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Flavobacterium scophthalmum sp. nov., a Pathogen of Turbot (*Scophthalmus maximus* L.)

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Fifty orange-pigmented, gram-negative, rod-shaped isolates were recovered from healthy and diseased turbot and from coastal waters (collected in Scotland). On the basis of the results of an examination of 125 phenotypic characteristics and the results of DNA-DNA and DNA-rRNA hybridization experiments, we concluded that these isolates are members of a new species in the genus *Flavobacterium*, for which the name *Flavobacterium scophthalmum* is proposed. The type strain is CCM 4109 (= LMG 13028).

Several species of orange- or yellow-pigmented, rod-shaped bacteria that have low DNA guanine-plus-cytosine (G+C) contents (i.e., *Cytophaga*, *Flavobacterium*, and *Flexibacter* species) have been associated with gill diseases of freshwater and marine fish (3). In 1987, during investigations of the microflora of healthy and diseased turbot (*Scophthalmus maximus* L.), members of a new group of bacteria that have thick cell walls were isolated (19). These organisms caused gill hyperplasia and systemic hemorrhagic septicemia in turbot. Externally, hemorrhaging was apparent in the eyes, skin, and jaw. Internally, there was necrosis or hemorrhaging in the brain, stomach, intestinal tract, liver, and kidney. These bacterial isolates were studied taxonomically, and we propose that they are members of a new species of the genus *Flavobacterium*, *Flavobacterium scophthalmum*.

MATERIALS AND METHODS

Bacterial isolates. Fifty gram-negative, orange-pigmented bacterial strains were isolated during 1987 from healthy and diseased turbot and from coastal water samples (collected in Scotland) on dilution plates containing medium K, which contains (per liter) 1.0 g of yeast extract (Oxoid, Basingstoke, England), 5.0 g of beef extract (Oxoid), 6.0 g of casein (Oxoid), 2.0 g of tryptone (Oxoid), 1.0 g of anhydrous calcium chloride, 15.0 g of agar (Oxoid no. 1), and 750 ml of seawater (aged for 30 days); the pH of this medium is 7.2 (18). Pure cultures were maintained on marine 2216E agar (Difco Laboratories, Detroit, Mich.), medium K, and tryptone soya agar (TSA) (Oxoid) at 4°C, with subculturing every 6 to 8 weeks. The hypothetical median organism, designated strain MM1^T (= CCM 4109^T = LMG 13028^T) (T = type strain), as calculated by the method of Liston et al. (15), was used for electron microscopic studies. Strain MM1^T and six other representative isolates (strains MM1A [= LMG 13029], MM1B [= LMG 13030], MM1D [= LMG 13031], MM2B [= LMG 13032], MM2C [= LMG 13033], and MM4 [= LMG 13034]) were used to determine the DNA base composition and for DNA-DNA and DNA-rRNA hybridization experiments, fatty acid analyses, and API ZYM tests (BioMérieux SA, Marcy l'Etoile, France). Forty-eight authentic cultures representing *Cytophaga*, *Flavobacterium*, *Flexibacter*, *Microscilla*, and *Weeksella* species were also used for DNA-DNA hybridization experiments, and some of these strains were used for DNA-rRNA hybridization experiments and API ZYM tests. The reference cultures used were type strains or authentic strains obtained from major collections and were grown at 18 to 25°C on Anacker-Ordal medium (2), Dubos medium (21), nutrient agar (Oxoid), TSA, the medium for marine flexibacteria (14), or marine 2216E agar, as appropriate (4).

Characterization of the isolates. All of the isolates obtained from turbot and coastal waters were examined for 125 phenotypic characteristics, as described previously (19). The tests used were those considered to have differential value for identification of gram-negative yellow- or orange-pigmented bacteria (12). The methods used were adapted from the methods of Christensen (6), Cowan (7), van der Meulen et al. (27), and Gerhardt et al. (11). The characteristics studied included colonial morphology (including the presence of flexirubin-like pigments, as determined by the development of a red color after the addition of 20% [wt/vol] potassium hydroxide [19]); cell morphology; the presence of gliding motility on Anacker-Ordal medium (2); oxidative or fermentative metabolism of glucose; production of ammonia from arginine, arginine dihydrolase, catalase, H_2S, indole, lysine and ornithine decarboxylases, oxidase, phenylalanine deaminase, and phosphatase; the methyl red test; nitrate reduction; the Voges-Proskauer reaction; production of acid from arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose, and xylose; degradation of esculin, agar, blood (beta-hemolysis), casein, cellulose, chitin, DNA, gelatin, starch, tributyrin, Tween 20, Tween 40, Tween 60, Tween 80, Tween 85, tyrosine, and urea; utilization of alginate, L-arabinose, L-arabitol, carboxymethyl cellulose, cellobiose, erythritol, ethanol, D-fructose, D-glucose, glycerol, heparin, inositol, inulin, L-leucine, lysine, maltose, mannitol, methanol, L-methionine, pectin, L-phenylalanine, polypectate, raffinose, D-ribose, sodium benzoate, sodium citrate, sodium succinate, sorbitol, sucrose, *m*-xylitol, and D-xylose as sole sources of carbon for energy and growth; utilization of sodium nitrate, vitamin-free Casamino Acids and yeast extract as sources of nitrogen; growth in the presence of 0 to 5% (wt/vol) NaCl; growth at 4 to 42°C; growth on MacConkey agar (Oxoid); and susceptibility to ampicillin (10 and 25 μg), carbenicillin (100 μg), chloramphenicol (10 and 50 μg), chlortetracycline (10 μg), cephaloridone (25 μg), cloxacillin (5 μg), colistin sulfate (10 μg), cotrimoxazole (25

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μg), erythromycin (10 μg), furazolidone (50 μg), fusidic acid (10 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), methicillin (10 μg), nalidixic acid (5 and 30 μg), neomycin (10 μg), nitrofurantoin (200 μg), novobiocin (5 μg), oleandomycin (5 μg), oxytetracycline (10 μg), penicillin G (1.5 IU), streptomycin (10 and 25 μg), sulfadiazine (50 μg), sulfafurazole (100 and 500 μg), and tetracycline (10 and 50 μg). When possible, medium K was used as the basal medium, and test results were usually recorded after incubation at 25°C for 14 days. In addition, seven representative isolates were used to determine the hydrolysis of 19 substrates in the API ZYM system following incubation at 22°C for 12 h. Hydrolysis resulted in the production of color, the intensity of which was measured on a scale of 0 to 5 (4).

DNA base composition. The methods used to extract and purify DNAs have been described previously (5). The base compositions were determined by thermal denaturation and were calculated by using the equation of Marmur and Doty (16), as modified by De Ley (8).

DNA-DNA hybridization. The degree of DNA-DNA binding, expressed as a percentage, was determined spectrophotometrically by using the initial renaturation rate method (9). The renaturation rates were measured in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0) at the optimal renaturation temperature (64.9°C). Each experiment was repeated at least once.

DNA-rRNA hybridization. rRNAs from *Flavobacterium indologenes* LMG 8337^T, *Flavobacterium meningosepticum* LMG 12279^T, and *Weeksella zoohelcum* LMG 8351^T were radioactively labelled in vivo by adding [^{3}H]adenine to early-log-phase broth cultures. Labelled 23S rRNAs were then prepared by using a procedure slightly modified from the procedure of Aiba et al. (1), as described by Vandamme et al. (26). Fixation of single-stranded DNA on cellulose nitrate filters, DNA-rRNA hybridization, RNase treatment, and determination of the thermostability of the hybrids were performed as previously described (10).

Gas chromatographic analysis of cellular FAMEs. Cultures of strains CCM 4109^T, LMG 13029, LMG 13030, LMG 13031, LMG 13032, LMG 13033, and LMG 13034 were grown for 24 h at 28°C on TSA and were harvested, and the cells were used for extracting fatty acid methyl esters (FAMEs). The FAMEs were then separated by gas-liquid chromatography (28). FAME fingerprints were identified by using the Microbial Identification System software package (MIS version no. 3.7, obtained from Microbial ID, Inc., Newark, Del.) and a calibration mixture of known standards (Hewlett-Packard). FAME profiles were compared by performing a numerical analysis (28).

Transmission electron microscopy. Dense suspensions containing approximately 10^9 cells per ml in 0.85% (wt/vol) saline were prepared from 48-h plate cultures. The suspensions were fixed overnight in 0.5% (vol/vol) buffered (pH 7.4) glutaraldehyde (EMscope Laboratories, Watford, England). The preparations were stained with 1% (vol/vol) phosphotungstic acid (EMscope) and then examined to determine cell morphology. Thin sections were also prepared and stained by using the methods of Watson (29), Millonig (17), and Reynolds (22). All specimens were examined with an AEI model EM6G transmission electron microscope.

Identification of the orange chromogens. We attempted to identify the isolates by using the diagnostic schemes described by Holmes et al. (12) and by using the descriptions of all relevant taxa published in the Approved Lists of Bacterial Names (24) and the supplements to the Approved Lists.

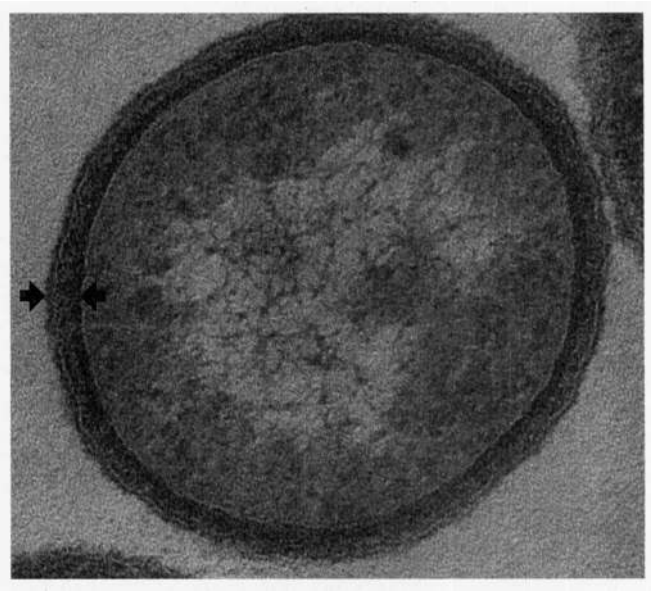

FIG. 1. Transmission electron micrograph of a cross section of a strain MM1^T cell. The arrows indicate the thick wall.

RESULTS AND DISCUSSION

Phenotypic characterization of the isolates. All of the isolates were members of a homogeneous group and exhibited marked uniformity in their characteristics (19). Round, raised, entire, shiny, smooth, orange-pigmented (nondiffusing, flexirubin-like pigment) colonies that were 5 to 6 mm in diameter were formed within 48 h of inoculation at 25°C on medium K, marine 2216E agar, and TSA. Fresh isolates, examined in 1987, appeared to exhibit gliding motility on Anacker-Ordal medium. However, gliding motility could not be confirmed during a subsequent examination in 1993. Consequently, we considered the cultures nonmotile. The cultures contained uniformly shaped rods that were approximately 2.0 by 0.5 μm and had thick (50-nm) cell walls (Fig. 1) after incubation for 18 h. Shorter rods (approximately 1.0 by 0.5 μm) (Fig. 2) were apparent in late-exponential-phase cultures after incubation for 48 h. Resting stages were not observed. The results of the API ZYM tests are shown in Table 1; the results of other tests are described below.

DNA base composition. The G+C ratios of the DNAs were calculated to be 34.1, 34.2, 33.5, 34.7, 34.5, 34.7, and 33.8 mol% for strains CCM 4109^T, LMG 13029, LMG 13030, LMG 13031,

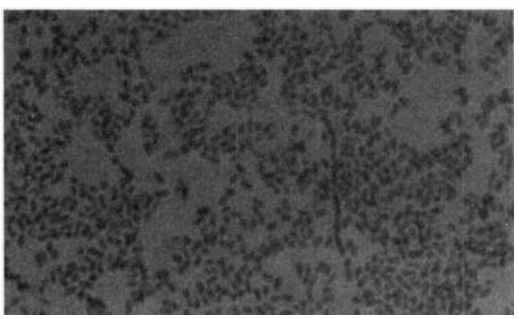

FIG. 2. Photomicrograph of Gram-stained MM1^T cells from a 48-h culture on medium K, showing the presence of short rods and some filaments.

TABLE 1. Profiles for *Flavobacterium* strains obtained by using the API ZYM system

Taxon	Hydrolysis of the following substrates[a]:																	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Flavobacterium scophthalmum[b]	5	3	4	1	5	4	2	4	1	5	5	0	0	0	3	1	2	0	0
Flavobacterium balustinum LMG 8329^T	5	1	3	1	5	5	2	2	1	5	5	0	0	0	3	4	2	0	0
Flavobacterium gleum LMG 8334^T	5	2	3	1	5	5	2	2	2	5	5	0	0	0	4	5	4	0	0
Flavobacterium indologenes LMG 8337^T	5	2	3	1	5	4	2	2	2	5	5	0	0	0	5	0	4	0	0
Flavobacterium indoltheticum LMG 4025^T	5	2	3	1	5	4	1	0	0	4	4	0	0	0	4	0	3	0	0

[a] A, 2-naphthyl-phosphate; B, 2-naphthyl-butyrate; C, 2-naphthyl-caprylate; D, 2-naphthyl-myristate; E, L-leucyl-2-naphthylamide; F, L-valyl-2-naphthylamide; G, L-cystyl-2-naphthylamide; H, *N*-benzoyl-DL-arginine-2-naphthylamide; I, *N*-glutaryl-phenylalanine-2-naphthylamide; J, 2-naphthyl-phosphate; K, naphthol-AS-BI-phosphate; L, 6-Br-2-naphthyl-α-D-galactopyranoside; M, 2-naphthyl-β-D-galactopyranoside; N, naphthol-AS-BI-β-D-glucuronide; O, 2-naphthyl-α-D-glucopyranoside; P, 6-Br-2-naphthyl-β-D-glucopyranoside; Q, 1-naphthyl-*N*-acetyl-β-D-glucosaminide; R, 6-Br-2-naphthyl-α-D-mannopyranoside; S, 2-naphthyl-α-L-fucopyranoside. The values are API ZYM reaction scores.

[b] Data for strain LMG 13028^T and six other strains.

LMG 13032, LMG 13033, and LMG 13034, respectively. The mean for the seven strains was 34.2 mol%.

DNA-DNA hybridization. The DNA of one of the isolates obtained from turbot (LMG 13028^T) was closely related to the DNAs of six other fresh isolates (levels of homology, 91 to 103%). The DNAs of *Flavobacterium scophthalmum* strains were moderately closely related to the DNAs of *Flavobacterium balustinum* LMG 8329^T (38 to 44%) and *Flavobacterium indoltheticum* LMG 4025^T (33 to 38%), but not to the DNAs of *Flavobacterium gleum* LMG 8334^T (10%) and *Flavobacterium indologenes* LMG 8337^T (13%) (Table 2).

Preliminary DNA-DNA hybridization experiments were performed at 60°C by using the S1-DE81 method (20) and labelled DNAs of *Flavobacterium scophthalmum* LMG 13028^T, other *Flavobacterium scophthalmum* strains (Table 2), and reference strains (3a). Comparable binding values were found between the *Flavobacterium scophthalmum* strains (85 to 104%). No significant binding values were obtained when labelled DNA from *Flavobacterium scophthalmum* LMG 13028^T was hybridized with DNAs from *Cytophaga agarovorans* NCIMB 2217^T (National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland), "*Cytophaga allerginae*" ATCC 35408 (American Type Culture Collection, Rockville, Md.), *Cytophaga aprica* ATCC 23126^T, *Cytophaga aquatilis* DSM 2063^T (Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany), "*Cytophaga arvensicola*" JCM 2836 (Japan Collection of Microorganisms, Saitama, Japan), *Cytophaga aurantiaca* NCIMB 8628^T, *Cytophaga diffluens* NCIMB 1402^T, *Cytophaga fermentans* NCIMB 2218^T, *Cytophaga flevensis* DSM 1076^T, "*Cytophaga heparina*" NCIMB 9290^T, *Cytophaga hutchinsonii* NCIMB 9469^T, *Cytophaga johnsonae* DSM 2064^T, *Cytophaga latercula* NCIMB 1399^T, *Cytophaga lytica* NCIMB 1423^T, *Cytophaga marinoflava* NCIMB 397^T, *Cytophaga pectinovora* NCIMB 9059^T, *Cytophaga saccharophila* NCIMB 2072^T, *Cytophaga salmonicolor* NCIMB 2216^T, *Cytophaga succinicans* NCIMB 2277^T, *Cytophaga uliginosa* NCIMB 1863^T, *Flavobacterium aquatile* NCIMB 8694^T, *Flexibacter aurantiacus* NCIMB 1382^T, *Flexibacter canadensis* ATCC 29591^T, *Flexibacter columnaris* NCIMB 2248^T, *Flexibacter elegans* NCIMB 1385^T, *Flexibacter filiformis* ATCC 29495^T, *Flexibacter flexilis* NCIMB 12853^T, *Flexibacter litoralis* NCIMB 1366^T, *Flexibacter maritimus* NCIMB 2154^T, *Flexibacter ovolyticus* NCIMB

TABLE 2. Levels of DNA-DNA relatedness for *Flavobacterium scophthalmum* strains and strains of other *Flavobacterium* species belonging to the same rRNA branch

Strain	% of DNA-DNA binding with[a]:										
	Flavobacterium scophthalmum LMG 13028^T	*Flavobacterium scophthalmum* LMG 13034	*Flavobacterium scophthalmum* LMG 13030	*Flavobacterium scophthalmum* LMG 13031	*Flavobacterium scophthalmum* LMG 13029	*Flavobacterium scophthalmum* LMG 13033	*Flavobacterium scophthalmum* LMG 13032	*Flavobacterium balustinum* LMG 8329^T	*Flavobacterium indoltheticum* LMG 4025^T	*Flavobacterium gleum* LMG 8334^T	*Flavobacterium indologenes* LMG 8337^T
Flavobacterium scophthalmum LMG 13028^T	100										
Flavobacterium scophthalmum LMG 13034	103	100									
Flavobacterium scophthalmum LMG 13030	102		100								
Flavobacterium scophthalmum LMG 13031	101			100							
Flavobacterium scophthalmum LMG 13029	99				100						
Flavobacterium scophthalmum LMG 13033	95					100					
Flavobacterium scophthalmum LMG 13032	91						100				
Flavobacterium balustinum LMG 8329^T	44	44				41	38	100			
Flavobacterium indoltheticum LMG 4025^T	37	38				33	32	35	100		
Flavobacterium gleum LMG 8334^T	10								14	100	
Flavobacterium indologenes LMG 8337^T	13									25	100

[a] Each value is the average of the values from at least two experiments.

TABLE 3. $T_{m(e)}$ values for DNA-rRNA hybrids

DNA from:	$T_{m(e)}$ (°C) with rRNA from[a]:		
	Flavobacterium indologenes LMG 8337^T	*Flavobacterium meningosepticum* LMG 12279^T	*W. zoohelcum* LMG 8351^T
Flavobacterium scophthalmum LMG 13028^T	75.0	71.3	71.8
Flavobacterium scophthalmum LMG 13032	75.9		
Flavobacterium balustinum LMG 8329^T	73.7		70.6
Flavobacterium gleum LMG 8334^T	76.2	73.3	73.8
Flavobacterium indologenes LMG 8337^T	78.1	70.6	72.5
Flavobacterium indoltheticum LMG 4025^T	75.3		73.1
Flavobacterium meningosepticum LMG 12279^T	72.2	75.4	71.9
W. zoohelcum LMG 8351^T	71.7	71.8	75.0

[a] Some of the data are from reference 23.

13127^T, *Flexibacter polymorphus* ATCC 27820^T, *Flexibacter psychrophilus* NCIMB 1947^T, *Flexibacter roseolus* NCIMB 1433^T, *Flexibacter ruber* NCIMB 1436^T, *Flexibacter sancti* NCIMB 1379^T, "*Microscilla aggregans*" NCIMB 1443^T, "*Microscilla arenaria*" NCIMB 1413^T, "*Microscilla furvescens*" NCIMB 1419^T, *Microscilla marina* NCIMB 1400^T, "*Microscilla sericea*" NCIMB 1403^T, and "*Microscilla tractuosa*" NCIMB 1408^T.

DNA-rRNA hybridization. The results of the DNA-rRNA hybridization experiments are shown in Table 3 and are presented as a dendrogram based on the melting temperature of elution [$T_{m(e)}$] values of the hybrids in Fig. 3; each DNA-rRNA hybrid was characterized by its $T_{m(e)}$ value, the temperature at which 50% of the hybrid was denatured. The $T_{m(e)}$ values for the reciprocal hybridizations between all of the strains on each branch were used to calculate the average linkage level for each pair of strains. After the results of a screening study revealed that the environmental isolates gave low levels of hybridization with the rRNA probes for the branches containing most *Cytophaga* and *Flexibacter* species (4a), the DNAs of these organisms were tested with the rRNA probes for *Flavobacterium indologenes*, *Flavobacterium meningosepticum*, and *W. zoohelcum*. Previous studies had revealed that these three bacterial species belong to the same rRNA homology branch within rRNA superfamily V, together with *Flavobacterium gleum*, *Flavobacterium balustinum*, *Flavobacterium indoltheticum*, and *Riemerella anatipestifer* (23). The data from the DNA-rRNA hybridization experiments performed with the DNAs from two environmental isolates and three rRNA probes revealed that the environmental isolates are most closely related to *Flavobacterium indologenes* and are related at lower levels to *Flavobacterium meningosepticum* and *W. zoohelcum*. The $T_{m(e)}$ values of the environmental isolates with *Flavobacterium indologenes* placed these organisms in the tight genomic cluster containing *Flavobacterium balustinum*, *Flavobacterium gleum*, and *Flavobacterium indoltheticum*.

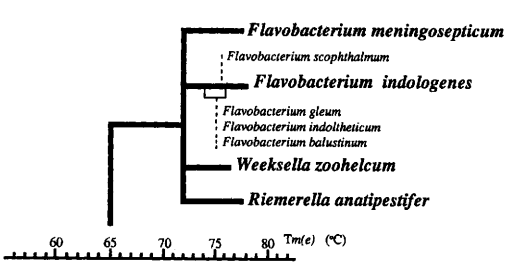

FIG. 3. Position of *Flavobacterium scophthalmum* on a partial dendrogram of rRNA superfamily V.

Gas chromatographic analysis of cellular FAMEs. The fatty acid profiles of seven environmental isolates were determined and compared with the profiles of their closest phylogenetic neighbors. The average fatty acid compositions of the isolates and of the reference strains are shown in Table 4. At least 15 fatty acids occurred in all of the environmental isolates. Branched fatty acids were dominant in the fatty acids of all of the strains studied. The four major fatty acids (fatty acids with mean levels greater than 5%) were 15:0 iso, iso 17:1 ω9c, 17:0 iso-3-OH, and summed feature 4 (i.e., 15:0 iso-2-OH and/or 16:1 ω7t, which could not be separated from each other by gas chromatography). There were few differences between the fatty acid compositions of the environmental isolates and the fatty acid compositions of the other species studied.

Evidence for assignment to the genus *Flavobacterium* and proposal for a new species. The environmental isolates had the overall characteristics of the genus *Flavobacterium* (12); viz., the cells were aerobic, short, (orange-) pigmented (the pigment was shown to be flexirubin by the development of a red color after the addition of 20% [wt/vol] potassium hydroxide), gram-negative, apparently nonmotile rods without resting stages, which had low DNA G+C contents (30 to 42 mol%) and produced catalase, oxidase and phosphatase. The chemotaxonomy data, which revealed the presence of branched-chain fatty acids and branched-chain hydroxy fatty acids, are also consistent with identification of these bacteria as flavobacteria or related organisms (12, 25). On the basis of the results of DNA hybridization experiments and phenotypic characterization tests, the environmental isolates could not be placed in any of the species included on the Approved Lists of Bacterial Names (24) and the supplements to the Approved Lists or in taxa now regarded as incertae sedis. Consequently, we propose that these isolates should be placed in a new species, *Flavobacterium scophthalmum* (scoph. thal' mum. N.L. n. *Scophthalmus*, turbot; N.L. adj. *scophthalmum*, pertaining to turbot).

Description of *Flavobacterium scophthalmum* sp. nov. Uniformly shaped gram-negative rods that are approximately 2.0 by 0.5 μm and have rounded ends after incubation for 18 h. Shorter rods, approximately 1.0 by 0.5 μm, are present in late-exponential-phase cultures after incubation for 48 h.

Fresh isolates appear to exhibit gliding motility; flagella are not produced. However, after storage, the presence of gliding motility cannot be confirmed. Therefore, isolates may be considered nonmotile.

The cell wall is approximately 50 nm thick (Fig. 1).

Resting stages are not observed.

Colonies on medium K (18), marine 2216E agar, and TSA are shiny, smooth, round, raised, entire, and 5 to 6 mm in diameter after incubation for 48 h at 25°C.

TABLE 4. Fatty acid compositions of *Flavobacterium scophthalmum* and allied bacteria[a]

Fatty acid	% in:						
	Flavobacterium scophthalmum (7 strains)	*Flavobacterium balustinum* LMG 8329^T	*Flavobacterium indoltheticum* LMG 4025^T	*Flavobacterium indologenes* LMG 8337^T	*Flavobacterium gleum* LMG 8334^T	*Flavobacterium meningosepticum* LMG 12279^T	*W. zoohelcum* LMG 8351^T
13:0 iso	Tr	Tr	ND	Tr	Tr	1.4	1.7
Unknown ECL 13.566[b]	3.3 ± 0.5	1.6	Tr	1.2	1.6	1.5	1.0
15:0 iso	34.9 ± 0.5	32.3	29.5	30.8	31.4	41.2	49.1
15:0 iso-3-OH	2.9 ± 0.1	2.7	2.2	2.7	2.5	3.5	4.0
15:0 anteiso	Tr	Tr	4.9	Tr	Tr	2.2	ND
16:0	1.1 ± 0.1	1.6	Tr	Tr	Tr	Tr	Tr
16:0 3-OH	1.3 ± 0.1	1.4	Tr	Tr	1.1	2.2	ND
16:0 iso-3-OH	Tr	Tr	1.2	ND	Tr	Tr	ND
Unknown ECL 16.580[b]	1.7 ± 0.2	1.3	1.3	1.7	1.9	1.7	Tr
17:0 2-OH	Tr	Tr	2.7	ND	ND	Tr	ND
17:0 iso	1.1 ± 0.1	1.0	Tr	Tr	Tr	Tr	ND
17:0 iso-3-OH	17.1 ± 0.2	16.8	15.4	18.1	19.0	16.3	11.5
iso 17:1 ω9c	21.3 ± 0.4	27.1	24.6	27.7	24.9	7.0	18.4
18:1 ω5c	Tr	Tr	Tr	Tr	Tr	Tr	1.5
Summed feature 4[c]	11.8 ± 0.6	9.2	12.0	12.9	12.8	18.0	9.6
Summed feature 5[d]	ND	Tr	ND	Tr	Tr	Tr	1.6

[a] Fatty acids which accounted for <1% of the total fatty acids in all of the strains studied were not included. For *Flavobacterium scophthalmum*, the means and standard deviations are given. Tr, trace (<1%); ND, not detected.

[b] The identities of the fatty acids which have equivalent chain lengths (ECL) of 13.566 and 16.580 are not known.

[c] 15:0 iso-2-OH and 16:1 ω7t could not be separated from each other by gas chromatography by using the MIS system and together were considered summed feature 4.

[d] 17:1 iso 1 and 17:1 anteiso B could not be separated from each other by gas chromatography by using the MIS system and together were considered summed feature 5.

A nondiffusible, nonfluorescent orange (flexirubin-like) pigment is produced.

Broth cultures are uniformly turbid.

Cultures are chemoorganotrophic (metabolism is respiratory and fermentative).

Nitrates are not reduced.

Ammonia is produced from arginine; catalase, oxidase, phenylalanine deaminase, and phosphatase are produced, but arginine dihydrolase, H_2S, indole, and lysine and ornithine decarboxylases are not produced.

The methyl red test and the Voges-Proskauer reaction are negative.

Esculin, blood (beta-hemolysis), casein, DNA, gelatin, tributyrin, Tween 20, Tween 40, Tween 60, Tween 80, Tween 85, tyrosine, and urea are degraded, but agar, cellulose, chitin, and starch are not degraded.

Acid is not readily produced from carbohydrates (arabinose, cellobiose, glucose, lactose, mannitol, raffinose, salicin, sucrose, and xylose).

Cellobiose, ethanol, D-fructose, D-glucose, maltose, pectin, polypectate, and *m*-xylitol are utilized as sole sources of carbon for energy and growth, but alginate, L-arabinose, L-arabitol, carboxymethyl cellulose, erythritol, glycerol, heparin, inositol, inulin, L-leucine, lysine, mannitol, methanol, L-methionine, L-phenylalanine, D-ribose, raffinose, sodium benzoate, sodium citrate, sodium succinate, sorbitol, sucrose, and D-xylose are not utilized.

Hydrolyzes the following substrates (as determined with the API ZYM system; scores of 2 to 5 are considered positive): 2-naphthyl-phosphate, 2-naphthyl-butyrate, 2-naphthyl-caprylate, L-leucyl-2-naphthylamide, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, 2-naphthyl-phosphate, naphthol-AS-BI-phosphate, 2-naphthyl-α-D-glucopyranoside, and 1-naphthyl-*N*-acetyl-β-D-glucosaminide.

Does not hydrolyze the following substrates (as determined with the API ZYM system; scores 0 and 1 are considered negative): 2-naphthyl-myristate, *N*-glutaryl-phenylalanine-2-naphthylamide, 6-Br-2-naphthyl-α-D-galactopyranoside, 2-naphthyl-β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronide, 6-Br-2-naphthyl-β-D-glucopyranoside, 6-Br-2-naphthyl-α-D-mannopyranoside, and 2-naphthyl-α-L-fucopyranoside.

Sodium nitrate, vitamin-free Casamino Acids, and yeast extract are utilized as nitrogen sources.

Growth occurs at 15 to 25°C (weak growth occurs at 4°C and no growth occurs at 35°C) and in the presence of 0 to 4% NaCl, but not in the presence of 5% (wt/vol) NaCl, or on MacConkey agar.

All of the strains which we studied are characterized by the following fatty acids: 15:0 iso, 15:0 iso-3-OH, 16:0, 16:0 3-OH, 17:0 iso, 17:0 iso-3-OH, iso 17:1 ω9c, summed feature 4 (i.e., 15:0 iso-2-OH and/or 16:1 ω7t), and two unidentified fatty acids with equivalent chain lengths of 13.556 and 16.580.

Cultures are found in coastal waters and are capable of producing gill disease and hemorrhagic septicemia in turbot.

The G+C content of the DNA is 33 to 35 mol%.

The type strain is MM1, which was isolated in 1987 from the gills of diseased turbot in Scotland; a culture of this strain has been deposited in the Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia, as CCM 4109 and in the culture collection of the Laboratorium voor Microbiologie, Ghent, Belgium, as LMG 13028. The characteristics of the type strain conform to the description given above.

Six additional strains of *Flavobacterium scophthalmum*, MM1A (isolated from the gills of diseased turbot), MM1B (isolated from diseased turbot), M1D (isolated from diseased turbot), MM2B (isolated from seawater), MM2C (isolated from seawater), and MM4 (isolated from the gills of diseased turbot), have been deposited in the culture collection of the Laboratorium voor Microbiologie, Ghent, Belgium, as LMG

TABLE 5. Differentiating characteristics of *Flavobacterium scophthalmum* and other taxa on the same rRNA homology branch in rRNA superfamily V

Taxon	Acid produced from glucose	Growth at 36°C on agar	Growth on MacConkey agar	Nitrate reduction	Phenylalanine deaminase	Urea degradation	Indole production
Flavobacterium scophthalmum	−[a]	−	−	−	+	+	−
Flavobacterium balustinum	+	−	+	+	−	−	+
Flavobacterium gleum	+	+	+	v	−	v	+
Flavobacterium indologenes	+	+	v	v	−	−	+
Flavobacterium indoltheticum	+	+	+	−	ND	−	+

[a] +, positive; −, negative; v, variable; ND, not determined. Data are from references 12, 13, and 30 and this study.

13029, LMG 13030, LMG 13031, LMG 13032, LMG 13033, and LMG 13034, respectively.

Differential characteristics for *Flavobacterium scophthalmum* and allied bacteria are shown in Tables 1 and 5.

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