PROPOSALS OF *SPHINGOBACTERIUM FAECIUM* SP.
NOV., *SPHINGOBACTERIUM PISCII* SP. NOV.,
SPHINGOBACTERIUM HEPARINUM COMB. NOV.,
SPHINGOBACTERIUM THALPOPHILUM COMB. NOV.
AND TWO GENOSPECIES OF THE GENUS
SPHINGOBACTERIUM, AND SYNONYMY
OF *FLAVOBACTERIUM YABUUCHIAE* AND
SPHINGOBACTERIUM SPIRITIVORUM

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Seventeen strains of *Cytophaga*, *Flavobacterium* and *Actinobacillus* species were taxonomically characterized. DNA base composition of these strains ranged from 39.0 to 42.3 mol%, and the menaquinone system was MK-7. All the strains contained sphingolipids with long-chain bases, which are characteristic of the genus *Sphingobacterium*. Based on their chemotaxonomic and physiological characteristics together with DNA/DNA hybridization studies, we propose two new species and two new combinations, *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalpophilum* comb. nov. and two genospecies of the genus *Sphingobacterium*. *Flavobacterium yabuuchiae* was found to be a synonym of *Sphingobacterium spiritivorum*.

The genus *Sphingobacterium* was established by Yabuuchi et al. (30) for the three species, *Sphingobacterium spiritivorum*, *Sphingobacterium multivorum* and *Sphingobacterium mizutae*. Recently, new species, *Sphingobacterium antarcticus* (23) has been reported. *Sphingobacterium* species are characterized by their low guanine-plus-cytosine (G+C) contents of DNA (approximately 39 to 42 mol%), isoprenoid quinones with MK-7 and sphingolipids with long-chain base dihydro-sphingosin.

However, Holmes et al. (8) proposed that *S. spiritivorum* and *S. mizutae* should

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be retained in the genus *Flavobacterium* as *Flavobacterium spiritivorum* and *Flavobacterium mizutaii*, respectively. Besides these 2 species, 11 species, namely *F. aquatile*, *F. breve*, *F. balustinum*, *F. meningosepticum*, *F. odoratum*, *F. thermophilum*, *F. branchiophila*, *F. gleum*, *F. indologenes*, *F. thalpophilum* and *F. yabuuchi-ae* are recognized in the genus *Flavobacterium* (3, 5–8, 23, 28, 30). Among the species, *F. thermophilum* should be excluded from the genus *Flavobacterium* on the basis of its high G + C content of DNA (62 mol%).

On the other hand, *Cytophaga* was established in 1929 by Winogradsky (29) for aerobic, cellulose-decomposing, water-insoluble pigment-producing organisms with a peculiar gliding motility. In Bergey's Manual of Systematic Bacteriology, Vol. 3 (21), *Cytophaga* species were reported to have low G + C contents from 30 to 45 mol% (many strains occurring around 35 mol%), the quinone system MK-6 or MK-7, and cellular fatty acids consist of branched-chain non-polar and hydroxy acids. However, the genera *Cytophaga* and *Flavobacterium* share chemotaxonomic characteristics, and the only criterion for differentiating these genera is gliding motility.

Cytophaga heparina is an organism described by Payza and Korn (20) as *Flavobacterium heparinum* and later re-classified as *C. heparina* by Christensen (2) for the reason that it had been shown to glide. On the other hand, "*Cytophaga keratolytica*" was first described under the name "*Flavobacterium keratolyticus*" by Kitamikado and Ito (12) and then re-classified as a species of the genus *Cytophaga* by Imai (10), despite the absence of obvious gliding movement. In Bergey's Manual of Systematic Bacteriology, Vol. 3 (21), it is suggested that these two species be excluded from the genus *Cytophaga* for the reason that they do not glide. Therefore, their taxonomic positions have remained uncertain (26).

Phenovar "4–5" strains of *C. heparina*, *Flavobacterium ferrugineum*, "*Flavobacterium sewanense*" and *Flavobacterium* sp. reported by Oyaizu et al. (17, 18), and *Actinobacillus* sp. IFO 13310 in the IFO culture collection, a keratan sulfate-degrading bacterium (12, 16), were found to be strains of the *Flavobacterium-Cytophaga* complex on the basis of G + C contents of DNA, quinone system, and cellular fatty acid composition. Furthermore, the presence of sphingolipid in the cells suggested that these might be strains of the genus *Sphingobacterium*.

In this paper, we examined the phenotypic and chemotaxonomic characteristics of 17 strains of named *Flavobacterium*, *Cytophaga* or *Actinobacillus*, and compared them with those of previously-reported species of the genus *Sphingobacterium*, and we propose two new species, two new combinations and two genospecies of the genus *Sphingobacterium* on the basis of chemotaxonomic, phenotypic and DNA/DNA hybridization studies.

MATERIALS AND METHODS

Microorganisms and culture conditions. The strains used are listed in Table 1. For the comparison of taxonomic characteristics, type strains of *Flavobacterium*

Table 1. Bacterial strains studied.

Species	IFO	Other strain designations	Reference	Re-classified as
<i>Cytophaga heparina</i>	12017 ^T	ATCC 13125	KS 0426 ^a	<i>Sphingobacterium heparinum</i> (T)
<i>Cytophaga heparina</i>	15299		KS 0470	<i>Sphingobacterium faecium</i> (T)
<i>Cytophaga heparina</i>			KS 0463	<i>Sphingobacterium faecium</i>
<i>Cytophaga heparina</i>			KS 0482	<i>Sphingobacterium</i> genospecies 2
" <i>Cytophaga keratolytica</i> "	14087	IAM 12650	(12, 13)	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium ferrugineum</i>	14992 ^T	ATCC 13524	(24)	
<i>Flavobacterium ferrugineum</i>	19483	IAM 1493	KS 0418	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium ferrugineum</i>	14984	JCM 7453	KS 0419	<i>Sphingobacterium piscium</i>
<i>Flavobacterium ferrugineum</i>	14985	JCM 7454	KS 0420	<i>Sphingobacterium piscium</i> (T)
" <i>Flavobacterium sewanense</i> "	14986	JCM 7461	KS 0433	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium</i> sp.	14987	JCM 7463	KS 0435	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium</i> sp.			KS 0506	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium</i> sp.			KS 0507	<i>Sphingobacterium</i> genospecies 1
<i>Flavobacterium</i> sp.			KS 0427	<i>Sphingobacterium piscium</i>
<i>Actinobacillus</i> sp.	13310		(17)	<i>Sphingobacterium multivorum</i>
<i>Flavobacterium thalophilum</i>	14963 ^T		(12, 13)	
<i>Flavobacterium yabuuchiae</i>	14975 ^T	ATCC 43320	(3, 5)	<i>Sphingobacterium multivorum</i>
<i>Sphingobacterium spiritivorum</i>	14948 ^T	NCTC 12113	(8)	<i>Sphingobacterium thalophilum</i>
<i>Sphingobacterium multivorum</i>	14947 ^T	GIFU 3101	(8, 30)	(T)
<i>Sphingobacterium mizutae</i>	14946 ^T	GIFU 2812	(30)	<i>Sphingobacterium spiritivorum</i>
		GIFU 1203	(8, 30)	

^T and T: type strain.

Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; GIFU, Department of Microbiology, Gifu University School of Medicine, Gifu, Japan; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; NCTC, National Collection of Type Cultures, Central Public Health Lab. Service, London, U.K.

^a Strain number by Oyaizu et al. (17).

ferrugineum (24) and species of the genus *Sphingobacterium* were used. All strains were cultured at 28°C with aerobic shaking in PY medium containing 1% peptone, 0.2% yeast extract, 0.2% NaCl and 0.2% glucose, pH 7.0.

Determination of phenotypic characteristics. Cultures were grown on PY medium containing 1.5% agar. API 20NE (Appareils et Procédés d'Identification; La Balme les Grottes, Montalieu Vercieu, France) was used for physiological and biochemical characteristics. API 50CH and API ZYM were used to determine the carbohydrate assimilation pattern and the enzymatic activities of test organisms. DNase test agar (Catalog No. 0632; Difco Lab., Detroit, Michigan, U.S.A.) was used for DNase activity.

Enzyme assay of endo- β -galactosidase. The reaction mixture contained 750 μ g of keratan sulfate (bovine cornea, Seikagaku Kogyo Co., Ltd., Tokyo, Japan) and an appropriate amount of the enzyme in 100 μ l of 0.05 M sodium acetate buffer, pH 6.0. After incubation at 37°C for 5 to 30 min, 250 μ l of carbonate cyanide solution (13) was added to stop the reaction. An appropriate amount of the reaction mixture was then withdrawn and reducing sugars liberated were determined by the method of Park and Johnson (19).

Cellular lipids and fatty acid analysis. Cells harvested after culturing 24 h in PY medium were dried, mixed with 2 ml of 5% HCl in methanol and heated at 100°C for 3 h. Fatty acid methyl esters extracted with *n*-hexane/diethyl ether (1:1, v/v) were separated by thin layer chromatography (TLC), extracted with diethyl ether, and analyzed by gas-liquid chromatography (GLC). The long-chain base components of the cellular sphingolipids were obtained from acid hydrolysates of dried cells by the method described by Yano et al. (31). The long-chain bases were developed by TLC, and their trimethylsilyl ether derivatives were analyzed by GLC and gas chromatography/mass spectrometry (GC-MS). GLC analyses were carried out using a GC-9A gas chromatograph (Shimadzu Corp., Kyoto, Japan). Columns employed were 10% diethyleneglycol succinate (5 m) at 180°C and 3% OV-1 (2 m) at 165°C for fatty acid analysis and 3% OV-1 (2 m) at 190°C for long-chain base analysis.

Abbreviations for fatty acids are as follows: In the shorthand numbering system used to identify fatty acids, the figures preceding the colon indicate the number of carbon atoms in the fatty acids, while those following the colon represent the number of double bonds present. *i* indicates *iso*-branched acid.

DNA base composition. DNA was isolated by the method of Saito and Miura (22). G+C contents of DNAs were determined by reversed phase high-performance liquid chromatography (HPLC) (15,27) after nuclease P₁ and alkaline phosphatase treatment.

DNA/DNA hybridization. DNA/DNA hybridization was carried out by fluorometric hybridization in microdilution wells (4) using biotinylated DNA.

Quinone system. Isoprenoid quinone was extracted from the dried cells twice with chloroform/methanol (2:1, v/v) for 4 h and analyzed by HPLC. HPLC was carried out using a Shimadzu liquid chromatograph LC-6AD with a Zorbax ODS

(4.6 mm $\times$ 15 cm) column. Menaquinone was detected by the absorbance at 270 nm using a Shimadzu spectrophotometric detector SPD-6A. Samples were eluted with methanol/isopropyl ether (4 : 1, v/v) at 1 ml/min at 30°C. The elution time and area of each peak were calculated with a Shimadzu Chromatopac C-R3A.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of whole cells. Wet cells (0.1 g) were suspended in 1.0 ml of 0.0625 M Tris-HCl buffer (pH 6.8) containing 2% SDS, 5% 2-mercaptoethanol and 10% glycerol and heated at 100°C for 10 min (11). After centrifuging at $13,000 \times g$ for 10 min, the supernatant was subjected to electrophoresis. SDS-PAGE was performed in SDS-containing alkaline gel (10%) using 0.025 M Tris-glycine buffer (pH 8.4) containing 0.1% SDS at a constant current of 25 mA. Proteins were visualized by staining in a stirred solution of Coomassie Blue.

RESULTS

Morphological and physiological characteristics

Phenotypic characteristics of 12 strains of phenovar "4-5" have been reported by Oyaizu et al. (18). Their physiological and biochemical data agreed with our results derived from API 20NE, API 50CH and API ZYM system, except for acid production from some carbohydrates of *C. heparina* IFO 12017^T (KS 0426). In our experiment, IFO 12017^T did not produce acid from D-glucose, D-fructose, cellobiose, trehalose and glycerol but weakly produced acids from D-xylose and L-arabinose, and these results were contrary to the results by Oyaizu et al. Data from API systems were taken in this paper.

All the strains studied were gram-negative rods, and colonies on nutrient agar after 48 h were circular, 1 to 3 mm in diameter, low convex, smooth and opaque. The color of the colonies on PY agar was creamy white. The tests for which all 19 strains except for *F. ferrugineum* IFO 14992^T gave uniform results are summarized in Table 2.

G + C content of DNA and DNA/DNA homology

The G + C contents of DNA of the strains except for *F. ferrugineum* IFO 14992^T ranged from 39.0 to 42.5 mol%, while that of IFO 14992^T was 48.4 mol%. As indicated by the data in Table 3, the strains studied could be separated into six distinct DNA relatedness groups. DNA/DNA homology values between *C. heparina* IFO 12017^T and the other 18 strains were below 22%. High homology values were obtained between *C. heparina* IFO 15299 and KS 0463 (group 2) and among *Flavobacterium* sp. KS 0427, *F. ferrugineum* IFO 14984 and IFO 14985 (group 3). On the other hand, DNA/DNA homology values among "*C. keratolytica*" IFO 14087, *F. ferrugineum* IFO 14983, *Flavobacterium* sp. KS 0506 and IFO 14987, "*F. sewanense*" IFO 14986, *Actinobacillus* sp. IFO 13310 and *S. multivorum* IFO 14947^T were 30 to 76%, but the values between these 7 strains and strains of other groups were low (0 to 30%). Based on the results of DNA/DNA hybridization, these 7

Table 2. Tests for which all 19 strains gave positive or negative results.

Positive results	Negative results
Catalase production	Indole production
Oxidase production	Acid production
Enzymatic activity (API ZYM)	D-Fucose
Alkaline phosphatase	Gluconate
Esterase lipase (C ₈)	2-Keto-gluconate
Acid phosphatase	Erythritol
Naphthol-AS-B1-phosphohydrazase	L-Xylose
Leucine arylamidase	Adonitol
β -Galactosidase	Dulcitol
N-Acetyl- β -glucosaminidase	Inositol
β -Glucosidase	L-Arabitol
Esterase (C ₄)	D-Tagatose
Cystine arylamidase	
α -Glucosidase	
α -Mannosidase	
Acid production (API 50CH)	
Galactose	
Glucose	
D-Fucose	
Mannose	
Esculin	
Cellobiose	
Maltose	
Lactose	
Saccharose	
D-Turanose	

strains are considered to be included in the same DNA/DNA homology group (group 4). *Flavobacterium* sp. KS 0507 (group 5) and *C. heparina* KS 0482 (group 6) did not show homology with any other strains tested.

Chemotaxonomic characteristics

As shown in Table 4, all 20 strains contained menaquinone 7, and all except *F. ferrugineum* IFO 14992^T contained relatively large amounts of long-chain bases, one of which was identified as dihydrosphingosin (d17:0) by GC-MS analysis. It showed a characteristic molecular mass of m/z 73, 116, 132, 234, 299, 326, 328, 416 and 431. Keratan sulfate-degrading enzyme (*endo*- β -galactosidase) activity was detected in group 4 strains: "*C. keratolytica*" IFO 14087, "*F. sewanense*" IFO 14986, *Flavobacterium* sp. IFO 14987 and *Actinobacillus* sp. IFO 13310. This activity was not found in the strains of other DNA/DNA homology groups. As shown in Table 4, all strains except for *C. heparina* IFO 12017^T and *F. ferrugineum* IFO 14992^T contained *i*-15:0 and 16:1 (9) fatty acids as major components. *C. heparina* IFO 12017^T contained *i*-15:0 and 17:0 as the major components of cellular acids, but the other 19 strains did not contain 17:0. In the strain IFO 14992^T, 14:0 and *i*-15:0 were the major fatty acids. As hydroxy fatty acids, all the

Table 3. DNA/DNA homology among the strains of the genus *Sphingobacterium*.

DNA/DNA homology group	Species	Strain number	G + C (mol%)	% Homology with labeled DNA from strain									
				IFO 12017	IFO 15299	IFO 14984	IFO 14087	IFO 14983	IFO 13310	IFO 14947	KS 0507	KS 0482	
Group 1	<i>C. heparina</i>	IFO 12017 ^T	42.3	100	3	14	15	10	26	12	19	4	
Group 2	<i>C. heparina</i>	IFO 15299	39.0	1	100	3	8	7	18	14	ND	6	
Group 3	<i>C. heparina</i>	KS 0463	40.4	1	82	18	12	9	26	15	20	7	
	<i>Flavobacterium</i> sp.	KS 0427	41.1	0	0	72	18	9	13	11	7	16	
	<i>F. ferrugineum</i>	IFO 14984	41.1	0	2	100	14	0	8	8	16	11	
	<i>F. ferrugineum</i>	IFO 14985	42.1	0	0	58	14	3	14	7	30	5	
Group 4	" <i>C. keratolytica</i> "	IFO 14087	41.4	15	2	22	100	76	62	45	ND	11	
	<i>F. ferrugineum</i>	IFO 14983	41.1	0	4	17	76	100	86	33	10	4	
	<i>Flavobacterium</i> sp.	KS 0506	40.4	0	3	15	69	49	62	40	16	8	
	" <i>F. sewanense</i> "	IFO 14986	42.0	1	0	19	45	41	43	51	23	16	
	<i>Flavobacterium</i> sp.	IFO 14987	39.5	0	0	23	30	37	36	53	15	4	
	<i>Actinobacillus</i> sp.	IFO 13310	41.4	0	0	14	34	34	100	36	25	6	
Group 5	<i>S. multivorum</i>	IFO 14947 ^T	39.6	0	3	27	39	34	37	100	25	6	
	<i>Flavobacterium</i> sp.	KS 0507	40.5	0	0	11	14	6	9	5	100	11	
	<i>C. heparina</i>	KS 0482	40.0	10	0	0	20	4	2	2	4	100	
	<i>F. spiritalium</i>	IFO 14948 ^T	41.4	0	0	13	5	4	8	1	7	2	
Group 6	<i>F. yabuuchiae</i>	IFO 14975 ^T	39.8	0	8	12	9	7	1	4	11	12	
	<i>S. mizutae</i>	IFO 14946 ^T	42.5	0	3	20	11	14	12	20	23	16	
	<i>S. thalophilum</i>	IFO 14963 ^T	41.7	22	0	20	9	9	8	9	13	4	

Table 4. Fatty acid composition of the strains^a of the genus *Sphingobacterium*.

Group	Species	Strain number	Sphingo-lipid	Non-polar acid					2-OH			3-OH		
				14:0	<i>i</i> -15:0	16:0	16:1 (Δ^9) ^b	<i>i</i> -17:0	<i>i</i> -15:0	17:0	<i>i</i> -15:0	14:0	<i>i</i> -15:0	<i>i</i> -17:0
Group 1	<i>C. heparina</i>	IFO 12017 ^T	+	—	24.4	16.9	18.4	9.7	100	30.6	—	5.3	20.1	2.3
Group 2	<i>C. heparina</i>	IFO 15299	+	1.3	43.2	7.1	48.4	—	100	—	—	12.9	21.4	13.2
	<i>C. heparina</i>	KS 0463	+	1.0	55.4	7.2	30.2	6.2	100	—	—	8.1	9.2	26.1
Group 3	<i>Flavobacterium</i> sp.	KS 0427	+	1.2	34.1	9.5	42.4	12.7	100	—	—	8.1	9.2	26.1
	<i>F. ferrugineum</i>	IFO 14984	+	—	48.7	—	51.3	—	100	—	—	—	25.7	8.2
	<i>F. ferrugineum</i>	IFO 14985	+	—	32.9	—	67.1	—	100	—	—	—	28.7	16.8
Group 4	" <i>C. keratolytica</i> "	IFO 14087	+	7.6	39.6	23.9	28.9	—	100	—	—	11.8	27.9	19.3
	<i>F. ferrugineum</i>	IFO 14983	+	5.8	40.7	14.5	39.0	—	100	—	—	13.9	23.2	36.8
	<i>Flavobacterium</i> sp.	KS 0506	+	5.6	34.9	10.2	49.3	—	100	—	—	7.4	24.5	35.6
	" <i>F. sewanense</i> "	IFO 14986	+	2.7	46.8	11.9	38.6	—	100	—	—	3.4	25.5	23.3
	<i>Flavobacterium</i> sp.	IFO 14987	+	3.0	42.9	13.4	40.7	—	100	—	—	3.7	26.7	23.2
	<i>Actinobacillus</i> sp.	IFO 13310	+	5.0	45.4	22.2	27.4	—	100	—	—	3.7	33.6	33.4
Group 5	<i>S. multivorum</i>	IFO 14947 ^T	+	—	60.0	4.4	28.9	6.7	100	—	—	—	28.6	—
	<i>Flavobacterium</i> sp.	KS 0507	+	2.1	43.3	8.1	46.5	—	100	—	—	19.3	8.0	16.8
Group 6	<i>C. heparina</i>	KS 0482	+	1.1	57.4	2.8	29.7	9.0	100	—	—	3.1	8.5	13.8
	<i>S. spiritivorum</i>	IFO 14948 ^T	+	6.3	52.6	16.4	24.7	—	100	—	—	—	33.3	22.2
	<i>F. yabuuchiae</i>	IFO 14975 ^T	+	—	58.8	14.4	26.8	—	100	—	—	—	25.6	20.1
	<i>S. mizutae</i>	IFO 14946 ^T	+	—	54.2	6.3	35.4	4.1	100	—	—	—	20.0	6.6
	<i>F. thalophilum</i>	IFO 14963 ^T	+	5.3	52.6	8.8	33.3	—	100	—	—	—	19.0	19.0
	<i>F. ferrugineum</i>	IFO 14992 ^T	—	33.1	61.4	5.5	—	—	100	—	—	—	8.3	7.1

^a Percentage of the total non-polar, 2-hydroxy (OH) and 3-hydroxy (OH) acids.^b Position of double bond.

Fig. 1. SDS-PAGE of the whole-cell proteins of the genus *Sphingobacterium*.

Lanes: 1, (group 1) IFO 12017^T; 2, (group 2) (a, IFO 15299, b, KS 0463); 3, (group 3) (a, KS 0427, b, IFO 14984, c, IFO 14985); 4, (group 4) (a, IFO 14087, b, IFO 14986, c, IFO 14987, d, IFO 13310, e, IFO 14947^T, f, IFO 14983, g, KS 0506); 5, (a, IFO 14975^T, b, IFO 14948^T); 6, IFO 14946^T; 7, IFO 14963^T; 8, (group 5) KS 0507; 9, (group 6) KS 0482.

strains contained 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

Whole-cell SDS-PAGE protein pattern

Comparison of the whole-cell protein pattern revealed that the strains belonging to the same DNA relatedness group showed similar patterns (Fig. 1). Strains of DNA relatedness group 4 also showed high similarity in this pattern. The whole-cell protein patterns of *F. yabuuchiae* IFO 14975^T and *S. spiritivorum* IFO 14948^T were found to be quite similar.

Phenotypic differentiation

The results presented above indicated that the 6 DNA relatedness groups constitute separate taxa. Differential characteristics among the 6 groups and the 4 previously-known species of the genus *Sphingobacterium* are summarized in Table 5. *C. heparina* IFO 12017^T was clearly differentiated from the other 19 strains in many physiological characteristics, especially the ability to produce acid from carbohydrates which is very weak or negative. The biochemical characteristics of *F. spiritivorum* IFO 14948^T are very similar to those of *F. yabuuchiae* IFO 14975^T.

DISCUSSION

In Bergey's Manual of Systematic Bacteriology, Vol. 1 (6), Holmes et al. describe the genus *Flavobacterium* as historically and taxonomically heterogeneous. The wide range of G + C content of DNA between 30 to 45 mol% suggests that the

Table 5. Biochemical characteristics of the strains of the genus *Sphingobacterium*.

Characteristics	Group 1 <i>C. heparina</i> IFO 12017 ^T	Group 2 (2 strains)	Group 3 (3 strains)	Group 4 (7 strains)	Group 5 <i>F. sp.</i> KS 0507	Group 6 <i>F. sp.</i> KS 0482	<i>F. thalpo- philum</i> IFO 14963 ^T	<i>S. mizu- tae</i> IFO 14946 ^T	<i>F. spiriti- vorum</i> IFO 14948 ^T	<i>F. yabu- uchiae</i> IFO 14975 ^T
Growth at 5°C	+	100	100	0	+	+	-	-	-	-
37°C	+	100	0	100	+	-	+	+	+	+
42°C	-	0	0	0	-	-	+	-	-	-
Nitrate reduction	-	0	0	0	-	-	+	-	-	-
Nitrite reduction	-	0	0	0	-	-	-	-	-	-
Gelatin hydrolysis	-	0	0	14	-	+	-	-	-	-
Urease	-	100	0	100	+	+	+	+	+	+
DNase	+	100	100	100	-	+	-	+	+	+
Enzyme activity (API ZYM)										
α-Galactosidase	+	100	100	100	+	+	+	-	+	+
Valine arylamidase	-	100	100	100	+	+	+	+	+	+
Trypsin	-	0	100	100	+	+	+	+	+	+
Chymotrypsin	-	100	100	100	+	-	+	+	+	+
α-Fucosidase	-	50	0	100	+	+	+	-	+	+
β-Glucuronidase	-	50	33	71	-	+	+	-	+	+
Lipase (C ₁₄)	-	100	66	86	-	+	-	+	+	+
Acid production (API 50CH)										
α-Methyl-D-glucoside	-	100	100	100	+	+	+	+	+	+
Amygdalin	-	100	100	100	+	+	+	+	+	+
Arbutin	-	100	100	100	+	+	+	+	+	+

genus is heterogeneous. G+C content of the strains of the genus *Flavobacterium* are around 34 mol%, and the species which have G+C content of DNA higher than 39 mol% should be set apart from the genus *Flavobacterium*. On the other hand, the respiratory quinone pattern appears to be particularly useful for the purpose of subdividing the genus *Flavobacterium* (1, 17). The menaquinone system of the strains belonging to the genus *Flavobacterium* is MK-6, and strains with MK-7 can be excluded from the genus *Flavobacterium*. Yabuuchi et al. proposed the new genus *Sphingobacterium* for the strains named *Flavobacterium* having approximately 39 to 42 mol% G+C contents of DNA, isoprenoid quinones with MK-7 and sphingolipids with the long-chain base dihydrosphingosin, which are absent in other *Flavobacterium* species (30).

In this paper, we characterized 17 strains of *Cytophaga* and *Flavobacterium*. On the basis of the characteristics described above, all the strains studied were identified as organisms belonging to the genus *Sphingobacterium*, and were divided into 6 DNA homology groups. Each DNA relatedness group except for group 4, differed from previously-known species of this genus. Based on these DNA/DNA hybridization studies and the chemotaxonomic and physiological characteristics, we propose two new species, *S. faecium* for the group 2 strains and *S. piscium* for the group 3 strains, a new combination, *S. heparinum*, for the group 1 strain and two genospecies for group 5 and 6 strains. Strains of group 5 and 6 are genotypically distinct but are difficult to phenotypically differentiate from already-known species and other groups, and furthermore, only one strain was detected in the respective group until now. Therefore, we were reluctant to propose new species for these strains. We also propose transferring *F. thalpophilum* to the genus *Sphingobacterium* as a species of a new combination, *S. thalpophilum*. Differential characteristics among the species and two genospecies are listed in Table 6.

The new species *S. antarcticus*, recently proposed by Shivaji et al. (23), grows between 2 and 30°C and not at temperatures above 30°C. Strains of *S. piscium* grow at 5°C and not at 37°C. However, as shown in Table 6, *S. antarcticus* differs from *S. piscium* and other *Sphingobacterium* species in many physiological characteristics.

Steyn et al. (25) have recently reported the taxonomic relationship of heparinase-producing strains including the type strain of *C. heparina*. Their DNA/rRNA hybridization studies revealed that these isolates display considerable genotypic and phenotypic heterogeneity, and were divided into three rRNA similarity groups. They suggested that these strains probably constitute a separate genus from *Sphingobacterium*, *Flavobacterium* and *Cytophaga* because of *Tm*(*e*) of more than 7°C and separate protein electrophoretic profiles. However, we consider that *C. heparina* should be placed in the genus *Sphingobacterium* because of the homogeneity in morphological, physiological and chemotaxonomic characteristics among the strains mentioned above and already-known species of the genus *Sphingobacterium*. Much additional work, including analysis of 16S rRNA sequences between *S. heparina* and representatives of *Sphingobacterium* species, will be necessary to

Table 6. Differential characteristics among the seven *Sphingobacterium* species and two *Sphingobacterium* genospecies.

Characteristics	<i>S. heparinum</i> IFO 12017	<i>S. faecium</i> IFO 15299	<i>S. piscium</i> IFO 14985	<i>S. multivorum</i> IFO 14947	<i>S. spiritivorum</i> IFO 14948	<i>S. thalophilum</i> IFO 14963	<i>S. mizutae</i> IFO 14946	<i>Sphingo.</i> genosp. 1 KS 0507	<i>Sphingo.</i> genosp. 2 KS 0482	<i>S. antarcticus</i> ^a 4BY (MTCC ^c 675)
Growth at 5°C	+	+	+	— ^d	—	—	—	+	+	+
37°C	+	+	—	+	+	+	+	—	—	—
42°C	—	—	—	—	—	+	—	—	—	—
Nitrate reduction	—	—	—	—	—	—	+	—	—	—
Nitrite reduction	—	—	—	—	—	—	—	—	—	—
Gelatin hydrolysis	—	—	—	—	—	—	—	—	—	—
Urease	—	+	—	+	+	+	+	+	+	+
DNase	+	+	—	+	+	—	+	+	+	NT ^f
Enzyme activity (API ZYM)										
α-Galactosidase	+	+	+	+	+	+	—	+	+	NT
Valine arylamidase	—	+	+	+	+	+	+	+	+	NT
Chymotrypsin	—	+	—	—	+	—	—	—	—	NT
Acid production from ethanol	—	—	—	—	—	—	—	—	—	—
Acid production (API 50CH)	—	—	—	—	—	—	—	—	—	—
Melezitose	—	—	—	—	—	—	—	—	—	—
Salicin	—	+	v	+	v	+	+	+	+	—
D-Raffinose, trehalose	—	+	+	+	+	+	+	+	+	—
D-Arabitol	—	+	+	—	—	—	—	—	—	NT
D-Fructose	+	+	+	+	+	+	+	+	+	—
Sucrose	+	+	+	+	+	+	+	+	+	—
L-Arabinose	+	+	v	+	+	+	+	—	—	—
D-Xylose	+	—	v	+	+	+	+	—	—	—
Galactose	+	+	+	+	+	+	+	+	+	—
Maltose	+	+	+	+	+	+	+	+	+	—
Sorbitol	+	—	—	—	—	—	—	—	—	—
L-Fucose	+	+	+	+	—	+	—	+	+	NT
5-Keto-gluconate	+	+	—	—	—	—	—	—	—	NT

^a Data from Shivaji et al. (23), where Hugh and Leifson's medium was used for the determination of acid production.^b MTCC, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.^c +, all strains positive.^d —, all strains negative.^e v, variable.^f NT, not tested.

determine the relationship of this new species with respect to existing species of the *Flavobacterium-Cytophaga-Sphingobacterium* group.

In addition, chemotaxonomic data presented here and their DNA/rRNA hybridization studies (25) indicate that the type strain of *F. ferrugineum* probably constitutes a separate genus.

Holmes et al. (8) reported that *F. yabuuchiae* does not hydrolyze 2-naphthyl phosphate at pH 5.4, and this characteristic differentiates *F. yabuuchiae* from *F. spiritivorum* and other *Sphingobacterium* species, despite the high homology value of DNA/DNA hybridization (63%) between them. However, in our experiment, type strains of both species hydrolyzed 2-naphthyl phosphate at pH 5.4, and the two strains showed high similarity in whole-cell protein pattern in SDS-PAGE and physiological characteristics. Based on these results, we conclude that these two species are the same species, and therefore, *F. yabuuchiae* should be included in *S. spiritivorum*.

The re-classification of test strains is summarized in Table 1. Descriptions for the new species, new combination and new genospecies are given below.

Description of *Sphingobacterium faecium* sp. nov.

Sphingobacterium faecium (fae'ci.um. L. n. *faecis*, dregs; L. gen. pl. n. *faecium*, of the dregs, of feces) cells are gram-negative, nonsporing rods $0.4\text{--}0.5 \times 0.5\text{--}1.0$ μm . Nonmotile, nongliding (no sliding translocation occurs). Aerobic. Circular, entire, low convex, smooth and opaque colonies developing on nutrient agar after 2 days; on nutrient agar, a yellow or creamy white, nonfluorescent pigment is produced. Grows at 5°C and 37°C but not at 42°C. Catalase, oxidase, urease, extracellular deoxyribonuclease and phosphatase (alkaline and acid) are produced, but gelatinase is not produced. Indole is not produced. Esculin is hydrolyzed. Nitrate and nitrite are not reduced. D-Xylose, L-arabinose, D-glucose, D-fructose, cellobiose, maltose, sucrose, raffinose, starch and inulin are utilized. Heparin, succinoglucan, pectin, polypectate, cellulose, arginate, chitin and agar are not utilized. Acid is produced from salicin, trehalose, raffinose, D- and L-arabinose, L-fucose, inulin, melibiose and melezitose but not from L-xylose, sorbose, rhamnose, xylitol, mannitol, D-ribose or ethanol (3%). β -Glucosidase and valine arylamidase are produced but trypsin and β -glucuronidase are not. G+C content of DNA is 39.0 to 40.4 mol%. The major isoprenoid quinone is MK-7. The major non-polar fatty acids from whole cells are *i*-15:0 and 16:1 and 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

The type strain is IFO 15299 (KS 0470).

Description of *Sphingobacterium piscium* sp. nov.

Sphingobacterium piscium (pis'ci.um. L. n. *piscis*, a fish; L. gen. pl. *piscium*, of fishes) cells are gram-negative, nonsporing rods $0.4\text{--}0.5 \times 0.5\text{--}1.0$ μm . Nonmotile, nongliding (no sliding translocation occurs). Aerobic. Circular, entire, low convex, smooth and opaque colonies developing on nutrient agar after 2 days; on nutrient

agar, a yellow or creamy white, nonfluorescent pigment is produced. Grows at 5 °C but not at 37 °C or 42 °C. Catalase, oxidase, extracellular deoxyribonuclease and phosphatase (alkaline and acid) are produced, but gelatinase and urease are not. Indole is not produced. Esculin is hydrolyzed. Nitrate and nitrite are not reduced. D-Xylose, L-arabinose, D-glucose, D-fructose, cellobiose, maltose, sucrose, raffinose and inulin are not utilized. Acid is produced from salicin, trehalose and D-raffinose but not from L-fucose, D- and L-arabinose, melezitose or ethanol (3%). Esterase, valine arylamidase, trypsin and chymotrypsin are produced; α -galactosidase, α -fucosidase and β -glucuronidase are not. G + C content of DNA is 41.1 to 42.1 mol %. The major isoprenoid quinone is MK-7. The major non-polar fatty acids from whole cells are *i*-15:0 and 16:1 and 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

The type strain is IFO 14985 (JCM 7454).

Description of *Sphingobacterium heparinum* (Christensen) comb. nov.

Sphingobacterium heparinum (he.pa.ri'num. Gr. n. *hepar*, liver; M. L. neut. adj. *heparinum*, connected with degrading heparin, acidic mucoheteropolysaccharides with sulfate groups from various animal tissues) cells are gram-negative, nonsporing rods 0.4–0.5 × 0.5–1.0 µm. Nonmotile, nongliding (no sliding translocation occurs). Aerobic. Circular, entire, low convex, smooth and opaque colonies developing on nutrient agar after 2 days; on nutrient agar, a yellow or creamy white, nonfluorescent pigment is produced. Grows at 5 °C and at 37 °C but not at 42 °C. Catalase, oxidase, extracellular deoxyribonuclease and phosphatase (alkaline and acid) are produced, but gelatinase and urease are not. Indole is not produced. Esculin is hydrolyzed. Nitrate and nitrite are not reduced. D-Xylose, L-arabinose, D-glucose, D-fructose, cellobiose, maltose, sucrose, raffinose, starch and inulin, heparin and polypectate are utilized. Succinoglucan, pectin, cellulose, chitin and agar are not utilized. Acid is produced from D-xylose, L-fucose, 5-keto-gluconate and sorbitol but not from D- and L-arabinose, salicin, trehalose and D-raffinose, inulin, xylitol, melezitose, mannitol, D-ribose or ethanol (3%). β -Glucosidase, esterase (C₄) and α -galactosidase are produced, but cystine arylamidase, valine arylamidase, trypsin, chymotrypsin, β -glucuronidase and lipase (C₁₄) are produced. G + C content of DNA is 42.3 mol%. The major isoprenoid quinone is MK-7. The major non-polar fatty acids from whole cells are *i*-15:0, 16:0, 16:1, *i*-17:0 and 17:0 and 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

The type strain is IFO 12017 (ATCC 13125).

Description of *Sphingobacterium thalpophilum* (Holmes) comb. nov.

Sphingobacterium thalpophilum (thal.po'phi.lum. Gr. n. *thalps*, warmth; Gr. n. adj. *philon*, loving; M. L. n. adj. *thalpophilum*, warmth-loving) cells are gram-negative, nonsporing rods 0.4–0.5 × 0.5–1.0 µm. Nonmotile, nongliding (no sliding translocation occurs). Aerobic. Circular, entire, low convex, smooth and opaque

colonies developing on nutrient agar after 2 days; on nutrient agar, a yellow or creamy white, nonfluorescent pigment is produced. Grows at 37°C but not at 5°C or 42°C. Catalase, oxidase, urease and phosphatase (alkaline and acid) are produced, but gelatinase and extracellular deoxyribonuclease are not. Indole is not produced. Esculin is hydrolyzed. Nitrate is reduced to nitrite, but nitrite is not reduced. D-Xylose, L-arabinose, D-glucose, D-fructose, cellobiose, maltose, sucrose, raffinose, starch and inulin are utilized, but heparin is not utilized. Acid is produced from salicin, L-fucose, trehalose, inulin, D-raffinose, D- and L-arabinose and rhamnose but not from L-lyxose, sorbose, xylitol, mannitol, D-ribose or ethanol (3%). β -Glucosidase, esterase (C_4), cystine arylamidase, valine arylamidase, α -mannosidase and α -fucosidase are produced, but chymotrypsin, β -glucuronidase and lipase (C_{14}) are not. G+C content of DNA is 41.7 mol%. The major isoprenoid quinone is MK-7. The major non-polar fatty acids from whole cells are *i*-15:0 and 16:1 and 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

The type strain is IFO 14963 (ATCC 43320).

Description of *Sphingobacterium* genospecies 1 and 2

Two strains, KS 0507 and KS 0482, are assigned to genospecies 1 and 2, respectively. Cells are all gram-negative, nonsporing rods $0.4\text{--}0.5 \times 0.5\text{--}1.0\ \mu\text{m}$. Nonmotile, nongliding (no sliding translocation occurs). Aerobic. Circular, entire, low convex, smooth and opaque colonies developing on nutrient agar after 2 days; on nutrient agar, a yellow or creamy white, nonfluorescent pigment is produced, and possess all of the characteristics of the genus *Sphingobacterium*. The strain of genospecies 1, KS 0507, does not utilize D-xylose, L-arabinose, D-glucose, D-fructose, cellobiose, maltose, sucrose, raffinose, starch or inulin, but the strain of genospecies 2, KS 0482, does, and they do not utilize heparin, succinoglucan, pectin, polypectate, cellulose, chitin or agar. Both genospecies are positive for acid production from α -methyl-D-glucoside, amygdalin, arbutin, salicin, trehalose, β -gentiobiose, D- and L-arabinose, L-fucose, melibiose, melezitose, starch and glycogen but not xylitol, rhamnose, L-sorbose, mannitol or D-ribose. Both produced β -glucosidase, esterase (C_4), cystine arylamidase, α -mannosidase and α -fucosidase. KS 0482 produces α -galactosidase, valine arylamidase and β -glucuronidase, but KS 0507 does not. G+C content of DNA of KS 0507 and KS 0482 is 40.5 and 40.0 mol%, respectively. The major isoprenoid quinone is MK-7. The major non-polar fatty acids from whole cells are *i*-15:0 and 16:1 and 2-hydroxy *i*-15:0, 3-hydroxy *i*-15:0 and 3-hydroxy *i*-17:0.

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