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Sphingobacterium gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: Glucose-Nonfermenting Gram-Negative Rods in CDC Groups IIK-2 and IIb

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A new genus, *Sphingobacterium*, two new combinations, *Sphingobacterium spiritivorum* (Holmes, Owen, and Hollis) and *Sphingobacterium multivorum* (Holmes, Owen, and Weaver), and two new species, *Sphingobacterium mizutae* and *Flavobacterium indologenes*, are described. The genus *Sphingobacterium* is characterized and distinguished from the genus *Flavobacterium* by the presence in its strains of high concentrations of sphingophospholipids as cellular lipid components. *S. spiritivorum* is designated the type species of the genus *Sphingobacterium*. *S. mizutae* ATCC 33299 (= GIFU 1203) and *F. indologenes* ATCC 29893 (= CDC 3716 = GIFU 1347 = NCTC 10796 = RH 542) are designated the type strains of the two new species. Emended descriptions of the type strains of *S. spiritivorum* and *S. multivorum* are presented.

During the course of a cellular lipid analysis of glucose-nonfermenting gram-negative rods, Yano et al. (I. Yano et al., Proc. Jpn. Conf. Biochem. Lipids **19**:19–22, 1977) found that the cells of six strains of *Pseudomonas*-like group IIk-2 (28) contained unique sphingophospholipids. The major ceramide species of these compounds was identified as *N*-2-hydroxy-13-methyltetradecanoly-15(?)-methylhexadecasphinganine (36). Yabuuchi et al. (34) compared the phenotypic characteristics, cellular lipid compositions, and guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of 12 strains of group IIK-2, 41 strains of five species of *Flavobacterium*, and the type strain of *Cytophaga johnsonae*. On the bases of these results and the presence of the unique sphingophospholipids, *Sphingobacterium* gen. nov. and *Sphingobacterium versatilis* sp. nov. were proposed for group IIK-2 strains, and the species was tentatively divided into three biovars; strain ATCC 33300 (= KM 2138) was designated the type strain of *S. versatilis*. Detailed studies of the three biovars of *S. versatilis* led to recognition of each biovar as a separate species; these species were *S. versatilis* sp. nov., *S. phingobacterium multivorum* (Holmes, Owen, and Weaver) comb. nov. (11, 30), and *Sphingobacterium mizutae* (30).

Before valid publication of these new names, Holmes et al. (10) proposed the new name *Flavobacterium spiritivorum* for a group of organisms which correspond to *S. versatilis*. In this paper we propose, in accordance with the Bacteriological code (1975 Revision), *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* (Holmes, Owen, and Hollis 1982) comb. nov. (type species of the genus), *Sphingobacterium multivorum* (Holmes, Owen, and Weaver 1981) comb. nov., and *Sphingobacterium mizutae* sp. nov.

Because of its frequent isolation from human specimens and from hospital environments and its deep orange pigment, a nonmotile, gram-negative, rod-shaped bacterium is well known among clinical bacteriologists as King group IIb (27). King group IIa is *Flavobacterium meningosepticum* (14). Group IIb has not been assigned nomenclatural status, despite numerous descriptions of its phenotypic and genotypic characteristics (19, 21–23, 25, 28, 32, 35). We compared strains of group IIb with strains of four other *Flavobacterium* species including *Flavobacter-*

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TABLE 1. Histories and designations of the 77 strains studied[a]

Species	GIFU strain no.[b]	Received as:	Other designation(s)	Received from:	Source of isolation
S. spiritivorum	2134	*Flavobacterium* sp. no. 2		Minamide	Vaginal secretion (41 years old)
	2138	*Flavobacterium* sp. no. 6	ATCC 33300	Minamide	Vaginal secretion (46 years old)
	2148	*Flavobacterium* sp. no. 9		Minamide	Vaginal secretion (19 years old)
	2289	*Flavobacterium* sp. no. 28		Kosakai	Sputum
	3101^T	*F. spiritivorum* CDC E7288^T	ATCC 33861^T, NCTC 11386^T, JCM 1277^T	Hollis	Intrauterine specimen
	3102	*F. spiritivorum* CDC D3221		Hollis	Skin trap of ambulance
	3103	*F. spiritivorum* CDC D7211	ATCC 33859	Hollis	Urine
	3104	*F. spiritivorum* CDC E6209	ATCC 33869	Hollis	Bone marrow
S. multivorum	926	Group IIK-2 strain CDC B3159	ATCC 33298, NCTC 11034, RH 2850	Hugh	Blood
	2087	*Xanthomonas campestris* AJ 2779		Komagata	Turnip
	2532	Group IIK-2 strain CDC 8895	NCTC 11033, RH 2851	Hugh	Cavity fluid
	2812^T	*F. multivorum* NCTC 11343^T	ATCC 33613^T	Holmes	Spleen
	2813	Urease-negative group IIK-2 strain T567/79	CDC E3540	Holmes	Blood
S. mizutae	1203^T	*F. meningosepticum* Teraoka^T	ATCC 33299^T	Mizuta	Ventricular fluid
	2055	*Flavobacterium* sp. no. 22		Karino	Clinical specimen
	2120	*F. meningosepticum* no. 3		Minamide	Synovial fluid
	2274	*Flavobacterium* sp. no. 7		Kosakai	Clinical specimen
	2789	*Sphingobacterium* sp.		Yamanaka	Urine
C. johnsonae	2500^T	*C. johnsonae* ATCC 17061^T		ATCC	Not recorded, maybe soil or mud
	2584	*C. johnsonae* ATCC 29583		ATCC	Soil
	2585	*C. johnsonae* ATCC 29584		ATCC	Alfalfa roots
	2586	*C. johnsonae* ATCC 29589		ATCC	Soil
	2587	*C. johnsonae* ATCC 29595		ATCC	Soil
F. indologenes	1347^T	*F. meningosepticum* RH 542^T	ATCC 29897^T, NCTC 10796^T, CDC 3716^T	Hugh	Trachea at autopsy
	1389	*Flavobacterium* sp. no. 2		Oguri	Sputum
	1390	*Flavobacterium* sp. no. 10		Oguri	Urine
	1391	*Flavobacterium* sp. no. 11		Oguri	Urine
	1392	*Flavobacterium* sp. no. 20		Oguri	Pharynx
	1393	*Flavobacterium* sp. no. 179		Oguri	Urine

Continued on next page

TABLE 1—*Continued*

Species	GIFU strain no.[b]	Received as:	Other designation(s)	Received from:	Source of isolation
	1394	*Flavobacterium* sp. no. 182		Oguri	Sputum
	1395	*Flavobacterium* sp. no. 184		Oguri	Pharynx
	1396	*Flavobacterium* sp. no. 186		Oguri	Pharynx
	1397	*Flavobacterium* sp. no. 196		Oguri	Sputum
	1398	*Flavobacterium* sp. no. 198		Oguri	Pus
	1399	*Flavobacterium* sp. no. 207		Oguri	Sputum
	2007	*Flavobacterium* sp. group IIb NCTC 10795	ATCC 29896, CDC 1858	NCTC	Sputum
F. meningosepticum	505	*F. meningosepticum*		Takeda	Spinal fluid (E)[c]
	506[T]	*F. meningosepticum* ATCC 13253[T] (A)[c]		ATCC	Spinal fluid
	507	*F. meningosepticum* ATCC 13254 (B)		ATCC	Blood
	508	*F. meningosepticum* ATCC 13255 (C)		ATCC	Spinal fluid
	515	*F. meningosepticum* CDC 6925 (D)		Weaver	Spinal fluid
	516	*F. meningosepticum* CDC 8388 (E)		Weaver	Blood
	517	*F. meningosepticum* CDC 8708 (F)		Weaver	Spinal fluid
	1113	*F. meningosepticum* Shimizu		Goto	Spinal fluid (C)
	1199	*F. meningosepticum* Otomo		Tomiyama	Spinal fluid (NAG)
	1204	*F. meningosepticum* Hirose		Mizuta	Spinal fluid (E)
	1321	*F. meningosepticum* Narita		Oguri	Spinal fluid (C)
	1346	*F. meningosepticum* RH 541	CDC 3552	Hugh	Spinal fluid (B)
	1606	*F. meningosepticum* C-20		Okuda	Pus (NAG)
	2270	*F. meningosepticum* no. 1		Kosakai	Urine (NAG)
	2271	*F. meningosepticum* no. 2		Kosakai	Urine (NAG)
	2272	*F. meningosepticum* no. 3		Kosakai	Sputum (NAG)
	2276	*Flavobacterium* sp. no. 10		Kosakai	Urine (NAG)
	2277	*Flavobacterium* sp. no. 11		Kosakai	Urine (NAG)
F. odoratum	1357[T]	*F. odoratum* ATCC 4651[T]		RIMD	Not known
	2237	*Flavobacterium* sp.		Kosakai	Clinical specimen
	2278	*Flavobacterium* sp. no. 12		Kosakai	Clinical specimen
	2283	*Flavobacterium* sp. no. 19		Kosakai	Clinical specimen
	2407	*Flavobacterium* sp. no. 80		Kosakai	Clinical specimen
	2884	*F. odoratum* no. 1		Tamagawa	Urine
	2885	*F. odoratum* no. 2		Tamagawa	Urine
	2886	*F. odoratum* no. 3		Tamagawa	Urine
	2887	*F. odoratum* no. 5		Tamagawa	Urine
	2888	*F. odoratum* no. 6		Tamagawa	Urine
	2889	*F. odoratum* no. 7		Tamagawa	Urine

	2890	*F. odoratum* no. 8		Tamagawa	Urine
	2891	*F. odoratum* no. 9		Tamagawa	Urine
	2892	*F. odoratum* no. 11		Tamagawa	Urine
	2893	*F. odoratum* no. 12		Tamagawa	Urine
	2894	*F. odoratum* no. 13		Tamagawa	Urine
	2895	*F. odoratum* no. 14		Tamagawa	Urine
F. balustinum	3158[T]	*F. balustinum* JCM 1278[T]	NCTC 11212[T]	JCM	Halibut
F. breve	2292	*Flavobacterium* sp. no. 35		Kosakai	Clinical specimen
	2299	*Flavobacterium* sp. no. 41		Kosakai	Clinical specimen
	2316	*Flavobacterium* sp. no. 59		Kosakai	Clinical specimen
	2318	*Flavobacterium* sp. no. 63		Kosakai	Clinical specimen
	3159[T]	*F. breve* JCM 1276[T]	NCTC 11099[T]	JCM	Bronchial secretion

[a] ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Disease Control, Atlanta, Ga.; GIFU, Department of Microbiology, Gifu University School of Medicine, Tsukasa-machi, Gifu, Japan; JCM, Japan Collection of Microorganisms, Wako, Saitama, Japan; NCTC, National Collection of Type Cultures, London, England; RH, Rudolph Hugh, George Washington University Medical Center, Washington, D.C.; RIMD, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan.

[b] Before 30 April 1982, strains (numbers below 2896) were designated with the prefix KM instead of GIFU.

[c] The letters in parentheses indicate serogroups. NAG, Not agglutinated by any of the six antisera tested.

ium balustinum Harrison 1929 (8), and found that the group IIb strains constitute a separate species, for which we propose the name *Flavobacterium indologenes* sp. nov.

The type strains of *S. mizutae* and *F. indologenes* are designated.

MATERIALS AND METHODS

Bacterial strains. The 77 strains of nine species examined in this study are listed in Table 1. All strains except the following were isolated from clinical materials: all of the *C. johnsonae* strains, *Flavobacterium odoratum* GIFU 1357[T] (type strain), *F. balustinum* GIFU 3158[T], *S. spiritivorum* GIFU 3102, and *S. multivorum* GIFU 2087. The reference strains used for DNA homology studies included *Cytophaga lytica* GIFU 2490, "*Cytophaga marinoflava*" GIFU 2502, *Cytophaga* sp. strain GIFU 2506, *Legionella pneumophila* GIFU 2522[T] (= ATCC 33152[T]), and *Bacillus cereus* ATCC 14597[T].

Determination of phenotypic characters. Cultures for all tests were incubated aerobically at 30°C unless stated otherwise. To observe spreading growth, semisolid motility medium (12) in a petri dish was inoculated at the center, and the culture was incubated at room temperature. The cellular arrangement at the edge of the spreading growth of a 0.5% agar medium culture on a glass microscope slide was determined by phase-contrast microscopy. Cell size was determined from phase-contrast micrographs.

Indole production was determined in 1% tryptone broth supplemented with 0.5% L-tryptophan (29). After 20 h of incubation, indole was detected by a distinct red color after Kovacs reagent was added. Filter paper impregnated with a 5.5% aqueous solution of lead acetate was air dried, cut into strips (5 by 50 mm), and autoclaved in a glass petri dish. A lead acetate strip was suspended over a Kligler iron agar (Nissui, Tokyo, Japan) slant immediately after inoculation and then observed daily for 5 days for development of a dark brown color at the lower end. Oxidative acid production from carbohydrates (concentration, 1%) or from 3% ethanol was determined in oxidation-fermentation basal medium (catalog no. 0688; Difco Laboratories, Detroit, Mich.). Skim acetate agar was prepared by the method of Christensen (6). The surface of a Mueller-Hinton agar plate was flooded with Gram iodine solution. A clear halo surrounding a culture indicated starch hydrolysis. Acylamidase activity was determined on slants of acetamide medium (Eiken, Tokyo, Japan) which contained (per liter) 0.5 g of yeast extract, 0.2 g of glucose, 5.0 g of NaCl, 1.0 g of KH_2PO_4, 3.0 g of acetamide, 0.03 g of phenol red, and 15 g of agar; the pH of this medium was 6.3. Deoxyribonuclease (DNase) activity was determined on slants of DNase medium (Eiken), which contained (per liter) 15.0 g of casein peptone, 5.0 g of soy peptone, 5.0 g of NaCl, 2.0 g of DNA, 0.1 g of toluidine blue, and 15.0 g of agar; the pH of this medium was 7.3. One loopful of a 20-h broth culture was streaked onto each slant, and the preparation was incubated with the screw cap loosened. After 24 to 48 h of incubation, the development of a red color indicated acylamidase activity, and a purple color indicated DNase activity. The egg yolk reaction was recorded as positive if a clear zone which gradually became turbid to form a grayish white halo

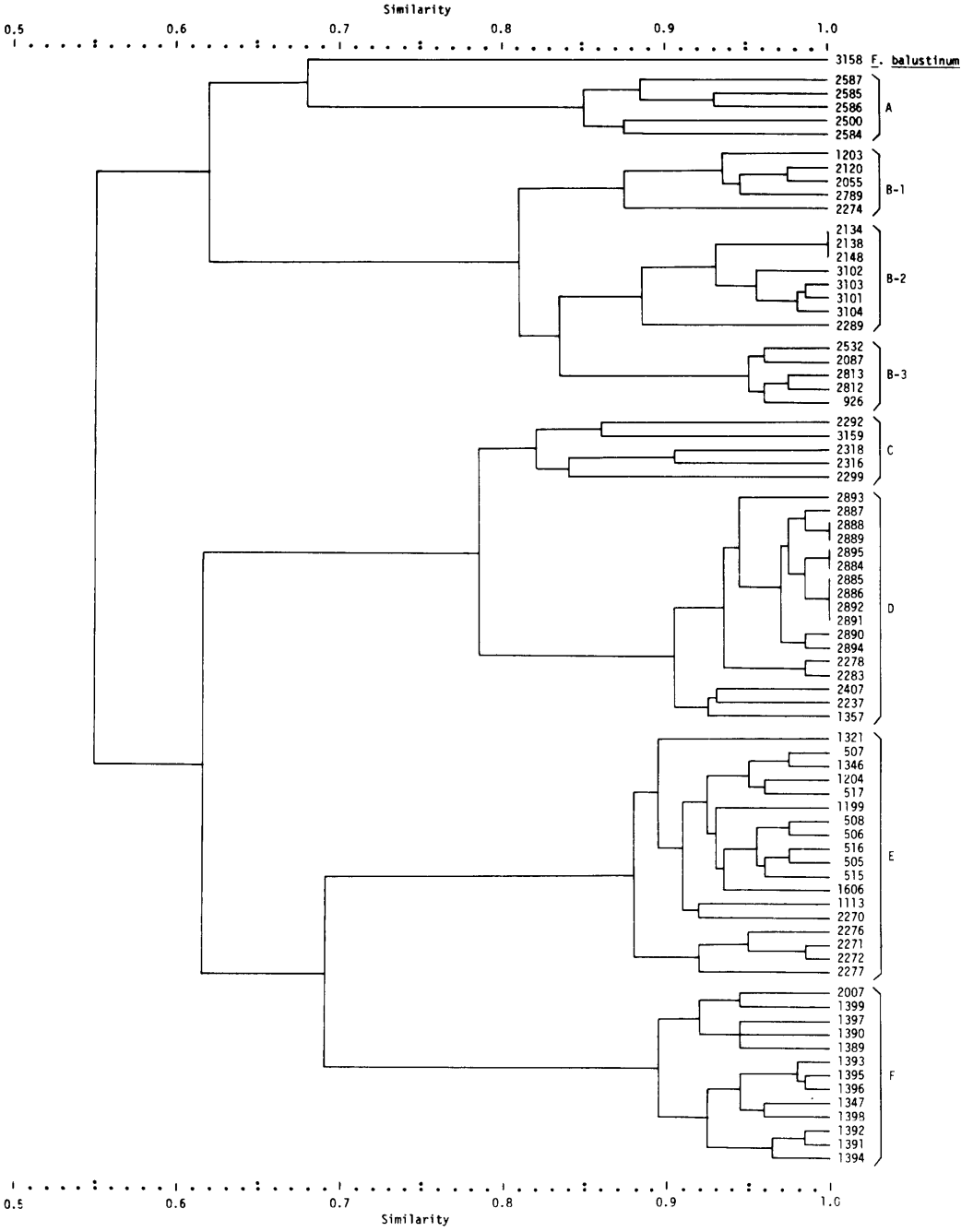

FIG. 1. Dendrogram prepared by simple matching and average-linkage methods for 73 effective data, using 107 phenotypic characters of 77 strains of nine species. *F. balustinum* GIFU 3158^T is at the top of the dendrogram. Cluster A corresponded to *C. johnsonae*. Cluster B was divided into the following three subclusters: *S. mizutae* (subcluster B-1), *S. spiritivorum* (subcluster B-2), and *S. multivorum* (subcluster B-3). Cluster C corresponded to *Flavobacterium breve*; cluster D corresponded to *F. odoratum*; cluster E corresponded to *F. meningosepticum*; and cluster F corresponded to *F. indologenes*.

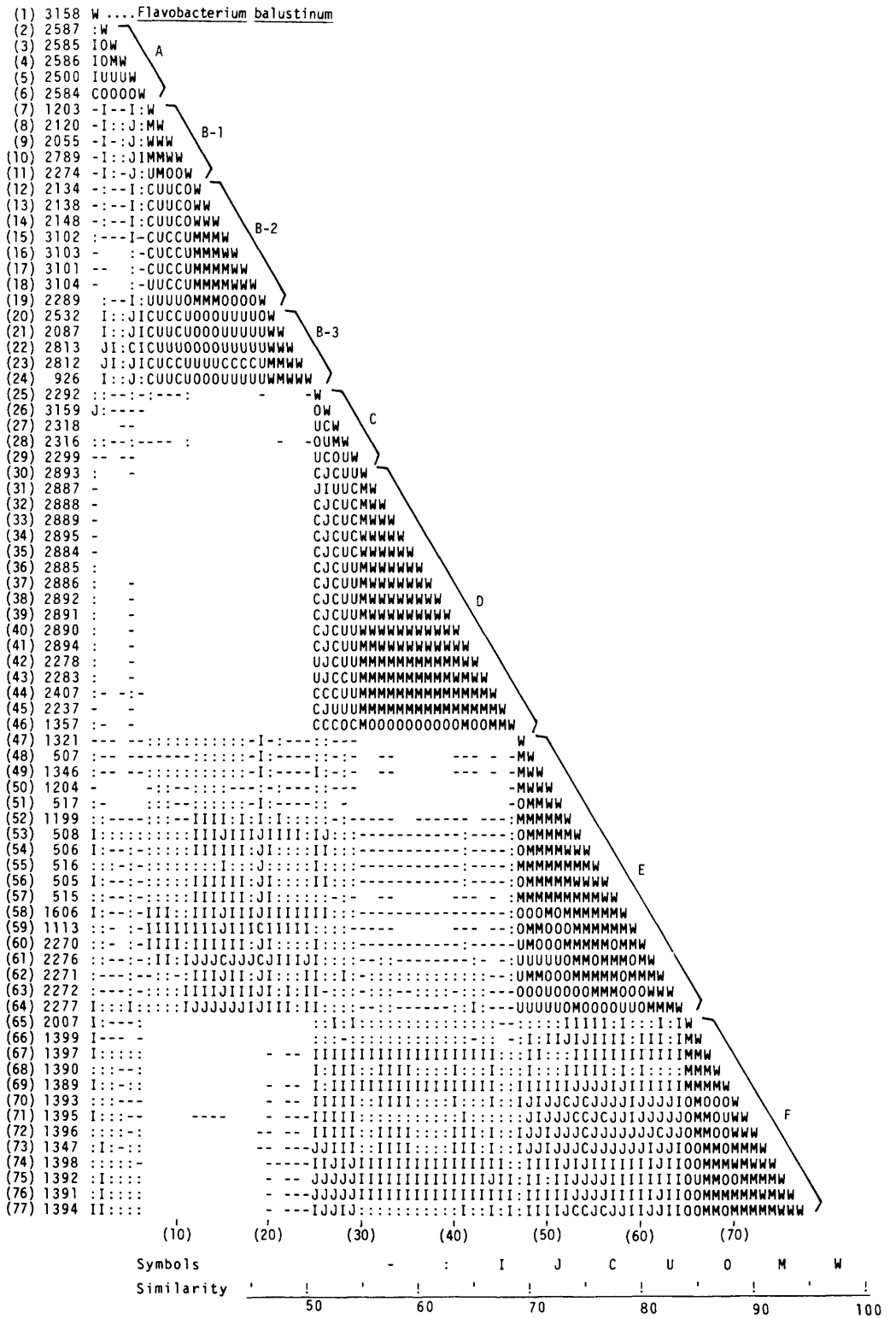

FIG. 2. Similarity matrix prepared by using the same method and data used to prepare Fig. 1. For an explanation of the clusters, see the legend to Fig. 1. The range of similarity percentages is symbolized as: W, >95%; M, 90.1 to 95.0%; O, 85.1 to 90.0%; U, 80.1 to 85.0%; C, 75.1 to 80.0%; J, 70.1 to 75.0%; I, 65.1 to 70.0%; :, 60.1 to 65.0%; −, 55.1 to 60.0%; open space, <55%.

TABLE 2. Base ratios and DNA homologies of *S. spiritivorum*, *S. multivorum*, *S. mizutae*, *Cytophaga* spp., *F. indologenes*, and four other *Flavobacterium* species

Source of unlabeled DNA	GIFU strain no.	G+C content (mol%)	Rebinding ratio with labeled DNA from strain:[b]					
			GIFU 3101^T (prepn A)	GIFU 2138		GIFU 2812^T (prepn B)	GIFU 926 (prepn B)	GIFU 1203^T (prepn B)
				Prepn A	Prepn B			
S. spiritivorum	3101^T	40.2	100		74			
	2134	40.0	81	94	90	19		9
	2138	40.3	91	100	100		14	
	2148	40.0	73	89	88			12
	2289	40.3	80	81	74	7		
	3102	40.5	85		73			
	3103	40.2	97		70			
	3104	39.9	82		64			
S. multivorum	2812^T	42.2		34	18	100	78	10
	2532	41.7				100	97	
	2813	40.8		24	18	89	79	
	2087	42.0		27	15	74	83	15
	926	39.8				66	100	10
S. mizutae	1203^T	40.0		19	11		10	100
	2055	41.5		28	19	8		
	2789	41.4		17	12			79
	2120	39.0				6		32
	2274	40.5	11		7	6		
C. johnsonae	2500^T	36.8						
	2584	37.1				3		
"*C. lytica*" ATCC 23178^T	2490^T	33.9		17	10			
C. marinoflava ATCC 19326^T	2502^T	39.5		8	4	2		3
Cytophaga sp. NCMB 249	2506	40.3						
F. meningosepticum	506^T	37.1					4	3
	507	36.4		11	6			6
F. indologenes	1347^T	37.6	4		7			
	1389							
	1391							
	1395							
	1397	38.3				5		6
	1399							
	2007	37.3		14	8	4	4	
F. balustinum	3158^T	34.7	2		5			
F. odoratum	1357^T	37.6		7	4			5
	2283	34.2						4
F. breve	3159^T	33.8	4		7			
	2318	35.0				6		
L. pneumophila ATCC 33152^T	2522^T	37.6				1		
B. cereus ATCC 14579^T		34.5				2		

[a] G+C contents were estimated from melting temperatures in 1× SSC.

[b] Homologies were estimated from DNA-DNA hybridization ratios by a membrane filter method. In each case, for preparation A normal hybridization was allowed to proceed for 40 h at 55°C; for preparation B, after normal hybridization was allowed to proceed for 40 h at 55°C, the filters were heated for 30 min at 65°C to eliminate unstable hybrids.

around the growth was produced. Anaerobic respiration of nitrate, nitrite, or fumarate was determined in 1% Casitone (Difco)–0.3% yeast extract (Difco) broth supplemented with 0.2% nitrate, 0.2% nitrite, or 0.08% sodium fumarate (3). These cultures were incubated in an anaerobic glove box (Forma Scientific). Other biochemical properties were determined by methods described previously (31, 33).

API ZYM chromogenic enzyme substrate strips (Appareils et Procédés d'Identification; La Balme les Grottes, Montalieu Vercieu, France) were used to determine the enzymatic activities of test organisms.

TABLE 2—*Continued*

GIFU 2055		GIFU 2274	GIFU 2500[T]	GIFU 2490[T]	GIFU 506[T]		GIFU 1347[T]	GIFU 2007		GIFU 3158[T]	GIFU 1357[T]
Prepn A	Prepn B	(prepn B)	(prepn A)	(prepn A)	Prepn A	Prepn B	(prepn B)	Prepn A	Prepn B	(prepn B)	(prepn B)
										5	
										5	3
27	13	15	7		8					6	
								9		5	4
26	12									7	
										5	
										5	
										5	
29	12				9						
44	15	18									
		16		7							3
25	13	15						7			5
18	9			6							4
69	69	35		5							4
100	100	42		6							
76	71	33			7			5			
40	30	35						5			4
49	35	100	11								
15	5		100	18							7
12	4		43	16	10						
			11	100							3
7	3		8	13							3
9	3		11	7							3
11	4		8		100	100	18		20		
					46						5
						13	100		43	26	
							39		47		
							35		43		
						14			49		
10	5			10			87	49			2
							43		49		
						11	48	100	100		3
										100	
9	4							9			100
			10		9			6			15
										10	
14	7							16			7
8	2		5		5						
7	2		4								

Cultures were grown on heart infusion agar for 20 h and then suspended in sterile distilled water at a turbidity equal to a MacFarland no. 5 standard. Then 2 drops of each suspension were distributed into each of the 20 cupules on a strip with a sterile Pasteur pipette. After incubation for 4 h at 37°C in a humid atmosphere, 1 drop each of two reagents (ZYM A and ZYM B) was added. Then the strips were exposed to the light from two 500-W bulbs for about 20 s to reduce the residual yellow color in negative reactions. Scores for enzymatic activity (0 through 5) were recorded according to the color table of the manufacturer; reactions which gave scores of 2 through 5 were recorded as positive.

Susceptibilities to antimicrobial agents. Susceptibilities to 17 antimicrobial agents (see Table 5) were estimated by the method of Bauer et al. (2). To do this, Sensi-Discs and Mueller-Hinton II agar plates (BBL Microbiology Systems, Cockeysville, Md.) were used. *Escherichia coli* GIFU 3005 (= ATCC 25922), *Pseudomonas aeruginosa* GIFU 3006 (= ATCC 27853), and *Staphylococcus aureus* GIFU 3007 (=ATCC 25923)

TABLE 3. Percent compositions of long-chain bases in the total lipids extracted from 10 strains of three *Sphingobacterium* species

Long-chain base[a]	% In total lipids									
	S. spiritivorum				*S. multivorum*				*S. mizutae*	
	GIFU 2134	GIFU 2138	GIFU 2148	GIFU 3101^T	GIFU 926	GIFU 2087	GIFU 2812^T	GIFU 2813	GIFU 1203^T	GIFU 2789
d-15:0br	t[b]	t	t	t	t	t	t	t	t	t
d-16:1(?)	t	t	t	t	2	t	t	t	t	t
d-16:0	4	3	5	4	5	4	8	5	t	t
d-17:0br	82	68	82	80	78	79	90	85	98	96
d-18:1(?)	1	t	2	1	2	2	t	t	t	t
d-18:0	13	25	11	15	14	15	2	10	3	4
d-19:0br	t	4	t	t	t	t	t	t	t	t

[a] d, Dihydroxy or two hydroxyl groups in the long-chain base. The number to the left of the colon indicates the number of carbon atoms in the chain, and the number to the right of the colon indicates the number of double bonds; br indicates a methyl branched-chain group in the long-chain base, and ? indicates a tentative identification.

[b] t, Trace (less than 1.0%).

were used as the reference cultures to standardize the tests, according to the instructions of the manufacturer.

Cellular lipids and fatty acid analysis. The long-chain base components of the cellular sphingolipids were obtained from acid hydrolysates of acetone-dried powders of the test strains by the method used to obtain the sphingoglycolipids of *Pseudomonas paucimobilis* (31). The long-chain bases were demonstrated by thin-layer chromatography and identified by gas-liquid chromatography and gas chromatography-mass spectrometry of their trimethylsilyl ether derivatives (36). To estimate the contents of cellular sphingolipids, the amounts of alkaline-stable lipids in the total lipids and the phosphorus contents of both total lipids and alkaline-stable lipids were quantitated with strain GIFU 2138. Cells of strain GIFU 2138 grown on PYG medium (1.0% Bacto-Peptone [Difco], 0.5% yeast extract [Difco], 0.5% glucose, 1.5% Bacto-Agar [Difco]) were harvested and lyophilized after 20 h of incubation at 30°C. The cellular lipids from 3.9 g of lyophilized cells were extracted twice with 80 ml of chloroform-methanol (2:1, vol/vol) and washed by the method of Folch et al. (7). After the preparation was reduced to dryness, the lipids were hydrolyzed by shaking with 0.5 N KOH in 50 ml of chloroform-methanol (2:1, vol/vol) for 3 h at room temperature. After neutralization with acetic acid and washing with 0.2 volume of water, the bottom layer was dried and dissolved in 50 ml of chloroform-methanol (2:1, vol/vol). The reaction mixture was washed twice with 0.2 volume of water. The bottom layer was evaporated, dried, and weighed; this fraction represented the alkaline-stable lipids. The phosphorus contents of the total lipids and alkaline-stable lipids were estimated by the method of Allen (1).

Cellular fatty acid analyses of *S. spiritivorum*, *F. indologenes*, and *F. meningosepticum* strains were performed with a gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with a fused silica capillary column, as described previously (30).

Cell-bound orange pigments. The solubilities of the cell-bound orange pigments of three *F. indologenes* strains (GIFU 1347^T, GIFU 1359, and GIFU 2007) and *C. johnsonae* GIFU 2500^T were tested with organic solvents after growth on PYG plates. Cells were removed from each plate and treated with ethanol, methanol, diethyl ether, acetone, *n*-hexane, or chloroform. The visible absorption spectra of the acetone extracts of the four strains mentioned above and *F. balustinum* GIFU 3158^T were determined with a Hitachi model 340 recording spectrophotometer. Spectra were determined before and after 20% KNO was added to the acetone extracts.

DNA base composition and DNA homology. DNA was prepared and purified by phenol extraction from cells grown in heart infusion broth. The G+C content of each DNA was determined by melting (denaturing) the DNA contained in 1× SSC (0.15 M NaCl plus 15 mM trisodium citrate buffer) in a model 2400S spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a model 2527 thermoprogrammer. The melting temperature was converted to G+C content by the formula of Marmur and Doty (16). Crude preparations of *F. indologenes* DNA contained unusual amounts of impurities, which made the solution turbid and strongly stimulated nonspecific binding of labeled DNA to filters. Nonspecific binding was prevented by heating the DNA in 0.1× SSC, which denatured the DNA and markedly decreased its viscosity. After centrifugation at 30,000 × *g* for 10 min, 0.33 volume of 10× SSC was added to the clear supernatant. The mixture was incubated at 60°C for several hours for annealing, and the DNA was then spooled out.

Tritium-labeled DNA was extracted from cells grown in the presence of [^{3}H]thymidine or [^{3}H]uridine. DNA-DNA hybridization experiments were performed as described previously (20). Incubation was performed at 55°C (which is significantly lower than the denaturation temperature minus 25°C) to detect relatedness between relatively different organisms (T. Kaneko, unpublished data). The heat stabilities of the hybrids were examined by reheating the filters at 65°C for 30 min after counting (20).,

Numerical analysis. Similarity values were calculated before and after omitting the 36 phenotypic characters for which all 77 test strains gave the same results

(either uniformly positive or uniformly negative). The susceptibilities of the 77 test strains to 17 antimicrobial agents were excluded in every calculation.

RESULTS AND DISCUSSION

Evidence for distinct taxonomic groups. (i) Numerical analysis. The dendrogram in Fig. 1, which was prepared by simple matching and average-linkage methods for effective data, illustrates the phenotypic relationships among the 77 strains which we studied.

Cluster B, which is composed of three subclusters of *Sphingobacterium*, is clearly separated from cluster A, which represents *C. johnsonae*. Subclusters B-1, B-2, and B-3 each corresponds to a separate species. Although the final within-species level of similarity was 87.3% for subcluster B-1 (*S. mizutae*), the average level of similarity among four strains (excluding strain GIFU 2274) was 93.6%, and the level of similarity between strains GIFU 2274 and GIFU 2120 was 90% or more (Fig. 2). The within-species level of similarity for subcluster B-2 (*S. spiritivorum*) was 88.5%, because strain GIFU 2289 was incorporated at this level. However, the levels of similarity for strain GIFU 2289 compared with strains GIFU 2134, GIFU 2138, and GIFU 2148 were between 90 and 95% (Fig. 2). The average level of similarity among the five strains of *S. multivorum* (subcluster B-3) was 95.1%.

The 13 strains of group IIb formed a well-defined cluster (cluster F) at a similarity level of 89.2%. The level of similarity between group IIb (cluster F) and *F. meningosepticum* (cluster E) was 68.2%, and the level of similarity between group IIb and *C. johnsonae* (cluster A) was 53.7%. *F. balustinum* GIFU 3158^T is associated primarily with cluster A (68%), not cluster F. As a result of chaining, the dendrogram suggests the presence of subclusters in cluster F. However, two- and three-dimensional coordination of the dendrogram indicated no substantial subclusters in cluster F (T. Kaneko, N. Niki, unpublished data), and the similarity matrix (Fig. 2) also did not indicate any clear subclusters.

Our numerical analysis (Fig. 1 and 2) indicates that the genus *Sphingobacterium* should be placed in the family *Cytophagaceae* together with the genus *Flavobacterium*, as proposed by Callies and Mannheim (4).

(ii) Base ratios and relative binding ratios of DNAs. The G+C contents of the DNAs of strains of three *Sphingobacterium* species, five *Flavobacterium* species, four *Cytophaga* species, *L. pneumophila* GIFU 2522^T, and *B. cereus* ATCC 12579^T and DNA-DNA homology values are shown in Table 2.

The G+C contents of the 18 strains of three *Sphingobacterium* species studied are slightly higher than those of the *Flavobacterium* species, *C. johnsonae* and "*C. lytica*." The G+C content of *F. balustinum* GIFU 3158^T is about 3 mol% lower than that of the *F. indologenes* strains tested.

High levels of homology were observed among the eight strains of *S. spiritivorum* and five strains of *S. multivorum* studied. The levels of homology between *S. spiritivorum* GIFU 2289 and *S. spiritivorum* GIFU 3101^T and GIFU 2138 were 80 and 74%, respectively, although the average level of similarity for strain GIFU 2289 in the numerical analysis of 107 phenotypic characteristics was lower than the average levels of similarity among the other four *S. spiritivorum* strains tested. Among the five strains of *S. mizutae* studied, both strain GIFU 2120 and strain GIFU 2274 gave homology values of only 30 to 42% compared with the other three strains. The lower levels of homology within this species may reflect biochemical dissimilarities between these two strains and the other *S. mizutae* strains (see Table 5) and suggest that strains GIFU 2120 and GIFU 2274 may represent a fourth species of *Sphingobacterium* which has not been described yet.

The levels of homology among the seven strains of *F. indologenes* studied ranged from 35 to 49%. However, some genetic relatedness is evident, based on the values of 11 to 14% among three of these strains and *F. meningosepticum* GIFU 506^T and 26% between *F. indologenes* GIFU 1347^T and *F. balustinum* GIFU 3158^T. Because of this evidence and the major phenotypic similarities of these strains, we assigned them to a single species. A much lower level of homology between *F. balustinum* and group IIb (21) was obtained by using different experimental conditions (22).

(iii) Cellular lipid composition. A total of 18 strains belonging to three *Sphingobacterium* species contained major amounts of long-chain bases in their cellular lipids, as determined by thin-layer chromatography. These components were not detected in the other 59 strains studied. Cells of *C. johnsonae* reportedly contain sulfonolipids (15). The relative amounts of the long-chain base components of nine strains belonging to three *Sphingobacterium* species are summarized in Table 3. The amount of total lipid extracted from 3.9 g of freeze-dried cells of strain GIFU 2138 was 466.5 mg, of which 295 mg (63.2%) was alkaline stable. The phosphorus contents of the total lipids and alkaline-stable lipids were 9.5 and 2.0 mg, respectively.

The amounts of branched-chain fatty acids in *F. indologenes* GIFU 1347^T and *F. meningosepticum* GIFU 506^T were more than 80% of the total cellular fatty acid contents, but the percentage of branched-chain acids in *C. johnsonae*

TABLE 4. Cellular fatty acid compositions of the type and reference strains of the three *Sphingobacterium* species, *C. johnsonae*, and two species of *Flavobacterium*

Fatty acid[a]	% Of total lipids										
	S. spiritivorum					*S. multivorum*		*S. mizutae*		*C. johnsonae*	
	GIFU 3101[T]	GIFU 2138	GIFU 3102	GIFU 3103	GIFU 3104	GIFU 2812[T]	GIFU 926	GIFU 1203[T]	GIFU 2055	GIFU 2500[T]	GIFU 2584
14:0	2	2	1	2	1	—[b]	5	—	—	4	5
15:0	—	—	—	—	—	—	—	—	—	1	1
16:1	20	24	12	16	5	13	29	17	15	27	35
16:0	4	5	3	3	2	2	11	3	1	23	16
17:0	—	—	—	—	—	—	—	—	—	—	—
18:2	—	—	1	—	1	—	—	—	—	—	—
18:1	—	—	—	—	—	—	—	—	—	—	—
18:0	—	—	—	—	—	—	—	—	—	—	—
i-13:0	—	—	—	—	—	—	—	—	—	—	—
i-15:0	36	25	42	40	50	27	18	26	32	32	15
a-15:0	—	—	—	—	1	—	—	—	—	—	—
i-16:0	—	—	—	—	—	—	—	—	—	3	4
i-17:1	—	—	2	1	2	3	—	2	4	1	—
i-17:0	—	—	—	—	—	—	—	—	—	—	1
2-OH-14:0	—	1	—	—	—	—	1	—	—	—	—
3-OH-14:0	—	—	—	—	—	—	—	—	—	3	4
3-OH-16:0	2	3	1	1	1	—	2	1	—	5	6
i-2-OH-15:0	24	28	26	25	25	35	19	30	29	2	1
i-3-OH-15:0	3	3	2	3	4	4	7	3	2	5	6
i-3-OH-17:0	4	3	6	4	5	10	1	11	11	1	1

[a] The number to the left of the colon indicates the number of carbon atoms, and the number to the right of the colon indicates the number of double bonds. i, A methyl group is present at the penultimate (iso) carbon atom; a, a methyl group is present at the antepenultimate (anteiso) carbon atom; 2-OH and 3-OH, a hydroxyl group is present at carbon atoms 2 and 3, respectively.

[b] —, Not detected or present at a level less than 1%.

TABLE 5. Antimicrobial agent susceptibilities of 18 strains of three *Sphingobacterium* species and 54 strains of five *Flavobacterium* species[a]

Drug	Concn	*S. spiritivorum* (n = 8)[b]			*S. multivorum* (n = 5)			*S. mizutae* (n = 5)		
		S[c]	I[c]	R[c]	S	I	R	S	I	R
Sulfadiazin	25 μg[d]	7[e]	0	1	4[e]	0	1	4[e]	0	1
Sulfamethoxazole-trimethoprim	23.75 μg and 1.25 μg	7[e]	1	0	4[e]	0	1	4[e]	0	1
Nalidixic acid	30 μg	0	4	4[e]	2	3[e]	0	2	2[e]	1
Erythromycin	15 μg	0	0	8[e]	2	3[e]	0	2[e]	0	3
Clindamycin	2 μg	0	1	7[e]	2	0	3[e]	2	0	3[e]
Streptomycin	10 μg	0	0	8[e]	0	1[e]	4	0	1	4[e]
Amikacin	10 μg	0	0	8[e]	0	0	5[e]	0	0	5[e]
Kanamycin	30 μg	0	0	8[e]	0	0	5[e]	0	0	5[e]
Gentamicin	10 μg	0	0	8[e]	3[e]	0	2	0	0	5[e]
Tetracycline	30 μg	0	1	7[e]	2	2[e]	1	3[e]	1	1
Chloramphenicol	30 μg	0	3[e]	5	3	1[e]	1	3[e]	0	2
Cephalothin	30 μg	0	0	8[e]	1	0	4[e]	1	1	3[e]
Penicillin	10 U[d]	0	0	8[e]	0	0	5[e]	1	1	3[e]
Ampicillin	10 μg	0	0	8[e]	0	0	5[e]	2	1	2[e]
Carbenicillin	100 μg	0	0	8[e]	3[e]	2	0	4[e]	0	1
Colistin	10 μg	0	0	8[e]	0	0	5[e]	0	0	5[e]
Polymyxin B	300 μg	0	0	8[e]	0	0	5[e]	0	0	5[e]

[a] The strains tested are listed in Table 1.

[b] Number of strains tested.

[c] S, Number of strains susceptible; I, number of strains with intermediate susceptibility; R, number of strains resistant.

[d] Amount of drug per disk.

[e] Category which included the type strain.

TABLE 4—*Continued*

F. indologenes								*F. meningosepticum*		
GIFU 1347[T]	GIFU 1389	GIFU 1391	GIFU 1394	GIFU 1395	GIFU 1397	GIFU 1399	GIFU 2007	GIFU 506[T]	GIFU 507	GIFU 1606
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
1	—	2	—	2	2	2	2	2	2	2
3	2	2	1	2	1	1	1	2	1	3
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
2	1	2	2	1	—	1	1	2	3	2
49	44	47	54	43	37	45	42	40	46	39
—	—	—	—	1	—	—	—	3	1	2
3	3	2	—	2	3	—	2	2	2	4
14	18	13	14	12	14	16	12	3	3	3
1	3	1	1	2	2	—	2	1	—	1
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	—	—	1	1	4	3	4
10	5	11	7	15	19	16	16	22	22	20
5	5	4	7	5	5	5	5	6	4	5
11	12	12	9	12	12	11	12	11	7	8

TABLE 5—*Continued*

F. indologenes (*n* = 13)			*F. meningosepticum* (*n* = 18)			*F. balustinum* (*n* = 1)			*F. odoratum* (*n* = 17)			*F. breve* (*n* = 5)		
S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
7[e]	0	6	14[e]	1	3	1[e]	0	0	1[e]	1	15	1[e]	0	4
12[e]	0	1	17[e]	0	1	1[e]	0	0	1[e]	0	16	1[e]	0	4
12[e]	1	0	7	4[e]	7	1[e]	0	0	0	4[e]	13	0	1[e]	4
1	0	12[e]	13[e]	0	5	1[e]	0	0	2[e]	0	15	4[e]	0	1
1	0	12[e]	4	2	12[e]	1[e]	0	0	1[e]	0	16	4[e]	0	1
1	2	10[e]	0	0	18[e]	0	1[e]	0	0	0	17[e]	0	1	4[e]
0	2	11[e]	1	1	16[e]	0	0	1[e]	1[e]	0	16	0	0	5[e]
0	0	13[e]	0	0	18[e]	0	0	1[e]	0	0	17[e]	0	0	5[e]
7[e]	0	6	3	0	15[e]	1[e]	0	0	0	0	17[e]	1	0	4[e]
0	0	13[e]	1[e]	1	16	0	0	1[e]	0	0	17[e]	1	1[e]	3
4	2	7[e]	2	3	13[e]	0	0	1[e]	1	2	14[e]	4[e]	0	1
0	0	13[e]	0	1	17[e]	1[e]	0	0	0	0	17[e]	0	1	4[e]
0	0	13[e]	1	0	17[e]	0	0	1[e]	0	1[e]	16	0	0	5[e]
0	0	13[e]	0	0	18[e]	1[e]	0	0	1[e]	0	16	0	2[e]	3
0	0	13[e]	3	3	12[e]	1[e]	0	0	0	1[e]	16	3[e]	0	2
0	0	13[e]	0	0	18[e]	0	0	1[e]	0	0	17[e]	0	0	5[e]
0	0	13[e]	0	0	18[e]	1[e]	0	0	0	0	17[e]	0	0	5[e]

GIFU 2500^T was approximately 34%. Moreover, strain GIFU 2500^T contained $C_{16:1}$ and $C_{16:0}$ acids in much higher concentrations than strains GIFU 1347^T and GIFU 506^T (Table 4). The major cellular fatty acid component of eight *F. indologenes* strains and three *F. meningosepticum* strains was i-$C_{15:0}$. However, cells of *F. indologenes* strains contained larger amounts of i-$C_{17:1}$ and smaller amounts of i-2-OH-$C_{15:0}$ than cells of *F. meningosepticum*.

For convenience of comparison with two *Flavobacterium* species, some of the cellular fatty acid data (30) for three *Sphingobacterium* species and *C. johnsonae* are also shown in Table 4.

(iv) Cell-bound orange pigments. The orange pigments of *F. indologenes*, which are similar to those of *C. johnsonae* strains and *F. balustinum* strain GIFU 3158^T, never diffused into the agar medium. The orange pigment immediately turned deep red when the culture was exposed to 1 drop of a 3% KOH solution and returned to the original orange color when 1 drop of 1.5 N HCl was added. The growth of *F. indologenes* at the top of semisolid Voges-Proskauer medium (Eiken) became red after Voges-Proskauer reagent B was added; this color change could be confused with a positive reaction. The orange pigments of *F. indologenes* and *C. johnsonae* were extracted from cells with methanol, ethanol, and acetone.

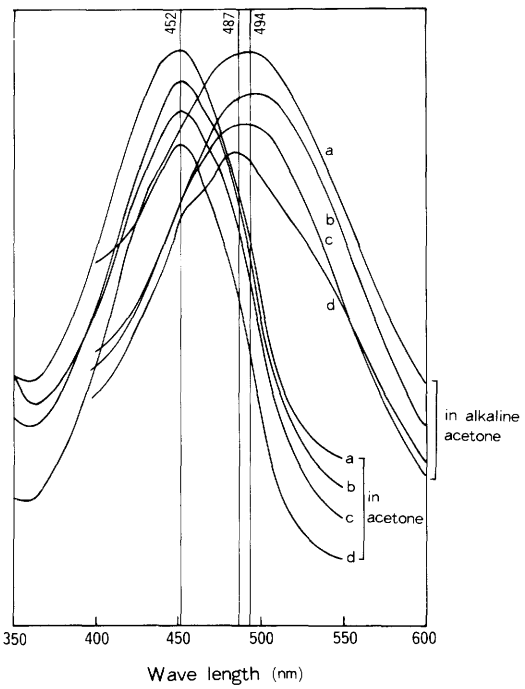

FIG. 3. Visible absorption spectra of acetone-extracted orange pigments from *F. indologenes* strains GIFU 1347^T (lines a), GIFU 1395 (lines b), and GIFU 2007 (lines c) and *C. johnsonae* GIFU 2500^T (lines d). Absorption spectra were recorded before and after treatment of acetone extracts with 20% KOH.

The visible absorption spectra of acetone extracts of strains GIFU 1347^T, GIFU 1395, GIFU 2007, and GIFU 2500^T had single peaks at approximately 451 nm, which shifted to 490 to 494, 494, 489 to 494, and 487 nm, respectively, in alkalinized acetone (Fig. 3). The characteristic reversible color change of growth on solid media indicates that the intracellular orange pigment of *F. indologenes* strains, as well as the pigments of *F. balustinum* and *C. johnsonae* strains, may be of the flexirubin type (26).

(v) Antimicrobial agent susceptibilities. All eight strains of *S. spiritivorum* were resistant to 11 of the 17 antimicrobial agents tested. All five strains of *S. multivorum* were resistant to six drugs, and the five strains of *S. mizutae* studied were resistant to five drugs. Sulfadiazin (25 μg) and sulfamethoxazole (23.75 μg)–trimethoprim (1.25 μg) were the only drugs to which most strains of these three *Sphingobacterium* species were susceptible (Table 5).

Description of *Sphingobacterium* gen. nov. On the basis of the results described above and the phenotypic characteristics shown in Table 6, we propose the new genus *Sphingobacterium* (sphin · go · bac · te'rium. Gr. adj. *sphingos* of sphinx; Gr. neut. n. *bakterion* a small rod; N.L. neut. n. *Sphingobacterium* a sphingosine-containing bacterium).

The cells of species in this genus are gram-negative, nonsporeforming, straight rods that have no flagella but may exhibit sliding motility. Catalase is produced. These organisms are chemoorganotrophs without specialized growth factor requirements. Colonies usually become yellowish after several days at room temperature. Indole and acetylmethylcarbinol are not produced. The cells are nonproteolytic, and gelatin is not hydrolyzed. Acid is produced from carbohydrates oxidatively but not fermentatively. The major characteristics for generic differentiation of *Sphingobacterium* from *Cytophaga* and *Flavobacterium* are shown in Table 6.

Sphingobacterium cellular lipids contain sphingophospholipids whose ceramide moieties are chiefly branched-chain dihydrosaturated $C_{17:0}$ sphingosin, and the major acid is i-2-OH-$C_{15:0}$.

Antisera against *F. meningosepticum* serogroups A through F do not agglutinate cells of strains of the *Sphingobacterium* species described here.

The DNA nucleotide base ratios (G+C contents) of described species range from 39 to 42 mol% (Table 2). The type species if *Sphingobacterium spiritivorum* (Holmes, Owen, and Hollis) comb. nov.

Description of the three species assigned to *Sphingobacterium* gen. nov. Cells of the three species are 0.3 by 1.3 to 1.6 μm, occur singly or in pairs, and remain the same under different cultural conditions.

Strains of the three species share the following characteristics. Indophenol oxidase is produced. No growth occurs at pH 4.5, at 4 or 41°C, or on MacConkey agar (Nissui) or nalidixic acid (15 mg/liter)-cetrimide (0.2 g/liter) agar medium (Eiken). No growth is observed in media containing 5% NaCl or 0.0075% KCN. Hydrogen sulfide production is usually detected by lead acetate-impregnated paper strips suspended over Kligler iron agar slants, but not in agar butts. Citrate, acetate, and malonate are not utilized. Esculin is hydrolyzed. Acid is produced from D-arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, melezitose, melibiose, raffinose, salicin, sucrose, trehalose, and xylose. Acid is not produced from adonitol, dulcitol, inositol, or sorbitol. Nitrate is not reduced to nitrite. L-Lysine decarboxylase, L-arginine dihydrolase, and L-orgnithine decarboxylase are not produced. Two-keto-gluconate is not produced from gluconate, and 3-ketolactonate is not produced from lactose.

The characteristics that are useful for differen-

TABLE 6. Phenotypic characteristics of the three species of *Sphingobacterium*, five species of *Flavobacterium*, and *C. johnsonae*

Character	% Of strains positive								
	S. spiritivorum (*n* = 8)[a]	*S. multivorum* (*n* = 5)	*S. mizutae* (*n* = 5)	*C. johnsonae* (*n* = 5)	*F. meningosepticum* (*n* = 18)	*F. indologenes* (*n* = 13)	*F. balustinum* (*n* = 1)	*F. odoratum* (*n* = 17)	*F. breve* (*n* = 5)
Flexirubin type of pigment[b]	0	0	0	100	0	100	100	0	0
Fruity odor[b]	0	0	0	0	0	46	0	100	100
Gliding translocation[b]	0	0	0	100	0	0	0	0	0
Growth in SMB containing 0.2% glucose and 0.1% ammonium sulfate[c]	100	100	100	40	0	0	0	0	0
Growth on skim acetate agar	0	0	0	100	94	100	100	76	60
Indole production[b]	0	0	0	0	100	100	100	0	100[d]
Gelatinase[b]	0	0	0	100	100	100	100	100	100
Oxidative acidity from:									
Galactose[b]	100	100	100	100	0	0	0	0	0
Melezitose[b]	100	100	100	0	0	0	0	0	0
Salicine[b]	100	100	100	20	0	0	0	0	0
Inulin[b]	38	100	0	80	0	0	0	0	0
Ethanol (3%)[e]	100	0	0	20	100	46	100	0	0
Mannitol[e]	100	0	0	40	100	31	0	0	0
Rhamnose[e]	100	0	100	0	0	0	100	0	0
Glycogen[e]	0	100	0	100	0	100	0	0	80
D-Arabinose[f]	100	100	100	100	100	0	100	0	0
Fructose, mannose[f]	100	100	100	100	100	100	100	0	0
Glucose[f]	100	100	100	100	100	100	100	0	100
Cellobiose[f]	100	100	100	100	100	0	0	0	0
Lactose[f]	100	100	100	0	100	0	0	0	0
Maltose[f]	100	100	100	100	100	100	0	0	100
Sucrose[f]	100	100	100	100	0	0	100	0	0
DNase[b,e]	100	100	0	0	100	100	100	100	100
Urease[f]	100	100	20	20	0	0	0	100	100
Starch hydrolysis[f]	100	0	0	100	0	100	0	6	60
Malonate utilization[f]	0	0	0	0	0	100	0	18	0
Esculin hydrolysis[f]	100	100	100	100	100	100	100	0	0
Egg yolk reaction[f]	0	0	0	0	0	100	0	0	0

[a] Number of strains studied.

[b] Characteristics useful for differentiating the three genera *Sphingobacterium*, *Cytophaga*, and *Flavobacterium*.

[c] SMB, Standard mineral base medium (12).

[d] The four strains other than type strain produced very weak reactions.

[e] Characteristics useful for differentiating the three *Sphingobacterium* species.

[f] Characteristics useful for differentiating the five *Flavobacterium* species.

TABLE 7. Variable characteristics among the strains of three *Sphingobacterium* species

Species	No. of strains	Character	Strain(s) producing the less common result	
			Positive reaction	Negative reaction
S. spiritivorum	8	Citrate (Christensen)	2289[a]	
		Acid from L-arabinose	2289, 3104	
		Acid from inulin	2134, 2138, 2148	
		Green discoloration on rabbit blood agar	2289	
		Fumarate respiration	2289	
		Valine arylamidase[b]	2289	
		Sliding translocation		2289, 3101^T
		Slant acid (Kligler iron agar)		3102
S. multivorum	5	Growth on MacConkey agar	2087	
		Green discoloration on rabbit blood agar	926	
		Fumarate respiration	2532	
		β-Glucosidase[b]	926, 2812^T	
		H_2S (lead acetate paper)		2813
		Tween 80 hydrolysis		2087
		Growth on 40% bile agar		2812^T
S. mizutae	5	Citrate (Christensen)	2274, 2789	
		Tween 80	2274	
		Urease	2274	
		Acid from glycogen	2789	
		Slant acid (Kligler iron agar)		1203^T
		H_2S (lead acetate paper)		2274
		Acid from ribose		2274
		Green discoloration on rabbit blood agar		2274, 2789
		Nitrite reduced to nitrogen gas		2120, 2274
		Valine arylamidase[b]		2120, 2274

[a] GIFU strain numbers (see Table 1).
[b] As determined by an API ZYM test.

tiating the three *Sphingobacterium* species are summarized in Table 6, and the characteristics that are variable among the strains in each species are shown in Table 7.

(i) *Sphingobacterium spiritivorum* (Holmes, Owen, and Hollis) comb. nov. The characteristics of *S. spiritivorum* (spi · ri · ti'vo · rum. L. n. *spiritus* spirit; L. adj. suffix *vorus* devouring, eating; M.L. adj. *spiritivorus* spirit-devouring, intended to refer to the ability of the organism to attack spirits [i.e., alcohol], producing acid in the process [10]) are as described above for the genus. Three of eight strains show distinctive spreading growth on 0.3% agar medium (Fig. 4a), but little or no spreading growth on medium containing 0.5% agar. At the edge of the spreading growth, the cells are arranged in a monolayer, which is not gliding (5, 9, 17, 24) but is typical for sliding translocation (9) (Fig. 4b). Three other strains show moderate spreading of semisolid agar medium.

This organism grows in the presence of 40% bile and produces acid in oxidation-fermentation medium from ethanol, glycerol, mannitol, and rhamnose. No acid is produced from D-ribose or glycogen. Urease and DNase are produced. Nitrite is not reduced to nitrogen gas.

The type strain is susceptible to sulfadiazin and sulfamethoxazole-trimethoprim but resistant to nalidixic acid and 14 other drugs (Table 5).

Isolated from human clinical specimens and hospital environments. The G+C content of the DNA is 40.0 mol%, as determined by the thermal denaturation method. The type strain is ATCC 33861 (= CDC E7288 = GIFU 3101 = JCM 1277 = NCTC 11386).

(ii) *Sphingobacterium multivorum* (Holmes, Owen, and Weaver) comb. nov. In addition to the generic characteristics described above, *S. multivorum* (mul · ti · vo'rum. L. adj. *multus* many; L. vt. *vorare* to swallow; M.L. adj. *multivorus* intended to mean produces acid from many carbohydrates [11]) has the following features. No sliding translocation occurs on semisolid agar medium. A weak acid reaction is observed on Kligler iron agar slants. Starch and urea are hydrolyzed. Acid is produced from L-arabinose, inulin, and glycogen but not from ethanol, man-

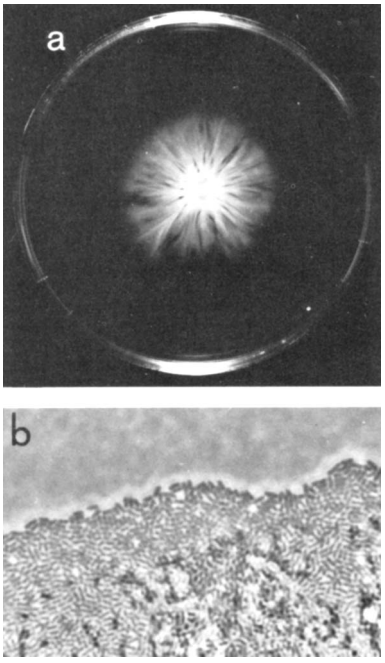

FIG. 4. (a) Spreading growth of *S. spiritivorum* GIFU 2138 on a semisolid agar motility medium (0.3% agar) after 70 h of incubation at 25°C. (b) Phase-contrast micrograph of *S. spiritivorum* GIFU 2138 cells in a monolayer at the edge of spreading growth on semisolid agar medium (0.5% agar) after 20 h at 25°C. ×830.

nitol, rhamnose, or D-ribose. Citrate is not utilized even on Christensen citrate agar medium. Nitrite is not reduced to nitrogen gas. Growth occurs in heart infusion broth adjusted to pH 5.0.

The major fatty acids extracted from saponified whole cells of the type strain are i-2-OH-$C_{15:0}$, i-$C_{15:0}$, $C_{16:1}$, and i-3-OH-$C_{17:0}$. The type strain is susceptible to sulfadiazin, sulfamethoxazole-trimethoprim, carbenicillin and gentamicin but resistant to penicillin, ampicillin, cephalothin, amikacin, kanamycin, colistin, and polymyxin B. Intermediate levels of susceptibility are observed with streptomycin, tetracycline, chloramphenicol, erythromycin, and nalidixic acid.

The G+C content is 42.2 mol%. The type strain is ATCC 33613 (= GIFU 2812 = NCTC 11033).

(iii) *Sphingobacterium mizutae* sp. nov. S. mizutae (mi · zu'tae. N.L. adj. referring to Shunsuke Mizuta (18), Japanese pediatrician, who first reported a case of meningitis in a premature baby from whose spinal fluid the type strain of the species was isolated) has the characteristics described above for the genus, and in addition strains of this species tolerate 40% bile and produce acid from rhamnose in oxidation-fermentation medium. No acid is produced from ethanol, mannitol, or inulin. No growth occurs at pH 5.0. Sliding translocation on semisolid agar medium and fumarate respiration are not observed. Starch hydrolysis and DNase production are negative. Most strains do not oxidize glycogen or hydrolyze urea. Nitrate is not reduced to nitrite, but the type strain and some other strains reduce nitrite to nitrogen gas.

The type strain is susceptible to carbenicillin, chloramphenicol, tetracycline, erythromycin, sulfadiazin, and sulfamethoxazole-trimethoprim but is resistant to penicillin, ampicillin, cephalothin, streptomycin, amikacin, kanamycin, gentamicin, clindamycin, colistin, and polymyxin B.

Pathogenicity has not been defined. The natural habitat of this organism is not known, but it has been isolated from clinical specimens. The G+C content of the DNA is in the range from 39.0 to 41.5 mol%. The type strain is ATCC 33299 (= GIFU 1203).

Description and discussion of *Flavobacterium indologenes* sp. nov. *F. indologenes* (in · do · lo' ge · nes. M.L. neut. n. *indolum* indole; Gr. v.

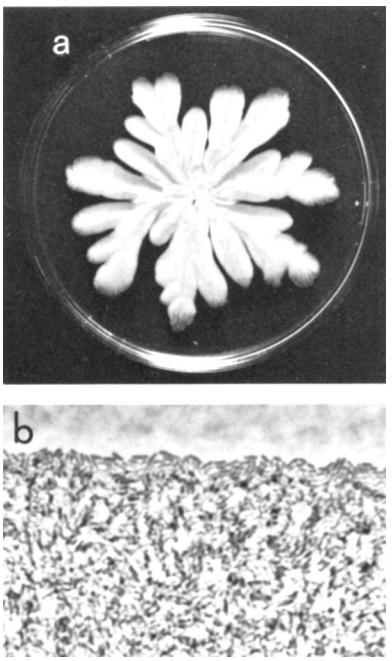

FIG. 5. (a) Spreading growth of *F. indologenes* GIFU 2007 on semisolid agar motility medium (0.3% agar) after 20 h at 25°C. (b) Phase-contrast micrograph of cells of *F. indologenes* GIFU 2007 in a monolayer at the edge of spreading growth on semisolid agar medium (0.5% agar) after 20 h at 25°C. ×830.

TABLE 8. Variable characteristics among the 13 strains of *F. indologenes*

Character	Strain(s) producing the less common result	
	Positive reaction	Negative reaction
Growth on MacConkey agar	1389, 1390, 1393, 1398, 1399, 2007[a]	
Fruity odor	1391, 1393, 1394, 1396, 1399, 2007	
Nitrate respiration	1389, 1390, 1397, 1399, 2007	
Acid from:		
L-Arabinose	1399, 2007	
Ethanol (3%)	1393, 1394, 1395, 1396, 1399, 2007	
Mannitol	1391, 1393, 1394, 1396, 1399, 2007	
Growth at pH 4.5		1389, 1391, 1392, 1394, 1397, 2007
Zinc dust test in negative NO_2 test		1389, 1390, 1397, 1399, 2007
API ZYM tests		
Esterase lipase (C8)		1390, 1391, 1392, 1394
Valine arylamidase		1393, 1395, 1396, 1397, 1398
β-Glucosidase	2007	
N-Acetyl-β-D-glucosaminidase	1347^T	

[a] GIFU strain numbers (see Table 1).

gennaio to produce; N.L. adj. *indologenes* indole producing) cells are straight rods 0.5 by 1.3 to 2.5 μm when the organism is grown on solid agar medium. In broth culture, many long, thread-like cells are present. Nonmotile and asporogenous. Colonies on heart infusion agar after 20 h of incubation at 30°C are 1 mm in diameter and have smooth surfaces and entire margins. Colonies of some strains become rhizoid within 20 h. Colonies on any medium are deep orange due to intracellular pigments of the flexirubin type, which are present from the very beginning of the appearance of colonies. The visible absorption spectra of the orange pigments in acetone and in alkalinized acetone are shown in Fig. 3.

Growth on a plate of motility medium (0.3% agar) spreads like a dahlia flower and almost covers the medium in a petri dish (diameter, 9 cm) within 20 h at room temperature (Fig. 5a). The spreading growth on 0.5% agar medium in a petri dish is moderate, and almost no spreading occurs on 1% agar medium. The cells are arranged in a monolayer at the edge of the spreading growth on 0.5% agar medium (Fig. 5b). Young cultures of many strains have a strong fruity odor similar to that of *F. odoratum*.

Chemoorganotrophic. Luxurious growth is produced on ordinary peptone media, but no growth is produced in standard mineral base medium (12) supplemented with glucose and ammonium sulfate as the sole sources of carbon and nitrogen. Nearly 50% of the strains are inhibited on MacConkey agar (Nissui). Metabolism is respiratory and not fermentative. This organism can grow under anaerobic conditions in the presence of fumarate. Some strains reduce nitrate to nitrogen gas; other strains reduce neither nitrate nor nitrite. Catalase, indophenol oxidase, amylase, DNase, gelatinase, lipase, and tryptophanase are produced. On egg yolk agar a clear zone around colonies is produced within 20 h; upon continued incubation this zone becomes increasingly turbid, and a grayish white halo is produced in the medium. Acid is produced oxidatively from fructose, glucose, glycogen, maltose, mannose, and trehalose, but not from D-arabinose, rhamnose, ribose, xylose, galactose, cellobiose, lactose, melibiose, sucrose, raffinose, melezitose, adonitol, dulcitol, inulin, or salicin. Urease, acylamidase, phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, and ornithine decarboxylase are not produced. The major biochemical characteristics of *F. indologenes*, *F. meningosepticum*, and other organisms used in this study are summarized in Table 6. Selected characteristics that are useful for differentiating *F. indologenes* among the five *Flavobacterium* species are also shown in Table 6. Table 8 shows the variable characteristics among the 13 strains of *F. indologenes* studied.

Four major components of the cellular fatty acids of strain GIFU 1347^T are i-$C_{15:0}$, i-$C_{17:1}$, i-3-OH-$C_{17:0}$, and i-2-OH-$C_{15:0}$ (Table 4). The G+C content of the DNA is 37.7 mol%.

The type strain is ATCC 17898 (= CDC 3716 = GIFU 1347 = NCTC 10796).

Although this species as currently described represents a genetically rather heterogenous group of organisms, it is assigned a proper scientific name with a detailed description, both for convenience and to encourage further taxonomic research on the genus *Flavobacterium*.

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