Reclassification of *Flavobacterium odoratum* (Stutzer 1929) Strains to a New Genus, *Myroides*, as *Myroides odoratus* comb. nov. and *Myroides odoratimimus* sp. nov.

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On the basis of genotypic, chemotaxonomic, and phenotypic data, *Flavobacterium odoratum* was excluded from the emended genus *Flavobacterium*. In the present study, the known heterogeneity within this species was examined by a polyphasic approach that included DNA-rRNA hybridizations, a determination of DNA base ratios, DNA-DNA hybridizations, a numerical analysis of whole-cell protein patterns, a determination of cellular fatty acid compositions, and a phenotypic analysis. All of the methods revealed the presence of two distinct species. We propose that *F. odoratum* strains should be placed in a new genus, the genus *Myroides*, which comprises two species, *Myroides odoratus* comb. nov. and *Myroides odoratimimus* sp. nov.

The genus *Flavobacterium* was created in 1923 (1) for gram-negative, nonsporulating, yellow-pigmented rods that weakly produced acid from carbohydrates. In 1984 Holmes et al. (10) redefined the genus and limited it to pigmented, strictly aerobic, nonmotile bacteria with guanine-plus-cytosine (G+C) contents ranging from 30 to 42 mol%. Since then, several *Flavobacterium* species have been reclassified in new or other genera, including the genera *Chryseobacterium* (21), *Cytophaga* (19), *Empedobacter* (21), and *Sphingobacterium* (9, 28). Furthermore, it has been shown that differentiation of the genera *Cytophaga*, *Flexibacter*, and *Flavobacterium* based on the presence of gliding motility is taxonomically irrelevant (19, 27). In a polyphasic study, Bernardet et al. (3) restricted the genus *Flavobacterium* to validly described species on the *Flavobacterium aquatile* rRNA branch which have the following features: gram-negative rods that are motile by gliding, produce cream to yellow colonies on agar, are chemoorganotrophic and aerobic, and decompose several polysaccharides but not cellulose. These organisms are widely distributed in soil and freshwater habitats, and some are pathogenic for fish. The G+C contents of *Flavobacterium* DNAs range from 32 to 37 mol%. The emended genus contains the following 10 species: *Flavobacterium aquatile*, *Flavobacterium branchiophilum*, *Flavobacterium columnare*, *Flavobacterium flevense*, *Flavobacterium hydati*, *Flavobacterium johnsoniae*, *Flavobacterium pectinovorum*, *Flavobacterium psychrophilum*, *Flavobacterium saccharophilum*, and *Flavobacterium succinicans*.

Bernardet et al. (3) excluded *Flavobacterium odoratum* from the emended genus *Flavobacterium* because of its clinical origin, its lack of gliding motility, its good growth at 37°C, its halotolerance, and several differences in its fatty acid profile. The conclusion of these authors was supported by 16S rRNA sequencing data which grouped the soil and freshwater organisms on a single branch, whereas *Flavobacterium odoratum* occupied a clearly independent position (7, 13, 22). Holmes et al. (10) had suggested previously that *Flavobacterium odoratum*

should be classified in a separate genus on the basis of its unique characteristic phenotypic features. Strains of this species differed from the majority of *Flavobacterium* species in being nonsaccharolytic and in failing to produce indole. These characteristics routinely differentiated *Flavobacterium odoratum* from *Chryseobacterium meningosepticum* and other medically important flavobacteria (26). Despite its phenotypically and phylogenetically separate position, several studies demonstrated that the taxonomic status of *Flavobacterium odoratum* required further examination (15, 17, 18).

Owen and Holmes (15) observed heterogeneity among *Flavobacterium odoratum* strains with respect to colonial morphology and genomic relatedness. Two groups which exhibited less than 30% DNA homology were recognized. The members of the first group, which included the type strain, had G+C contents of 34 to 36 mol% and exhibited DNA binding values greater than 48%, and the members of the second group had G+C contents of 31 to 32 mol% and were more homogeneous (levels of DNA homology, >80%). No other phenotypic characteristics differentiated the two groups.

In addition, Rasoamananjara et al. (17) recognized two subgroups among *Flavobacterium odoratum* strains on the basis of nutritional features. These authors also characterized *Flavobacterium odoratum* strains by performing a gas-liquid chromatographic analysis of the volatile fatty acids produced in the culture medium, and the resulting data revealed three subgroups (18). However, these data could not be compared with the data obtained by Owen and Holmes (15) because of a lack of reference strains.

In the present study, we performed an extensive polyphasic taxonomic analysis in order to clarify the taxonomic relationships of 19 strains classified as *Flavobacterium odoratum*, and in this paper we describe a new genus, the genus *Myroides*, to accommodate these strains. We found that 4 strains conformed to the description of *Myroides odoratus* comb. nov. and that 15 isolates belonged to a novel species, for which we propose the name *Myroides odoratimimus* sp. nov.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains studied and their sources are listed in Table 1. Strains were grown on Trypticase soy agar (catalog

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TABLE 1. Strains used in this study

Strain ^a	Other designation ^a	Source
<i>M. odoratus</i> strains		
LMG 1233 ^T	NCTC 11036 ^T	Not known
LMG 4028	NCTC 11179	Urine (United Kingdom)
LMG 12841	CCUG 22182	Serum specimen (Denmark)
LMG 13344	CL 44/74 ^b	Urine (United Kingdom)
<i>M. odoratimimus</i> strains		
LMG 4029 ^T	NCTC 11180 ^T	Wound (United Kingdom)
LMG 12835	CCUG 469A	Urine (Göteborg, Sweden, 1969)
LMG 12836	CCUG 3837	Urine, woman (Göteborg, Sweden, 1974)
LMG 12837	CCUG 10230	Man (Göteborg, Sweden, 1980)
LMG 12838	CCUG 12700	Urine, man (Göteborg, Sweden, 1982)
LMG 12839	CCUG 12901	Urine, man (Göteborg, Sweden, 1982)
LMG 12840	CCUG 22181	Water sample (Denmark)
LMG 14956	CIP 100062	Not known (Guéret, France, 1983)
LMG 14961	CIP 101113	Human leg slough (Bondy, France, 1984)
LMG 14968	CIP 103057	Pus (Colmar, France, 1979)
LMG 14969	CIP 103058	Urine (St-Maurice, France, 1978)
LMG 14970	CIP 103059	Human leg slough (Beaune, France, 1981)
LMG 14973	CIP 103073	Urine (Bourg-en-Bresse, France, 1975)
LMG 14974	CIP 103075	Urine (Bourg-en-Bresse, France, 1975)
LMG 14981	CIP 78.62	Urine (France, 1978)

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; LMG, Laboratorium voor Microbiologie Gent Culture Collection, University of Ghent, Ghent, Belgium; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom.

^b Strain received from the National Collection of Type Cultures.

no. 11768; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and were incubated aerobically at 28°C, unless otherwise indicated.

PAGE of whole-cell proteins. After incubation for 24 h, whole-cell protein extracts were prepared, and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described by Pot et al. (16). A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the Gelcompar software package, version 3.1 (Applied Maths, Kortrijk, Belgium) (25).

Fatty acid methyl ester analysis. After incubation for 24 h, a loopful of well-grown cells was harvested, and fatty acid methyl esters were prepared, separated by gas-liquid chromatography, and identified by using the Microbial Identification System software package, version 3.8 (Microbial ID, Inc., Newark, Del.), as described previously (23).

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described previously (23).

Determination of DNA base compositions. All of the mean G+C values were determined by the thermal denaturation method and were calculated by using the equation of Marmur and Doty (12), as modified by De Ley (5).

DNA-DNA hybridization experiments. The degrees of DNA-DNA binding, expressed as percentages, were determined spectrophotometrically by the initial renaturation rate method of De Ley et al. (6). DNA binding values of 30% and less indicate that the level of DNA relatedness is not significant.

DNA-rRNA hybridization experiments. ³H-labeled rRNA of *M. odoratus* LMG 1233^T (T = type strain) was isolated and purified as described previously (22). Labeled rRNAs of *Flavobacterium aquatile* and the generically misclassified species *Flexibacter maritimus* were prepared previously (3, 20). Hybridization between rRNA probes and single-stranded filter-fixed DNA was carried out as described previously by Van Landschoot and De Ley (24). Each DNA-rRNA

hybrid was characterized by determining the temperature at which one-half of the hybrid was thermally denatured [$T_{m(e)}$]. A homologous duplex was a duplex formed with DNA and rRNA of the same strain; a heterologous hybrid was a hybrid formed with DNA and rRNA of different strains. The average level of linkage between each pair of rRNA branches was calculated by using the $T_{m(e)}$ values from reciprocal hybridization experiments and including the values for all strains on both rRNA branches.

Phenotypic characterization. All of the strains studied were characterized biochemically by using the following four different commercial galleries: API 20NE, API ZYM, Biotype 100 (bioMérieux, La Balme-Les-Grottes, France), and Biolog (Biolog, Inc., Hayward, Calif.). The cells used for these tests were pre-cultivated on nutrient agar (catalog no. CM3; Oxoid) plates for 24 h at 28°C. The inoculation procedure, incubation, and visual reading of the galleries were performed according to the instructions of the manufacturers.

RESULTS

Comparative protein gel electrophoresis. The whole-cell protein profiles of all strains studied and the corresponding dendrogram obtained after an average-linkage cluster analysis are shown in Fig. 1. At a correlation coefficient (r) of 0.75, two clusters (clusters I and II) were delineated. As discussed below, the 4 cluster I strains belong to *M. odoratus*, whereas the 15 cluster II strains belong to *M. odoratimimus*. Considerable heterogeneity was observed among the profiles of the *M. odoratus* strains ($r \geq 0.79$). More similar protein patterns were found for the strains of *M. odoratimimus* ($r \geq 0.88$).

Gas chromatographic analysis of methylated fatty acids. The cellular fatty acid compositions of *Myroides* strains are shown in Table 2. The dominant fatty acids were 15:0 iso, 15:0 iso 3OH, 16:0 3OH, 17:0 iso 3OH, and 17:1 iso ω 9c. Characteristic of *M. odoratimimus* strains was the presence of significant relative amounts of 13:0 iso and 15:0. *M. odoratus* strains contained appreciable amounts of 15:0 anteiso and had a higher amount of 15:0 iso and a lower amount of summed feature 4 (Table 2) than *M. odoratimimus* strains.

DNA base compositions. The DNA base ratios determined in this study are shown in Table 3. The *M. odoratus* strains had G+C contents between 37 and 38 mol%; the DNA base contents of *M. odoratimimus* strains were significantly lower, ranging from 30 to 35 mol%.

DNA-DNA hybridizations. DNA-DNA hybridization data are shown in Table 3, and these data revealed that the four *M. odoratus* strains studied exhibited DNA binding values ranging from 55 to 85%. Five representative strains of *M. odoratimimus* constituted a homogeneous genospecies with DNA binding values greater than 80%. No significant level of DNA relatedness between *M. odoratus* and *M. odoratimimus* strains was found (Table 3).

DNA-rRNA hybridizations. The results of the DNA-rRNA hybridization experiments are shown in Table 4, and a dendrogram based on these results is shown in Fig. 2. The DNA-rRNA hybridization data show that the two *Myroides* species form distinct taxonomic groups with separate positions on the *Myroides* rRNA branch.

Phenotypic characterization. The biochemical characteristics of all strains were determined by using an API 20NE gallery. All strains were positive for urease activity, gelatinase activity, and oxidase production. Of the 15 *M. odoratimimus* strains, 10 exhibited a weak positive reaction for growth on malate. None of the strains reduced nitrate, produced indole, acidified glucose, exhibited arginine dihydrolase or β -galactosidase activity, hydrolyzed esculin, or grew on glucose, L-arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate, adipate, citrate, or phenyl-acetate.

When we used a Biotype 100 gallery, in which assimilation of 99 different carbon sources (carbohydrates, amino acids, and organic acids) was tested, none of the *M. odoratus* strains yielded a single positive reaction. The same negative results

FIG. 1. SDS-protein electropherograms for the strains studied and corresponding dendrogram based on an average linkage cluster analysis of correlation coefficients (r values). The molecular weight markers (track MWM) used were (from left to right) trypsin inhibitor (molecular weight, 20,100), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), bovine albumin (66,000), and β -galactosidase (116,000). The roman numerals are cluster numbers.

were obtained when we used the two prescribed inoculation media or a third standardized inoculation medium described by Kersters et al. (11). Strains of *M. odoratimimus* yielded positive reactions for growth on L-aspartate, L-glutamate, and L-proline and variable reactions for growth on L-malate, succinate, and fumarate.

All of the strains exhibited similar reactions for hydrolysis of 18 different substrates in the API ZYM system (see below). Only one test, hydrolysis of L-leucyl-2-naphthylamide, slightly differentiated *M. odoratus* strains from *M. odoratimimus* strains; the intensities of hydrolysis (which was measured by using a scale of 0 to 5 [2]) were 1 and 3 for *M. odoratus* and *M. odoratimimus*, respectively.

Biolog MicroPlates were used to test the ability of the strains to oxidize a panel of 95 different carbon sources. Strains of *M. odoratus* exhibited positive reactions in all wells, including the negative control well, after a few hours of incubation. No oxidation occurred in the negative control well with strains of *M. odoratimimus*. Strains of the latter species yielded a characteristic profile (see below).

DISCUSSION

Genus delineation. Using a combination of genotypic and phenotypic criteria, Bernardet et al. (3) redefined the genus *Flavobacterium*. All validly described species of the *Flavobac-*

TABLE 2. Fatty acid compositions of the *Myroides* taxa studied^a

Taxon	% of total fatty acids							ECL 16.580 ^b	17:0 iso 3OH	17:1 iso ω 9 ^c	Summed feature 4 ^c
	13:0 iso	15:0	15:0 iso	15:0 iso 3OH	15:0 anteiso	16:0	16:0 3OH				
<i>M. odoratus</i> LMG 1233 ^T	Tr ^d	Tr	52.1	6.7	2.5	Tr	1.2	1.6	10.8	20.7	Tr
<i>M. odoratus</i> (3 strains) ^e	Tr	Tr	60.8 $\pm$ 2.5	4.5 $\pm$ 1.1	1.3 $\pm$ 0.8	Tr	5.1 $\pm$ 1.7	1.1 $\pm$ 0.2	11.0 $\pm$ 1.7	9.4 $\pm$ 1.1	2.8 $\pm$ 0.4
<i>M. odoratimimus</i> (15 strains)	6.6 $\pm$ 1.7	2.4 $\pm$ 0.7	45.5 $\pm$ 1.3	4.1 $\pm$ 0.3	Tr	1.1 $\pm$ 0.3	5.2 $\pm$ 0.8	Tr	9.7 $\pm$ 1.6	10.3 $\pm$ 1.7	9.5 $\pm$ 1.6

^a Fatty acids that accounted for less than 1.0% of the total fatty acids in all of the strains studied are not shown.

^b ECL, equivalent chain length. The identity of the fatty acid is not known.

^c Summed feature 4 consisted of one or more of the following fatty acids which could not be separated by the Microbial Identification System: 15:0 iso 2OH, 16:1 ω 7c, and 16:1 ω 7t.

^d Tr, trace (less than 1.0%).

^e Strains LMG 4028, LMG 12841, and LMG 13344.

TABLE 3. DNA base compositions of and DNA-DNA hybridization results for *Myroides* strains

Strain	G+C content (mol%)	% DNA-DNA homology with:								
		<i>M. odoratus</i> LMG 1233 ^T	<i>M. odoratus</i> LMG 4028	<i>M. odoratus</i> LMG 12841	<i>M. odoratus</i> LMG 13344	<i>M. odoratimimus</i> LMG 4029 ^T	<i>M. odoratimimus</i> LMG 12837	<i>M. odoratimimus</i> LMG 14973	<i>M. odoratimimus</i> LMG 12839	<i>M. odoratimimus</i> LMG 14961
<i>M. odoratus</i> LMG 1233 ^T	38-36 ^a	100								
<i>M. odoratus</i> LMG 4028	38-36 ^a	75-80 ^a	100							
<i>M. odoratus</i> LMG 12841	37	71	85	100						
<i>M. odoratus</i> LMG 13344	37-35 ^a	55-48 ^a	57	55	100					
<i>M. odoratimimus</i> LMG 4029 ^T	34-32 ^a	14				100				
<i>M. odoratimimus</i> LMG 12837	34		9			98	100			
<i>M. odoratimimus</i> LMG 14973	34						91	100		
<i>M. odoratimimus</i> LMG 12839	30						89	89	100	
<i>M. odoratimimus</i> LMG 14961	35	14				80	81	83	83	100

^a Data from reference 15.

terium aquatile rRNA cluster except *Flavobacterium odoratum* were included in the emended genus. *Flavobacterium odoratum* was excluded because of considerable genomic and phenotypic differences. 16S rRNA sequencing data grouped the soil and freshwater organisms on one lineage, while the clinical organism *Flavobacterium odoratum* formed a clearly distinct lineage (7, 13, 14, 22). Moreover, small-subunit rRNA sequence signatures have been found which clearly differentiate *Flavobacterium odoratum* from its neighbors (7). A detailed overview of phenotypic characteristics which differentiate *Flavobacterium odoratum* from other taxa belonging to the emended family *Flavobacteriaceae* has been published previously (3). This organism was distinguished from most members of the emended genus *Flavobacterium* by its lack of gliding motility, its good growth at 37°C, its halotolerance, and several differences in its fatty acid profile (3). These findings clearly warranted reclassification of *Flavobacterium odoratum* in a new genus. However, nomenclatural modifications were not proposed pending

further examination of the demonstrated heterogeneity within *Flavobacterium odoratum* (15, 17, 18). In the present study, 19 *Flavobacterium odoratum* strains representing the reported heterogeneity were extensively characterized. These strains formed two distinct species belonging to a single phylogenetic branch and were assigned to the new genus *Myroides*.

TABLE 4. $T_{m(e)}$ values of DNA-rRNA hybrids

Source of DNA ^a	$T_{m(e)}$ (°C) of hybrid with rRNA from:	
	<i>Myroides odoratus</i> LMG 1233 ^T	<i>Flavobacterium aquatile</i> LMG 4008 ^T
<i>Myroides odoratus</i> LMG 1233 ^T	76.3	72.9
<i>Myroides odoratus</i> LMG 4028	77.0	73.5
<i>Myroides odoratimimus</i> LMG 4029 ^T	74.1	73.3
<i>Myroides odoratimimus</i> LMG 12837	75.7	
<i>Myroides odoratimimus</i> LMG 12839	73.2	
<i>Myroides odoratimimus</i> LMG 14973	75.3	
<i>Flavobacterium aquatile</i> LMG 4008 ^T	72.2	77.2
<i>Flavobacterium columnare</i> NCIMB 2248 ^T	71.5	71.0 ^b
<i>Flavobacterium psychrophilum</i> NCIMB 1947 ^T	69.5	71.5 ^b
<i>Flavobacterium saccharophilum</i> NCIMB 2072 ^T	68.8	72.3 ^b
<i>Flexibacter maritimus</i> NCIMB 2158	66.3	68.4

^a NCIMB, National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland, United Kingdom.^b Data from reference 3. FIG. 2. Simplified rRNA dendrogram based on the $T_{m(e)}$ values of DNA-rRNA hybrids with members of rRNA superfamily V. Data from this study and from references 3 and 20 were used to construct the dendrogram.

Taxonomic characterization of the genus *Myroides*. Owen and Holmes (15) demonstrated that there was considerable genomic heterogeneity among 10 strains of *Flavobacterium odoratum*. Three strains, including the type strain, formed one group whose members had G+C contents of 34 to 36 mol% and exhibited intermediate levels of DNA homology (>48%), and seven strains formed a second group whose members had G+C contents of 31 to 32 mol% and exhibited high levels of DNA homology (>80%). These two taxonomic groups were less than 30% related to each other. Owen and Holmes did not propose nomenclatural changes as colonial morphology was the only phenotypic characteristic which differentiated the two DNA hybridization groups. In the present study, we included all three strains (LMG 1233^T, LMG 4028, and LMG 13344) of the first group and one strain (LMG 4029) of the second group. A total of 15 additional strains that were isolated from human clinical samples or hospital environments in Denmark, France, and Sweden and were identified as *Flavobacterium odoratum* were also investigated. A polyphasic approach confirmed the subdivision of *Flavobacterium odoratum* strains into two species.

All of the strains were first compared by performing an SDS-PAGE analysis of whole-cell proteins (Fig. 1). We identified two major clusters, which contained 4 and 15 strains. The members of cluster I, which included all members of the first group of Owen and Holmes (15) and one Danish isolate, exhibited considerable protein electrophoretic heterogeneity. The lowest level of similarity ($r = 0.79$) observed was the level of similarity for strain LMG 13344, which was consistent with the rather low levels of DNA binding (55 to 57%) between this strain and the other strains belonging to the cluster (Table 3). The levels of protein electrophoretic similarity for the remaining cluster I strains were more than 0.88 and were comparable to the values obtained for cluster II strains (Fig. 1). The levels of DNA binding for the cluster II strains were more than 80% (Table 3). The DNA binding data of Owen and Holmes (15) and the data obtained in the present study (Table 3) were similar and revealed that the levels of binding between strains belonging to clusters I and II were not significant.

The phylogenetic affiliations of representative strains of the two clusters were determined by performing DNA-rRNA hybridization experiments (Table 4; Fig. 2). The data obtained demonstrated that the two taxa are located on the same branch and that the emended genus *Flavobacterium* is its nearest neighbor. The two clusters occupy distinct positions on the *Myroides* rRNA branch. The DNA base compositions of representative strains of the two clusters were determined (Table 3), and these data extended the data of Owen and Holmes (15). Slightly higher values were observed in the present study as cluster I strains had G+C contents between 37 and 38 mol% and the values for cluster II strains varied between 30 and 35 mol%.

The fatty acid compositions of all strains were determined to evaluate the usefulness of this characteristic for differentiation and identification of the taxa (Table 2). Cluster II strains were easily differentiated from cluster I strains by the presence of significant amounts of 13:0 iso and 15:0. Only cluster I strains contained appreciable amounts of 15:0 anteiso, and these strains had higher levels of 15:0 iso and lower levels of summed feature 4. Unexpectedly, the fatty acid content of the type strain of *Flavobacterium odoratum* was quantitatively aberrant; this strain contained less 15:0 iso and summed feature 4 and considerably more 17:1 iso ω 9c than the other cluster I strains. Qualitatively, our fatty acid data corroborated the data of Dees et al. (4), but Dees et al. did not distinguish subgroups within *Flavobacterium odoratum*.

Four different commercial galleries were used to phenotypically characterize all of the strains investigated. The API 20NE and API ZYM galleries did not discriminate between the two groups. As determined with the Biotype 100 gallery, all of the cluster II strains grew on three amino acids, L-aspartate, L-glutamate, and L-proline, whereas the cluster I strains did not. Previously, Rasoamananjara et al. (17) subdivided *Flavobacterium odoratum* strains into two subgroups on the basis of nutritional properties. A comparison of the data of these authors and our data is rather difficult because of a lack of corresponding reference strains. However, the phenotypic characteristics of the cluster I and II strains resembled the phenotypic characteristics of the two subgroups of Rasoamananjara et al.; strains belonging to the first subgroup did not grow on any carbon source, and strains belonging to the second subgroup utilized a variety of amino acids. In the present study, the Biolog assay results distinguished the two taxa easily. The cluster I strains produced a purple color in all wells; even the negative control well yielded a false-positive reaction after a few hours of incubation. The possible causes of this include utilization of extracellular polysaccharides, utilization of stored endogenous substrates, and utilization of lysed cell material. No oxidation occurred in the negative control wells containing cluster II strains. A characteristic profile (see below) was obtained for the cluster II strains.

All of the genomic, chemotaxonomic, and phenotypic data demonstrated that strains of the former species *Flavobacterium odoratum* should be placed in two species. We propose that cluster I strains, including the type strain of *Flavobacterium odoratum*, should be placed in *M. odoratus* comb. nov. and that cluster II strains should be placed in a new taxon, for which the name *M. odoratimimus* is proposed. The latter species name reflects the paucity of appropriate phenotypic characteristics for differentiating the two *Myroides* species. The genus and species descriptions below are based on data from references 4, 8, and 10 and the present study.

Description of *Myroides* gen. nov. *Myroides* (My.roi'des. Gr. n. *myron*, perfume; Gr. adj. suffix *-oides*, similar to; N. L. masc. n. *Myroides*, resembling perfume). Cells are gram-negative rods that are 0.5 μ m in diameter and 1 to 2 μ m long, but longer rods and long chains (containing 4 to 10 cells) may occur in broth medium. Cells lack flagella. Neither cellular gliding movement nor swarming growth is observed. Various colony types may occur, but most colonies are yellow pigmented. A characteristic fruity odor is produced by most strains. Strictly aerobic with good growth on nutrient agar and MacConkey agar. Nonhemolytic on blood agar. Growth occurs at room temperature (18 to 22°C) and at 37°C, but not at 5 and 42°C. Oxidase, catalase, urease, and gelatinase positive. Indole is not formed. Nitrite is characteristically reduced, but nitrate is not reduced. None of the strains exhibit arginine dihydrolase or β -galactosidase activity or hydrolyze esculin, and all strains are nonsaccharolytic.

As determined by API ZYM galleries, all strains hydrolyze 2-naphthyl-phosphate, 2-naphthyl-butyrate, 2-naphthyl-caprylate, L-leucyl-2-naphthylamide, and naphthol-AS-BI-phosphate. No strain hydrolyzes *N*-benzoyl-DL-arginine-2-naphthylamide, 6-Br-2-naphthyl- α -D-galactopyranoside, 2-naphthyl- β -D-galactopyranoside, naphthol-AS-BI- β -D-glucuronide, 2-naphthyl- α -D-glucopyranoside, 6-Br-2-naphthyl- β -D-glucopyranoside, 1-naphthyl-*N*-acetyl- β -D-glucosaminide, 6-Br-2-naphthyl- α -D-mannopyranoside, and 2-naphthyl- α -L-fucopyranoside. Very weak or no hydrolysis occurs with the substrates 2-naphthyl-myristate, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, and *N*-glutaryl-phenylalanine-2-naphthylamide.

The genus belongs to rRNA superfamily V. The closest

phylogenetic neighbor as determined by DNA-rRNA hybridization is the emended genus *Flavobacterium*. As in the genus *Flavobacterium*, menaquinone 6 is the major isoprenoid quinone, and homospermidine is the dominant polyamine component. The dominant fatty acids are 15:0 iso, 15:0 iso 3OH, 16:0 3OH, 17:0 iso 3OH, and 17:1 iso ω 9c. Strains are isolated from human clinical specimens or hospital environments. The G+C contents range from 30 to 38 mol%. The type species is *M. odoratus*.

Description of *Myroides odoratus* comb. nov. (basonym, *Flavobacterium odoratum* Stutzer 1929). The description of *M. odoratus* is the same as that given above for the genus, with the following additions. No positive reaction is observed in Biotype 100 galleries. In the Biolog GN MicroPlate assay, all strains, even the negative control, oxidize all wells after a few hours of incubation. The levels of DNA relatedness range from 55 to 100%. The major fatty acids are 15:0 iso, 15:0 iso 3OH, 15:0 anteiso, 16:0 3OH, 17:0 iso 3OH, and 17:1 iso ω 9c. The type strain is LMG 1233 (= NCTC 11036). The G+C content is 35 to 38 mol%.

Description of *Myroides odoratimimus* sp. nov. The description of *Myroides odoratimimus* (o.do.ra.ti'mi.mus. Gr. nom. n. mimos, imitator; N. L. n. *odoratimimus*, imitator of *M. odoratus*) is the same as that given above for the genus, with the following additions.

As determined by Biotype 100 galleries, *M. odoratimimus* strains grow on L-aspartate, L-glutamate, and L-proline and exhibit variable reactions for growth on L-malate, succinate, and fumarate. When the Biolog GN MicroPlate assay is used, all strains oxidize Tween 40, Tween 80, methylpyruvate, monomethylsuccinate, acetic acid, α -hydroxybutyric acid, α -ketovaleric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, alaninamide, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, inosine, uridine, and thymidine. Variable reactions are detected for oxidation of L-fucose, formic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketobutyric acid, α -ketoglutaric acid, L-histidine, L-phenylalanine, D-serine, urocanic acid, phenyl ethylamine, 2,3-butanediol, and glycerol. None of the strains oxidizes α -cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, meso-erythritol, D-fructose, D-galactose, gentiobiose, α -D-glucose, meso-inositol, α -D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, D-alanine, hydroxy-L-proline, L-pyrogutamic acid, DL-carnitine, γ -aminobutyric acid, putrescine, 2-aminooethanol, DL- α -glycerolphosphate, glucose 1-phosphate, or glucose 6-phosphate. The levels of DNA relatedness range from 80 to 100%. The major fatty acids are 13:0 iso, 15:0, 15:0 iso, 15:0 iso 3OH, 16:0 3OH, 17:0 iso 3OH, 17:1 iso ω 9c, and summed feature 4. The type strain is LMG 4029 (= NCTC 11180). The G+C content is 30 to 35 mol%.

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REFERENCES

- Bergey, D. H., F. C. Harrison, R. S. Breed, B. W. Hammer, and F. M. Huntoon. 1923. Bergey's manual of determinative bacteriology, 1st ed. The Williams and Wilkins Co., Baltimore.
- Bernardet, J.-F., and P. A. D. Grimont. 1989. Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida, and Masumura 1986. Int. J. Syst. Bacteriol. 39:346-354.
- Bernardet, J.-F., P. Segers, M. Vancanneyt, F. Berthe, K. Kersters, and P. Vandamme. 1996. Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). Int. J. Syst. Bacteriol. 46:128-148.
- Dees, S. B., C. W. Moss, D. G. Hollis, and R. E. Weaver. 1986. Chemical characterization of *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium*-like groups IIe, IIh, and IIi. J. Clin. Microbiol. 23:267-273.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. J. Bacteriol. 101:738-754.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. Eur. J. Biochem. 12:133-142.
- Gherna, R., and C. R. Woese. 1992. A partial phylogenetic analysis of the "flavobacter-bacteroides" phylum: basis for taxonomic restructuring. Syst. Appl. Microbiol. 15:513-521.
- Hamana, K., and S. Matsuzaki. 1990. Occurrence of homospermidine as a major polyamine in the authentic genus *Flavobacterium*. Can. J. Microbiol. 36:228-231.
- Holmes, B. 1992. The genera *Flavobacterium*, *Sphingobacterium* and *Weeksellia*, p. 3620-3630. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed., vol. 4. Springer-Verlag, Berlin.
- Holmes, B., R. J. Owen, and T. A. McMeekin. 1984. Genus *Flavobacterium* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 97^{AL}, p. 353-361. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore.
- Kersters, K., K.-H. Hinz, A. Hertle, P. Segers, A. Lievens, O. Siegmann, and J. De Ley. 1984. *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. Int. J. Syst. Bacteriol. 34:56-70.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5:109-118.
- Nakagawa, Y., and K. Yamasato. 1993. Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. J. Gen. Microbiol. 139:1155-1161.
- Nakagawa, Y., and K. Yamasato. 1996. Emendation of the genus *Cytophaga* and transfer of *Cytophaga agarovorans* and *Cytophaga salmonicolor* to *Mari-nilibilia* gen. nov.: phylogenetic analysis of the *Flavobacterium*-*Cytophaga* complex. Int. J. Syst. Bacteriol. 46:599-603.
- Owen, R. J., and B. Holmes. 1978. Heterogeneity in the characteristics of deoxyribonucleic acid from *Flavobacterium odoratum*. FEMS Microbiol. Lett. 48:41-46.
- Pot, B., P. Vandamme, and K. Kersters. 1994. Analysis of electrophoretic whole-organism protein fingerprints, p. 493-521. In M. Goodfellow and A. G. O'Donnell (ed.), Chemical methods in prokaryotic systematics. J. Wiley and Sons, Chichester, United Kingdom.
- Rasoamananjara, D., B. Korošec, and H. Monteil. 1987. Classification and identification of *Flavobacterium* species by carbon source utilization. J. Clin. Microbiol. 25:1285-1290.
- Rasoamananjara, D., F. Peladan, J. C. Turlot, H. Monteil, and C. Richard. 1986. Characterization of *Flavobacterium* species by analysis of volatile fatty acid production. J. Gen. Microbiol. 132:2723-2732.
- Reichenbach, H. 1989. Order I. *Cytophagales* Leadbetter 1974, 99^{AL}, p. 2011-2013. In J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. The Williams and Wilkins Co., Baltimore.
- Segers, P., W. Mannheim, M. Vancanneyt, K. De Brandt, K.-H. Hinz, K. Kersters, and P. Vandamme. 1993. *Riemerella anatipestifer* gen. nov., the causative agent of septicemia anserum exsudativa, and its phylogenetic affiliation within the *Flavobacterium*-*Cytophaga* rRNA homology group. Int. J. Syst. Bacteriol. 43:768-776.

21. Vandamme, P., J.-F. Bernardet, P. Segers, K. Kersters, and B. Holmes. 1994. New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. Int. J. Syst. Bacteriol. 44:827-831.
22. Vandamme, P., P. Segers, M. Vancanneyt, K. Van Hove, R. Muttters, J. Hommez, F. E. Dewhirst, B. J. Paster, K. Kersters, E. Falsen, L. A. Devriese, M. Bisgaard, K.-H. Hinz, and W. Mannheim. 1994. Description of *Omnithobacterium rhinotracheale* gen. nov., sp. nov. isolated from the avian respiratory tract. Int. J. Syst. Bacteriol. 44:24-37.
23. Vandamme, P., M. Vancanneyt, B. Pot, L. Mels, B. Hoste, D. Dewettinck, L. Vlaes, C. Van den Borre, R. Higgins, J. Hommez, K. Kersters, J.-P. Butzler, and H. Goossens. 1992. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. Int. J. Syst. Bacteriol. 42:344-356.
24. Van Landschoot, A., and J. De Ley. 1983. Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other gram-negative bacteria. J. Gen. Microbiol. 129:3057-3074.
25. Vauterin, L., and P. Vauterin. 1992. Computer-aided objective comparison of electrophoresis patterns for grouping and identification of microorganisms. Eur. Microbiol. 1:37-41.
26. von Graevenitz, A. 1995. *Acinetobacter*, *Alcaligenes*, *Moraxella*, and other nonfermentative Gram-negative bacteria, p. 520-532. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
27. Woese, C. R., D. Yang, L. Mandelco, and K. O. Stetter. 1990. The *Flexibacter-Flavobacter* connection. Syst. Appl. Microbiol. 13:161-165.
28. Yabuuchi, E., T. Kaneko, I. Yano, C. W. Moss, and N. Miyoshi. 1983. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting gram-negative rods in CDC groups IIk-2 and IIb. Int. J. Syst. Bacteriol. 33:580-598.