INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1989, p. 346–354 Vol. 39, No. 3
0020-7713/89/030346-09$02.00/0

Deoxyribonucleic Acid Relatedness and Phenotypic Characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida, and Masumura 1986

JEAN-FRANCOIS BERNARDET[1]* AND PATRICK A. D. GRIMONT[2]

Laboratoire d'Ichtyopathologie, Institut National de la Recherche Agronomique, Station de Virologie Immunologie Moléculaires, Centre de Recherches de Jouy-en-Josas, Domaine de Vilvert, 78350 Jouy-en-Josas, France[1]; and Unité des Entérobactéries, Institut National de la Santé et de la Recherche Médicale, Unité 199, Institut Pasteur, F-75724 Paris Cédex 15, France[2]

Eight unidentified fish pathogens and 10 strains received as "*Flexibacter columnaris*," "*Cytophaga psychrophila*," and *Flexibacter maritimus* were compared with the type strains of all previously described species in the genera *Cytophaga* and *Flexibacter* and with seven *Flavobacterium* species by determining levels of deoxyribonucleic acid (DNA) relatedness (S1 nuclease method) and by performing phenotypic tests. The name *Flexibacter columnaris* sp. nov. is revived for a DNA relatedness group comprising eight strains that are 75 to 100% related to strain TG 39/87 and 0 to 8% related to all of the other species studied. These strains produce flat rhizoid colonies which adhere to agar, show strong gliding movement, absorb Congo red, reduce nitrate to nitrite, and produce H_2S. The guanine-plus-cytosine content of the DNA is 32 mol%. The type strain is strain NCMB 2248 (= ATCC 23463). The name *Flexibacter psychrophilus* sp. nov. is revived for a DNA relatedness group comprising seven strains that are 90 to 100% related to strain NCMB 1947^T (T = type strain) and 0 to 5% related to all of the other species studied. These strains produce circular, convex colonies that have regular or spreading margins and do not adhere to agar, show very slow gliding movement, do not absorb Congo red, do not reduce nitrate, and do not produce H_2S. The guanine-plus-cytosine content of the DNA is 33 mol%. The type strain is strain NCMB 1947. Both *F. columnaris* and *F. psychrophilus* produce the flexirubin type of pigments, are strongly proteolytic, and do not hydrolyze (or produce acid from) any carbohydrate. *Flexibacter maritimus* (three strains) was shown to constitute a DNA relatedness group that is 0 to 8% related to all of the other species tested. Furthermore, this organism can be differentiated by phenotypic tests. The presence of *F. maritimus* in Europe is shown for the first time.

Since they were first described by Davis in 1922 (9), bacteria that are pathogenic for fish and belong to the order *Cytophagales* have frequently been isolated from diseased marine and freshwater fishes worldwide (1). These organisms have been responsible for substantial fish mortality and economic losses.

The class *Flexibacteriae* has recently been defined by Reichenbach and Dworkin (25) to include unicellular bacteria that are capable of gliding movement on wet surfaces (gliding bacteria) but are devoid of flagella. This class is composed of two orders; the members of the order *Myxobacterales* have high deoxyribonucleic acid (DNA) guanine-plus-cytosine (G+C) contents (67 to 71 mol%) and a typical life cycle with fruiting bodies and microcysts, and the members of the order *Cytophagales* have lower G+C contents with a very wide range (28 to 67 mol%), reflecting the heterogeneity of these organisms, and do not produce fruiting bodies.

The gliding bacteria isolated from diseased fish belong to the order *Cytophagales* and to the genera *Flexibacter* and *Cytophaga*. However, the taxonomy of these groups remains in a state of confusion. One of the main problems is the relationship of these organisms to the genus *Flavobacterium*; the phenotypic characteristics and G+C contents of the DNAs are rather similar, and the published data concerning DNA or ribonucleic acid relatedness are not comprehensive enough to allow clear distinction between the two groups (7, 24, 29). The feature considered to be of major importance is that members of the order *Cytophagales* are capable of gliding, whereas *Flavobacterium* species are not. However, the taxonomic reliability of gliding is uncertain (26). Thus, several authors prefer to use the name "*Flavobacterium-Cytophaga* group" and think that these bacteria should be studied together (13). Another very important problem concerns the definitions of the genera *Cytophaga* and *Flexibacter* and the distinction between these two taxa (26).

Among the members of the order *Cytophagales* isolated from diseased fish, the following three species are considered to be pathogenic: "*Flexibacter columnaris*," "*Cytophaga psychrophila*," and *Flexibacter maritimus*.

In 1922, Davis (9) observed in the external lesions of diseased fish a bacterium which he named "*Bacillus columnaris*." Ordal and Rucker (21) isolated this organism and classified it among the "gliding bacteria" under the name "*Chondrococcus columnaris*" because they thought that microcysts and fruiting bodies were produced. Garnjobst (11) isolated this bacterium in 1945, found it to be devoid of fruiting bodies and microcysts, and renamed it "*Cytophaga columnaris*." In *Bergey's Manual of Determinative Bacteriology*, 8th ed., Leadbetter (16) considered this organism a true species of the genus *Flexibacter* and thus changed its name to "*Flexibacter columnaris*." This name was not listed on the Approved Lists of Bacterial Names (30) and has not

* Corresponding author.

been validated since 1980 by publication or announcement in the International Journal of Systematic Bacteriology. The disease caused by "*Flexibacter columnaris*" is called columnaris disease. It occurs worldwide in numerous species of freshwater fish where the water temperature exceeds 14°C. The infection may be restricted to the gills or body surface, causing extensive necrosis and ulcers, or it may be generalized (3).

"*Cytophaga psychrophila*" was discovered by Davis in diseased fish in 1946 (10). Borg (A. F. Borg, Ph.D. thesis, University of Washington, Seattle, 1948) isolated this bacterium, named it (5), and studied the disease which it provokes. Leadbetter (16) considered this species to be incertae sedis, close to the genus *Flexibacter*. The name "*Cytophaga psychrophila*" did not appear on the Approved Lists (30) and has not been validated since 1980. The disease which this organism induces in fish is called cold-water disease or low-temperature disease, because it occurs where the water temperature is below 15°C. It is restricted to salmonids, and until now it has been reported only in the northern states of the United States and in Canada. Fry and young fish are preferentially infected, but adults are also susceptible. Various clinical signs may occur, the most frequent being erosions of the peduncle area (hence, the name peduncle disease, which is sometimes given to the disease) (R. A. Holt, M.Sc. and Ph.D. theses, Oregon State University, Corvallis, 1972 and 1987).

Flexibacter maritimus has recently been isolated from several species of marine fish reared in seawater in Japan. This new species has been validly published (32). Infected fish have eroded mouths and fins and ulcerated skin lesions (31).

"*Flexibacter columnaris*" (4) and "*Cytophaga psychrophila*" (J. F. Bernardet and B. Kerouault, Appl. Environ. Microbiol., in press) were recently isolated in France for the first time. In this study, French isolates were compared with isolates from other countries, with the type strains of each valid species belonging to the genera *Flexibacter* and *Cytophaga*, and with seven *Flavobacterium* species. The two strains of *Flexibacter maritimus* deposited in the National Collection of Marine Bacteria (NCMB), Aberdeen, Scotland, were included in the study and compared with a "*Flexibacter columnaris*"-like bacterium (strain NCMB 2158) that was isolated from marine fish in Scotland (8). The results of our phenotypic and DNA relatedness studies indicate that these three fish-pathogenic bacteria are independent species. Consequently, the names *Flexibacter columnaris* and *Flexibacter psychrophilus* are revived, and the identification of strain NCMB 2158 as *Flexibacter maritimus* proves the presence of this species in Europe for the first time.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains which we studied are listed in Table 1. The type strains of the valid species of the genera *Flexibacter* and *Cytophaga* and the seven *Flavobacterium* species were obtained from collections, and other strains were isolated from diseased fish in France and in other countries.

Most bacterial strains were grown in Anacker-Ordal (2) broth or agar, which contain (per liter) 0.5 g of tryptone (Difco Laboratories, Detroit, Mich.), 0.5 g of yeast extract (Difco), 0.2 g of sodium acetate (Prolabo, Paris, France), and 0.2 g of beef extract (Difco); Anacker-Ordal agar also contains 9 g of agar (Difco) per liter. The pH was adjusted to 7.2 to 7.4.

Some strains, which did not grow on the media described above, were grown on other media. *Cytophaga hutchinsonii* was grown in Dubos medium (26) supplemented with 30% (wt/vol) D-cellobiose; *Flexibacter roseolus* and *Flexibacter ruber* were grown in nutrient agar and in nutrient broth (Oxoid Ltd., London, England). All of the marine species, except *Flexibacter polymorphus* and *Cytophaga latercula*, were grown in Anacker-Ordal broth and Anacker-Ordal agar prepared with artificial seawater (26) instead of distilled water; *Flexibacter polymorphus* was cultivated on *Flexibacter* medium (17), and *Cytophaga latercula* was cultivated on a medium for marine flexibacteria (18). *Flavobacterium odoratum*, *Flavobacterium breve*, *Flavobacterium spiritivorum*, *Flavobacterium meningosepticum*, and *Flavobacterium multivorum* were grown in Trypticase soy agar and broth (bioMérieux, Charbonnières-les-Bains, France).

Most strains were cultivated at 22°C, with the following exceptions: "*Cytophaga psychrophila*," 18 to 20°C; *Flexibacter roseolus*, *Flexibacter ruber*, and most marine species, 25°C; *Flexibacter polymorphus*, *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium spiritivorum*, 30°C; and *Flavobacterium meningosepticum* and *Flavobacterium multivorum*, 37°C.

DNA-DNA hybridization. We used previously described methods to extract, purify, and shear unlabeled DNAs (6). The exact procedures used for in vitro labeling of DNA (nick translation) with tritium-labeled nucleotides (Amersham International, Amersham, England) and for hybridization experiments (S1 nuclease-trichloroacetic acid method) have been described previously (12).

G+C content of DNA. The melting temperatures (T_m) of 50-μg/ml DNA solutions in 0.1× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate) were determined optically by thermal denaturation (20) with a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The G+C contents of DNAs were determined from melting temperatures by using the following equation of Owen et al. (22): G+C content = $(2.08 \times T_m) - 106.4$. The DNA of *Escherichia coli* K-12 was used as a standard (G+C content, 50.6 mol%); in all cases, it was dialyzed together with the DNAs to be tested against the same buffer solution in the same flask to avoid any difference in salt concentration.

Morphological and physiological studies. The morphological and physiological studies were performed as described elsewhere (4). The morphology of colonies on solid medium was studied by visual observation and by light microscopy (magnification, ×20). Gliding movement was checked under a microscope (magnification, ×100) by using a hanging drop preparation of a 48-h broth culture; the bacterial cells were observed under a microscope (magnification, ×100) after Gram staining. The tolerance of the strains to different temperatures (3, 6, 10, 12, 15, 18, 20, 22, 25, 28, 30, 33, 35, 37, and 40°C) and to different NaCl concentrations (0.5, 1, 2, and 3%, wt/vol) was studied in the liquid medium used for growing each strain (see above) and in Trypticase soy broth.

Biochemical tests. Biochemical characteristics were determined as previously described (4). Tests were performed at the temperatures and in the media selected for the growth of each strain (see above).

In addition, deoxyribonuclease was tested by spot inoculation of agar containing DNA (Diagnostics Pasteur, Marnes-la-Coquette, France); DNA hydrolysis was visualized by flooding the plate with 1 N HCl. Production of acid from carbohydrates was tested by using API 50CH galleries (API System, La Balme-les-Grottes, France) and ammonium salt-

TABLE 1. Bacterial strains included in this study

Name as received	Strain[a]	Source
Cytophaga aquatilis	DSM 2063^T	Gills of diseased salmon, Michigan
Cytophaga arvensicola	JCM 2836^T	Soil, Osaka, Japan
Cytophaga fermentans[b]	NCMB 2218^T	Marine mud, California
Cytophaga flevensis	DSM 1076^T	Lake Ijsselmeer, The Netherlands
Cytophaga heparina	NCIB 9290^T	Soil
Cytophaga hutchinsonii	NCIB 9469^T	Soil
Cytophaga johnsonae	DSM 2064^T	Soil or mud, Rothamsted or Cambridge, England
	ATCC 29585	Diseased freshwater fish, Manitoba, Canada
	ATCC 29586	Diseased freshwater fish, Manitoba, Canada
Cytophaga latercula[b]	NCMB 1399^T	Outflow of marine aquarium, California
Cytophaga lytica[b]	NCMB 1423^T	Beach mud, Limon, Costa Rica
Cytophaga salmonicolor[b]	NCMB 2216^T	Marine mud, California
"*Cytophaga allerginae*"	ATCC 35408	Water in industrial air-cooling unit, United States
Flexibacter aggregans[b]	NCMB 1443^T	Beach sand, Tema, Ghana
Flexibacter aurantiacus	NCMB 1382^T	Garden soil, Minnesota
	NCMB 1455	United States
Flexibacter canadensis	ATCC 29591^T	Soil, Canada
Flexibacter flexilis	NCMB 1377^T	Lily pond, San Jose, Costa Rica
Flexibacter litoralis[b]	NCMB 1366^T	Outflow of marine aquarium, California
Flexibacter polymorphus[b]	ATCC 27820^T	Decaying ascidian, La Paz, Mexico
Flexibacter roseolus	NCMB 1433^T	Hot spring, Agua Caliente, Costa Rica
Flexibacter ruber	NCMB 1436^T	Hot spring, Geysir, Iceland
Flexibacter sancti	NCMB 1379^T	Buenos Aires, Argentina
Flexibacter tractuosus[b]	NCMB 1408^T	Sand, Nhatrang, Vietnam
Flexibacter maritimus[b]	NCMB 2154^T	Diseased red sea bream (*Pagrus major*) kidney, Hiroshima, Japan
	NCMB 2153	Diseased black sea bream (*Acanthopagrus schlegeli*) kidney, Hiroshima, Japan
Flavobacterium aquatile	NCIB 8694^T	Deep well, Kent, England
Flavobacterium balustinum	LA 724^T	Heart blood of fish, France
Flavobacterium breve	NCTC 11099^T	Human bronchial secretion, Switzerland
Flavobacterium meningosepticum	NCTC 10016^T	Cerebrospinal fluid from premature infant, United States
Flavobacterium odoratum	NCTC 11036^T	Unknown
Flavobacterium multivorum	NCTC 11343^T	Human spleen, Washington, D.C.
Flavobacterium spiritivorum	NCTC 11386^T	Human uterus, Kansas
"*Flexibacter columnaris*"	NCMB 1038	Salmonid, United States
	NCMB 2248^T	Morphological variant of strain NCMB 1038
	Holt DD3-69	Adult chinook salmon (*Oncorhynchus tshawytscha*) gill lesions, Oregon
	Holt IC8-69	Young catfish (*Ictalurus* sp.) kidney, Idaho
	Wakabayashi EK28	Japanese eel (*Anguilla japonica*) gill lesions, Japan
	Farkas H82/7	Carp (*Cyprinus carpio*) skin ulcer, Hungary
"*Cytophaga psychrophila*"	NCMB 1947^T	Coho salmon (*Oncorhynchus kisutch*) kidney, Washington
	Holt SH3-81	Coho salmon kidney, Oregon
Unidentified isolates	TG 39/87	Adult black bullhead (*Ictalurus melas*) skin ulcer, France
	TG 44/87	Brown trout fry (*Oncorhynchus mykiss*) skin lesions, France
	TG 02/86	Rainbow trout fry (*Salmo gairdneri*) kidney, France
	TG 28/86	Adult rainbow trout skin lesions, France
	LNPAA P01/88	Rainbow trout fry spleen, France
	TG P02/88	Rainbow trout fry spleen, France
	LNPAA P03/88	Rainbow trout fry spleen, France
	NCMB 2158[b,c]	Dover sole (*Solea solea*) skin lesions, Scotland

[a] ATCC, American Type Culture Collection, Rockville, Md.; NCIB and NCMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; JCM, Japanese Collection of Microorganisms, Tokyo, Japan; LA, Centre de Collection des Types Microbiens, Lausanne, Switzerland; NCTC, National Collection of Type Cultures, London, England; Holt, R. A. Holt, Department of Microbiology, Oregon State University, Corvallis; Wakabayashi, H. Wakabayashi, Department of Fisheries, Faculty of Agriculture, University of Tokyo, Tokyo, Japan; Farkas, J. Farkas, Fisheries Research Institute, Szarvas, Hungary; TG, Laboratoire d'Ichtyopathologie, Institut National de la Recherche Agronomique, Thiverval-Grignon, France; LNPAA, Laboratoire National de Pathologie des Animaux Aquatiques, IFREMER, Centre de Brest, Plouzané, France.

[b] Halophile or marine species requiring media supplemented with NaCl or seawater for growth.

[c] Strain NCMB 2158 (a *Flexibacter* species) was isolated by A. C. Campbell and J. A. Buswell, Department of Biology, Paisley College of Technology, Paisley, Scotland.

sugar broth. Hydrolysis of 19 substrates was tested by using API ZYM galleries incubated at the temperature selected for each strain; previous trials (Bernardet, unpublished data) showed that incubation for 12 h was preferable when the temperature was 18 to 30°C instead of 37°C. Hydrolysis of a given substrate resulted in production of a given color, whose intensity was scored on a scale from 1 to 5.

RESULTS

DNA-DNA hybridization. The DNA relatedness results obtained with labeled reference DNAs from strains TG 39/87, NCMB 1947^T, and NCMB 2153 are shown in Table 2.

Strain TG 44/87 and the six "*Flexibacter columnaris*" strains formed a tight genomic species, whose members

TABLE 2. Levels of DNA relatedness among *Flexibacter columnaris*, *Flexibacter psychrophilus*, *Flexibacter maritimus*, and other species belonging to the genera *Cytophaga*, *Flexibacter*, and *Flavobacterium*

Source of unlabeled DNA[a]		% Reassociation at 60°C with labeled DNA from:		
Species or group	Strain	Strain TG 39/87	Strain NCMB 1947^T	Strain NCMB 2153
Flexibacter columnaris	TG 39/87	100[b]	3	0
	NCMB 1038	95	4	0
	NCMB 2248^T	99	3	0
	Holt DD3-69	87	2	8
	Holt IC8-69	76	2	0
	Wakabayashi EK28	78	3	0
	Farkas H82/7	96	3	0
	TG 44/87	101	3	0
Flexibacter psychro philus	NCMB 1947^T	4	100	0
	Holt SH3-81	2	92	5
	TG 02/86	4	103	0
	TG 28/86	3	105	0
	LNPAA P01/88	2	105	1
	TG P02/88	2	107	1
	LNPAA P03/88	3	90	1
Flexibacter maritimus	NCMB 2153	1	3	100
	NCMB 2154^T	1	1	73
	NCMB 2158	2	2	77
Cytophaga spp. (13 strains)[c]		1 to 6	0 to 3	0 to 8
Flexibacter spp. (11 strains)[d]		0 to 2	0 to 5	0
Flavobacterium spp. (7 strains)[e]		1 to 9	0 to 3	0 to 6

[a] Abbreviations are explained in Table 1, footnote *a*.

[b] The values are percentages of relatedenss.

[c] *Cytophaga aquatilis* DSM 2063^T, *Cytophaga arvensicola* JCM 2836^T, *Cytophaga fermentans* NCMB 2218^T, *Cytophaga flevensis* DSM 1076^T, *Cytophaga heparina* NCIB 9290^T, *Cytophaga hutchinsonii* NCIB 9469^T, *Cytophaga johnsonae* DSM 2064^T, ATCC 29585, and ATCC 29586, *Cytophaga latercula* NCMB 1399^T, *Cytophaga lytica* NCMB 1423^T, *Cytophaga salmonicolor* NCMB 2216^T, and "*Cytophaga allerginae*" ATCC 35408.

[d] *FLexibacter aggregans* NCMB 1443^T, *Flexibacter aurantiacus* NCMB 1382^T and NCMB 1455, *FLexibacter canadensis* ATCC 29591^T, *Flexibacter flexilis* NCMB 1377^T, *Flexibacter litoralis* NCMB 1366^T, *Flexibacter polymorphus* ATCC 27820^T, *Flexibacter roseolus* NCMB 1433^T, *Flexibacter ruber* NCMB 1436^T, *Flexibacter sancti* NCMB 1379^T, and *Flexibacter tractuosus* NCMB 1408^T.

[e] *Flavobacterium aquatile* NCIB 8694^T, *Flavobacterium balustinum* LA 724^T, *Flavobacterium breve* NCTC 11099^T, *Flavobacterium meningosepticum* NCTC 10016^T, *Flavobacterium odoratum* NCTC 11036^T, *Flavobacterium multivorum* NCTC 11343^T, and *Flavobacterium spiritivorum* NCTC 11386^T.

were more than 75% related to strain TG 39/87 and only 0 to 9% related to all of the other organisms studied belonging to the genera *Cytophaga*, *Flexibacter*, and *Flavobacterium*.

Strain Holt SH3-81 and unidentified isolates TG 02/86, TG 28/86, LNPAA P01/88, TG P02/88, and LNPAA P03/88 also formed a tight genomic species, whose members were more than 90% related to "*Cytophaga psychrophila*" NCMB 1947^T and only 0 to 5% related to all of the other organisms studied.

Flexibacter maritimus NCMB 2154^T and unidentified *Flexibacter* sp. strain NCMB 2158 also formed a tight genomic species; they were more than 73% related to *Flexibacter maritimus* NCMB 2153 and only 0 to 8% related to all of the other organisms studied.

DNA base compositions. The base compositions of the three "*Flexibacter columnaris*" strains tested (strains NCMB 2248^T, TG 39/87 and TG 44/87) were 32, 32.3, and 33.2 mol% G+C, respectively, and the base compositions of the three "*Cytophaga psychrophila*" strains tested (strains NCMB 1947^T, TG 02/86, and LNPAA P01/88) were 32.5, 33.8, and 33.8 mol% G+C, respectively.

Phenotypic characteristics common to all strains. All of the strains studied were gram-negative rods that were devoid of flagella. The length of the *Cytophaga* and *Flexibacter* spp. cells was usually in the range from 3 to 10 μm, but there were wide variations depending on the age of the culture, the medium, and the temperature. The following species had exceptionally long or short cells: *Flexibacter flexilis*, 15 to 20 μm; *Cytophaga arvensicola* and *Flexibacter sancti*, more than 30 μm; *Flexibacter roseolus* and *Flexibacter ruber*, 50 to 100 μm; *Flexibacter polymorphus*, filaments more than 100 μm long; and *Cytophaga heparina* and *Cytophaga flevensis*, 0.5 to 1.5 μm. *Flavobacterium meningosepticum* and *Flavobacterium odoratum* had cell lengths ranging from 0.2 to 2 μm and from 1 to 10 μm, respectively. The widths of the cells of all organisms were 0.3 to 0.5 μm.

Most strains belonging to the order *Cytophagales* exhibited gliding movement; gliding cells frequently bent and pivoted around one extremity fixed to the glass slide, and filamentous cells displayed active flexing movements. *Cytophaga heparina*, *Cytophaga flevensis*, *Cytophaga hutchinsonii*, and *Flexibacter sancti* did not glide; "*Cytophaga psychrophila*" exhibited very slow gliding movement involving only a few cells in the microscope field. Gliding motility was never observed in *Flavobacterium* species.

The *Flavobacterium* spp. colonies were always circular and convex with entire margins and did not adhere to the agar. Most *Cytophaga* and *Flexibacter* spp. strains produced flat, irregular to rhizoid colonies with undulate to filamentous margins. The colonies of "*Flexibacter columnaris*" (with the exception of strain NCMB 1038) adhered strongly to the agar, while *Flexibacter maritimus* and *Flexibacter sancti* colonies adhered moderately to the agar. The species belonging to the order *Cytophagales* frequently formed swarms which spread on the agar. The colonies of "*Cytophaga psychrophila*" were circular and convex with regular or spreading margins. Most strains produced a cream of yellow nondiffusible pigment, with the following notable exceptions: *Flexibacter roseolus* and *Flexibacter ruber*, bright orange pigment; *Cytophaga salmonicolor* and *Flexibacter litoralis*, pink pigment; and *Cytophaga latercula*, bright red pigment.

All strains were strictly aerobic and oxidase positive and hydrolyzed tributyrin. *Cytophaga hutchinsonii* was the only species that digested cellulose. Skim milk agar was cleared by all strains except *Cytophaga heparina* and *Cytophaga hutchinsonii* strains. Tween 20 was hydrolyzed by all strains except the *Cytophaga hutchinsonii* strain, and Tween 80 was hydrolyzed by all strains except *Cytophaga flevensis*, *Cytophaga hutchinsonii*, *Cytophaga arvensicola*, and *Cytophaga fermentans* strains. A total of 26 strains did not grow in decarboxylase liquid media, and the 46 other strains gave negative or uninterpretable results.

All strains grew in the liquid media selected for growth at 22 and 25°C. Most strains that grew in the presence of 2% NaCl were able to grow in the presence of 3% NaCl; exceptions were *Cytophaga aquatilis*, *Cytophaga arvensicola*, *Cytophaga johnsonae* DSM 2064^T, *Flavobacterium meningosepticum*, and *Flavobacterium multivorum*.

Antibiotic susceptibility was highly variable among the strains, but all strains were resistant to gentamicin and polymyxin B (no inhibition around disks).

TABLE 3. Differential characteristics of *Cytophaga*, *Flexibacter* and *Flavobacterium* species

Species and/or strain[a]	Test results																				
	Flexirubin type pigments	Congo red absorption	Catalase production	Nitrate reduction	H_2S production	ONPG test[b]	Carboxymethyl cellulose hydrolysis	Chitin hydrolysis	Starch hydrolysis	Agar hydrolysis	Gelatin hydrolysis	Tyrosine hydrolysis	Pigment on tyrosine agar	Precipitate on egg yolk agar	Growth in Trypticase soy broth	Growth at 15°C	Growth at 37°C	Growth with 0% NaCl	Growth with 0.5% NaCl	Growth with 1% NaCl	Growth with 2% NaCl
Flexibacter columnaris (8 strains)	+[c]	+	+	+	+	−	−	−	−	−	+	−	d	+	−	d	d	+	+	−	−
Flexibacter psychrophilus (7 strains)	+	−	(+)	−	−	−	−	−	−	−	+	+	−	+	−	+	−	+	+	−	−
Flexibacter maritimus (3 strains)	−	+	+	+	−	−	−	−	−	−	+	+	+	+	−	(+)	−	NA	NA	NA	NA
Cytophaga aquatilis DSM 2063^T	+	−	+	+	(+)	+	−	+	+	−	+	(+)	−	−	+	+	−	+	+	+	+
Cytophaga arvensicola JCM 2836^T	+	−	(+)	(+)	−	+	+	−	(+)	−	+	+	−	−	+	+	+	+	+	+	(+)
Cytophaga fermentans NCMB 2218^T	−	−	−	−	−	+	+	−	+	+	+	−	−	−	NT	+	−	−	−	(+)	+
Cytophaga flevensis DSM 1076^T	−	−	+	−	−	+	−	−	+	+	−	−	−	−	+	−	−	+	+	+	−
Cytophaga heparina NCIB 9290^T	−	−	+	+	−	+	−	−	−	−	−	+	−	−	+	+	(+)	+	+	+	−
Cytophaga hutchinsonii NCIB 9469^T	+	−	(+)	−	−	−	+	−	+	−	−	−	−	−	NT	+	−	+	+	+	−
Cytophaga johnsonae DSM 2064^T	−	−	+	−	−	+	+	+	+	−	+	+	−	−	+	(+)	−	+	+	+	(+)
Cytophaga johnsonae ATCC 29585	+	−	+	+	−	+	+	+	+	−	+	+	−	(+)	+	+	−	+	+	+	−
Cytophaga johnsonae ATCC 29586	+	−	+	+	−	+	+	+	+	−	+	+	(+)	(+)	+	+	−	+	+	+	−
Cytophaga latercula NCMB 1399^T	+	?[d]	−	+	+	−	+	−	+	+	+	+	+	−	NT	+	−	−	−	+	+
Cytophaga lytica NCMB 1423^T	−	−	+	−	−	+	+	−	+	+	−	+	+	−	NT	+	+	−	−	+	+
Cytophaga salmonicolor NCMB 2216^T	−	−	+	−	+	+	+	−	+	−	+	−	−	−	NT	(+)	+	−	−	+	+
"*Cytophaga allerginae*" ATCC 35408	+	−	+	−	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−
Flexibacter aggregans NCMB 1443^T	−	−	−	−	+	+	+	−	+	+	−	+	+	−	NT	−	+	−	−	+	+
Flexibacter aurantiacus NCMB 1382^T	+	−	+	−	−	+	+	+	+	−	+	+	−	−	+	+	(+)	+	+	+	−
Flexibacter aurantiacus NCMB 1455	+	−	+	−	−	+	+	+	+	−	+	+	−	−	+	+	(+)	+	+	+	−
Flexibacter canadensis ATCC 29591^T	−	−	+	+	−	+	+	−	+	−	+	+	−	−	+	−	+	+	+	(+)	−
Flexibacter flexilis NCMB 1377^T	−	−	−	−	−	−	−	−	+	−	(+)	+	(+)	−	−	−	−	+	−	−	−
Flexibacter litoralis NCMB 1366^T	−	−	−	−	+	−	−	−	−	−	+	+	(+)	+	NT	(+)	−	−	−	−	+
Flexibacter polymorphus ATCC 27820^T	−	−	−	+	−	+	−	−	+	(+)	(+)	+	−	+	NT	−	−	−	−	−	+
Flexibacter roseolus NCMB 1433^T	−	−	−	−	−	−	−	−	−	−	(+)	+	−	+	+	−	+	+	+	+	(+)
Flexibacter ruber NCMB 1436^T	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	+	+	+	+	(+)
Flexibacter sancti NCMB 1379^T	+	+	+	−	−	+	+	+	+	−	+	−	+	+	(+)	(+)	+	+	+	(+)	−
Flexibacter tractuosus NCMB 1408^T	−	−	(+)	−	+	−	−	−	+	−	+	+	+	+	NT	−	+	(+)	+	+	+
Flavobacterium aquatile NCIB 8694^T	−	−	(+)	+	(+)	+	−	−	+	−	+	+	−	+	+	+	+	+	−	−	−
Flavobacterium balustinum LA 724^T	+	−	+	+	+	−	−	−	(+)	−	+	+	+	+	+	+	−	+	+	+	+
Flavobacterium breve NCTC 11099^T	+	−	+	−	−	−	−	−	+	−	+	+	+	+	+	(+)	+	+	+	+	+
Flavobacterium meningosepticum NCTC 10016^T	−	−	+	−	−	+	−	−	−	−	+	(+)	(+)	−	+	(+)	+	+	+	+	+
Flavobacterium odoratum NCTC 11036^T	+	−	+	−	−	−	−	−	−	−	+	+	+	−	+	−	−	+	+	+	(+)
Flavobacterium multivorum NCTC 11343^T	−	−	(+)	(+)	(+)	+	−	−	+	−	−	(+)	(+)	−	+	(+)	+	+	+	+	(+)
Flavobacterium spiritivorum NCTC 11386^T	−	−	+	−	−	+	−	−	−	−	−	+	(+)	−	+	(+)	+	+	+	+	(+)

[a] Abbreviations are explained in Table 1, footnote *a*.
[b] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[c] +, All strains positive; −, all strains negative; (+), weakly positive; d, different reactions; NT, not tested; NA, not applicable (no growth in Anacker-Ordal broth supplemented with only NaCl).
[d] Undetermined due to the red coloration of colonies.

The following species did not produce acid from any carbohydrate (API 50CH galleries): "*Flexibacter columnaris*" (eight strains), "*Cytophaga psychrophila*" (seven strains), *Flexibacter maritimus* (three strains), *Flexibacter roseolus*, *Flexibacter ruber*, *Flexibacter litoralis*, and *Flexibacter polymorphus*. All of the other species tested produced acid from 4 to 34 carbohydrates in API 50CH galleries.

Other phenotypic properties which varied among the strains are shown in Tables 3 and 4.

Phenotypic characteristics of "*Flexibacter columnaris*." The properties that were common to the eight strains of "*Flexibacter columnaris*" are given below. The following properties varied among the strains: the aspect of the colonies on agar was more or less rhizoid; strains NCMB 1038, Farkas H82/7, Holt DD3, Holt IC8, TG 39/87, and TG 44/87 produced a brown color on tyrosine agar (probably due to an oxidized derivative of tyrosine); strains NCMB 1038, Holt DD3, and Wakabayashi EK28 were susceptible to sulfonamides; strains NCMB 1038, NCMB 2248^T, Wakabayashi EK28, TG 39/87, and TG 44/87 hydrolyzed *N*-benzoyl-DL-arginine-2-naphthylamide and *N*-glutaryl-phenylalanine-2-naphthylamide in API ZYM galleries; and strains NCMB 1038 and NCMB 2248^T did not grow at temperatures above 33°C, and four other strains grew scantily at 35 and 37°C.

Phenotypic characteristics of "*Cytophaga psychrophila*."

TABLE 4. Enzymatic patterns of *Cytophaga*, *Flexibacter* and *Flavobacterium* species in API ZYM galleries

Species and/or strain[a]	Hydrolysis of the following substrates[b]:																		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Flexibacter columnaris (8 strains)	5[c]	2	3	0	4	4	1	D[d]	D[d]	3	3	0	0	0	0	0	0	0	0
Flexibacter psychrophilus (7 strains)	5	2	3	1	5	1	0	0	0	3	3	0	0	0	0	0	0	0	0
Flexibacter maritimus (3 strains)	5	3	4	1	5	5	3	1	2	5	5	0	0	0	0	0	0	0	0
Cytophaga aquatilis DSM 2063^T	5	2	4	1	4	5	2	0	0	4	5	0	0	0	4	0	2	0	0
Cytophaga arvensicola JCM 2836^T	5	2	3	1	5	5	2	4	1	4	4	3	4	0	4	3	5	2	3
Cytophaga fermentans NCMB 2218^T	5	1	3	0	5	2	0	2	0	5	5	0	5	1	2	5	5	0	0
Cytophaga flevensis DSM 1076^T	5	1	2	1	5	1	1	0	0	3	3	1	5	0	3	0	4	0	0
Cytophaga heparina NCIB 9290^T	5	2	4	0	4	2	1	0	0	4	2	0	0	0	1	0	4	1	0
Cytophaga hutchinsonii NCIB 9469^T	4	2	4	1	4	4	3	0	0	3	2	0	0	0	0	0	0	0	0
Cytophaga johnsonae DSM 2064^T	5	1	3	1	5	5	2	1	1	4	5	0	3	0	4	1	4	0	0
Cytophaga johnsonae ATCC 29585	5	3	4	1	4	5	3	1	2	5	4	5	5	1	5	2	5	0	0
Cytophaga johnsonae ATCC 29586	5	2	3	1	5	5	3	1	2	5	4	3	3	1	5	2	5	0	0
Cytophaga latercula NCMB 1399^T	5	2	4	1	5	5	3	5	5	4	4	0	0	0	0	0	0	0	0
Cytophaga lytica NCMB 1423^T	5	3	4	1	5	4	2	3	1	5	5	1	1	0	3	2	5	2	0
Cytophaga salmonicolor NCMB 2216^T	5	1	2	0	0	0	0	0	4	2	1	3	0	1	5	5	5	0	0
"*Cytophaga allerginae*" ATCC 35408	5	2	4	1	5	5	2	1	3	5	5	3	3	2	5	5	4	0	0
Flexibacter aggregans NCMB 1443^T	5	3	4	1	5	4	3	3	0	5	5	1	3	0	4	2	5	3	3
Flexibacter aurantiacus NCMB 1382^T	5	2	4	1	5	5	3	0	0	5	3	0	1	0	5	3	3	0	0
Flexibacter aurantiacus NCMB 1455	5	2	4	1	5	5	3	0	0	5	4	0	0	0	5	3	4	0	0
Flexibacter canadensis ATCC 29591^T	5	2	3	1	5	5	3	4	1	5	5	4	1	0	5	3	4	0	3
Flexibacter flexilis NCMB 1377^T	5	3	4	0	5	4	3	1	2	3	1	0	0	0	3	0	0	0	0
Flexibacter litoralis NCMB 1366^T	5	2	4	1	5	5	3	5	1	5	5	0	0	0	0	0	0	0	0
Flexibacter polymorphus ATCC 27820^T	5	2	3	1	5	5	3	4	1	2	2	0	0	0	2	0	0	0	0
Flexibacter roseolus NCMB 1433^T	4	2	3	1	3	3	2	1	3	2	2	0	0	0	0	0	0	0	0
Flexibacter ruber NCMB 1436^T	5	3	4	2	3	3	2	1	3	3	3	0	0	0	0	0	0	0	0
Flexibacter sancti NCMB 1379^T	5	0	2	0	4	1	0	4	0	4	5	4	4	0	4	3	4	0	2
Flexibacter tractuosus NCMB 1408^T	5	2	3	1	5	4	3	1	4	5	4	0	0	0	5	1	0	0	0
Flavobacterium aquatile NCIB 8694^T	4	2	4	1	5	5	2	0	0	1	2	0	0	0	5	0	0	0	0
Flavobacterium balustinum LA 724^T	5	1	2	2	5	5	3	2	1	5	5	0	0	0	3	3	2	0	0
Flavobacterium breve NCTC 11099^T	5	3	4	0	5	5	3	3	2	5	5	0	0	0	4	0	0	0	0
Flavobacterium meningosepticum NCTC 10016^T	5	4	4	3	5	5	5	3	4	4	5	3	0	0	4	2	5	2	2
Flavobacterium odoratum NCTC 11036^T	5	2	3	0	2	0	0	1	0	5	5	0	0	0	0	0	0	0	0
Flavobacterium multivorum NCTC 11343^T	5	4	4	1	5	1	1	0	0	5	5	2	4	0	4	3	5	3	0
Flavobacterium spiritivorum NCTC 11386^T	5	2	4	0	5	1	1	5	2	5	5	2	4	1	3	2	5	3	4

[a] Abbreviations are explained in Table 1, footnote *a*.

[b] A, 2-Naphthyl-phosphate; B, 2-naphtyl-butyrate; C, 2-naphthyl-caprylate; D, 2-naphthyl-myristate; E, L-leucyl-2-naphthylamide; F, L-valyl-2-naphthylamide; G, L-cystyl-2-naphthylamide; H, *N*-benzoyl-DL-arginine-2-naphthylamide; I, *N*-glutaryl-phenylalanine-2-naphthylamide; J, 2-naphthyl-phosphate; K, naphthol-AS-BI-phosphate; L, 6-Br-2-naphthyl-α-D-galactopyranoside; M, 2-naphthyl-β-D-galactopyranoside; N, naphthol-AS-BI-β-D-glucuronide; O, 2-naphthyl-α-D-glucopyranoside; P, 6-Br-2-naphthyl-β-D-glucopyranoside; Q, 1-naphthyl-*N*-acetyl-β-D-glucosaminide; R, 6-Br-2-naphthyl-α-D-mannopyranoside; S, 2-naphthyl-α-L-fucopyranoside.

[c] The values 0 to 5 are API reaction scores.

[d] D, Different reactions. Strains NCMB 1038, NCMB 2248^T, Wakabayashi EK28, TG 39/87, and TG 44/87 gave positive results for substrates H (score 3) and I (score 1 or 2); other strains gave negative results for both of these substrates.

The properties that were common to the seven strains of "*Cytophaga psychrophila*" are given below. The following properties varied among the strains: all strains except strain NCMB 1947^T produced both compact colonies with regular edges and more or less spreading colonies with uneven margins (strain NCMB 1947^T produced only compact colonies); strain TG 28/86 was resistant to kanamycin; strains TG 02/86, LNPAA P01/88, and LNPAA P03/88 were resistant to nalidixic acid; strains TG 28/86 and TG P02/88 were resistant to actinomycin D; and strains TG 02/86, TG 28/86, LNPAA P01/88, TG P02/88, and LNPAA P03/88 were resistant to sulfonamides.

Phenotypic characteristics of *Flexibacter maritimus*. All of the properties studied were found to be identical among the three *Flexibacter maritimus* strains and in agreement with the results of Wakabayashi et al. (32). Some additional characteristics are given below.

DISCUSSION

Although the epithet *columnaris* has been associated with a fish-pathogenic gliding bacterium since 1922 (9) and has been used in various combinations, including "*Bacillus columnaris*" (9), "*Chondrococcus columnaris*" (21), "*Cytophaga columnaris*" (11), and "*Flexibacter columnaris*" (16), by fish pathologists for 66 years, no name with this epithet was included on the Approved Lists (30), and no such name has been validated by announcement or publication in the International Journal of Systematic Bacteriology. In this study we found that six strains labeled "*Flexibacter columnaris*" and two unlabeled isolates constitute a genomic species which can be identified by phenotypic properties. This taxon should be recognized as a species (33), which we call provisionally "species *columnaris*."

The epithet *psychrophila*, for an organism belonging to the *Cytophaga-Flexibacter-Flavobacterium* complex, has been associated with a fish-pathogenic gliding bacterium since 1960 (5) and has been used in the combinations "*Cytophaga psychrophila*" (5) and "*Flexibacter psychrophila*" (sic) (27, 28) by fish pathologists for 28 years; this organism has been remarkably characterized by Pacha (23) and by Holt (M.Sc. and Ph.D. theses). No name with this epithet was included on the Approved Lists (30), and no such name has been

validated since 1980. In this study we showed that two strains labeled "*Cytophaga psychrophila*" and five other unlabeled isolates constitute a genomic species which can be identified by phenotypic properties. This organism should be recognized as a species (33), which we call provisionally "species *psychrophila*."

Strain NCMB 1455 does not belong in species *psychrophila*. Many phenotypic characteristics of this organism differ from those of the species, and strain NCMB 1455 is only 5% related to strain NCMB 1947^T. Strain NCMB 1455 was received from E. J. Ordal as "*Cytophaga psychrophila*" by Lewin and Lounsbery (18), who classified it as *Flexibacter aurantiacus*. Results of our DNA study confirmed the opinion of Holt (Ph.D. thesis) that strain NCMB 1455 is not a member of "*Cytophaga psychrophila*," as demonstrated by serological and biochemical tests.

The name *Flexibacter maritimus* has been validly published (32). In this study we found that this taxon constitutes a genomic species which can be identified by phenotypic properties, thus justifying the proposal of Wakabayashi et al. (32).

One of the major problems in the taxonomy of the *Cytophaga-Flexibacter-Flavobacterium* phylogenetic branch (26, 29) is the delineation of genera. The ribosomal ribonucleic acid sequence data which have been published are of no help in the delineation of the genus *Flexibacter* since the only species of that genus which has been studied is an invalid one, "*Flexibacter elegans*" (24).

The three genomic species which we studied (species *columnaris*, species *psychrophila*, and *Flexibacter maritimus*) are composed of gliding bacteria and thus cannot belong in the genus *Flavobacterium* (14). Their G+C contents are 32 to 33 mol%, which excludes them from the genus *Lysobacter* (G+C content, around 65 mol%) (26). Strict aerobic metabolism is incompatible with inclusion in the genus *Capnocytophaga* (26). Lack of microcyst production and cellulose digestion is incompatible with inclusion in the genus *Sporocytophaga* (16). Species *columnaris* and species *psychrophila* are neither marine nor halophilic and thus cannot belong in the genus *Microscilla* (26). Thus, the three genotypic groups which we studied can belong only in the genus *Cytophaga* or the genus *Flexibacter*.

Following the opinion of several authors, Leadbetter (16) proposed that the genera *Cytophaga* and *Flexibacter* should be differentiated on the basis of polysaccharide degradation (polysaccharides are degraded by *Cytophaga* species and not by *Flexibacter* species). Accordingly, the three genomic groups which we studied belong in the genus *Flexibacter*. Reichenbach and Dworkin (26) have cast doubt on such differentiation. Instead, these authors propose to give more weight to morphological characteristics (members of the genus *Cytophaga* have short to moderately long rod-shaped cells, some longer nonmotile threads appearing only occasionally in old cultures, whereas *Flexibacter* species have very long and agile threadlike cells, becoming very short in aging cultures) and to the G+C contents of the DNAs (*Cytophaga* spp., 30 to 35 mol%; *Flexibacter* spp., around 48 mol%). According to these criteria, our three genomic groups do not belong in the genus *Flexibacter*. However, since cell fragmentation in the late stationary phase is not restricted to the members of the order *Cytophagales* with high G+C contents and is highly dependent on the composition of the medium (15), and since the type species of the genera *Cytophaga* and *Flexibacter* have insignificantly different G+C contents (*Cytophaga hutchinsonii*, 39 to 40 mol% [6, 15]; *Flexibacter flexilis*, 39 to 44 mol% [19; H. Behrens, Ph.D. thesis, Technical University Braunschweig, Braunschweig, Federal Republic of Germany, 1978]), these arguments are weakened.

It is obvious that more work is needed to define the genera in the *Cytophaga-Flexibacter-Flavobacterium* phylogenetic branch. At the present state of knowledge, we propose that species *columnaris* and species *psychrophila* should be included in the genus *Flexibacter*, as *Flexibacter columnaris* and *Flexibacter psychrophilus*, pending further reorganization of the whole phylogenetic branch. Nine years have elapsed since the publication of the Approved Lists, and *Flexibacter columnaris* and *Flexibacter psychrophilus* (in these or other combinations) have not yet been validated. Such validation is formally proposed below.

Description of *Flexibacter columnaris* sp. nov., nom. rev. (ex Leadbetter 1974). *Flexibacter columnaris* (basionym, "*Bacillus columnaris*" Davis 1922) (co.lum.nar'is. L. adj. *columnaris*, rising as a pillar). The description below is based on eight strains and also includes data collected by Leadbetter (16) and Becker and Fujihara (3). Cells are nonsporulating, nonmotile, gram-negative rods that are 3 to 10 μm long by 0.3 to 0.5 μm wide in 48-h liquid cultures; some longer cells (15 to 25 μm) may occur. No formation of microcysts. In older cultures, spherical degenerative forms (spheroplasts) may appear. A clear gliding movement, as well as bending and pivoting, occur in hanging-drop preparations. Grows in Anacker-Ordal broth (2) supplemented with 0 or 0.5% NaCl and at 10 to 33°C; some strains tolerate 37°C, but the optimum temperature is 20 to 25°C. No growth in Trypticase soy broth. Colonies on Anacker-Ordal agar are flat, thin, spreading, greenish yellow, more or less rhizoid, and adherent to the agar. In shaken liquid cultures, numerous filamentous tufts of bacteria adhere to the glass. Nondiffusible flexirubin type pigments are present. Congo red is absorbed by colonies. Strictly aerobic. Catalase and cytochrome oxidase are produced. Nitrate is reduced to nitrite; nitrite is not reduced. Hydrogen sulfide is produced. *o*-Nitrophenyl-β-D-galactopyranoside is not hydrolyzed. Cellulose, carboxymethyl cellulose, chitin, starch, esculin, and agar are not hydrolyzed. No acid is produced from carbohydrates in ammonium salt-sugar medium (API 50CH galleries). Gelatin, casein (skim milk agar), and tyrosine are hydrolyzed. Lysine, arginine, and ornithine are not decarboxylated. Tributyrin, lecithin (egg yolk), Tween 20, and Tween 80 are hydrolyzed. Rapid and intense DNA hydrolysis. No inhibition zone is formed around the following types of disks (the concentration in each type of disk is indicated in parentheses): gentamicin (15 μg), neomycin (30 μg), kanamycin (30 μg), polymyxin B (30 μg), trimethoprim (5 μg), and actinomycin D (2.5 μg). Susceptible (inhibition zone, more than 9 mm) to vibriostatic compound O/129 (500 μg), ampicillin (10 μg), cephalothin (30 μg), streptomycin (10 IU), tetracycline (30 IU), chloramphenicol (30 μg), erythromycin (15 IU), novobiocin (30 IU), nalidixic acid (30 μg), and furans (300 μg). Hydrolyzes the following substrates (API ZYM galleries): 2-naphthyl-phosphate (at pH 8.5 and 5.4), 2-naphthyl-butyrate, 2-naphthyl-caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, and naphthol-AS-BI-phosphate. Does not hydrolyze the following substrates (API ZYM galleries): 2-naphthyl-myristate, L-cystyl-2-naphthylamide, 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, and 2-naphthyl-α-L-fucopyranoside.

The base composition of the DNA is 32 to 33 mol% G+C (three strains). All strains were isolated from freshwater fish suffering from columnaris disease in North America, Europe, and Japan.

The type strain is strain NCMB 2248 (= ATCC 23463).

Description of the type strain. Strain NCMB 2248^T has all of the characteristics given above for the species. In addition, it produces very rhizoid and strongly adherent colonies on Anacker-Ordal agar, but some less rhizoid and more spreading colonies may appear. Does not produce a brown color on tyrosine agar. Resistant to sulfonamides (200-μg disks). Hydrolyzes *N*-benzoyl-DL-arginine-2-naphthylamide and *N*-glutaryl-phenylalanine-2-naphthylamide in API ZYM galleries. No growth occurs at temperatures above 33°C; weak growth occurs at 15°C. The base composition of the type strain is 32 mol% G+C.

Description of *Flexibacter psychrophilus* sp. nov., nom. rev. *Flexibacter psychrophilus* (basionym, "*Cytophaga psychrophila*" ex Borg 1960) (psy.chro'phil.us Gr. adj. *psychros*, cold; Gr. adj. *philos*, loving; M.L. adj. *psychrophilus*, cold loving). The description below is based on seven strains and also includes data from Borg (5), Pacha (23), and Holt (M.Sc. and Ph.D. theses). Cells are nonsporulating, nonmotile, gram-negative rods that are 1 to 5 μm long by 0.3 to 0.5 μm wide in 48-h liquid cultures; a few longer cells (8 to 12 μm) may appear. Some cells have an enlarged end. In hanging-drop preparations, the gliding movement is frequently slow and weak and is noticed after prolonged observation. Some strains exhibit faster gliding. Good growth occurs in Anacker-Ordal broth (2) supplemented with 0 or 0.5% NaCl and at 10 to 20°C. The optimum temperature is 15 to 18°C, and scant, slow growth occurs at 6 and 22 to 25°C. No growth occurs in Trypticase soy broth. Growth on Anacker-Ordal agar is enhanced by an enriched formula (0.5% tryptone instead of 0.05% tryptone). Most colonies are smooth, glossy, and circular with regular edges, but most strains can also produce colonies with narrow and uneven spreading margins. The colonies are bright yellow and do not adhere to the agar. Nondiffusible flexirubin type pigments are present. Colonies do not absorb Congo red. Strictly aerobic. Catalase and cytochrome oxidase are produced. Nitrate is not reduced, and hydrogen sulfide is not produced. *o*-Nitrophenyl-β-D-galactopyranoside is not hydrolyzed. Cellulose, carboxymethyl cellulose, chitin, starch, esculin, and agar are not hydrolyzed. No acid is produced from carbohydrates in ammonium salt-sugar medium (API 50CH galleries). Gelatin, casein (skim milk agar), and tyrosine are hydrolyzed. No brown color is produced on tyrosine agar. Lysine, arginine, and ornithine are not decarboxylated. Tributyrin, lecithin (egg yolk), Tween 20, and Tween 80 are hydrolyzed. Weak, slow DNA hydrolysis. No inhibition zone is formed around the following types of disks (the concentration in each type of disk is indicated in parentheses): gentamicin (15 μg), neomycin (30 μg), polymyxin B (30 μg), and trimethoprim (5 μg). Susceptible (inhibition zone, more than 9 mm) to vibriostatic compound O/129 (500 μg), ampicillin (10 μg), cephalothin (30 μg), streptomycin (10 IU), tetracycline (30 IU), chloramphenicol (30 μg), erythromycin (15 IU), novobiocin (30 IU), and furans (300 μg). Hydrolyzes the following substrates (API ZYM galleries): 2-naphthyl-phosphate (at pH 8.5 and 5.4), 2-naphthyl-butyrate, 2-naphthyl-caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, and naphthol-AS-BI-phosphate. Does not hydrolyze the following substrates (API ZYM galleries): 2-naphthyl-myristate, L-cystyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, 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, and 2-naphthyl-α-L-fucopyranoside.

The base composition is 32.5 to 34 mol% G+C (three strains).

All strains were isolated from freshwater fish suffering from cold-water disease in North America and France.

The type strain is strain NCMB 1947.

Description of the type strain. Strain NCMB 1947^T has all of the characteristics given above for the species. In addition, it produces round colonies with regular edges. Colonies with narrow spreading margins are sometimes produced. The gliding movement is very slow. Susceptible (inhibition zone, more than 9 mm) to kanamycin (30 μg), sulfonamides (200 μg), nalidixic acid (30 μg), and actinomycin D (2.5 μg). The base composition of the type strain is 32.5 mol% G+C.

Addendum to the description of *Flexibacter maritimus*. In addition to the original description (32), the characteristics given below may be useful in identification. *o*-Nitrophenyl-β-D-galactopyranoside is not hydrolyzed. Agar is not hydrolyzed. No acid is produced from carbohydrates in ammonium salt-sugar medium (API 50CH galleries). A brown color is produced on tyrosine agar. Lecithin (egg yolk), Tween 20, and Tween 80 are hydrolyzed. Weak, slow DNA hydrolysis. No inhibition zone is formed around the following types of disks (the concentration in each type of disk is indicated in parentheses): gentamicin (15 μg), neomycin (30 μg), kanamycin (30 μg), streptomycin (10 IU), polymyxin B (30 μg), actinomycin D (2.5 μg), and nalidixic acid (30 μg). Susceptible (inhibition zone, more than 9 mm) to vibriostatic compound O/129, ampicillin (10 μg), cephalothin (30 μg), tetracycline (30 IU), chloramphenicol (30 μg), erythromycin (15 IU), novobiocin (30 IU), sulfonamides (200 μg), trimethoprim (5 μg), and nitrofurans (300 μg). The following substrates are hydrolyzed (API ZYM galleries): 2-naphthyl-phosphate (at pH 8.5 and 5.4), 2-naphthyl-butyrate, 2-naphthyl-caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, and naphthol-AS-BI-phosphate. Does not hydrolyze the following substrates (API ZYM galleries): 2-naphthyl-myristate, 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, and 2-naphthyl-α-L-fucopyranoside.

ACKNOWLEDGMENTS

We thank R. A. Holt (Oregon State University, Corvallis), H. Wakabayashi (Faculty of Agriculture, Tokyo, Japan), J. Farkas (Fisheries Research Institute, Szarvas, Hungary), and F. Baudin-Laurencin (Laboratoire National de Pathologie des Animaux Aquatiques, Brest, France) for supplying several strains included in this study. J.F.B. thanks L. Le Minor for hospitality in the Unité des Entérobactéries (Institut Pasteur, Paris). We acknowledge the excellent technical assistance of E. Ageron and B. Kerouault. We also thank M. C. Le Cochennec for typing the manuscript.

This work was supported by grant 88003 from the Conseil Supérieur de la Pêche.

LITERATURE CITED

1. **Amend, D. F.** 1982. Columnaris (*Flexibacter columnaris*) disease of freshwater fishes and a brief review of other flexibacte-

rial diseases of fish, p. 139–151. *In* D. P. Anderson, M. Dorson, and P. Dubourget (ed.), Antigens of fish pathogens. Symposium International de Talloires. Collection Fondation Marcel Mérieux, Lyon.
2. **Anacker, R. L., and E. J. Ordal.** 1955. Study of a bacteriophage infecting the myxobacterium *Chondrococcus columnaris*. J. Bacteriol. **70**:738–741.
3. **Becker, C. D., and M. P. Fujihara.** 1978. The bacterial pathogen *Flexibacter columnaris* and its epizootiology among Columbia River fish. A review and synthesis. Monograph no. 2. American Fisheries Society, Washington, D.C.
4. **Bernardet, J. F.** 1989. "*Flexibacter columnaris*": first description in France and comparison with bacterial strains from other origins. Dis. Aquat. Org. **6**:37–44.
5. **Borg, A. F.** 1960. Studies on myxobacteria associated with diseases in salmonid fishes. J. Wildl. Dis. **8**:1–85. (Two microcards.)
6. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15**:1133–1140.
7. **Callies, E., and W. Mannheim.** 1980. Deoxyribonucleic acid relatedness of some menaquinone-producing *Flavobacterium* and *Cytophaga* strains. Antonie van Leeuwenhoek J. Microbiol. Serol. **46**:41–49.
8. **Campbell, A. C., and J. A. Buswell.** 1982. An investigation into the bacterial aetiology of "black patch necrosis" in Dover sole (*Solea solea* L.). J. Fish Dis. **5**:495–508.
9. **Davis, H. S.** 1922. A new bacterial disease of fresh-water fishes. Bull. U.S. Bur. Fish. **38**:261–280.
10. **Davis, H. S.** 1946. Care and diseases of trout. Research report no. 12. U.S. Fish and Wildlife Service, Washington, D.C.
11. **Garnjobst, L.** 1945. *Cytophaga columnaris* (Davis) in pure culture: a myxobacterium pathogenic to fish. J. Bacteriol. **49**:113–128.
12. **Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Lemelin.** 1980. Reproducibility and correlation study of three deoxyribonucleic acid hydridization procedures. Curr. Microbiol. **4**:325–330.
13. **Holmes, B.** 1987. International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Flavobacterium* and *Cytophage*-like Bacteria minutes of the meeting, 10 September 1986, Manchester, England. Int. J. Syst. Bacteriol. **37**:301.
14. **Holmes, B., R. J. Owen, and T. A. McMeekin.** 1984. Genus *Flavobacterium*, p. 353–360. *In* N. R. Krieg (ed.), Bergeys' manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
15. **Humphrey, B. A., and K. C. Marshall.** 1980. Fragmentation of some gliding bacteria during the growth cycle. J. Appl. Bacteriol. **49**:281–289.
16. **Leadbetter, E. R.** 1974. Order II. *Cytophagales* nomen novum, p. 99. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
17. **Lewin, R. A.** 1974. *Flexibacter polymorphus*, a new marine species. J. Gen. Microbiol. **82**:393–403.
18. **Lewin, R. A., and D. M. Lounsbery.** 1969. Isolation, cultivation, and characterization of flexibacteria. J. Gen. Microbiol. **58**: 145–170.
19. **Mandel, M., and R. A. Lewin.** 1969. Deoxyribonucleic acid base composition of flexibacteria. J. Gen. Microbiol. **58**:171–178.
20. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
21. **Ordal, E. J., and R. R. Rucker.** 1944. Pathogenic myxobacteria. Proc. Soc. Exp. Biol. Med. **56**:15–18.
22. **Owen, R. J., L. R. Hill, and S. P. Lapage.** 1969. Determination of DNA base compositions from melting profiles in dilute buffers. Biopolymers **7**:503–516.
23. **Pacha, R. E.** 1968. Characteristics of *Cytophaga psychrophila* (Borg) isolated during outbreaks of bacterial coldwater disease. Appl. Microbiol. **16**:97–101.
24. **Paster, B. J., W. Ludwig, W. G. Weisburg, E. Stackebrandt, R. B. Hespell, C. M. Hahn, H. Reichenbach, K. O. Stetter, and C. R. Woese.** 1985. A phylogenetic grouping of the *Bacteroides*, cytophagas, and certain flavobacteria. Syst. Appl. Microbiol. **6**:34–42.
25. **Reichenbach, H., and M. Dworkin.** 1981. Introduction to the gliding bacteria, p. 315–327. *In* M. P. Starr et al. (ed.), The prokaryotes, vol. 1. Springer-Verlag, New York.
26. **Reichenbach, H., and M. Dworkin.** 1981. The order *Cytophagales* (with addenda on the genera *Herpetosiphon*, *Saprospira*, and *Flexithrix*), p. 356–379. *In* M. P. Starr et al. (ed.), The prokaryotes, vol. 1. Springer-Verlag, New York.
27. **Richards, R. H., and R. J. Roberts.** 1978. The bacteriology of teleosts, p. 183–204. *In* R. J. Robers (ed.), Fish pathology. Bailliere Tindall, London.
28. **Schneider, R., and B. L. Nicholson.** 1980. Bacteria associated with fin rot disease in hatchery-reared Atlantic Salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **37**:1505–1513.
29. **Shewan, J. M., and T. A. McMeekin.** 1983. Taxonomy (and ecology) of *Flavobacterium* and related genera. Annu. Rev. Microbiol. **37**:233–252.
30. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath. (ed.).** 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30**:225–420.
31. **Wakabayashi, H., M. Hikida, and K. Masumura.** 1984. *Flexibacter* infection in cultured marine fish in Japan. Helgol. Meeresunters. **37**:587–593.
32. **Wakabayashi, H., M. Hikida, and K. Masumura.** 1986. *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. Int. J. Syst. Bacteriol. **36**:396–398.
33. **Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper.** 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int. J. Syst. Bacteriol. **37**:463–464.