Flavobacterium gleum, a New Species Found in Human Clinical Specimens

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We propose a new species, *Flavobacterium gleum*. Each of the 12 strains placed in this new species was examined for 129 characteristics, including reactions in 58 enzyme tests (API ZYM System). These bacteria are rod shaped, aerobic, gram negative, and nonmotile and attack carbohydrates oxidatively. The mean guanine-plus-cytosine content of the deoxyribonucleic acids of six representative strains is 37.6 ± 1.0 mol%. Most strains of the new species were isolated from human clinical specimens; vaginal specimens were a common source. One of the strains included in the new species (strain NCTC 10795) is a reference strain of the unnamed taxon *Flavobacterium* species group IIb. The type strain is strain F93 (= NCTC 11432).

Tatum et al. (23) described various unnamed groups of bacteria, several of which conform to the genus *Flavobacterium* as redefined by Holmes and Owen (6) and either correspond to existing species or constitute new ones. Group M-4f was shown to correspond to *Flavobacterium odoratum* (11), and the name *Flavobacterium multivorum* was given to strains previously assigned to group IIk, biotype 2 (10). *Flavobacterium* species group IIb, which also corresponds to the revised description of *Flavobacterium* (6), remains the only unnamed *Flavobacterium* group of the groups originally described by Tatum et al. (23).

Group IIb was first described by King (14) when she proposed the name *Flavobacterium meningosepticum* for the taxon which until then had been known as group IIa. King (14) briefly described nine group IIb strains from human clinical sources and noted their similarities to *F. meningosepticum*. Since then various strains identified as group IIb have been isolated from human clinical material and other sources in hospital environments, although the clinical significance of the isolates was generally uncertain (24).

Price and Pickett (21), by performing various numerical taxonomic analyses of phenotypic data on their group IIb strains, found the taxon phenotypically somewhat heterogeneous, although distinct subgroups could not be discerned, and these authors concluded that most of the strains belonged to a single species. Deoxyribonucleic acid (DNA)-DNA hybridization data (18, 20) have shown that group IIb may comprise two or three separate DNA relatedness groups and thus may contain several species. However, Holmes and Owen (7), referring to an unpublished numerical taxonomic analysis, found little phenotypic data which correlated with the possible DNA relatedness groups within group IIb. The type strain of *Flavobacterium balustinum* was also included in the cluster of group IIb strains, so the relationship between this species and group IIb was a further consideration (7).

In this study 12 group IIb strains were shown by their phenotypic characteristics, DNA base compositions, and DNA-DNA relatedness levels to constitute a homogeneous taxon which was distinct from other group IIb strains, from

F. balustinum, and from the other named species of *Flavobacterium*. For this new taxon the name *Flavobacterium gleum* is proposed, with strain F93 (= NCTC 11432) as the type strain.

MATERIALS AND METHODS

Bacterial strains. The 12 bacterial strains studied and the sources from which they were isolated are given in Table 1. The strains were isolated over a period of several years from diverse geographical locations.

Bacteriological investigations. The methods which we used have been described previously (10).

DNA relatedness. The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been described previously (3). DNA from *F. gleum* strain F93^T (T = type strain) was labeled with ³²P₄ in vitro (4).

Centrotpe. The centrotpe was determined by the method of Lapege and Willcox (15).

RESULTS

Phenotypic characteristics of *F. gleum*. The 12 strains which we studied were strictly aerobic, gram-negative, uniformly staining rods that were 2.0 to 3.0 μ m long and had parallel sides and rounded ends (Fig. 1). After 24 h the colonies on nutrient agar were circular, about 2 mm in diameter, entire, and viscid or butyrous in consistency. A nondiffusible, nonfluorescent bright yellow pigment, which turned red upon addition of 20% KOH, was produced by all strains. All of the strains also produced a water-soluble dark brown pigment on tyrosine agar. The strains grew at room temperature and at 30 and 37°C but failed to grow at 5°C; nine strains grew at 42°C. Hemolysis was not present after aerobic growth for 24 h on 5% (vol/vol) horse blood agar.

A total of 108 characteristics were common to all strains (either all positive or all negative; see below), and there were 21 characteristics in which one or more of the strains differed (Table 2). Strain F93^T was the centrotpe (there was no median organism). Strains CL344/74, A121/68, and NCTC 10795 were the least typical members of *F. gleum*, and each differed from the other 11 strains in one biochemical characteristic (but not the same one). Nine strains (Table 2) of *F. gleum* produced β -D-galactosidase as determined by the conventional *o*-nitrophenyl- β -D-galactopyranoside test, but

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TABLE 1. Strains of *Flavobacterium gleum* sp. nov. studied

Strain	Isolated from: ^a
CL344/74	Catheter, Birmingham
CL542/74	Abdominal tap, London
CL223/76	Dialysis fluid and catheter tip, London
A6/68 (= F7)	High vaginal swab, Rochdale
A121/68 (= F61)	High vaginal swab, Liverpool
A134/68 (= F66)	Unknown, Rochdale
F93 ^T (= NCTC 11432 ^T)	High vaginal swab, London
F127	Vaginal discharge, Rochdale
F128	Cerebrospinal fluid, London
F129 (= NCTC 11433)	Wound swab, London
F130	Peritoneal swab, London
NCTC 10795 (= 3531)	Sputum, South Carolina, United States

^a Geographical locations in the United Kingdom unless otherwise specified.

none of these strains produced β -D-galactosidase as determined by the API ZYM method (see below).

G+C content of *F. gleum* DNA. The guanine-plus-cytosine (G+C) contents of the DNAs of six phenotypically representative strains of *F. gleum* have been published previously (19). These values were between 36.0 and 38.5 mol%, with a mean of 37.6 mol% and a standard deviation of ± 1.0 mol%.

DNA relatedness. Labeled DNA from *F. gleum* type strain F93 was 60 to 97% related (average, 75%) to unlabeled DNAs from eight other *F. gleum* strains in hybridization reactions done at 60°C, a temperature that is close to the

FIG. 1. Electron micrograph of a cell of *F. gleum* type strain NCTC 11432. Cell size is 2.6 by 0.7 μ m.

optimum for DNA reassociation, and 63 to 92% related (average, 78%) to 11 *F. gleum* strains in reactions done at 75°C, at which only very closely related DNA sequences can reassociate (Table 3). The divergence (percentage of unpaired bases) within related sequences ranged from 1.0 to 3.5%. The level of relatedness of *F. gleum* to strains of *F. meningosepticum* and *Flavobacterium breve* was 14 to 23%.

DISCUSSION

Our results indicate that the 12 strains of gram-negative yellow-pigmented bacteria for which we propose the name *Flavobacterium gleum* (gle'um. Gr. neu. adj. gloion slip-

TABLE 2. Characteristics in which the 12 strains of *F. gleum* differed from each other

Characteristic	No. of strains positive	Result of type strain F93 ^T	Strains that gave the less common result
Tween 80 hydrolysis	11	+	A121/68
S-Benzyl-L-cysteyl- β -naphthylamide hydrolysis ^a	11	+	NCTC 10795
Deoxyribonuclease production	10	+	A121/68, A134/68
Acid from ammonium salt-arabinose	10	+	F127, F130
β -D-Galactosidase production (ONPG test) ^b	9 ^c	+	A134/68, F128, NCTC 10795
Growth at 42°C	9	+	CL542/74, CL223/76, A6/68
Starch hydrolysis	9	+	F127, F129, F130
2-Naphthyl caprylate hydrolysis ^{a,d}	9	+	CL344/74, CL223/76, F128
Alkali production on Christensen citrate	8	+	CL344/74, CL542/74, A6/68, NCTC 10795
Nitrate reduction	7 ^e	—	A6/68, A121/68, A134/68, F93, F127
Urease production (Christensen medium)	7	—	CL344/74, CL223/76, A6/68, F93 ^T , NCTC 10795
Tyrosine hydrolysis	7	+	A121/68, A134/68, F127, F129, F130
Nitrite reduction	7	+	A6/68, A121/68, A134/68, F127, F129
Oxidative in Hugh-Leifson O-F test	6	—	CL223/76, A121/68, A134/68, F93 ^T , F127, F129 ^f
Acid from ammonium salt-glycerol	6	+	A121/68, A134/68, F127, F128, F129, F130 ^f
Acid from ammonium salt-xylose	4	—	CL344/74, CL542/74, A6/68, A134/68
Hydrolysis of:			
6-Bromo-2-naphthyl- β -D-xylopyranoside ^a	4	—	CL344/74, CL542/74, A121/68, F127
L-Tyrosyl- β -naphthylamide ^a	4	—	CL344/74, F127, F128, F130
L-Pyrrolidonyl- β -naphthylamide ^a	2	—	CL344/74, F128
N-Carbobenzoxyl-L-arginine-4-methoxy- β -naphthylamide hydrochloride ^a	2	—	F127, F128
Acid from ammonium salt-ethanol	1	—	CL344/74

^a Tested by using various API ZYM galleries.

^b ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

^c A positive reaction required 18 to 24 h of incubation.

^d Enzyme test included in the standard API ZYM gallery.

^e These strains could denitrify (i.e., grow anaerobically in the presence of nitrate by using nitrate as a terminal electron acceptor and reducing it to N₂).

^f Strains which gave negative results.

TABLE 3. DNA relatedness among *F. gleum* strains

Source of unlabeled DNA	³² PO ₄ -labeled DNA from <i>F. gleum</i> strain F93 ^T (= NCTC 11432 ^T)		
	Relative binding index at 60°C ^a	Divergence (%) ^b	Relative binding index at 75°C
<i>F. gleum</i> F93 ^T	100	0.0	100
<i>F. gleum</i> NCTC 10795	97	3.5	82
<i>F. gleum</i> F128	92	2.0	92
<i>F. gleum</i> CL344/74	78	2.0	85
<i>F. gleum</i> CL542/74	72	2.0	82
<i>F. gleum</i> F129	71	1.5	74
<i>F. gleum</i> A134/68	65	2.0	75
<i>F. gleum</i> F130	64	2.0	70
<i>F. gleum</i> F127	60	1.0	74
<i>F. gleum</i> CL223/76	NT ^c	NT	75
<i>F. gleum</i> A121/68	NT	NT	66
<i>F. gleum</i> A6/68	NT	NT	63
<i>F. meningosepticum</i> B422 (= ATCC 13254)	23	NT	18
<i>F. breve</i> KC 1428 ^T (= NCTC 11099 ^T)	17	NT	9
<i>F. meningosepticum</i> A14 ^T (= ATCC 13253 ^T)	14	NT	4

^a The relative binding index was calculated as follows: (percentage of heterologous DNA bound to hydroxyapatite/percentage of homologous DNA bound to hydroxyapatite) × 100.

^b Divergence was calculated on the assumption that each 1°C decrease in the thermal stability of a heterologous DNA compared with the thermal stability of the homologous DNA duplex was caused by 1% unpaired bases within the duplex. Divergence was calculated to the nearest 0.5%. All reactions were done at least twice. The values shown are averages. Before normalization to 100%, the percentage of DNA bound to hydroxyapatite in homologous reactions was approximately 40%. The amount of labeled DNA that bound to hydroxyapatite in control reaction mixtures that did not contain unlabeled DNA was 0.6 to 1.7%. These control values were subtracted from all reassociation reaction values before normalization.

^c NT, Not tested.

pery, sticky; M. L. neu. adj. *gleum* sticky) constitute a homogeneous taxon. This new species conforms to the definition of *Flavobacterium* as emended by Holmes and Owen (6). Characteristics that are useful for the practical identification of the new species are shown in Table 4. *Pseudomonas paucimobilis* is included in Table 4 because this species has biochemical properties that are similar to those of some *Flavobacterium* species. Table 4 shows that the new species is more similar in phenotypic characteristics to *F. balustinum*, *Flavobacterium indoltheticum*, and *F. meningosepticum* than to other members of the genus. *F. gleum* differs from *F. balustinum* in producing acid from maltose and trehalose and in hydrolyzing the substrates L-glutamine-β-naphthylamide hydrochloride and 2-naphthyl-α-D-glucopyranoside (API ZYM System; Holmes and Owen, unpublished data). Also, the G+C content of the DNA of *F. balustinum* (33.1 mol% [17]) is slightly lower than the G+C contents of *F. gleum* strains. Furthermore, Owen and Holmes (18) have shown by DNA-DNA hybridization that *F. balustinum* is distinct from representatives of group IIb. Similarly, *F. indoltheticum* has a lower G+C content (33.8 mol%) than *F. gleum*. In addition, *F. indoltheticum* differs from the new species in failing to produce acid from fructose and trehalose and in growing at 5°C. In contrast, there are few biochemical tests by which *F. gleum* and *F. meningosepticum* can be distinguished, other than colony pigmen-

tion. The new species forms colonies that are pigmented bright yellow, whereas the colonies of *F. meningosepticum* are pale yellow or even nonpigmented. This pigmentation is the single most useful characteristic for distinguishing these two species.

Although Owen and Lapage (19) suggested that group IIb strains might constitute a subspecies of *F. meningosepticum*, subsequent DNA-DNA hybridization (18, 20) showed that group IIb and *F. meningosepticum* are distinct taxa. Owen and Snell (20) showed that three strains (F93^T, F129, and NCTC 10795) now placed in *F. gleum* are closely related and could constitute a species. The DNA of strain F93^T displayed 100% relative binding with the DNA of strain NCTC 10795, and the corresponding value for strain F129 was 99% (20). In the present study DNA relatedness determinations with 11 strains showed that *F. gleum* is a single species that is distinct from *F. breve* and *F. meningosepticum*. The level of relatedness of the type strain to three other *F. gleum* strains was less than the 70% or more relatedness usually found among strains of a single species. The very low level of sequence divergence among strains (1.0 to 2.0%) and the high level of relatedness at the stringent 75°C incubation temperature (70 to 75%) attest to the validity of assigning all 12 strains to a single species, *F. gleum*. The identification of additional new species within group IIb and the differentiation of these taxa from *F. gleum* are currently being examined by us because DNA data (18) show that *F. gleum* is distinct from other strains belonging to group IIb.

Few clinical details were available for the strains of *F. gleum*, and the clinical significance of these organisms could not be assessed. The clinical role of *Flavobacterium*, including group IIb, has been reviewed by von Graevenitz (24), and several reports have drawn attention specifically to the role of group IIb strains in a case of meningitis (2) and in various cases of bacteremia (5, 22). The resistance of group IIb strains to a wide range of antimicrobial agents (25) indicates that any infection due to a member of this group could prove difficult to treat. Resistance to antimicrobial agents is a feature that group IIb strains share with most other *Flavobacterium* species (1, 9–14).

Description of the species and the type strain. The characteristics for which all 12 strains of *F. gleum* gave either positive results or negative results are listed below. The characteristics for which the 12 strains gave different results are listed in Table 2.

Gram-negative, nonsporeforming rods 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 (18 to 22°C).

Circular, entire colonies that are viscid or butyrous in consistency develop on nutrient agar, becoming mucoid and translucent after incubation for 5 days. Colonies nonhemolytic on 5% (vol/vol) horse blood agar. On nutrient agar, a bright yellow pigment is produced, which is not fluorescent and turns red upon addition of 20% KOH. A water-soluble brown pigment is produced on tyrosine agar.

Aerobic.

Growth at room temperature and at 37°C but not at 5°C.

Production of catalase and cytochrome oxidase.

Does not tolerate KCN at a concentration of 0.0075% (wt/vol).

Hydrolysis of Tween 20.

Production of opalescence on lecithovitellin agar.

Production of indole (Ehrlich reagent); no production of indole (Kovacs reagent).

No production of hydrogen sulfide (lead acetate paper and

TABLE 4. Characteristics for practical identification and differentiation of *F. gleum* from other *Flavobacterium* species and from *Pseudomonas paucimobilis*^a

Test	<i>F. gleum</i> (12 strains)	<i>F. balustinum</i> (1 strain)	<i>F. breve</i> (7 strains)	<i>F. indoltheticum</i> (1 strain)	<i>F. meningosepticum</i> (49 strains)	<i>F. odoratum</i> (28 strains) ^b	<i>F. multivorum</i> (28 strains) ^c	<i>F. spiritivorum</i> (13 strains)	<i>P. paucimobilis</i> (29 strains) ^d
Acid from ASS-glucose ^e	+	+	6/7 ^f	+	42/49	—	+	+	+
Acid from ASS-arabinose	10/12	—	—	—	1/49	—	+	3/13	+
Acid from ASS-cellobiose	—	—	—	—	4/49	—	+	+	+
Acid from ASS-ethanol	1/12	+	—	—	28/49	—	—	+	26/29
Acid from ASS-fructose	+	+	—	—	28/49	—	+	+	28/29
Acid from ASS-glycerol	6/12	—	—	—	38/49	—	27/28	+	6/29
Acid from ASS-lactose	—	—	—	—	27/49	—	+	+	+
Acid from ASS-maltose	+	—	6/7	+	46/49	—	+	+	+
Acid from ASS-mannitol	—	—	—	—	31/49	—	—	+	—
Acid from ASS-raffinose	—	—	—	—	—	—	+	+	28/29
Acid from ASS-salicin	—	—	—	—	—	—	+	+	26/29
Acid from ASS-sucrose	—	—	—	—	—	—	+	+	+
Acid from ASS-trehalose	+	—	—	—	42/49	—	+	+	+
Acid from ASS-xylose	4/12	—	—	—	3/49	—	+	+	+
Casein digestion	+	+	+	+	+	+	—	—	—
Esculin hydrolysis	+	+	—	+	47/49	—	+	+	+
Growth at 5°C	—	—	—	+	1/49	1/28	—	—	—
Growth on MacConkey agar	+	+	+	+	+	+	+	+	—
Indole production (Ehrlich reagent)	+	+	+	+	24/49	—	—	—	—
Nitrite reduction	7/12	—	—	—	18/49	+	—	—	—
Urease production (Christensen medium)	7/12	—	—	—	16/49	+	27/28	+	—
β-D-Galactosidase production (ONPG test) ^g	9/12	—	—	—	48/49	—	+	+	+
Guanine-plus-cytosine content (mol%)	37.6 ± 1.0 (6) ^h	33.1 (1)	32.4 ± 0.6 (10)	33.8 (1)	37.0 ± 0.5 (8)	31.4–36.1 (10)	39.6 ± 0.5 (11)	41.4 ± 0.4 (6)	65.3 ± 1.0 (12)

^a +, All strains tested positive; —, all strains tested negative. The phenotypic results for *F. gleum* were from this study, and the phenotypic results for the other taxa were obtained from previous work performed at the National Collection of Type Cultures, as follows: *F. balustinum*, Holmes and Owen, unpublished data; *F. breve*, reference 12; *F. indoltheticum* and *F. meningosepticum*, Holmes and Owen, unpublished data; *F. multivorum*, reference 10; *F. odoratum*, reference 13; *F. spiritivorum*, reference 9; and *P. paucimobilis*, reference 8. The guanine-plus-cytosine values for *F. gleum* were obtained previously (19), and the values for the other taxa were obtained as follows: *F. balustinum*, reference 17; *F. breve*, reference 17; *F. indoltheticum*, Holmes and Owen, unpublished data; *F. meningosepticum*, references 19 and 20; *F. odoratum*, reference 16; *F. multivorum*, reference 10; *F. spiritivorum*, reference 9; and *P. paucimobilis*, reference 8.

^b Formerly known as group M-4f (11, 23).

^c Formerly known as group IIk, biotype 2 (10, 23).

^d Formerly known as group IIk, biotype 1 (8, 23).

^e ASS, Ammonium salt-sugar medium.

^f Number of strains positive/number of strains tested.

^g ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

^h Mean ± standard deviation. The numbers in parentheses are the numbers of strains tested for guanine-plus-cytosine contents.

triple sugar iron agar methods).

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

Casein digested.

Hydrolysis of esculin and tributyrin.

No utilization of citrate (Simmons medium) or malonate.

No oxidation of gluconate.

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

No production of 3-ketolactose.

No reduction of selenite and no deamination of phenylalanine.

Production of phosphatase.

Production of acid in ammonium salt medium under aerobic conditions from glucose, fructose, maltose, and trehalose.

No production of acid in ammonium salt medium under aerobic conditions from adonitol, cellobiose, dulcitol, inosi-

tol, lactose, mannitol, raffinose, rhamnose, salicin, sorbitol, or sucrose.

No production of acid from 10% (wt/vol) glucose or 10% (wt/vol) lactose.

No production of gas from glucose in peptone-water medium.

Hydrolysis of the following substrates (using API ZYM galleries): 2-naphthyl phosphate at pH 8.5; L-leucyl-2-naphthylamide; L-valyl-2-naphthylamide; 2-naphthyl phosphate at pH 5.4; naphthol-AS-B1-phosphodiamide; 2-naphthyl-α-D-glucopyranoside; 6-bromo-2-naphthyl-β-D-glucopyranoside; 1-naphthyl-N-acetyl-β-D-glucosaminide; bis-(*p*-nitrophenyl)phosphate; 4-methylumbelliferylcellobiopyranoside; L-phenylalanyl-β-naphthylamide; L-lysyl-β-naphthylamide; L-histidyl-β-naphthylamide; glycyl-β-naphthylamide; L-aspartyl-β-naphthylamide; L-arginyl-β-naphthylamide; L-alanyl-β-naphthylamide; α-L-glutamyl-β-naphthylamide; DL-methionyl-β-naphthylamide; glycyl-glycyl-β-naphthylamide hydrobromide; glycyl-L-

phenylalanyl- β -naphthylamide; glycyl-L-prolyl- β -naphthylamide; L-leucyl-glycyl- β -naphthylamide; L-seryl-L-tyrosyl- β -naphthylamide; L-glutamine- β -naphthylamide hydrochloride; α -L-glutamyl- β -naphthylamide; L-isoleucyl- β -naphthylamide; L-ornithyl- β -naphthylamide; L-prolyl- β -naphthylamide hydrochloride; L-seryl- β -naphthylamide; L-threonyl- β -naphthylamide; L-tryptophyl- β -naphthylamide; N-carbobenzoxy-glycyl-glycyl-L-arginine- β -naphthylamide.

No hydrolysis of the following substrates (using API ZYM galleries): 2-naphthyl butyrate; 2-naphthyl myristate; L-cystyl-2-naphthylamide; N-benzoyl-DL-arginine-2-naphthylamide; N-glutaryl-phenylalanine-2-naphthylamide; 6-bromo-2-naphthyl- α -D-galactopyranoside; 2-naphthyl- β -D-galactopyranoside; naphthol-AS-B1- β -D-glucuronic acid; 6-bromo-2-naphthyl- α -D-mannopyranoside; 2-naphthyl- α -L-fucopyranoside; p-nitrophenyl- α -D-xylopyranoside; p-nitrophenyl- β -D-fucopyranoside; p-nitrophenyl- β -L-fucopyranoside; o-nitrophenyl-N-acetyl- α -D-glucosaminide; p-nitrophenyl-lactoside; p-nitrocatechol-sulfate; 4-methylumbelliferyl-arabinopyranoside; L-hydroxyprolyl- β -naphthylamide; N-benzoyl-L-leucyl- β -naphthylamide.

The G+C content of the DNA is 37.6 ± 1.0 mol%, and the G+C content of the DNA of the type strain is 37.0 mol%, as estimated by the thermal denaturation method.

The following two additional strains of *F. gleum* have been deposited in the National Collection of Type Cultures: group IIB strain 3531 (= NCTC 10795) and strain F129 (= NCTC 11433).

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