *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed;

and the organism is susceptible to vibriostatic compound O/129 (8).

Description of *Flavobacterium psychrophilum* comb. nov. *Flavobacterium psychrophilum* (basonym, *Flexibacter psychrophilus* (Borg 1960) Bernardet and Grimont 1989) was described as *Cytophaga psychrophila* by Borg in 1960 (11); this combination was also used in *Bergey's Manual of Systematic Bacteriology* (68), but in a 1989 study Bernardet and Grimont proposed that this taxon should be transferred to the genus *Flexibacter* (10). The description of this taxon is the same as that given previously (10, 68). This species is frequently isolated from internal organs and superficial lesions of diseased fish, and pathogenicity for fish has been demonstrated by experimental infection tests (18).

Description of *Flavobacterium saccharophilum* comb. nov. *Flavobacterium saccharophilum* (basonym, *Cytophaga saccharophila* Agbo and Moss 1979). The description of this taxon is the same as that given previously (1, 68). Catalase and cytochrome oxidase activities were listed as positive in the original description of the species (1), but they were not observed in a later study (68). We clearly observed catalase activity, but no cytochrome oxidase activity was detected when we used both dimethylparaphenylene diamine (oxidase discs; bioMérieux, Marcy-l'Etoile, France) and tetramethylparaphenylene diamine (oxidase liquid reagent; bioMérieux). Good growth occurs on nutrient agar and Trypticase soy agar; esculin and tyrosine are degraded, but no pigment is produced on tyrosine agar; no precipitate is formed on egg yolk agar; and *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed (8).

Description of *Flavobacterium succinicans* comb. nov. *Flavobacterium succinicans* (basonym, *Cytophaga succinicans* Anderson and Ordal 1961). The combination *Flexibacter succinicans* has also been used for this species (49). The description of this taxon is the same as that given previously (4, 68). Good growth occurs on nutrient agar and Trypticase soy agar; esculin and DNA are degraded; tyrosine is not degraded; no precipitate is formed on egg yolk agar; *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed; the organism is susceptible to vibriostatic compound O/129; and H_2S is not produced (8). The three *Flavobacterium succinicans* strains currently available were isolated from superficial lesions on diseased fish and from water in a fish tank, but the pathogenicity of these strains was not tested (4).

Differentiation of *Flavobacterium* species. The main characteristics that differentiate the 10 valid species that belong to the emended genus *Flavobacterium* are shown in Table 5. Since the data in this table were collected from several references, it is possible that different procedures used in the different studies resulted in apparent phenotypic differences. Additional differentiating characteristics for the *Flavobacterium* species include their API ZYM profiles (Table 6) and their polyamine distribution patterns (see above).

We determined the fatty acid and protein profiles of all valid species and invalid taxa belonging to the *Flavobacterium aquatile* rRNA cluster (Table 4 and Fig. 2). Some differences in the fatty acid profiles of species are described above, and these differences are valuable characteristics for differentiating several species. From Fig. 2, it is obvious that whole-cell protein analysis combined with a computer-assisted numerical comparison of the patterns is a useful technique for differentiating *Flavobacterium* strains. As mentioned above, the different [*Flexibacter*] *columnaris* and [*Flexibacter*] *psychrophilus* strains which we studied are identical as determined by protein electrophoresis; this finding confirms that these two species are homogeneous genotypically, as determined by DNA-DNA hybridization (10). In this study, several species were represented by only one strain. However, other species exhibited various levels of protein electrophoretic heterogeneity (Fig. 2), as discussed below.

In the case of *Flavobacterium succinicans*, the protein profile of strain NCIMB 2279 is clearly different from the protein profiles of the two other strains, whereas the results of the fatty acid analysis can be used to differentiate strain NCIMB 2278 from the two other strains. These data confirm the previous observations that there are phenotypic differences in this species (68). The G+C contents of the three strains are very similar (68) (Table 2), but no DNA-DNA hybridization study has been performed yet.

The seven *Flavobacterium branchiophilum* strains which we tested all produce very similar fatty acid profiles, but they can be separated into two very distinct groups when their protein profiles are compared. As there are no obvious differences in the phenotypic characteristics of these strains and they have very similar G+C contents, additional studies (including DNA-DNA hybridization analyses) will be necessary to investigate the taxonomic structure of *Flavobacterium branchiophilum.*

The two strains belonging to the invalid taxon "[*Sporocytophaga*] *cauliformis*" clearly produce different fatty acid and protein profiles, which is consistent with the differences in G+C contents and phenotypic characteristics noticed previously (68).

[*Flexibacter*] *aurantiacus* Lewin 1969 contains only two strains (50). Type strain NCIMB 1382 was previously considered a *Cytophaga aurantiaca* strain, while strain NCIMB 1455 was previously classified as a strain of [*Cytophaga*] *psychrophila* (synonym, [*Flexibacter*] *psychrophilus* [see above]). Both strains are phenotypically indistinguishable from the *Flavobacterium johnsoniae* type strain but very clearly different from both bona fide *Cytophaga aurantiaca* and [*Flexibacter*] *psychrophilus* strains (9, 10). In the 1990 National Collection of Industrial and Marine Bacteria *Catalogue of Strains* (57a) both strains are listed as "possibly [*Cytophaga*] *johnsonae*." Moreover, the two [*Flexibacter*] *aurantiacus* strains were the closest relatives of the *Flavobacterium johnsoniae* type strain when the fatty acid profiles were analyzed numerically by a principal-component analysis (data not shown). The data obtained from DNA-DNA hybridization studies (Table 3) confirmed that the two [*Flexibacter*] *aurantiacus* strains belong to the same species; their high levels of DNA-DNA relatedness and low ΔT_m with the DNA of the *Flavobacterium johnsoniae* type strain showed that these three strains form a tight genomic species. Thus, [*Flexibacter*] *aurantiacus* Lewin 1969 appears to be a junior synonym of [*Cytophaga*] *johnsonae* Stanier 1947, and we propose that it should be included in *Flavobacterium johnsoniae*. In general, *Flavobacterium johnsoniae* appears to be very heterogeneous. The type strain, two additional strains, and the former [*Flexibacter*] *aurantiacus* strains make up two separate electrophoretic subgroups. Similar intraspecific protein electrophoretic subgroups have been described for several other bacteria (87, 89). However, four additional strains, two of which (ATCC 29585 and ATCC 29586) form a single cluster, occupy distinct positions on the dendrogram (Fig. 2). DNA-DNA hybridization experiments performed with strains ATCC 29585 and ATCC 29586 revealed that these organisms exhibit a high level of DNA homology, which confirmed the general rule that the results of whole-cell protein electrophoresis can be used to group closely related strains. However, these strains and one of the other strains (LMG 11391) did not exhibit significant levels of DNA homology with other *Flavobacterium johnsoniae* strains. The fourth strain which occupied a separate position on the dendrogram was not included in the DNA-DNA hy-

TABLE 6. API ZYM profiles of the type strains of the 10 valid species belonging to the genus *Flavobacterium*[a]

Strain[b]	Hydrolysis of the following substrates[c]:																	
	2-Naphthyl-phosphate	2-Naphthyl-butyrate	2-Naphthyl-caprylate	2-Naphthyl-myristate	L-Leucyl-2-naphthylamide	L-Valyl-2-naphthylamide	L-Cystyl-2-naphthylamide	*N*-Benzoyl-DL-arginine-2-naphthylamide	*N*-Glutaryl-phenylalanine-2-naphthylamide	2-Naphthyl-phosphate	Naphthol-AS-BI-phosphate	6-Br-2-naphthyl-α-D-galactopyranoside	2-Naphthyl-β-D-galactopyranoside	Naphthol-AS-BI-β-D-glucuronide	2-Naphthyl-α-D-glucopyranoside	6-Br-2-naphthyl-β-D-glucopyranoside	1-Naphthyl-*N*-acetyl-β-D-glucosaminide	6-Br-2-naphthyl-α-D-mannopyranoside	2-Naphthyl-α-L-fucopyranoside
Flavobacterium aquatile LMG 4008^T	4	2	4	1	5	5	2	0	0	1	2	0	0	0	5	0	0	0	0
Flavobacterium branchiophilum ATCC 35035^T	5	2	3	0	5	4	2	2	0	4	4	0	0	0	1	0	0	0	0
Flavobacterium columnare NCIMB 2248^T	5	2	3	0	4	4	1	3	1	3	3	0	0	0	0	0	0	0	0
Flavobacterium flevense DSM 1076^T	5	1	2	1	5	1	1	0	0	3	3	1	5	0	3	0	4	0	0
Flavobacterium hydatis DSM 2063^T	5	2	4	1	4	5	2	0	0	4	5	0	0	0	4	0	2	0	0
Flavobacterium johnsoniae LMG 1341^T	5	1	3	1	5	5	2	1	1	4	5	0	3	0	4	1	4	0	0
Flavobacterium pectinovorum NCIMB 9059^T	5	3	3	0	4	4	2	0	0	4	4	0	2	1	4	5	3	0	0
Flavobacterium psychrophilum NCIMB 1947^T	5	2	3	1	5	1	0	0	0	3	3	0	0	0	0	0	0	0	0
Flavobacterium saccharophilum NCIMB 2072^T	5	3	4	0	4	4	2	0	0	5	3	2	4	0	5	0	4	0	0
Flavobacterium succinicans NCIMB 2277^T	5	3	3	0	4	4	2	1	0	5	5	0	0	0	4	2	4	0	0

[a] Data from references 8 and 10.
[b] See Table 1, footnote *a*.
[c] The values are API ZYM reaction scores.

bridization analysis. Our results confirm the previous conclusion concerning the phenotypic and genomic diversity of *Flavobacterium johnsoniae* (68). We concluded that clearly protein electrophoretic heterogeneity occurs in this species, but that most of the strains that produce aberrant protein patterns are misidentified field isolates.

Finally, the taxa "[*Cytophaga*] *allerginae*," "[*Cytophaga*] *xantha*," "[*Flexibacter*] *aurantiacus* subsp. *excathedrus*," "[*Promyxobacterium*] *flavum*," and "[*Sporocytophaga*] *cauliformis*" clearly belong to the emended genus *Flavobacterium*, as shown by their phenotypic characteristics, rRNA homology data (Fig. 1 and Table 2), and fatty acid analysis data (Table 4). However, these names have not been validly published, and each of them except "[*Sporocytophaga*] *cauliformis*" is represented by only a single strain; two rather different strains of "[*Sporocytophaga*] *cauliformis*" are available (68). No 16S rRNA sequence data have been published for any of these organisms except one "[*Sporocytophaga*] *cauliformis*" strain (30). Thus, instead of creating five new *Flavobacterium* species, each represented by one or two poorly characterized strains, we decided to refer to these isolates as *Flavobacterium* sp., pending isolation of new strains. A larger collection of strains should enable researchers to properly describe these five taxa and to determine if each of them really represents a distinct species belonging to the genus *Flavobacterium*. Preliminary DNA-DNA hybridization studies have shown that the DNAs from "[*Cytophaga*] *allerginae*," "[*Cytophaga*] *xantha*," "[*Promyxobacterium*] *flavum*," and the two "[*Sporocytophaga*] *cauliformis*" strains do not exhibit significant levels of homology with DNAs from the *Flavobacterium aquatile* and *Flavobacterium johnsoniae* type strains (8).

Differentiation of *Flavobacterium* from related genera. The main characteristics that can be used to differentiate the emended genus *Flavobacterium* from several related taxa belonging to rRNA superfamily V are shown in Table 7. The following taxa were included in Table 7: (i) the closest phylogenetic relative of the *Flavobacterium* rRNA branch, as determined by DNA-rRNA hybridization (i.e., [*Flavobacterium*] *odoratum*) (Fig. 1); (ii) the genera to which the valid species included in the emended genus *Flavobacterium* were previously assigned (i.e., the genera *Cytophaga* and *Flexibacter*; these genera are represented by their type species, *Cytophaga hutchinsonii* and *Flexibacter flexilis*, respectively); (iii) the genera that now contain species that previously were considered *Flavobacterium* species (i.e., the genera *Bergeyella*, *Chryseobacterium*, *Empedobacter*, *Sphingobacterium*, and *Weeksella*; the genera *Bergeyella*, *Empedobacter*, and *Weeksella* are represented by their type and only species, *Bergeyella zoohelcum*, *Empedobacter brevis*, and *Weeksella virosa*, respectively); and (iv) other taxa belonging to the emended family *Flavobacteriaceae* (see below).

Delineation of the families belonging to the *Cytophaga-Flavobacterium-Bacteroides* rRNA homology group. In *Bergey's Manual of Systematic Bacteriology*, the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter* were placed in the order *Cytophagales* along with several other related taxa (68). This definition of the order took into account classical phenotypic characteristics, recent data obtained from chemotaxonomic investigations, and the first results of rRNA studies. The following two families were included in the order *Cytophagales*: the family *Cytophagaceae* Stanier 1940 (including the genera *Cytophaga*, *Capnocytophaga*, *Flexithrix*, and *Sporocytophaga*; the genera *Flexibacter*, *Microscilla*, and *Chitinophaga* were considered close relatives of this family) and the proposed family "*Flavobacteriaceae*." The name of the latter family, which contained strictly aerobic, nonmotile, nongliding, free-living or parasitic organisms, was subsequently validated (44a). The following other taxa were considered rather close relatives of the order *Cytophagales*: the family *Bacteroidaceae* Pribram 1933

TABLE 7. Characteristics that can be used to differentiate the emended genus *Flavobacterium*, related taxa belonging to rRNA superfamily V, and the taxa included in the emended family *Flavobacteriaceae*[a]

Characteristic	*Flavobacterium*	[*Flavobacterium*] *odoratum*	*Cytophaga hutchinsonii*	*Flexibacter flexilis*	*Sphingobacterium*	*Chryseobacterium*
Habitat	Free living or saprophytic	Free living or saprophytic	Free living (soil)	Free living (freshwater)	Free living or saprophytic	Free living or parasitic
Pigmented colonies	+[b]	+	+	+	(+)	+
Gliding motility	+[c]	−	+	+	−	−
Menaquinone[e]	MK-6	MK-6	MK-7	ND	MK-7	MK-6
Capnophilic metabolism	−	−	−	−	−	−
Presence of sphingophospholipids	−	−	ND	ND	+	−
Growth at 37°C	−	+	−	−	v	+[f]
Growth at 42°C	−	−	−	−	−[g]	v
Growth on MacConkey agar	ND	+	ND	ND	+	+[h]
Growth on β-hydroxybutyrate	ND	+	ND	ND	ND	+
Acid production from glucose	v	−	+	+	+	+[i]
Acid production from sucrose	v	−	ND	ND	+	−
DNase activity	v	+	−	ND	v	+
Urease activity	v	+	+	ND	v	v
Catalase activity	+	+	+	−	+	+
Production of indole	−	−	−	−	−	v[i]
Degradation of cellulose	−	−	+	−	−	−
Degradation of esculin	v	−	ND	ND	+	+
Degradation of gelatin	+[c]	+	+	+	v	+
Resistance to penicillin G	v	ND	ND	ND	ND	+
G+C content (mol%)	32–37	37	40	40–43	39–45	33–38

[a] Data from references 9, 10, 19, 20, 24, 37, 41–44, 45, 46, 61, 68, 72, 84, 85, 88, and 102 and this study. Additional phenotypic characteristics that differentiate the *Capnocytophaga* species, *Ornithobacterium rhinotracheale*, and *Riemerella anatipestifer* are described in reference 88.

[b] +, positive reaction; −, negative reaction; (+), weak positive reaction; v, variable within and between species; ND, not determined or determined for some species only.

[c] See exceptions in Table 5.

[d] Positive for all members of the rRNA cluster except [*Flavobacterium*] *gondwanense* and [*Flavobacterium*] *salegens.*

[e] MK-6, menaquinone 6; MK-7, menaquinone 7.

[f] Most strains are positive for this characteristic.

[g] Negative for all *Sphingobacterium* species except *Sphingobacterium thalpophilum.*

[h] Strain dependent for *Chryseobacterium indologenes*. Positive for all other *Chryseobacterium* species except *Chryseobacterium scophthalmum.*

[i] Strain dependent for *Chryseobacterium meningosepticum*. Positive for all other *Chryseobacterium* species except *Chryseobacterium scophthalmum.*

[j] Positive for all *Capnocytophaga* species except *Capnocytophaga canimorsus.*

(36) and the ring-forming bacteria, which were later placed in a fourth family, the *Spirosomaceae* Larkin and Borrall 1978 (48). The general structure of the order *Cytophagales* and related taxa was later confirmed and completed by new data obtained from rRNA studies (30, 56, 58, 72, 101). Thus, this taxonomic entity seems to correspond to the phylogenetic entity called superfamily V or the "flavobacter-bacteroides" phylum depending on the authors and the method used for rRNA homology studies (30, 73).

Because of the taxonomic and nomenclatural changes proposed in this study and in other recent publications (85), there are many new problems concerning the definition of the families *Flavobacteriaceae* and *Cytophagaceae* (38). An emended genus *Cytophaga* (i.e., a genus restricted to *Cytophaga hutchinsonii* and a few other cellulolytic taxa) must remain the type genus of the family *Cytophagaceae*. Most other misclassified *Cytophaga* and *Flexibacter* species have already been placed in other genera or new genera. The emended genus *Flavobacterium* proposed in this paper is the type genus of the family *Flavobacteriaceae*, but the current description of this genus is indeed much more similar to the description of "noncellulolytic cytophagas" than to its previous description.

The levels of genotypic divergence corresponding to differences in $T_{m(e)}$ of 14°C or more (Fig. 1 and Table 2) between former members of the genus *Flavobacterium* are far too great to include all of these bacteria in a single family. On the other hand, from a phenotypic and chemotaxonomic point of view, it would not be logical to separate some of the taxa belonging to different major rRNA clusters or solitary rRNA branches, such as the genera *Chryseobacterium* and *Empedobacter* or the genera *Ornithobacterium* and *Riemerella*. Three of the rRNA clusters (the *Chryseobacterium-Bergeyella-Riemerella* rRNA cluster, the *Empedobacter-Weeksella* rRNA cluster, and the *Flavobacterium aquatile* rRNA cluster) and two solitary rRNA branches (the *Ornithobacterium* and *Capnocytophaga* rRNA branches) are linked at an average $T_{m(e)}$ of about 65°C, which corresponds to a difference in $T_{m(e)}$ of about 14°C. The differences in $T_{m(e)}$ within most well-characterized families of bacteria are in the range from 8 to 12°C. In our opinion, the phenotypic similarities of all of the taxa that clustered above a value of 65°C in this study overcame the obviously high levels of genotypic divergence. A major problem in the chemotaxonomic description of this group was the previously reported presence of menaquinone 7 as the sole respiratory quinone in *Ornithobacterium* and *Riemerella* strains (27), while menaquinone 6 is the only quinone found in members of the genera *Capnocytophaga*, *Chryseobacterium*, *Empedobacter*, and *Flavobacterium* (as defined in this study), [*Flavobacterium*] *odoratum*, members of the [*Flexibacter*] *maritimus* rRNA branch, and *Weeksella virosa* (56, 85) (no data for *Bergeyella zoohelcum* are available). However, the menaquinone contents of two reference strains of *Ornithobacterium rhinotracheale* and two reference strains of *Riemerella anatipestifer* were determined by high-performance liquid chromatography and mass spectrometry, and all four strains were found to contain large amounts of menaquinone 6 and trace amounts of menaquinone 5 (47). Thus, the taxa

TABLE 7—*Continued*

Empedobacter brevis	*Weeksella virosa*	*Bergeyella zoohelcum*	*Capnocytophaga*	*Ornithobacterium rhinotracheale*	*Riemerella anatipestifer*	[*Flexibacter*] *maritimus* rRNA cluster
Free living or parasitic	Parasitic or saprophytic	Parasitic or saprophytic	Parasitic or saprophytic	Parasitic	Parasitic	Free living (marine environment) or saprophytic
+	−	−	+	−	−	+
−	−	−	+	−	−	+[d]
MK-6	MK-6	ND	MK-6	MK-6	MK-6	MK-6
−	−	−	+	+	+	−
−	−	−	ND	ND	ND	ND
+[f]	+	+	+	+	+	−
−	+	−	ND	+	+[f]	−
+	+	−	−	−	−	ND
+	+	−	ND	ND	ND	ND
+[f]	−	−	+	v	v	v
−	−	−	+[j]	−	−	v
+	−	−	ND	−	ND	ND
−	−	+	ND	+	v	v
+	+	+	v	−	+	v
+	+	+	−	−	−	−
−	−	−	−	−	−	−
−	−	−	+	−	ND	ND
+	+	+	v	−	+[f]	v
+	−	−	−	v	−	ND
31–33	35–38	35–37	34–40	37–39	29–35	33–42

belonging to each cluster and branch in this bacterial group contain the same respiratory quinone. The members of three genera (the genera *Capnocytophaga*, *Ornithobacterium*, and *Riemerella*) exhibit similar capnophilic metabolism (88), while the other taxa are considered obligately aerobic (68). In fact, within a genus whose members are for the most part aerobic, such as the emended genus *Flavobacterium* proposed above, some species may exhibit capnophilic behavior under certain conditions (this is true of *Flavobacterium hydatis* and *Flavobacterium succinicans*). Moreover, other well-defined bacterial families contain organisms that have rather different types of metabolism (86). Thus, we believe that the phenotypic similarities, as well as the genotypic similarities, of all of these organisms justify their inclusion in a single family. The emended description of the family *Flavobacteriaceae* below is a compilation of previously published phenotypic data (43, 44, 72, 85, 88) (Table 5).

Emended description of the family *Flavobacteriaceae* Reichenbach 1989. Cells are short to moderately long rods with parallel or slightly irregular sides and rounded or slightly tapered ends and are usually 0.3 to 0.6 μm wide and 1 to 10 μm long, but some species may form filamentous flexible cells under certain growth conditions. Cells in old cultures may form spherical or coccoid bodies. Gram negative. Spores are not formed. Flagella are absent. Nonmotile (*Bergeyella*, *Chryseobacterium*, *Empedobacter*, [*Flavobacterium*] *odoratum*, *Ornithobacterium*, *Riemerella*, and *Weeksella* strains) or motile by gliding (*Capnocytophaga* and *Flavobacterium* strains and members of the [*Flexibacter*] *maritimus* rRNA branch). Growth is aerobic (*Bergeyella*, *Chryseobacterium*, *Empedobacter*, *Flavobacterium*, [*Flavobacterium*] *odoratum*, and *Weeksella* strains and members of the [*Flexibacter*] *maritimus* rRNA branch) or microaerobic to anaerobic (*Capnocytophaga*, *Ornithobacterium*, and *Riemerella* strains). The optimum temperature is usually in the range from 25 to 35°C. Colonies are nonpigmented (*Bergeyella*, *Ornithobacterium*, *Riemerella*, and *Weeksella* strains) or pigmented by carotenoid or flexirubin types of pigments or both (*Capnocytophaga*, *Chryseobacterium*, *Empedobacter*, *Flavobacterium*, and [*Flavobacterium*] *odoratum* strains and members of the [*Flexibacter*] *maritimus* rRNA branch). Menaquinone 6 is the only respiratory quinone or the major respiratory quinone. Chemoorganotrophic. Intracellular granules of poly-β-hydroxybutyrate are absent. Sphingophospholipids are absent. Homospermidine is the major polyamine, and agmatine and putrescine are frequently present as minor components. Cellulose is not decomposed. The DNA base composition ranges from 29 to 45 mol%. Mostly saprophytic in terrestrial and aquatic habitats. Several members of the family are commonly isolated from diseased humans or animals; some species are considered true pathogens.

The type genus is *Flavobacterium* Bergey, Harrison, Breed, Hammer, and Huntoon 1923, as emended in this study. Other taxa included in the family are the genera *Bergeyella*, *Capnocytophaga*, *Chryseobacterium*, *Empedobacter*, *Ornithobacterium*, *Riemerella*, and *Weeksella*, [*Flavobacterium*] *odoratum*, and the taxa belonging to the [*Flexibacter*] *maritimus* rRNA branch. Differential characteristics for the taxa belonging to the family *Flavobacteriaceae* are shown in Table 7.

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