Flavobacterium multivorum, a New Species Isolated from Human Clinical Specimens and Previously Known as Group IIk, Biotype 2

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Thirty-two gram-negative bacterial strains, most of which were yellow-pigmented and isolated from clinical sources, were compared with 17 culture collection strains representing 13 named strains of four *Flavobacterium* species and 4 unnamed strains of *Flavobacterium* species. Twenty-nine of the strains were originally received as or subsequently assigned to group IIk, biotype 2. All 49 strains were examined for 127 characteristics, including reactions to 58 enzyme tests (API ZYM system), and the data were analyzed by numerical taxonomic methods involving estimation of Gower's general similarity coefficient and clustering by unweighted pair-group average linkage and single-linkage analyses. Twenty-eight of the group IIk, biotype 2, strains formed a single, well-defined cluster (92.0% similarity level). These 28 strains were aerobic, gram-negative, nonmotile, oxidative rods; the mean base composition of the deoxyribonucleic acids of 11 strains was 39.6 ± 0.5 mol% guanine plus cytosine. Distinctive features of the 28 strains were the ability to produce acid from a range of carbohydrates and their resistance to antimicrobial agents. The 28 strains were classified in the genus *Flavobacterium*, but they could not be assigned to any previously defined species. Therefore a new species, with the name *Flavobacterium multivorum*, is proposed for these 28 strains; the type strain is B5533 (= NCTC 11343).

Tatum et al. (25) described 140 strains of gram-negative, yellow-pigmented, rod-shaped bacteria thought to resemble *Pseudomonas* or possibly *Xanthomonas* species isolated from human and other sources of clinical interest. These bacteria were referred to as "group IIk," and two biotypes were recognized (although the word "biovar" is now recommended in place of "biotype" [16], we continue to use "biotype" here because of its historical association with group IIk). The 100 strains of biotype 1 were deeply yellow pigmented, whereas the 40 strains of biotype 2 were usually pale to light yellow. The motility observations of the group IIk strains in semisolid agar medium were sometimes equivocal; hence, wet-mount preparations were recommended. Five percent of the biotype 2 strains were reported as motile, and for both biotypes of group IIk, strains were described as monotrichous (25).

Holmes et al. (10) studied various yellow-pigmented isolates originally received at the National Collection of Type Cultures (NCTC), London, United Kingdom, for identification and showed, by numerical analysis of phenotypic characteristics, that 27 strains formed a cluster with two reference strains of group IIk, biotype 1. The name *Pseudomonas paucimobilis* was proposed by Holmes et al. (10) for these 29

strains because most motile strains contained few actively motile cells. Motile strains had a single polar flagellum. Holmes et al. excluded two reference strains of group IIk, biotype 2, and two biochemically similar strains from the *P. paucimobilis* cluster. Deoxyribonucleic acid (DNA) base compositions (moles percent guanine plus cytosine [G+C] provided clear evidence that biotypes 1 and 2 were unrelated: the biotype 2 strains had G+C contents of 39.6 mol%, whereas those of *P. paucimobilis* (biotype 1) were notably higher (ca. 65.0 mol%). The biotype 2 strains were also resistant to a wider range of antibiotics than were the biotype 1 strains. The lack of motility and the G+C contents of 39% of the group IIk, biotype 2, strains was confirmed by E. Yabuuchi, I. Yano, E. Tanimura, and A. Ohyaama [Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)32, p. 351], who believed, on the basis of these characteristics as well as of 90 phenotypic tests and cellular lipid analyses, that biotype 2 should not be regarded as *Pseudomonas*-like but should be placed in a "new" genus. Dees et al. (3) reported that group IIk, biotype 2, was readily distinguishable from *P. paucimobilis* by cellular fatty acids and suggested that it might be an additional species of *Flavobacterium*.

In the present study, a numerical analysis of

phenotypic characteristics, determinations of DNA base compositions, and determinations of susceptibilities to antimicrobial agents were used to investigate the taxonomic relationships between group IIk, biotype 2, strains and species of the genus *Flavobacterium* as redefined by Holmes and Owen (9). Evidence is presented that 28 of 29 strains originally assigned at the Centers for Disease Control (Atlanta, Ga.), NCTC, or Madigan Army Medical Center (Tacoma, Wash.) to group IIk, biotype 2, constitute *Flavobacterium multivorum*, a new species.

MATERIALS AND METHODS

Bacterial strains. The 49 bacterial strains studied (Table 1) comprised: 28 strains of *F. multivorum*, 4 unnamed strains resembling *F. multivorum*, and 17 culture collection strains representing named and unnamed *Flavobacterium* species.

Bacteriological investigations. The strains were maintained on nutrient agar (25 g of Oxoid nutrient broth powder CM 67 [Oxoid Ltd., Basingstoke, United Kingdom], and 12 g of New Zealand agar per liter of distilled water) under aerobic conditions and were tested at their optimum growth temperatures, usually 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 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 through 4, and the methods used have been described by Holmes et al. (8), but the following changes or additions were made. Indole production was tested with Kovács reagent and with Ehrlich 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 (6). Phosphatase production was determined by method 1 of Cowan (2). Tests for the production of 58 specific enzymes (listed in Tables 2 through 4) were performed with a commercially produced kit system (API System, La Balme-les-Grottes, Montalieu-Vercieu, France). The structure of the API 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, Lyons, France, 1978). Nineteen of the enzyme tests that constitute the standard API ZYM gallery are widely available. The remaining 39 enzyme tests constitute four experimental API galleries: ZYM II, AP 1, AP 2, and AP 3. A 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 6- by 5/8-in. [ca. 15.2 by 1.6-cm] 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 5 by 1/2-in. (ca. 12.7- by 1.2-cm) 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 range 1.0 to 2.0. Suspensions with an 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 was 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 through 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 through 5 according to the intensity of color change and 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.

Susceptibilities to antimicrobial agents were tested in selected strains by an agar dilution method. Twofold dilutions of antimicrobial agents were added to Diagnostic Sensitivity Test agar (Oxoid CM 261) to give a range of concentrations of 128 to 0.006 µg/ml. Lysed horse blood (2%, vol/vol) was added to medium containing sulfonamide or co-trimoxazole. The medium was dispensed into petri dishes, which were inoculated, using a replicating device, with 0.003-ml volumes of overnight broth cultures of strains diluted to contain about 10⁵ cells per ml. Each plate was also inoculated with three control strains of known susceptibilities: *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662, and *Staphylococcus aureus* NCTC 6571. The minimum inhibitory concentration of an antimicrobial agent for each strain was defined as the lowest concentration completely inhibiting growth after overnight incubation at 30°C.

Computation of data. A total of 127 characteristics (listed in Tables 2 through 4) were used in the numerical analysis. The majority of tests were treated as two-state characters (all-or-none characters, such as present or absent, which may be symbolized by + and -), and the results were coded as 1 (+) or 0 (-) (moderately positive results, recorded as ±, were treated as +; weakly positive results, recorded as ∓, were treated as -). For two-state related tests, implied negative results were coded as no comparison (24). The Hugh and Leifson oxidation-fermentation test was treated as a qualitative multistate character (states cannot be ordered; in a series, such as A, B, C, one can only say that any two are the same or different; there is no implication that the difference between A and B is greater or less than that between A and C, for example). The test results for gelatin hydrolysis (the stab and plate methods) were combined to give a single quantitative multistate character (has states that may be ordered in magnitude: 0, both methods negative; 1, plate only positive; 2, both methods posi-

TABLE 1. *Strains used in this study*

Strain no. ^a	Received as (name or other designation):	Source of isolation	
		Anatomic	Geographic ^b
Strains assigned to <i>F. multivorum</i> :			
CL201/75	Unidentified	Human clinical	United Kingdom
CL23/79 ^c	Group IIk, biotype 2	Lung (autopsy)	Washington
CL24/79 ^c	Group IIk, biotype 2	Eye	Washington
CL25/79 ^c	Group IIk, biotype 2	Serum	Washington
CL26/79 ^c	Group IIk, biotype 2	Throat	Washington
CL406/79 ^d	Unidentified	Peritoneal fluid	Illinois
8354	Group IIk, biotype 2	Blood	Oregon
A561	Group IIk, biotype 2	Abdominal fluid	New York
A1327	Group IIk, biotype 2	Blood	Connecticut
A7317	Group IIk, biotype 2	Urine	Wisconsin
A7371	Group IIk, biotype 2	Blood	New York
A7543	Group IIk, biotype 2	Throat	Missouri
A7810	Group IIk, biotype 2	Skin	Texas
A8895 (= NCTC 11033)	Group IIk, biotype 2	Serous cavity fluid	Georgia
B2623	Group IIk, biotype 2	Blood	Washington
B3159 (= NCTC 11034)	Group IIk, biotype 2	Blood	New Jersey
B3392	Group IIk, biotype 2	Blood	Illinois
B5147	Group IIk, biotype 2	Urine	Texas
B5367	Group IIk, biotype 2	Cerebrospinal fluid	Egypt
B5533 (= NCTC 11343) ^e	Group IIk, biotype 2	Spleen	Washington
B5759	Group IIk, biotype 2	Abdominal hematoma	Zaire
B9042	Group IIk, biotype 2	Catheter tip, kidney transplant	Texas
D3833	Group IIk, biotype 2	Wound	Georgia (probably)
D8510	Group IIk, biotype 2	Blood	New York
D8864	Group IIk, biotype 2	Urine	Washington
E2786	Group IIk, biotype 2	Eye discharge	Pennsylvania
E3089	Group IIk, biotype 2	Cerebrospinal fluid	Missouri
E3540	Group IIk, biotype 2	Blood	Pennsylvania
Unnamed strains resembling <i>F. multivorum</i> :			
CL404/79	Unidentified	Blood	United Kingdom
A14/65	Unidentified	Wound swab	United Kingdom
E894	Unidentified	Unknown	United Kingdom
8291	Group IIk, biotype 2	Blood	Louisiana
CIP 12.75	<i>Flavobacterium</i> sp.	Unknown	Unknown
Reference strains:			
La 724 (= NCTC 11212) ^e	<i>F. balustinum</i>	Heart blood of fish	France
CL88/76 (= NCTC 11099)	" <i>F. breve</i> "	Bronchial secretion	Switzerland
CL626/75 (= NCTC 11162)	" <i>F. breve</i> "	Eye swab	Eire
CL669/76 (= NCTC 11163)	" <i>F. breve</i> "	Urine	Czechoslovakia
ATCC 13253 (= NCTC 10016) ^e	<i>F. meningosepticum</i>	Spinal fluid	Massachusetts
ATCC 13254 (= NCTC 10585)	<i>F. meningosepticum</i>	Blood	Florida
ATCC 13255 (= NCTC 10586)	<i>F. meningosepticum</i>	Spinal fluid and throat	South Carolina
6925 (= NCTC 10587)	<i>F. meningosepticum</i>	Cerebrospinal fluid	Sri Lanka
8388 (= NCTC 10588)	<i>F. meningosepticum</i>	Blood	New York
8707 (= NCTC 10589)	<i>F. meningosepticum</i>	Spinal fluid	New York
ATCC 4651 (= NCTC 11036) ^e	<i>F. odoratum</i>	Unknown	Unknown
ATCC 29979 (= NCTC 11179)	<i>F. odoratum</i>	Urine	United Kingdom
CL229/67 (= NCTC 11180)	<i>F. odoratum</i>	Wound swab	United Kingdom
3531 (= NCTC 10795)	<i>Flavobacterium</i> sp. group IIb	Sputum	South Carolina
3716 (= NCTC 10796)	<i>Flavobacterium</i> sp. group IIb	Trachea at autopsy	Unknown
B1858 (= NCTC 10797)	<i>Flavobacterium</i> sp. group IIb	Drinking fountain spout	Arkansas

^a Key to sources: NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection de l'Institut Pasteur, Paris, France; La, International Center for Information on and Distribution of Type-Cultures, Lausanne, Switzerland; strains A14/65 and E894 and those prefixed by CL are strains referred for identification to the NCTC Computer Identification Laboratory; all other strains with three- or four-digit numbers and with or without a single-letter prefix are strains referred for identification to the Special Bacteriology Section, Centers for Disease Control, Atlanta, Ga.

^b Country, or state in the United States.

^c Strain obtained from Madigan Army Medical Center, Tacoma, Wash., and presumed to have been isolated in Washington.

^d A case report on this strain has been published (4).

* Type strain.

TABLE 2. Characteristics for which all 28 strains of *F. multivorum* are positive

Acid from the following carbohydrates (tested in an ammonium salt medium):	<i>N</i> -Acetyl- β -D-glucosaminidase ^c
Glucose	β -D-Cellobiosidase
Arabinose	L-Phenylalanyl-2-naphthylamide hydrolase
Cellobiose	L-Lysyl-2-naphthylamide hydrolase
Fructose	L-Histidyl-2-naphthylamide hydrolase
Lactose	Glycyl-2-naphthylamide hydrolase
Maltose	α -L-Aspartyl-2-naphthylamide hydrolase
Raffinose	L-Arginyl-2-naphthylamide hydrolase
Salicin	L-Alanyl-2-naphthylamide hydrolase
Sucrose	<i>S</i> -Benzyl-L-cysteyl-2-naphthylamide hydrolase
Trehalose	L-Methionyl-2-naphthylamide hydrolase
Xylose	L-Glycyl-glycyl-2-naphthylamide hydrolase
Catalase production	Glycyl-L-phenylalanyl-2-naphthylamide hydrolase
Cytochrome <i>c</i> oxidase production	Glycyl-L-prolyl-2-naphthylamide hydrolase
Esculin hydrolysis	L-Leucyl-glycyl-2-naphthylamide hydrolase
Growth at 37°C	L-Seryl-tyrosyl-2-naphthylamide hydrolase
Growth at room temperature ^a	L-Glutaminyl-2-naphthylamide hydrolase
Growth on MacConkey agar	α -L-Glutamyl-2-naphthylamide hydrolase
Oxidative in Hugh and Leifson oxidation-fermentation test	L-Ornithyl-2-naphthylamide hydrolase
Phosphatase production	L-Seryl-2-naphthylamide hydrolase
Production of the following enzymes: ^b	L-Tryptophyl-2-naphthylamide hydrolase
Alkaline phosphatase ^c	<i>N</i> -Carbobenzoxy-glycyl-glycyl-L-arginyl-2-naphthylamide hydrolase
L-Leucyl-2-naphthylamide hydrolase ^c	Production of yellow pigment
Acid phosphatase ^c	Tributylin hydrolysis
Phosphoamidase ^c	Tween 20 hydrolysis
	β -D-Galactosidase production

^a Room temperature = 18 to 22°C.^b Tested by using various API ZYM galleries.^c Enzyme test included in the standard API ZYM gallery.

tive). A similar procedure was adopted for the test results for H₂S production (the lead acetate paper and triple sugar iron agar methods). Test results for indole production (as determined with Ehrlich reagent) and for the API ZYM tests were also treated as quantitative multistate characters; for indole production, test results were coded as 3 (+), 2 ($\pm$), 1 ($\mp$), or 0 (-); for the ZYM tests, the color grade (0 to 5) was used as the result code (e.g., color grade 4 was coded as 4). The between-strain similarities were calculated by using Gower's (5) general similarity coefficient (S_G in the notation of Sneath and Sokal [24]), and negative matches were included for the two-state characters. The strains were clustered by the method of unweighted pair-group average linkage (24) and by single-linkage cluster analysis (24). All calculations were carried out on the ICL 1900 computer at Nottingham University (Nottingham, United Kingdom) with a program developed by M. J. Sackin of the Department of Microbiology, University of Leicester (Leicester, United Kingdom). The median organism of the new taxon was determined (17), and the within- and between-taxon average similarities were calculated as arithmetic means of the relevant similarities.

DNA isolation. Cultures were grown overnight at 30°C in nutrient broth with aeration at 200 rpm in an orbital incubator (Gallenkamp Ltd., London, United Kingdom). The bacterial cells were collected by centrifugation and were suspended in 0.15 M NaCl-0.10 M ethylenediaminetetraacetic acid at pH 8.0. Pronase

(Koch-Light Laboratories Ltd., Colnbrook, United Kingdom) was added to a final concentration of 200 μ g/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 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 (18), but it was supplemented by treatment twice with 50 μ g of ribonuclease (bovine pancreas; BDH Chemicals Ltd., Poole, United Kingdom) per ml and final precipitation with 2-ethoxyethanol.

DNA base composition. The DNA base composition, expressed as moles percent G+C to total bases, was estimated from the thermal denaturation temperature, which was determined by the method of Marmur and Doty (19). 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 (7). The G+C content was calculated from the thermal denaturation temperature determined in 0.05 M NaCl buffered with 0.005 M trisodium citrate (1/3 SSC) at pH 7.0 by the equation moles percent G+C = $52.0 + 2.24(\text{thermal denaturation temperature} - 84.4)$; this was derived using the

TABLE 3. Characteristics for which all 28 strains of *F. multivorum* are negative

Acid from the following carbohydrates (tested in an ammonium salt medium):	Nitrite reduction
Adonitol	Ornithine decarboxylase
Dulcitol	Phenylalanine deamination
Ethanol	Poly- β -hydroxybutyrate inclusion granules
Inositol	Production of the following enzymes: ^d
Mannitol	Esterase (C-4) ^e
Sorbitol	Lipase (C-14) ^e
Alkali production on Christensen's citrate	L-Cystyl-2-naphthylamide hydrolase ^e
Arginine deiminase	N-Benzoyl-DL-arginyl-2-naphthylamide hydrolase ^e
Arginine deiminase	N-Glutaryl-L-phenylalanyl-2-naphthylamide hydro- lase ^e
Casein digestion	β -D-Glucuronidase ^e
Fluorescence on King medium B	α -D-Mannosidase ^e
Gas from peptone-water-glucose	β -D-Fucosidase
Gluconate oxidation	β -L-Fucosidase
Growth at 5°C	N-Acetyl- α -D-glucosaminidase
Growth at 42°C	Arylsulfatase
Growth on cetrimide	L-Hydroxypropyl-2-naphthylamide hydrolase
Growth on Simmons citrate	N-Benzoyl-L-leucyl-2-naphthylamide hydrolase
Hydrogen sulfide production ^a	N-Carbobenzoxyl-L-arginyl-4-methoxyl-2-naphthyl- amide hydrolase
Indole production ^b	L-Prolyl-2-naphthylamide hydrolase
Lysine decarboxylase	Reduction of 0.4% (wt/vol) selenite
Malonate utilization	Starch hydrolysis
Motility at 37°C	Tyrosine hydrolysis
Motility at room temperature ^c	3-Ketolactose production
Nitrate reduction	

^a By both lead acetate paper and triple sugar iron agar methods.^b Tested with Kovács and Ehrlich reagents.^c Room temperature = 18 to 22°C.^d Tested by using various API ZYM galleries.^e Enzyme test included in the standard API ZYM gallery.

general equation of Owen et al. (20), and it expressed the G+C content relative to reference DNA from *E. coli* B NCTC 10537 (52.0 mol% G+C).

RESULTS

Numerical taxonomic analysis. The similarities between the strains studied are illustrated in a dendrogram (Fig. 1) derived by single-linkage cluster analysis. The strains of *F. multivorum*, which comprised 28 of 29 strains originally assigned at the Centers for Disease Control, NCTC, or Madigan Army Medical Center to group IIk, biotype 2, formed a homogeneous cluster at the 92.0% similarity level, and the majority of strains (26/28) joined within the cluster at a similarity level of 93.5%. Table 5 lists the average within- and between-taxon similarities of *F. multivorum*, of the five strains phenotypically similar to *F. multivorum*, and of the clusters formed by the various reference strains at the 92.0% similarity level. The average within-taxon similarity of 91.2% for *F. multivorum* was just slightly lower than the range of values determined for "*Flavobacterium breve*" and *F. odoratum* (Table 5) and was higher than the range of values determined for *F. meningosepticum* and *Flavobacterium* sp. group IIb. The

latter two taxa were phenotypically heterogeneous and failed to form discrete clusters at or above the 92.0% similarity level (Fig. 1). In the *F. multivorum* cluster, strain B5533 was the centrostrain but was not the median organism because it produced acid from rhamnose in ammonium salt-sugar medium. Strains CL201/75, CL23/79, B3159, D8510, and E3540 were the least typical members of *F. multivorum*, and each differed from others of the cluster in two, but not the same two, biochemical characteristics (see Table 4). The numerical analysis demonstrated that *F. multivorum* was quite distinct from the other recognized *Flavobacterium* species: *F. balustinum*, "*F. breve*," *F. meningosepticum*, *F. odoratum* (9), and *Flavobacterium* sp. group IIb. The name "*F. breve*" is in quotation marks because it is not on the Approved Lists of Bacterial Names (23). 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).

Characteristics of *F. multivorum*. The 28 of 29 strains originally assigned at the Centers for Disease Control, NCTC, or Madigan Army Medical Center to group IIk, biotype 2, were strictly aerobic, gram-negative, uniformly

TABLE 4. Characteristics for which the 28 strains of *F. multivorum* give different results

Characteristic	No. of strains positive	Result for type strain (B5533)	Strain(s) that gave the less common result
Acid from ammonium salt-glycerol	27	+	B5367
Acid from 10% (wt/vol) glucose	27	+	CL201/75
Acid from 10% (wt/vol) lactose	27	+	CL201/75
Growth on β -hydroxybutyrate	27	+	A7810
Tween 80 hydrolysis	27	+	D8510
Urease production	27	+	E3540
α -D-Glucosidase production ^{a,b}	27	+	CL23/79
Esterase-lipase (C-8) production ^{a,b}	25	+	CL24/79, E2786, E3089
L-Pyroglyutamyl-2-naphthylamide hydrolase production ^a	25	+	CL25/79, A561, B2623
α -L-Glutamyl-2-naphthylamide hydrolase production ^a	24	+	CL201/75, CL25/79, A7543, B3159
L-Threonyl-2-naphthylamide hydrolase production ^a	24	+	CL25/79, B3159, B9042, D8864
Lactosidase production ^a	21	+	CL406/79, A7810, A8895, B3159, B3392, B5147, E2786
Deoxyribonuclease production	17	+	CL201/75, CL406/79, 8354, A1327, A7543, B5147, B5367, D3833, E2786, E3089, E3540
L-Valyl-2-naphthylamide hydrolase production ^{a,b}	15	+	CL201/75, CL24/79, CL25/79, CL26/79, CL406/79, A7810, A8895, B3159, B3392, B9042, D8510, D8864, E3540
β -D-Galactosidase production ^{a,b}	15	+	CL201/75, CL23/79, CL24/79, CL25/79, CL26/79, 8354, A1327, A7317, A7810, B3392, B5367, B5759, B9042
Acid from ammonium salt-rhamnose	13	+	CL201/75, CL23/79, CL25/79, 8354, A561, A1327, A7317, A7543, B5367, B5533, B5759, D8510, E3540
β -D-Glucosidase production ^{a,b}	10	—	8354, A1327, A7317, A7371, A7543, A7810, B5759, B9042, D3833, E2786
α -L-Arabinosidase production ^a	8	—	CL24/79, A561, A7371, A7543, A8895, B3392, E2786, E3540
L-Tyrosyl-2-naphthylamide hydrolase production ^a	8	—	CL201/75, CL23/79, A1327, A8895, B3159, B9042, D8510, D8864
Gelatin hydrolysis ^c	4	—	A561, B5759, B9042, D8510
β -D-Xylosidase production ^a	4	—	A561, A7371, A7543, E3089
Acid from peptone-water-glucose	3	—	CL201/75, CL406/79, A7543
KCN tolerance	1	—	D8510
Opalescence on lecithovitellin agar	1	—	B5759
Phosphatase production ^a	1	—	CL24/79
Pigment production on tyrosine agar	1	—	E3540
α -D-Galactosidase production ^{a,b}	1	—	B3159
α -L-Fucosidase production ^{a,b}	1	—	B3159
α -D-Xylosidase production ^a	1	—	CL23/79
L-Isoleucyl-2-naphthylamide hydrolase production ^a	1	—	8354

^a Tested by using various API ZYM galleries.^b Enzyme test included in the standard API ZYM gallery.^c By plate method; all strains tested failed to liquefy a nutrient gelatin stab.

stained rods, 1.0 μ m in length, with parallel sides and rounded ends. The colonies on nutrient agar after 48 h were circular, 1 mm in diameter, low convex, smooth, and opaque (translucent at 24 h). A pale yellow pigment was produced which did not diffuse in nutrient agar or fluoresce on

King medium B; pigment was clearly yellow at 48 h. Only one strain (E3540) produced a dark brown pigment on tyrosine agar. The colonies were butyrous in consistency and were easily emulsified in distilled water to give uniform suspensions. Colonies of strains A1327, A8895, and

E3089 appeared rough when first subcultured on nutrient agar, but the colonies appeared smooth on further subculture. The strains grew opti-

mally at 30°C, and all biochemical tests, unless specified otherwise, were carried out at this temperature. The strains grew at room temperature

FIG. 1. Dendrogram based on single-linkage cluster analysis. Percent similarity was calculated with the general similarity coefficient of Gower (S_G), negative matches included.

TABLE 5. Within- and between-taxon average similarities of the taxa included in this study

Taxon	No. of strains	Avg similarities (% S _C)																
(1) <i>F. balustinum</i>	1	— ^a																
(2) " <i>F. breve</i> "	3	85.6	94.8															
(3) <i>F. meningosepticum</i> NCTC 10016	1	78.8	81.4	—														
(4) <i>F. meningosepticum</i> NCTC 10585	1	78.5	79.6	90.5	—													
(5) <i>F. meningosepticum</i> NCTC 10586	1	83.1	85.4	87.6	89.0	—												
(6) <i>Flavobacterium</i> group IIb NCTC 10796	1	87.1	86.9	83.1	84.6	89.7	—											
(7) <i>F. meningosepticum</i> NCTC 10588	1	78.0	78.1	88.7	88.0	89.2	81.3	—										
(8) <i>F. meningosepticum</i> NCTC 10587, 10589	2	79.2	82.0	85.5	85.9	87.7	84.7	86.2	93.2									
(9) <i>Flavobacterium</i> group IIb NCTC 10795	1	86.0	81.7	80.4	78.2	82.0	87.8	77.0	78.7	—								
(10) <i>F. odoratum</i>	3	78.9	83.3	70.3	68.2	72.8	74.7	66.7	70.1	73.9	95.2							
(11) <i>Flavobacterium</i> group IIb NCTC 10797	1	80.0	81.7	81.4	78.8	79.6	81.7	78.1	74.3	82.8	70.9	—						
(12) Unclassified strain E894	1	75.4	77.4	72.9	72.6	78.1	76.4	74.7	78.5	72.0	68.4	68.2	—					
(13) <i>Flavobacterium</i> sp. CIP 12.75	1	75.9	77.8	74.5	74.4	79.4	76.5	73.7	79.5	74.3	67.2	68.2	90.6	—				
(14) Unclassified strain A14/65	1	69.9	69.9	72.5	73.2	75.2	69.6	73.2	74.7	69.4	63.8	63.9	84.0	82.6	—			
(15) Unclassified strain CL404/79	1	74.6	72.4	75.0	74.8	80.0	72.7	75.9	75.9	71.5	66.4	67.4	82.0	82.2	91.6	—	91.2	
(16) <i>F. multivorum</i> ^b	28	72.7	73.4	74.2	73.5	75.1	72.8	74.2	74.7	74.0	66.4	69.9	81.2	84.1	84.5	86.9		
(17) Unclassified group IIk, biotype 2, strain 8291	1	71.3	71.6	74.0	72.0	73.5	70.7	72.5	73.1	74.4	64.6	69.4	81.6	85.5	81.9	84.7	88.9	—
Taxon no.		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)

^a —, Only one strain in group.^b Comprises 28 strains that were originally assigned at the Centers for Disease Control, NCTC, or Madigan Army Medical Center to group IIk, biotype 2.

and at 37°C, but not at 5 or 42°C. Hemolysis was not obtained from aerobic growth on 5% (vol/vol) horse blood agar.

The biochemical characteristics of the strains are listed in Tables 2 through 4. A total of 97 characteristics were common to all strains (either all positive [Table 2] or all negative [Table 3]), and there were 30 characteristics in which one or more of the strains differed (Table 4). The tests with the largest number of strain differences were acid from rhamnose in ammonium salt-sugar medium and production of L-valyl-2-naphthylamide hydrolase and β -D-galactosidase (tested by the API ZYM System) (Table 4). It should be noted that all strains of *F. multivorum* produced phosphatase and β -D-galactosidase by conventional methods (Table 2), whereas by the API ZYM method, only 1 strain produced phosphatase and 15 strains produced β -D-galactosidase (Table 4). These discrepancies between the test methods were attributed to the fact that in the API ZYM system, only grades of color change in the range 3 to 5 were treated as positive. However, in the phosphatase production (API ZYM) test, 23 of 27 strains scored as negative produced a slight color change (grades 1 and 2), and in the β -D-galactosidase production (API ZYM) test, 11 of 13 strains scored as negative produced a color change (grades 1 and 2). The susceptibilities of selected strains to antimicrobial agents are listed in Table 6. The 12 strains tested were resistant to therapeutic levels of streptomycin, kanamycin, gentamicin, tobramycin, amikacin, ampicillin, carbenicillin, cephaloridine, erythromycin, polymyxin B, and nalidixic acid and moderately susceptible to sulfamethoxazole, co-trimoxazole, trimethoprim, chloramphenicol, and tetracycline.

DNA base composition. Table 7 lists the base compositions of 11 phenotypically representative strains of *F. multivorum*. The values ranged from 38.6 to 40.1 mol% G+C, with a mean of 39.6 mol% and a standard deviation of ± 0.5 mol%.

Strains of uncertain taxonomic position. Several strains (taxon numbers 12, 13, 14, 15, and 17 in Table 5) had similarities to *F. multivorum* (Table 5 and Fig. 1) but were excluded from the species because they did not form a cluster with it at the 92.0% similarity level on the dendrogram derived from single-linkage cluster analysis. Strain 8291 was hitherto regarded as belonging to group IIk, biotype 2. On the dendrogram derived from unweighted pair-group average linkage, these strains, except for strain 8291, were again excluded from the *F. multivorum* cluster. The main characteristics that distinguish these unclassified strains from

F. multivorum are given in Table 8. The G+C contents determined on three of the five strains were slightly higher, in the range 40.7 to 43.4 mol% (Table 7), than those of *F. multivorum*. These strains may provide the bases of future taxa.

DISCUSSION

The results of the cluster analysis and the within-group similarities presented above show that the 28 strains of yellow-pigmented bacteria, for which the name *Flavobacterium multivorum* (mul.ti.vo'rum. L. adj. *multus* many; L. trans.v. *vorare* to swallow; M.L. adj. *multivorus* intended to mean "produces acid from many carbohydrates") is here proposed, constitute a homogeneous and distinctive taxon. These findings were corroborated by similarities among strains both with respect to the G+C contents of their DNAs and their patterns of susceptibilities to antimicrobial agents.

The 28 strains for which we propose the name *F. multivorum* conform to the definition of *Flavobacterium* as emended by Holmes and Owen (9), although they were not actively proteolytic, unlike most members of the genus, and did not produce indole, even in small quantities. We exclude these strains from *Pseudomonas* on the basis of the G+C contents of their DNAs. Tatum et al. (25) found 2 of their 40 group IIk, biotype 2, strains to be motile, but they cautioned that the results of tests for motility in semisolid medium may be equivocal. Consequently group IIk, biotype 2, was described as monotrichous. However, the group IIk, biotype 2, strains described here and those of Yabuuchi et al. [Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)32, p. 351], were nonmotile. The presence of flagella has been reported on cells of the type strain of *Flavobacterium aquatile* and of the type strain of *F. meningosepticum* (27). Weeks (28) described structures on *F. aquatile* strain F36 that did not appear to be true flagella and which he considered similar to pseudoflagella. These pseudoflagella were seen on cells after silver impregnation and on chromium-shadowed cells under the electron microscope, but they were not seen on cells stained by Leifson's procedure. In the case of *F. meningosepticum*, the results of Webster and Hugh (27) represent the first reported observation of motility in this species, and we believe further confirmation by electron microscopy of the nature of the structures observed is required.

The numerical taxonomic analysis showed that *F. multivorum* was quite distinct from the other main taxa that would be included in *Flavobacterium*: *F. balustinum*, "*F. breve*," *F. meningosepticum*, *F. odoratum* (9), and *Flavobac-*

TABLE 7. DNA base compositions of 11 strains of *F. multivorum* and 3 unclassified strains resembling *F. multivorum*

Strain	Designation	Buffer ^a	$T_m \pm SD$ (°C) ^b	G+C content (mol%) ^c
A561	<i>F. multivorum</i>	1/3 SSC	78.4 $\pm$ 0.00	38.6
B5367	<i>F. multivorum</i>	1/3 SSC	78.7 $\pm$ 0.10	39.2
E3540	<i>F. multivorum</i>	1/3 SSC	78.7 $\pm$ 0.00	39.2
B5533	<i>F. multivorum</i>	1/3 SSC	78.75 $\pm$ 0.05	39.3
CL23/79	<i>F. multivorum</i>	1/3 SSC	78.85 $\pm$ 0.05	39.6
CL406/79	<i>F. multivorum</i>	1/3 SSC	78.85 $\pm$ 0.25	39.6
A7317	<i>F. multivorum</i>	1/3 SSC	78.9 $\pm$ 0.10	39.7
B2623	<i>F. multivorum</i>	1/3 SSC	78.95 $\pm$ 0.15	39.8
A7810	<i>F. multivorum</i>	1/3 SSC	79.05 $\pm$ 0.15	40.0
A8895	<i>F. multivorum</i>	1/10 SSC	70.2 $\pm$ 0.05 ^d	40.1
B3159	<i>F. multivorum</i>	1/10 SSC	70.2 $\pm$ 0.05 ^d	40.1
E894	Unclassified strain	SSC	86.3 $\pm$ 0.20	40.7 ^d
A14/65	Unclassified strain	SSC	86.5 $\pm$ 0.05	41.1 ^d
8291	Unclassified strain	1/3 SSC	80.55 $\pm$ 0.05	43.4

^a Compositions of the buffers used: 1/3 SSC, 0.05 M NaCl-0.005 M trisodium citrate; 1/10 SSC, 0.015 M NaCl-0.0015 M trisodium citrate; SSC, 0.15 M NaCl-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 equations used to calculate moles percent G+C were as follows: 1/3 SSC, moles percent G+C = 52.0 + [2.24(T_m - 84.4)]; 1/10 SSC, moles percent G+C = 52.0 + [2.08(T_m - 75.9)]. The mean value for the 11 strains designated *F. multivorum* was 39.6 $\pm$ 0.5 mol% G+C.

^d This value was published previously (10).

TABLE 8. Characteristics for differentiation of five unclassified strains phenotypically resembling *F. multivorum*

Test	<i>F. multivorum</i> ^a	Strain no.				
		8291	E894	CIP 12.75	A14/65	CL404/79
Acid from ASS ^b -adonitol	-	+	+	+	-	-
Acid from ASS-arabinose	+	+	-	+	-	-
Acid from ASS-ethanol	-	-	-	-	+	+
Acid from ASS-mannitol	-	-	-	-	+	+
Acid from ASS-xylose	+	+	-	+	+	+
Growth at 42°C	-	+	-	-	-	-
Nitrate reduction	-	+	-	+	-	-
Production of yellow pigment	+	+	+	+	-	-
Production of the following enzymes ^c :						
L-Cystyl-2-naphthylamide hydrolase	-	-	-	+	-	-
N-Benzoyl-DL-arginyl-2-naphthylamide hydrolase	-	-	+	+	+	+
Arylsulfatase	-	-	+	-	+	-
L-Phenylalanyl-2-naphthylamide hydrolase	+	-	+	+	-	-
N-Carbobenzoxy-L-arginyl-4-methoxyl-2-naphthylamide hydrolase	-	-	+	+	+	-

^a Based on a study of 28 strains. +, All strains tested positive; -, all strains tested negative.

^b ASS, Ammonium salt-sugar medium.

^c Tested by using various API ZYM galleries.

terium sp. group IIb. Characteristics useful in the practical identification of *F. multivorum* and in its differentiation from the above-mentioned taxa are recorded in Table 9. *P. paucimobilis* is also included in Table 9 because of its apparent similarities to *F. multivorum*.

The strains of *F. multivorum* described here

were isolated from various clinical specimens, and one strain (CL406/79) was reported to be the cause of a spontaneous bacterial peritonitis (4). There is no other evidence of strains incriminated in infections in humans, but their resistance to a wide range of antimicrobial agents indicates that any infections due to this species

TABLE 9. *Characteristics for practical identification and differentiation of*

Taxon	No. of strains	Test result ^a											
		Acid from ammonium salt medium plus:											
		Glu- cose	Arabi- nose	Cello- biose	Etha- nol	Glyc- erol	Lac- tose	Mal- tose	Raffi- nose	Salic- in	Su- crose	Tre- halose	Xy- lose
<i>F. multivorum</i>	28	+	+	+	—	27/28	+	+	+	+	+	+	+
<i>F. balustinum</i>	1	+	—	—	+	—	—	—	—	—	—	—	—
" <i>F. breve</i> "	7	6/7	—	—	—	—	—	6/7	—	—	—	—	—
<i>F. meningosep- ticum</i>	9	8/9	—	1/9	4/9	8/9	6/9	+	—	—	—	+	1/9
<i>F. odoratum</i>	28	—	—	—	—	—	—	—	—	—	—	—	—
<i>Flavobacterium</i> group IIb	4	+	2/4	—	—	3/4	—	+	—	—	—	+	1/4
<i>P. paucimobilis</i>	29	+	+	+	26/29	6/29	+	+	28/29	26/29	+	+	+

^a +, All strains positive; —, all strains negative; fractions indicate numbers of strains positive over numbers of strains tested. The phenotypic results for *F. multivorum* and for *F. balustinum* were from this study, and the phenotypic results for the other taxa were derived from previously published work in NCTC: "*F. breve*" (12), *F. meningosepticum* (10, 12), *F. odoratum* (13), *Flavobacterium* group IIb (10, 12), and *P. paucimobilis* (10).

could prove difficult to treat. Resistance to antimicrobial agents is a characteristic of several other *Flavobacterium* and allied taxa, notably "*F. breve*" (12), *F. meningosepticum* (1, 14), *F. odoratum* (11, 13), and *Flavobacterium* sp. group IIb (26).

Description of the type strain. The characteristics of the type strain, B5533 (= NCTC 11343), also listed in Tables 2 through 4, are as follows.

Gram-negative, nonsporeforming rods, 1.0 μ m in length, of regular shape with rounded ends. Cells occurring singly or in pairs. 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, production of a yellow pigment which is not fluorescent. No production of brown pigment on tyrosine agar.

Aerobic; no growth under anaerobic conditions in an atmosphere of pure hydrogen, but possible growth in the presence of 7% (vol/vol) CO₂.

Growth at 37°C but not at 5 or 42°C. Optimum 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 lecithovitelin agar.

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

No production of indole and 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 and no hydrolysis of gelatin.

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

No utilization of citrate and malonate.

No oxidation of gluconate.

No production of arginine deiminase, arginine deiminase, lysine decarboxylase, and ornithine decarboxylase.

No production of 3-ketolactose.

No reduction of selenite and 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. [8]).

Production of acid in ammonium salt medium under aerobic conditions from glucose, arabinose, cellobiose, glycerol, fructose, lactose, maltose, raffinose, rhamnose, 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, dulcitol, ethanol, inositol, mannitol, or sorbitol.

No production of acid or 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, esterase-lipase (C-8), L-leucyl-2-naphthylamide hydrolase, L-valyl-2-naphthylamide

F. multivorum from other *Flavobacterium* taxa and from *P. paucimobilis*

Test result ^a										
Casein digestion	Esculin hydrolysis	Gelatin hydrolysis (plate method)	Growth on MacConkey agar	Motility at room temperature	Nitrite reduction	Poly- β -hydroxybutyrate inclusion granules	Starch hydrolysis	Urease production	β -D-Galactosidase production	Mol % G+C $\pm$ standard deviation (no. of strains tested) ^b
—	+	4/28	+	—	—	—	—	27/28	+	39.6 $\pm$ 0.5 (11)
+	+	+	+	—	—	—	—	—	—	33.1
+	—	+	+	—	—	—	—	—	—	32.4 $\pm$ 0.6 (10)
+	+	+	+	—	1/9	—	—	—	+	37.0 $\pm$ 0.5 (8)
+	—	+	+	—	+	—	—	+	—	31.4–36.1 (10)
+	+	+	+	—	1/4	—	+	—	1/4	37.5 $\pm$ 1.4 (9)
—	+	3/29	—	22/29	—	25/29	18/29	—	+	65.3 $\pm$ 1.0 (12)

^b Data for *F. multivorum* were obtained in this study, and those for the other taxa were derived from previously published work: *F. meningosepticum* and *Flavobacterium* group IIb (22), *F. odoratum* (21), *P. paucimobilis* (10), and "*F. breve*" and *F. balustinum* (R. J. Owen, unpublished data).

hydrolase, acid phosphatase, phosphoamidase, β -D-galactosidase, α -D-glucosidase, *N*-acetyl- β -D-glucosaminidase, lactosidase, β -D-cellobiosidase, L-pyroglyutamyl-2-naphthylamide hydrolase, L-phenylalanyl-2-naphthylamide hydrolase, 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, α -L-glutamyl-2-naphthylamide hydrolase, S-benzyl-L-cysteyl-2-naphthylamide hydrolase, L-methionyl-2-naphthylamide hydrolase, L-glycylglycyl-2-naphthylamide hydrolase, glycyl-L-phenylalanyl-2-naphthylamide hydrolase, glycyl-L-prolyl-2-naphthylamide hydrolase, L-leucyl-glycyl-2-naphthylamide hydrolase, L-seryl-L-tyrosyl-2-naphthylamide hydrolase, L-glutaminyl-2-naphthylamide hydrolase, α -L-glutamyl-2-naphthylamide hydrolase, L-ornithyl-2-naphthylamide hydrolase, L-seryl-2-naphthylamide hydrolase, L-threonyl-2-naphthylamide hydrolase, L-tryptophyl-2-naphthylamide hydrolase, *N*-carbobenzoyl-glycyl-glycyl-L-arginyl-2-naphthylamide hydrolase.

No production of the following enzymes (using various API ZYM galleries): esterase (C-4), lipase (C-14), L-cystyl-2-naphthylamide hydrolase, *N*-benzoyl-DL-arginyl-2-naphthylamide hydrolase, *N*-glutaryl-L-phenylalanyl-2-naphthylamide hydrolase, α -D-galactosidase, β -D-glucuronidase, β -D-glucosidase, α -D-mannosidase, α -L-fucosidase, β -D-xylosidase, phosphatase, α -D-xylosidase, β -D-fucosidase, β -L-fucosidase, *N*-acetyl- α -D-glucosaminidase, arylsulfatase, α -L-arabinosidase, L-tyrosyl-2-naphthylamide hydrolase, L-hydroxyprolyl-2-naphthylamide hydrolase, *N*-benzoyl-L-leucyl-2-naphthylamide hydrolase, *N*-carbobenzoyl-L-arginyl-4-meth-

oxyl-2-naphthylamide hydrolase, L-isoleucyl-2-naphthylamide hydrolase, and L-prolyl-2-naphthylamide hydrolase.

Resistant to streptomycin, kanamycin, gentamicin, tobramycin, amikacin, ampicillin, carbenicillin, cephaloridine, erythromycin, polymyxin B, and nalidixic acid and moderately susceptible to sulfamethoxazole, co-trimoxazole, trimethoprim, chloramphenicol, and tetracycline.

G+C content of DNA: 39.3 mol% (thermal denaturation temperature estimation).

Source: isolated from a spleen.

Two additional strains of *F. multivorum* have been deposited in the National Collection of Type Cultures: NCTC 11033 and NCTC 11034 (group IIk, biotype 2, strains A8895 and B3159, respectively).

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REPRINT REQUESTS

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