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Flavobacterium spiritivorum, a New Species Isolated from Human Clinical Specimens

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A new species, *Flavobacterium spiritivorum*, is proposed. Each of the 13 strains placed in the new species was examined for 129 characteristics, including 58 enzyme reactions (API ZYM system). These bacteria were rod shaped, aerobic, gram negative, and nonmotile, and oxidized glucose in oxidation-fermentation medium. The mean guanine-plus-cytosine content of the deoxyribonucleic acids of six selected strains was 41.4 ± 0.4 mol%. A distinguishing feature of the new species is its ability to produce acid from various carbohydrates and alcohols. In particular, the ability of *F. spiritivorum* strains to produce acid from ethanol and mannitol distinguishes them from all other *Flavobacterium* species. Eleven strains of the new species were isolated from human clinical specimens, of which blood and urine were common sources. The type strain is E7288 (= NCTC 11386).

The name *Pseudomonas paucimobilis* was proposed (9) for various yellow-pigmented, clinically isolated bacteria which were received at the National Collection of Type Cultures for identification and which were similar to strains of Centers for Disease Control group IIk biotype 1 (26). The name *Flavobacterium multivorum* was proposed (10) for strains that belonged to group IIk biotype 2. The degree of relatedness between biotype 1 and biotype 2 strains was confused in the past because of their apparent overall phenotypic similarity (26). However, guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) (9, 10) and cellular fatty acid compositions (3) demonstrated that the two biotypes were not closely related and belonged in different genera.

The present study describes the characteristics of 13 strains that resemble those of group IIk biotype 2 yet differ from *F. multivorum*, and we present evidence that they constitute a new species, herein named *Flavobacterium spiritivorum*. The strains included in the new species were previously referred to as "group IIk, type 3" (8), because they seemed likely to provide the basis of a new taxon (10).

MATERIALS AND METHODS

Bacterial strains. The 13 bacterial strains studied and the sources from which they were isolated are given in Table 1.

Bacteriological investigations. The strains were maintained on nutrient agar (Oxoid nutrient broth powder CM 67, 25 g, and New Zealand Agar, 12 g, per liter of distilled water) under aerobic conditions and were tested at their optimal growth temperature, about 30°C. Colonial morphology was described from aerobic growth on nutrient agar, and hemolysis was determined from aerobic growth on 5% (vol/vol) horse blood agar. Pigmentation was recorded from growth on nutrient and tyrosine agars, and fluorescence in ultraviolet light was tested for on medium B of King et al. (15). The Gram reaction was determined by Lillie's modification as described by Cowan (2). Motility was tested by the hanging-drop method on overnight cultures grown at room temperature (18 to 22°C) and at 37°C in nutrient broth (Oxoid CM 67).

The biochemical characteristics investigated are listed in Tables 2, 3, and 4, and the methods used were described by Holmes et al. (6), but with the following changes or additions. Indole production was tested with Kovacs' reagent and with Ehrlich's reagent, the latter by method 3 of Cowan (2). Tributyrin hydrolysis was tested for on nutrient agar containing 1% (vol/vol) glycerol tributyrate, as described by Hayes (4). Phosphatase production was determined by method 1 of Cowan (2). Tests for the detection of 58 specific enzymes (listed in Tables 2–4) were performed with a commercially produced kit system (API System, La Balme-les-Grottes, 38390 Montalieu-Vercieu, France). The structure of the ZYM gallery, the choice of substrates, how the enzymes are detected, and the various applications of the system are fully described elsewhere (D. Monget, Ph.D. thesis, Université Claude Bernard, Lyon, France, 1978). Nineteen of the enzyme tests that constitute the standard API ZYM gallery are available commercially. The remaining 39 enzyme tests constitute four experimental API galleries—ZYM II, AP 1, AP 2, and AP 3. The following standardized procedure was adopted to ensure that results of the enzyme tests were comparable between strains. Each strain was grown overnight on a nutrient agar slant (in 150- by 15-mm test tubes) at 30°C. The bacteria were washed off the slant and suspended in 6 ml of distilled water (API System #2011) in a 125- by 12.5-mm test tube. The absorbance, at 550 nm, of the resulting bacterial suspension was measured in a Pye Unicam model SP 600 spectrophotometer to check that it was in the 1.0 to 2.0 range. Suspensions with an

TABLE 1. Strains of *F. spiritivorum* sp. nov. studied

Strain	Source
CL404/79	Blood, UK
CL48/80	Blood, UK
E3438	Blood, USA (Pennsylvania)
A14/65	Wound swab, UK
D3221	Sink trap in ambulance, USA (Michigan)
D3250	Humidifier sponge in ambulance, USA (Michigan)
D7039	Peritoneal fluid, USA (Maine)
D7211	Urine, USA (Kansas)
E8472	Urine, USA (Hawaii)
D7529	Sputum, USA (Maine)
E6209	Bone marrow, New Zealand
E6826	Vaginal swab, New Zealand
E7288	Intrauterine specimen, USA (Kansas)

TABLE 2. Characteristics in which all 13 strains of *F. spiritivorum* were positive

Acid from the following alcohols and carbohydrates (tested in an ammonium salt medium):	
Glucose	Mannitol
Cellobiose	Raffinose
Glycerol	Salicin
Ethanol	Sucrose
Fructose	Trehalose
Lactose	Xylose
Maltose	
Acid from 10% (wt/vol) glucose	
Catalase production	
Cytochrome-oxidase production	
Deoxyribonuclease production	
Esculin hydrolysis	
Gelatinase production (plate method)	
Growth at 37°C	
Growth at room temperature (18 to 22°C)	
Growth on MacConkey agar	
Growth on β-hydroxybutyrate	
Oxidative in Hugh and Leifson oxidation-fermentation test	
Phosphatase production	
Production of β-D-cellobiosidase[a]	
Tributyrin hydrolysis	
Tween 20 hydrolysis	
Tween 80 hydrolysis	
Urease production	
β-D-Galactosidase production	

[a] Tested using an API ZYM gallery.

absorbance outside this range were not used because of possible weak color reactions. An absorbance of just over 1.0 was considered by the manufacturer to be most suitable. Two drops of the suspension were inoculated into each cupule of the test galleries, which were incubated at 30°C for 4 h. Reagents were then added to each cupule according to the manufacturer's instructions. Positive results in 56 enzyme tests were denoted by a color change. In two additional tests in the ZYM II gallery, positive results were detected by fluorescence under an ultraviolet light source. After reading the results of tests 2 to 10 in the ZYM II gallery, test 1 in that gallery and all the tests in the other four galleries were exposed to a 1,000-W light source. The results were graded 0 to 5 according to the intensity of color change and as based on the interpretation scheme provided by the manufacturer. A positive result in the present paper included all grades of color change in the range 3 to 5 as recommended by the manufacturer.

All 13 strains were tested for susceptibility to antimicrobial agents by methods described previously (19). Two control strains of known susceptibility were included: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Median organism or centrostrain. The median organism (or centrostrain if there is no median organism) was determined by the method of Lapage and Willcox (16).

DNA isolation. Cultures were grown overnight at 30°C in nutrient broth with aeration at 200 rpm in an orbital incubator (Gallenkamp Ltd., London, U.K.). The bacterial cells were collected by centrifuging and were suspended in 0.15 M NaCl and 0.10 M ethylenediaminetetraacetic acid at pH 8.0. Pronase (Koch-Light) was added to a final concentration of 200 μg/ml, and the bacteria were lysed with 2% (wt/vol) sodium lauryl sulfate at 60°C for 15 min. The lysate was incubated overnight at 37°C and was then mixed thoroughly with 1 M sodium perchlorate and deproteinized with an equal volume of a 50:50 (vol/vol) mixture of phenol and chloroform. Purification of the DNA was completed essentially by the method of Marmur (17), but it was supplemented by treatment twice with 50 μg of ribonuclease per ml (bovine pancreas, BDH) and final precipitation with 2-ethoxyethanol.

Estimation of G+C content of DNA. The G+C contents of the DNAs were estimated from the thermal denaturation temperatures (T_m), which were determined by the method of Marmur and Doty (18). Thermal denaturation of DNA was carried out in a Pye Unicam SP 1800 spectrophotometer equipped with an SP 876 series 2 temperature program controller and heated cell block. The absorbance changes at 260 nm were recorded on a Pye Unicam AR 25 linear recorder. The temperature of the DNA solution was measured in the cuvette with a thermistor thermometer (5). The G+C content was calculated from the T_m determined in the 0.05 M NaCl buffered with 0.005 M trisodium citrate (0.33× SSC) at pH 7.0 by the equation: mol% G+C = 52.0 + [2.24(T_m − 84.4)]. This was derived by using the general equation of Owen et al. (20), in which the G+C content was expressed relative to reference DNA from *E. coli* B NCTC 10537 (52.0 mol% G+C).

RESULTS

Phenotypic characteristics of *F. spiritivorum*. The 13 strains were strictly aerobic, gram-negative, uniformly stained rods, 1.0 μm in length, with parallel sides and rounded ends. The colonies on nutrient agar, after 48 h, were circular, 1 to 3 mm in diameter (except D7529, which formed pinpoint colonies), and low convex, smooth, and opaque. A pale yellow pigment was produced by all strains except A14/65 and

TABLE 3. Characteristics in which all 13 strains of *F. spiritivorum* were negative

Acid from the following alcohols (tested in an ammonium salt medium):
Adonitol Inositol
Dulcitol Sorbitol
Alkali production on Christensen's citrate
Arginine desimidase
Arginine dihydrolase
Casein digestion
Fluorescence on King medium B
Gas from glucose in peptone water sugar
Gelatin liquefaction
Gluconate oxidation
Growth at 5 and 42°C
Growth on cetrimide
Growth on Simmons' citrate
Hydrogen sulfide production[a]
Indole production[b]
Lysine decarboxylase
Malonate utilization
Motility (at both 37°C and room temperature)
Nitrate and nitrite reduction
Opalescence on lecithovitellin agar
Ornithine decarboxylase
Phenylalanine deamination
Pigment production on tyrosine agar
Poly β-hydroxybutyrate inclusion granules
Production of the following enzymes[c]:
Esterase (C-4)[d]
Lipase (C-14)[d]
L-Valyl-2-napthylamide hydrolase[d]
L-Cystyl-2-naphthylamide hydrolase[d]
N-Glutaryl-L-phenylalanyl-2-naphthylamide hydrolase[d]
α-D-Galactosidase[d]
β-D-Glucuronidase[d]
α-D-Mannosidase[d]
α-D-Xylosidase
β-D-Fucosidase
β-L-Fucosidase
N-Acetyl-α-D-glucosaminidase
Lactosidase
L-Tyrosyl-2-naphthylamide hydrolase
L-Phenylalanyl-2-naphthylamide hydrolase
L-Hydroxyprolyl-2-naphthylamide hydrolase
N-Benzoyl-L-leucyl-2-naphthylamide hydrolase
L-Isoleucyl-2-naphthylamide hydrolase
L-Prolyl-2-naphthylamide hydrolase
Reduction of 0.4% (wt/vol) selenite
Starch hydrolysis
Tyrosine hydrolysis
3-Ketolactose production

[a] By both lead acetate paper and triple sugar iron agar (TSI) methods.
[b] Tested with Kovács' and Ehrlich's reagent.
[c] Tested using various API ZYM galleries.
[d] Enzyme test included in the standard API ZYM gallery.

CL404/79, which were nonpigmented; the pigment neither diffused in nutrient agar nor fluoresced on King's medium B when exposed to ultraviolet light. None of the strains produced a dark-brown pigment on tyrosine agar. The strains grew optimally at 30°C, and all biochemical tests, unless specified otherwise, were carried out at this temperature. The strains grew at room temperature and at 37°C but not at 5 or 42°C. Hemolysis was not present after aerobic growth for 1 day on 5% (vol/vol) horse blood agar.

The biochemical characteristics of the strains are listed in Tables 2 to 4. Eighty-five characteristics were common to all strains (either all positive or all negative), and there were 44 characteristics in which one or more of the strains differed. Strain E7288 proved to be the centrostrain, as there was no median organism. Strains D3221 and E3438 were the least typical members of *F. spiritivorum*, and each differed from the other 12 strains in two, but not the same two, biochemical characteristics. In Table 2 it should be noted that all strains of *F. spiritivorum* produced phosphatase (method 1 of Cowan [2]) and β-D-galactosidase (2) by conventional methods, whereas in the API ZYM method, only one strain produced phosphatase and only one strain produced β-D-galactosidase (Table 4). These discrepancies between the test methods were attributed to the fact that, in the API ZYM method, only grades of color change in the range 3 to 5 were treated as positive. However, in the phosphatase production (API ZYM) test, 12 of 12 strains scored as negative produced a slight color change (grades 1 and 2), and in the β-D-galactosidase production (API ZYM) test, 10 of 12 strains scored as negative produced a color change (grades 1 and 2). The susceptibilities of the strains to antimicrobial agents are listed in Table 5. All 13 strains were resistant to therapeutic levels of ampicillin, carbenicillin, cephalothin, amikacin, gentamicin, kanamycin, tobramycin, and tetracycline; four strains were susceptible to chloramphenicol; and all were susceptible to rifampin.

G+C content of *F. spiritivorum* DNA. Table 6 lists the G+C contents of the DNAs of six phenotypically representative strains of *F. spiritivorum*. The values were between 40.8 and 41.9 mol%, with a mean of 41.4 mol% and a standard deviation of ±0.4 mol%.

DISCUSSION

The results presented above indicate that the 13 strains of gram-negative, yellow-pigmented bacteria for which the name *Flavobacterium spiritivorum* (spi.ri.ti'vo.rum. L. noun *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) is here proposed constitute a homogeneous taxon. This new species conforms to the defini-

TABLE 4. Characteristics in which the 13 strains of *F. spiritivorum* differ from one another

Strain differences	No. of strains positive	Result of type strain (E7288)	Reference no. of strains that gave the less common result
Alkaline phosphatase production[a,b]	12/13	+	E3438
Phosphoamidase production[a,b]	12/13	+	D7529
Acid from glucose in peptone water sugar	11/13	+	CL404/79, A14/65
Acid from 10% (wt/vol) lactose	11/13	+	A14/65, D7211
Production of yellow pigment	11/13	+	CL404/79, A14/65
Acid phosphatase production[a,b]	11/13	+	D7039, D7529
L-Histidyl-2-naphthylamide hydrolase production[a]	11/13	+	D7039, E6209
α-L-Aspartyl-2-naphthylamide hydrolase production[a]	11/13	+	D7039, E6209
α-D-Glucosidase production[a,b]	10/13	+	D7529, E3438, E8472
N-Acetyl-β-D-glucosaminidase production[a,b]	10/13	+	D7211, D7529, E6826
L-Alanyl-2-naphthylamide hydrolase production[a]	10/13	+	D7529, E6209, E6826
N-Benzoyl-DL-arginyl-2-naphthylamide hydrolase production[a,b]	9/13	+	D3250, D7529, E3438, E6209
L-Lysyl-2-naphthylamide hydrolase production[a]	9/13	+	D7529, E3438, E6209, E8472
Glycyl-2-naphthylamide hydrolase production[a]	9/13	+	D7039, D7529, E3438, E6209
L-Arginyl-2-naphthylamide hydrolase production[a]	9/13	+	D7039, D7529, E6209, E6826
L-Leucyl-glycyl-2-naphthylamide hydrolase production[a]	9/13	+	D7039, D7529, E6826, E8472
N-Carbobenzoxy-L-arginyl-4-methoxyl-2-naphthylamide hydrolase production[a]	9/13	−	CL404/79, D7039, E3438, E7288
Glycyl-L-prolyl-2-naphthylamide hydrolase production[a]	8/13	+	D7039, D7529, E6209, E6826, E8472
L-Glutaminyl-2-naphtylamide hydrolase production[a]	8/13	+	D7039, D7529, E3438, E6209, E6826
α-L-Glutamyl-2-naphthylamide hydrolase production[a]	8/13	+	D7529, E3438, E6209, E6826, E8472
N-Carbobenzoxy-glycyl-glycyl-L-arginyl-2-naphthylamide hydrolase production[a]	8/13	+	D7039, D7529, E3438, E6209, E8472
Esterase-lipase (C-8) production[a,b]	7/13	−	D3250, E3438, E6209, E6826, E7288, E8472
L-Glycyl-glycyl-2-naphthylamide hydrolase production[a]	7/13	−	CL48/80, D7039, D7529, E6209, E6826, E7288
Exo-1, 4-β-D-xylosidase production[a]	6/13	−	CL404/79, A14/65, D3221, D3250, E6209, E8472
α-L-Arabinofuranosidase production[a]	6/13	+	CL48/80, D3221, D7529, E6209, E7288, E8472
L-Methionyl-2-naphthylamide hydrolase production[a]	6/13	−	CL404/79, CL48/80, A14/65, D3221, D3250, D7211
L-Seryl-tyrosyl-2-naphthylamide hydrolase production[a]	6/13	−	CL404/79, CL48/80, A14/65, D3250, D7211, E3438
L-Ornithyl-2-naphthylamide hydrolase production[a]	6/13	−	CL404/79, CL48/80, A14/65, D3221, D3250, D7211
L-Seryl-2-naphthylamide hydrolase production[a]	6/13	−	CL404/79, A14/65, D3221, D3250, D7211, E6209
L-Tryptophyl-2-naphthylamide hydrolase production[a]	6/13	−	CL404/79, CL48/80, A14/65, D3221, D3250, D7211
Arylsulfatase production[a]	4/13	−	CL48/80, A14/65, E3438, E6826
γ-L-Glutamyl-2-naphthylamide hydrolase production[a]	4/13	−	CL404/79, A14/65, D3250, D7211
S-Benzyl-L-cysteyl-2-naphthylamide hydrolase production[a]	4/13	−	CL404/79, A14/65, D3250, D7211
L-Threonyl-2-naphthylamide hydrolase production[a]	4/13	−	CL404/79, A14/65, D3250, D7211

TABLE 4—*Continued*

Strain differences	No. of strains positive	Result of type strain (E7288)	Reference no. of strains that gave the less common result
Acid from arabinose (ammonium salt sugar medium)	3/13	–	D3250, E6209, E8472
L-Leucyl-2-naphthylamide hydrolase production[a,b]	3/13	–	CL404/79, CL48/80, A14/65
α-L-Fucosidase production[a,b]	3/13	–	CL48/80, A14/65, D3250
Glycyl-L-phenylalanyl-2-naphthylamide hydrolase production[a]	2/13	–	CL404/79, A14/65
Acid from rhamnose (ammonium salt sugar medium)	1/13	–	A14/65
KCN tolerance	1/13	–	E8472
β-D-Galactosidase production[a,b]	1/13	–	D3221
β-D-Glucosidase production[a,b]	1/13	–	D3221
Phosphatase production[a]	1/13	–	E3438
L-Pyroglutamyl-2-naphthylamide hydrolase production[a]	1/13	–	D3250

[a] Tested using various API ZYM galleries.
[b] Enzyme test included in the standard API ZYM gallery.

tion of *Flavobacterium* as emended by Holmes and Owen (7), although members of the species were not markedly yellow pigmented and they were not as actively proteolytic as most species in the genus (i.e., *F. balustinum*, "*F. breve*," *F. meningosepticum*, and *F. odoratum*). Furthermore, the G+C values of 40 to 42 mol% obtained for *F. spiritivorum* were higher than the values of other recognized species of *Flavobacterium* (see Table 7). These results indicate that the G+C range of *Flavobacterium* (7) should be widened to 31 to 42 mol%. Characteristics for the practical identification of the new species are recorded in Table 7. *P. paucimobilis* is included in Table 7 because it has biochemical properties similar to those of *F. multivorum* and *F. spiritivorum*. Table 7 shows that the new species was most similiar to *F. multivorum*. There were consistent differences between *F. multivorum* and *F. spiritivorum* in two conventional tests (production of acid, in ammonium salt medium, from ethanol and from mannitol) and in one API ZYM test (production of L-phenylalanyl-2-naphthylamide hydrolase). There was a small but significant (>2 × standard deviation) difference in the mean G+C contents of the two species (39.6 mol% G+C for *F. multivorum* compared with 41.4% G+C for *F. spiritivorum*).

Most strains of *F. spiritivorum* were isolated from clinical specimens, of which blood and urine were the most common sources. Their clinical significance cannot be assessed at present, but their resistance to a wide range of antimicrobial agents indicates that any infections in humans due to such bacteria could prove difficult to treat. Resistance to antimicrobial agents is a characteristic that *F. spiritivorum* shares with most other *Flavobacterium* species (1, 10–14, 27).

Description of the type strain. The characteristics of the type strain, E7288 (= NCTC 11386), also listed in Tables 2 through 6, are as follows:

Gram-negative, nonsporeforming rods, 1.0 μm in length, of regular shape with rounded ends. Nonmotile in hanging-drop preparations after overnight growth in nutrient broth incubated at either 37°C or room temperature.

Circular, low convex, smooth, and opaque colonies developing on nutrient agar after 2 days; colonies nonhemolytic on 5% (vol/vol) horse blood agar; on nutrient agar, a yellow, nonfluorescent pigment is produced. There is no production of brown pigment on tyrosine agar.

Aerobic.

Growth at 37°C but not at 5 or 42°C. Optimal temperature: about 30°C.

Production of catalase, cytochrome oxidase, and deoxyribonuclease.

Production of urease.

No toleration of KCN at a concentration of 0.0075% (wt/vol). Hydrolysis of Tween 20 and Tween 80.

No production of opalescence on lecithovitellin agar.

No reduction of nitrate to nitrite; no reduction of nitrite.

No production of indole or hydrogen sulfide.

Growth on β-hydroxybutyrate (without production of lipid inclusion granules) and on MacConkey agar, but not on cetrimide agar.

No digestion of casein.

Hydrolysis of esculin, gelatin, and tributyrin, but not starch or tyrosine.

No utilization of citrate and malonate.

TABLE 5. Susceptibilities of 13 strains of *F. spiritivorum* to antimicrobial agents

Antimicrobial agent	Minimal inhibitory concn (μg/ml) for:													
	F. spiritivorum strain												*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
	CL404/79	CL48/80	A14/65	D3221	D3250	D7039	D7211	D7529	E3438	E6209	E6826	E7288	E8472	
Ampicillin	32	32	32	32	32	32	32	32	32	32	32	32	16	4	>32
Carbenicillin	128	512	128	128	128	128	256	128	128	128	128	128	128	8	32
Cephalothin	>32	>32	>32	32	>32	>32	>32	>32	>32	>32	>32	>32	32	8	>32
Amikacin	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	1	4
Gentamicin	16	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	≤0.5	2
Kanamycin	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	2	>64
Tobramycin	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	0.5	1
Chloramphenicol	32	8	16	32	32	32	32	32	32	16	>32	32	8	4	>32
Tetracycline	16	>16	16	>16	>16	>16	>16	8	8	>16	>16	16	8	2	>16
Rifampin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.5	8	>32

TABLE 6. DNA base compositions of six strains of *F. spiritivorum*

Strain	Buffer[a]	T_m ± SD[b] (°C)	G+C content (mol%)
E8472	0.33× SSC	78.2 ± 0.20	40.8[c]
E7288	0.33× SSC	78.3 ± 0.20	41.0
CL48/80	0.33× SSC	78.4 ± 0.00	41.3
E6209	0.33× SSC	78.4 ± 0.10	41.3
A14/65	SSC	86.5 ± 0.05	41.8[d]
CL404/79	0.33× SSC	78.7 ± 0.30	41.9
Mean ± SD			41.4 ± 0.4

[a] Compositions of the buffers used: 0.33× SSC, 0.05 M NaCl and 0.005 M trisodium citrate; SSC, 0.15 M NaCl and 0.015 M trisodium citrate.

[b] The thermal denaturation temperature (T_m) is the mean of two or three determinations and the standard deviation (SD).

[c] The equation used to calculate G+C content was: mol% G+C = 52.0 + [2.24(T_m − 83.2)].

[d] This value was published previously (9).

No oxidation of gluconate.

No production of arginine desimidase, arginine dihydrolase, lysine decarboxylase, or ornithine decarboxylase.

No produciton of 3-ketolactose.

No reduction of selenite and no deamination of phenylalanine.

Production of phosphatase and β-D-galactosidase (when tested, respectively, by method 1 of Cowan [2] and the method given by Holmes et al. [6]).

Production of acid in ammonium salt medium under aerobic conditions from glucose, cellobiose, glycerol, ethanol, fructose, lactose, maltose, mannitol, raffinose, salicin, sucrose, trehalose, and xylose.

Production of acid from 10% (wt/vol) glucose and 10% (wt/vol) lactose.

No production of acid in ammonium salt medium under aerobic conditions from adonitol, arabinose, dulcitol, inositol, rhamnose, or sorbitol.

Production of acid, but not gas, from glucose in peptone-water medium; production of acid in only the open tube of Hugh and Leifson oxidation-fermentation glucose medium.

Production of the following enzymes (using various API ZYM galleries): alkaline phosphatase, *N*-benzoyl-DL-arginyl-2-naphthylamide hydrolase, acid phosphatase, phosphoamidase, α-D-glucosidase, *N*-acetyl-β-D-glucosaminidase, α-L-arabinofuranosidase, β-D-cellobiosidase, L-lysyl-2-naphthylamide hydrolase, L-histidyl-2-naphthylamide hydrolase, glycyl-2-naphthylamide hydrolase, α-L-aspartyl-2-naphthylamide hydrolase, L-arginyl-2-naphthylamide hydrolase, L-alanyl-2-naphthylamide hydrolase, glycyl-L-prolyl-2-naphthylamide hydrolase, L-leucyl-glycyl-2-

TABLE 7. Characteristics for the practical identification and differentiation of *F. spiritivorum* from other *Flavobacterium* taxa and from *P. paucimobilis*[a]

Test	*F. spiritivorum* (13 strains)	*F. balustinum* (1 strain)	"*F. breve*"[b] (7 strains)	*F. meningosepticum* (49 strains)	*F. multivorum*[c] (28 strains)	*F. odoratum*[d] (28 strains)	*Flavobacterium* sp. group IIb (55 strains)	*P. paucimobilis*[e] (29 strains)
Acid from ASS[f] glucose	+	+	6/7	42/49	+	−	+	+
Acid from ASS arabinose	3/13[g]	−	−	1/49	+	−	13/55	+
Acid from ASS cellobiose	+	−	−	4/49	+	−	3/55	+
Acid from ASS ethanol	+	+	−	28/49	−	−	9/55	26/29
Acid from ASS glycerol	+	−	−	38/49	27/28	−	35/55	6/29
Acid from ASS lactose	+	−	−	27/49	+	−	−	+
Acid from ASS maltose	+	−	6/7	46/49	+	−	+	+
Acid from ASS mannitol	+	−	−	31/49	−	−	3/55	−
Acid from ASS raffinose	+	−	−	−	+	−	−	28/29
Acid from ASS salicin	+	−	−	−	+	−	1/55	26/29
Acid from ASS sucrose	+	−	−	−	+	−	12/55	+
Acid from ASS trehalose	+	−	−	42/49	+	−	48/55	+
Acid from ASS xylose	+	−	−	3/49	+	−	9/55	+
Casein digestion	−	+	+	+	−	+	+	−
Esculin hydrolysis	+	+	−	47/49	+	−	52/55	+
Gelatinase production (plate method)	+	+	+	+	4/28	+	+	3/29
Growth on MacConkey agar	+	+	+	+	+	+	51/55	−
Indole production (Ehrlich's reagent)	−	+	+	24/49	−	−	53/55	−
Motility at room temperature	−	−	−	−	−	−	−	22/29
Nitrite reduction	−	−	−	18/49	−	+	14/55	−
Poly-β-hydroxybutyrate inclusion granules	−	−	−	−	−	−	−	25/29
Starch hydrolysis	−	−	−	−	−	−	36/55	18/29
Urease production	+	−	−	16/49	27/28	+	11/55	−
β-D-Galactosidase production	+	−	−	48/49	+	−	15/55	+
Mol% G+C ± standard deviation	41.4 ± 0.4 (6)	33.1 (1)	32.4 ± 0.6 (10)	37.0 ± 0.5 (8)	39.6 ± 0.5 (11)	31.4–36.1 (10)	35.0–38.5 (13)	65.3 ± 1.0 (12)

[a] Key: +, All strains tested positive; −, all strains tested negative. The phenotypic results for *F. spiritivorum* were from this study, and the phenotypic results for the other taxa were derived from previous work in the National Collection of Type Cultures: *F. balustinum* (B. Holmes and R. J. Owen, unpublished data), "*F. breve*" (12), *F. meningosepticum* (Holmes and Owen, unpublished data), *F. multivorum* (10), *F. odoratum* (13), *Flavobacterium* sp. group IIb (Holmes and Owen, unpublished data), and *P. paucimobilis* (9). The G+C values for *F. spiritivorum* were obtained in this study, and the results on the other taxa were derived as follows: *F. balustinum* (22), "*F. breve*" (22), *F. meningosepticum* (23, 24), *F. multivorum* (10), *F. odoratum* (21), *Flavobacterium* sp. group IIb (Holmes and Owen, unpublished data), and *P. paucimobilis* (9). The numbers of strains tested in each species for mol% G+C are given in parentheses.

[b] The name "*F. breve*" is in quotation marks because it is not on the Approved Lists of Bacterial Names (25) and therefore has no standing in the nomenclature. However, a proposal has been made to effect revival of "*F. breve*" with NCTC 11099 as the type strain (Holmes and Owen, submitted for publication).

[c] Formerly known as group IIk, biotype 2 (10, 26).

[d] Formerly known as group M-4f (11, 26).

[e] Formerly known as group IIk, biotype 1 (9, 26).

[f] ASS, Ammonium salt sugar medium.

[g] Number of strains showing characteristic/number of strains tested.

naphthylamide hydrolase, L-glutaminyl-2-naphthylamide hydrolase, α-L-glutamyl-2-naphthylamide hydrolase, *N*-carbobenzoxy-glycyl-L-arginyl-2-naphthylamide hydrolase.

No production of the following enzymes (using various API ZYM galleries): esterase (C-4), esterase-lipase (C-8), lipase (C-14), L-leucyl-2-naphthylamide hydrolase, L-valyl-2-naphthylamide hydrolase, L-cystyl-2-naphthylamide hydrolase, *N*-glutaryl-L-phenylalanyl-2-naphthylamide hydrolase, α-D-galactosidase, β-D-galactosidase, β-D-glucuronidase, β-D-glucosidase, α-D-mannosidase, α-L-fucosidase, exo-1,4-β-D-xylosidase, phosphatase, α-D-xylosidase, β-D-fucosidase, β-L-fucosidase, *N*-acetyl-α-D-glucosaminidase, lactosidase, arylsulfatase, L-tyrosyl-2-naphthylamide hydrolase, L-pyroglutamyl-2-naphthylamide hydrolase, L-phenylalanyl-2-naphthylamide hydrolase, L-hydroxyprolyl-2-naphthylamide hydrolase, γ-L-glutamyl-2-naphthylamide hydrolase, *N*-benzoyl-L-leucyl-2-naphthylamide hydrolase, *S*-benzyl-L-cysteyl-2-naphthylamide hydrolase, L-methionyl-2-naphthylamide hydrolase, L-glycyl-glycyl-2-naphthylamide hydrolase, glycyl-L-phe-nylal-alanyl-2-naphthylamide hydrolase, L-seryl-tyrosyl-2-naphthylamide hydrolase, *N*-carbobenzoxy-L-arginyl-4-methoxyl-2-naphthylamide hydrolase, L-isoleucyl-2-naphthylamide hydrolase, L-ornithyl-2-naphthylamide hydrolase, L-prolyl-2-naphthylamide hydrolase, L-seryl-2-naphthylamide hydrolase, L-threonyl-2-naphthylamide hydrolase, L-tryptophyl-2-naphthylamide hydrolase.

Resistant to ampicillin, carbenicillin, cephalothin, amikacin, gentamicin, kanamycin, tobramycin, chloramphenicol, and tetracycline. Susceptible to rifampin.

G+C content of DNA: 41.0 mol% (thermal denaturation temperature estimation).

Source: isolated from an intrauterine specimen.

Two additional strains of *F. spiritivorum* have been deposited in the National Collection of Type Cultures: A14/65 as NCTC 11387 and CL48/80 as NCTC 11388.

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REPRINT REQUESTS

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