Flavobacterium thalpophilum, a New Species Recovered from Human Clinical Material

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We propose a new species, *Flavobacterium thalpophilum*. Each of the seven strains placed in this new species was examined for 129 characteristics, including 58 enzyme reactions (API ZYM System). These bacteria are rodshaped, aerobic, gram negative, and nonmotile and oxidize glucose in oxidation-fermentation medium. The mean guanine-plus-cytosine content of the deoxyribonucleic acids of seven strains is 45.0 ± 0.8 mol%. The distinguishing features of the new species include an ability to reduce nitrate, an ability to grow at 42°C, and an ability to produce acid from various carbohydrates and alcohols. In particular, the ability of *F. thalpophilum* strains to produce acid from adonitol distinguishes them from all other *Flavobacterium* species. The seven strains of the new species were isolated from human clinical specimens. The type strain is strain K-1173 (= NCTC 11429).

The name *Flavobacterium multivorum* was proposed by Holmes et al. (11) for strains that belonged to group IIk, biotype 2 (21). Holmes et al. (11) also described two strains differing from *F. multivorum* in producing acid from ethanol and mannitol and in having a significantly higher proportion of guanine plus cytosine (G+C) in their deoxyribonucleic acid (DNA). For these 2 strains and 11 additional isolates the name *Flavobacterium spiritivorum* was proposed (10). Holmes et al. (11) also described a group IIk, biotype 2 strain (strain 8291) which could not be included in *F. multivorum*, as it differed from this species in producing acid from adonitol, growing at 42°C, reducing nitrate, and having a significantly higher proportion of G+C. Four strains with the latter characteristics were also described by Levine et al. (16), who also detected no DNA-DNA hybridization with an isolate representative of the majority of their strains belonging to group IIk, biotype 2. The characteristics of strain 8291 from the study of Holmes et al. (11), the four similar strains from the study of Levine et al. (16), and two additional isolates are described here. We present evidence that these strains constitute a new species, for which we propose the name *Flavobacterium thalpophilum*.

MATERIALS AND METHODS

Bacterial strains. The seven bacterial strains studied (all from the United States) and the sources from which they were isolated are given in Table 1.

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

G+C content. The G+C contents of DNAs were determined optically by thermal denaturation (5).

Escherichia coli B DNA (G+C content, 51.2 mol%) was used as a control.

DNA relatedness. The preparation and purification of DNA and the conditions used to determine DNA relatedness by the hydroxyapatite method have been described previously (2). DNA from *F. thalpophilum* strain K-1173^T (T = type strain) was labeled with ³²PO₄ in vitro (3).

RESULTS

Phenotypic characteristics of *F. thalpophilum*.

The seven strains which we studied were strictly aerobic, gram-negative, uniformly staining rods with parallel sides and rounded ends. After 48 h at 30°C, colonies on nutrient agar were circular, 0.5 to 2 mm in diameter (except strain K-1232, which formed pinpoint colonies), low convex, smooth, and opaque. A pale yellow pigment that was nondiffusible and nonfluorescent was produced by strains 8291, C499, and E8209 within 5 days and by the remaining strains after 5 days. None of the strains produced a dark brown pigment on tyrosine agar. The strains grew well at room temperature and at 30, 37, and 42°C but failed to grow at 5°C. Hemolysis was not present after aerobic growth for 24 h. A total of 112 characteristics were common to all strains (either all positive or all negative; see below), and there were 17 characteristics in which one or more of the strains differed (Table 2). Strain K-1173^T was the centrotype (there was no median organism). Strains 8291 and K-1232 were the least typical members of *F. thalpophilum*: the former differed from the other six strains in four biochemical characteristics, whereas the latter differed from the other six strains in two different biochemical characteristics.

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

Strain	Isolated from:
8291	Blood; Louisiana
B5832	Wound; New York City
C499 (= K-1207 = NCTC 11430)	Abscess; Tennessee
E8209	Blood; Texas
K-997	Leg wound; California
K-1173 ^T (= NCTC 11429 ^T)	Wound; New York City
K-1232 (= NCTC 11431)	Foot wound; California

All seven strains were resistant to therapeutic levels of the following antimicrobial agents (minimum inhibitory concentrations indicated in parentheses): ampicillin (64 µg/ml), carbenicillin (32 to 128 µg/ml), cephalothin (128 µg/ml), amikacin (>32 µg/ml), gentamicin (≥32 µg/ml), kanamycin (>32 µg/ml), tobramycin (>32 µg/ml), and chloramphenicol (>8 µg/ml). One strain was susceptible (1 µg/ml) and six were resistant (64 µg/ml) to tetracycline; all strains were susceptible to rifampin (0.5 to 2 µg/ml).

G+C content of *F. thalpophilum* DNA. G+C contents were determined for seven strains of *F. thalpophilum* and for the type strains of *F. multivorum*, and *F. spiritivorum*. The average % G+C content of *F. thalpophilum* was 45.0 ± 0.8 mol% (range, 43.8 to 46.0 mol%). The G+C contents of the type strains of *F. thalpophilum*, *F. multivorum*, and *F. spiritivorum* were 43.8,

40.8, and 41.6 mol%, respectively. The two latter values are in good agreement with previously published values (Table 3).

DNA relatedness. The levels of relatedness of labeled *F. thalpophilum* DNA to unlabeled DNAs from six other *F. thalpophilum* strains were 64 to 96% (average, 74%) in reactions done at a temperature that was close to optimal for DNA reassociation and 63 to 96% (average, 74%) in reactions done at 75°C, where only very closely related DNA sequences can reassociate (Table 4). The levels of divergence (percentage of unpaired bases) within related sequences were 0.5 to 1.5%. The levels of relatedness of *F. thalpophilum* to the type strains of *F. multivorum* and *F. spiritivorum* were 20 and 12%, respectively.

DISCUSSION

The results presented above indicate that the seven strains of gram-negative, yellow-pigmented bacteria for which the name *Flavobacterium thalpophilum* (thal.po'phi.lum. Gr. n. *thalpos* warmth; Gr. n. adj. *philon* loving; M. L. n. adj. *thalpophilum* warmth-loving) is here proposed constitute a homogeneous taxon. This new species conforms phenotypically to the definition of *Flavobacterium* as emended by Holmes and Owen (7), although members of the species are not markedly yellow pigmented and (like *F. multivorum* and *F. spiritivorum*) are less active proteolytically than other species in the genus (i.e., *Flavobacterium balustinum*, *Flavobacter-*

TABLE 2. Characteristics for which the seven strains of *F. thalpophilum* gave different results

Characteristic	No. of strains positive	Result for type strain K-1173	Strain(s) that gave the less common result
Acid from peptone-water-glucose	6	+	C499
Deoxyribonuclease production	6	+	K-1232
Gelatin hydrolysis (plate method)	6	+	8291
Hydrolysis of:			
L-Valyl-2-naphthylamide ^{a,b}	6	+	K-1232
6-Bromo-2-naphthyl-β-D-glucopyranoside ^{a,b}	5	+	8291, K-1232
L-Tyrosyl-β-naphthylamide ^a	5	+	8291, B5832
6-Bromo-2-naphthyl-α-D-galactopyranoside ^{a,b}	4	+	8291, B5832, K-997
2-Naphthyl-β-D-galactopyranoside ^{a,b}	4	+	8291, B5832, K-997
2-Naphthyl-α-L-fucopyranoside ^{a,b}	4	+	8291, B5832, K-997
p-Nitrocathechol sulfate ^a	4	+	8291, K-997, K-1232
4-Methylumbelliferylargininopyranoside ^a	3	—	8291, E8209, K-1232
N-Carbobenzoxyl-L-arginine-4-methoxy-β-naphthylamide hydrochloride ^a	3	+	C499, E8209, K-1173 ^T
Production of yellow pigment	3	—	8291, C499, E8209
Hydrolysis of:			
L-Phenylalanyl-β-naphthylamide ^a	2	—	E8209, K-997
2-Naphthyl caprylate ^{a,b}	1	—	8291
Bis-(p-nitrophenyl)-phosphate ^a	1	—	8291
p-Nitrophenyl lactoside ^a	1	—	8291

^a Tested by using various API ZYM galleries.

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

ium breve, *Flavobacterium meningosepticum*, and *Flavobacterium odoratum*). Furthermore, the G+C values of 44 to 46 mol% obtained for *F. thalophilum* are higher than the values obtained for other recognized species of *Flavobacterium* (Table 3). The inclusion of *F. thalophilum* in the genus thus widens the G+C range of *Flavobacterium* (7) to 31 to 46 mol%. Since this is a wide range for a bacterial genus, *F. multivorum*, *F. spiritivorum*, and *F. thalophilum* could be moved to a new genus along the lines suggested by Holmes and Owen (8). However, there are insufficient phenotypic differences to create a new genus. Characteristics for the practical identification of the new species are shown in Table 3. *Pseudomonas paucimobilis* is included in Table 3 because it has biochemical properties similar to those of *F. multivorum*, *F. spiritivorum*, and *F. thalophilum*. Table 3 shows that the new species is most similar to *F. multivorum*. There are consistent differences between *F. multivorum* and *F. thalophilum* in three conventional tests (production of acid in ammonium salt medium from adonitol; growth at 42°C, and reduction of nitrate) but not in any API ZYM test. The mean G+C content of the strains of the new species which we examined (45.0 mol%) is slightly higher than the previously published values for *F. multivorum* (39.6 mol% G+C [present study, 40.8 mol%]) and *F. spiritivorum* (41.4 mol% G+C [present study, 41.6 mol%]). DNA relatedness studies showed that the seven strains of *F. thalophilum* tested constitute a single species that is distinct from *F. multivorum* and *F. spiritivorum*. The levels of relatedness of the type strain to some other strains are 64 to 69%, values which are slightly lower than the levels of relatedness (70% or more) that are usually observed between strains of a single species (2). The very low levels of sequence divergence among these strains and the fact that the levels of relatedness remain high in reactions performed at the stringent 75°C incubation temperature (63 to 78%) insure the validity of assigning all of the strains to a single species. Previously, the DNAs of strains K-1173^T and K-1232 showed no hybridization with the DNA of a strain of *F. multivorum* (16).

All of the strains of *F. thalophilum* were isolated from clinical specimens, and blood and wounds were the most common sources. The clinical significance of these organisms cannot be assessed at present, but their resistance to a wide range of antimicrobial agents indicates that any infections in humans due to such bacteria could prove difficult to treat. Resistance to antimicrobial agents is a characteristic that *F. thalophilum* shares with the other *Flavobacterium* species (1, 11–15, 22).

Description of the species and the type strain.

The characteristics for which all seven strains of the species gave either positive results or negative results are given below. Characteristics for which the seven strains gave different results are given 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, low convex, smooth, opaque colonies develop on nutrient agar after 2 days; colonies nonhemolytic on 5% (vol/vol) horse blood agar; on nutrient agar, eventual production of a yellow pigment which is not fluorescent. No production of brown pigment on tyrosine agar.

Aerobic.

Growth at room temperature and at 37 and 42°C, but not at 5°C.

Production of catalase and cytochrome oxidase.

Production of urease.

Does not tolerate KCN at a concentration of 0.0075% (wt/vol). Hydrolysis of Tween 20 and Tween 80.

No production of opalescence on lecithovitelin agar.

Reduction of nitrate to nitrite; no reduction of nitrite.

No production of indole or hydrogen sulfide.

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

No digestion of casein.

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

No utilization of citrate 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 and β -D-galactosidase (when tested by method 1 of Cowan [4] and the method of Holmes et al. [6], respectively).

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

No production of gas from glucose in peptone-

TABLE 3. Characteristics for practical identification and differentiation of *F. thalophilum* from other *Flavobacterium* taxa and from *Pseudomonas paucimobilis*^a

Test	<i>F. thalophilum</i> (7 strains)	<i>F. multivorum</i> (28 strains)	<i>F. spiritorum</i> (13 strains)	<i>F. balustinum</i> (1 strain)	<i>F. breve</i> (7 strains)	<i>F. meningosepticum</i> (49 strains)	<i>Flavobacterium</i> species group IIb (55 strains)	<i>F. odoratum</i> (28 strains)	<i>P. paucimobilis</i> (29 strains)
Acid from ammonium salt-sugar medium containing:									
Glucose	+	+	+	+	6/7 ^c	42/49	+	—	+
Adonitol	+	—	—	—	—	—	—	—	—
Arabinose	+	+	3/13	—	—	1/49	13/55	—	+
Cellobiose	+	+	+	—	—	4/49	3/55	—	+
Ethanol	—	—	+	+	—	28/49	9/55	—	26/29
Glycerol	+	27/28	+	—	—	38/49	35/55	—	6/29
Lactose	+	+	+	—	—	27/49	—	—	+
Maltose	+	+	+	—	6/7	46/49	+	—	+
Mannitol	—	—	+	—	—	31/49	3/55	—	—
Raffinose	+	+	+	—	—	—	—	—	28/29
Rhamnose	+	13/28	1/13	—	—	—	1/55	—	5/29
Salicin	+	+	+	—	—	—	1/55	—	26/29
Sucrose	+	+	+	—	—	—	12/55	—	+
Trehalose	+	+	+	—	—	42/49	48/55	—	+
Xylose	+	+	+	—	—	3/49	9/55	—	+
Casein digestion	—	—	—	+	+	+	+	+	—
Esculin hydrolysis	+	+	+	+	—	47/49	52/55	—	+
Gelatinase production (plate method)	6/7	4/28	+	+	+	+	+	+	3/29
Growth at 42°C	+	—	—	—	—	6/49	15/55	—	—
Growth on MacConkey agar	+	+	+	+	+	+	51/55	+	—
Indole production (Ehrlich reagent)	—	—	—	+	+	24/49	53/55	—	—
Motility at room temperature	—	—	—	—	—	—	—	—	22/29
Nitrate reduction	+	—	—	+	—	2/49	16/55	—	—
Nitrite reduction	—	—	—	—	—	18/49	14/55	+	—
Poly-β-hydroxybutyrate inclusion granules	—	—	—	—	—	—	—	—	25/29
Starch hydrolysis	—	—	—	—	—	—	36/55	—	18/29
Urease production	+	27/28	+	—	—	16/49	11/55	+	—

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; naphthol-AS-B1- β -D-glucuronic acid; 6-bromo-2-naphthyl- α -D-mannopyranoside; 6-bromo-2-naphthyl- β -D-xylopyranoside; *p*-nitrophenyl- α -D-xylopyranoside; *p*-nitrophenyl- β -D-fucopyranoside; *o*-nitrophenyl-*N*-acetyl- α -D-glucosaminide; L-hydroxyprolyl- β -naphthylamide; *N*-benzoyl-L-leucyl- β -naphthylamide; L-isoleucyl- β -naphthylamide; L-prolyl- β -naphthylamide hydrochloride.

Resistant to ampicillin, carbenicillin, cephalothin, amikacin, gentamicin, kanamycin, tobramycin, chloramphenicol, and tetracycline (strain K-1232 is susceptible). Susceptible to rifampin.

The G+C content of the DNA: is 45.0 ± 0.8 mol%, and the G+C content of the DNA of the type strain is 43.8 mol%, as estimated by the thermal denaturation method.

Source of type strain: wound (human).

The following two additional strains of *F. thalpophilum* have been deposited in the National Collection of Type Cultures: C499 (= K-1207) as NCTC 11430 and K-1232 as NCTC 11431.

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LITERATURE CITED

1. Altmann, G., and B. Bogokovsky. 1971. In-vitro sensitivity of *Flavobacterium meningosepticum* to antimicrobial agents. *J. Med. Microbiol.* 4:296-299.
2. Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* 15:1133-1140.
3. Brenner, D. J., A. G. Steigerwalt, R. E. Weaver, J. E. McDade, J. C. Feeley, and M. Mandel. 1978. Classification of the Legionnaires' disease bacterium: an interim report. *Curr. Microbiol.* 1:71-75.
4. Cowan, S. T. 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, London.
5. DeLey, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* 101:738-754.
6. Holmes, B., S. P. Lapage, and H. Malnick. 1975. Strains of *Pseudomonas putrefaciens* from clinical material. *J. Clin. Pathol.* 28:149-155.
7. Holmes, B., and R. J. Owen. 1979. Proposal that *Flavobacterium breve* be substituted as the type species of the genus in place of *Flavobacterium aquatile* and emended description of the genus *Flavobacterium*: status of the named species of *Flavobacterium*. Request for an Opinion. *Int. J. Syst. Bacteriol.* 29:416-426.
8. Holmes, B., and R. J. Owen. 1981. Emendation of the genus *Flavobacterium* and the status of the genus. Developments after the 8th edition of *Bergey's Manual*, p. 17-26. In H. Reichenbach and O. B. Weeks (ed.), *The Flavobacterium-Cytophaga* group. Proceedings of the International Symposium on Yellow-Pigmented Gram-Negative Bacteria of the *Flavobacterium-Cytophaga* Group, Braunschweig, July 8 to 11, 1980. Verlag Chemie, Weinheim.
9. Holmes, B., R. J. Owen, A. Evans, H. Malnick, and W. R. Willcox. 1977. *Pseudomonas paucimobilis*, a new species isolated from human clinical specimens, the hospital environment, and other sources. *Int. J. Syst. Bacteriol.* 27:133-146.
10. Holmes, B., R. J. Owen, and D. G. Hollis. 1982. *Flavobacterium spiritivorum*, a new species isolated from human clinical specimens. *Int. J. Syst. Bacteriol.* 32:157-165.
11. Holmes, B., R. J. Owen, and R. E. Weaver. 1981. *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as group IIk, biotype 2. *Int. J. Syst. Bacteriol.* 31:21-34.
12. Holmes, B., J. J. S. Snell, and S. P. Lapage. 1977. Revised description, from clinical isolates, of *Flavobacterium odoratum* Stutzer and Kwaschnina 1929, and designation of the neotype strain. *Int. J. Syst. Bacteriol.* 27:330-336.
13. Holmes, B., J. J. S. Snell, and S. P. Lapage. 1978. Revised description, from clinical strains, of *Flavobacterium breve* (Lustig) Bergey et al. 1923 and proposal of the neotype strain. *Int. J. Syst. Bacteriol.* 28:201-208.
14. Holmes, B., J. J. S. Snell, and S. P. Lapage. 1979. *Flavobacterium odoratum*: a species resistant to a wide range of antimicrobial agents. *J. Clin. Pathol.* 32:73-77.
15. King, E. O. 1959. Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Am. J. Clin. Pathol.* 31:241-247.
16. Levine, M. G., M. J. Pickett, and M. Mandel. 1980. Taxonomy of nonfermentative bacilli: the IIk-2 group. *Curr. Microbiol.* 4:41-44.
17. Owen, R. J., and B. Holmes. 1978. Heterogeneity in the characteristics of deoxyribonucleic acid from *Flavobacterium odoratum*. *FEMS Microbiol. Lett.* 4:41-46.
18. Owen, R. J., and B. Holmes. 1980. Differentiation between strains of *Flavobacterium breve* and allied bacteria by comparisons of deoxyribonucleic acids. *Curr. Microbiol.* 4:7-11.
19. Owen, R. J., and S. P. Lapage. 1974. A comparison of strains of King's group IIb of *Flavobacterium* with *Flavobacterium meningosepticum*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 40:255-264.
20. Owen, R. J., and J. J. S. Snell. 1976. Deoxyribonucleic acid reassociation in the classification of flavobacteria. *J. Gen. Microbiol.* 93:89-102.
21. Tatum, H. W., W. H. Ewing, and R. E. Weaver. 1974. Miscellaneous gram-negative bacteria, p. 270-294. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
22. Von Graevenitz, A., and M. Grehn. 1977. Susceptibility studies on *Flavobacterium* II-b. *FEMS Microbiol. Lett.* 2:289-292.