Taxonomy of the Genus *Microcycclus* Orskov 1928: Reintroduction and Emendation of the Genus *Spirosoma* Migula 1894 and Proposal of a New Genus, *Flectobacillus*

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The type strains of the three currently recognized species of the genus *Microcycclus* Orskov 1928 (*M. aquaticus* Orskov, strain ATCC 25396; *M. major* Gromov, strain BKM 859; and *M. flavus* Raj, strain ATCC 23276), together with strains apparently belonging to each species, were examined to determine their taxonomic relationships. There were many differences between the type strains in their morphological and physiological characteristics as well as in the guanine-plus-cytosine (G+C) contents of their deoxyribonucleic acids (DNAs). Therefore, we propose to split the genus *Microcycclus* into three genera, each containing a single species. *M. aquaticus*, the type species of *Microcycclus*, remains as the only species in the genus. It is characterized by the formation of cellular rings which are produced when the ends of the curved cells overlap. It is nonpigmented and is respiratory in its metabolism; the G+C content of its DNA ranges from 66.3 to 68.4 mol%. *M. flavus* ATCC 23276 and three similar strains were found to possess characters in agreement with those given in the original description of *Spirosoma linguale*, and they were placed in that species. The name *M. flavus* Raj 1970 thus becomes a later subjective synonym of *Spirosoma linguale* (Eisenberg 1891) Migula 1894. This species is characterized by the formation of wavy and coiled filaments, yellow pigmentation on *Microcycclus*-*Spirosoma* (MS) agar, and respiratory metabolism. The G+C content of its DNA ranges from 51.0 to 52.9 mol%. Strain DSM 74 is designated as the neotype strain of *S. linguale*. *M. Major* BKM 859 and one similar strain were placed in a new genus, *Flectobacillus*. *F. major* (Gromov) comb. nov., the type species, is composed of rods which are straight to curved, the degree of curvature varying among individual cells within a culture. The cells may form long, sinuous filaments and rings. Colonies on MS agar are pale pink to rose-colored. Metabolism is respiratory, and the G+C content of its DNA ranges from 39.5 to 40.3 mol%. The type strain of *F. major* is strain BKM 859.

In the eighth edition of *Bergey's Manual* (19), the genus *Microcycclus* Orskov 1928 is a heterogeneous collection of three species: *M. aquaticus* Orskov (the type species), *M. flavus* Raj, and *M. major* Gromov. All three species were described and named on the basis of single strains, and these strains (see below) are, therefore, according to Rule 18C of the Bacteriological Code (12), type strains by monotypy. The lack of additional strains of these species has been a hindrance in conducting a comprehensive study of this genus. However, Claus (4) determined that two organisms isolated in Europe and designated as *Spirosoma* sp. were similar to *M. flavus*. On the basis of the base compositions of the DNAs, Claus et al. (5) suggested that the three *Microcycclus* species represented three different taxa and that *M. flavus* should be placed in the little-used genus *Spirosoma* Migula 1894. Recently, we isolated from

bodies of water near Baton Rouge, La., an organism which resembled *M. flavus*, another which resembled *M. major*, and several strains which resembled *M. aquaticus*. With these additional strains available, a comprehensive study of the taxonomic relationships of these three species was made.

MATERIALS AND METHODS

Bacterial strains. Cultures of the following type strains were obtained from the Deutsche Sammlung von Mikroorganismen (DSM) through the kindness of D. Claus of Göttingen, Germany: the Orskov strain of *M. aquaticus* (DSM 101 = ATCC 25396) and the Gromov strain of *M. major* (DSM 109 = BKM 859). *Spirosoma* sp. strains 1 and 2 were also obtained from D. Claus with the accession numbers DSM 74 and DSM 75, respectively. A culture of the type strain of *M. flavus* was obtained from the American Type Culture Collection (ATCC), Rockville, Md., under the number 23276. Strains 3 and 5

and strain 1C of *M. aquaticus* were isolated in this lab. Cultures of the above-mentioned strains were maintained on slants of *Microcycylus-Spirosoma* (MS) agar of the following composition: 0.1% each of glucose, peptone, and yeast extract plus 1.5% agar. Transfers were made at intervals of about 2 weeks with incubation at 25°C.

Utilization of carbohydrates. The production of acid by aerobic or anaerobic means from carbohydrates was determined by the method of Hugh and Leifson (11). Incubation was continued for 8 weeks for cultures which gave negative results.

Utilization of single carbon sources. The basal medium of Gordon and Mihm (8), to which was added 0.2% of the substrate or the sodium salt of the substrate, was used. If growth occurred through four successive subcultures, the results were considered positive even in the absence of a color change in the bromothymol blue indicator.

Hydrolysis of macromolecules. Gelatin and starch hydrolyses were detected by zones of hydrolysis surrounding colonies on plate cultures (10). Gelatin hydrolysis was also determined in stab tubes of nutrient gelatin. Casein hydrolysis was detected by zones of clearing around colonies on nutrient agar containing 10% (vol/vol) of skim milk. Cellulose and chitin hydrolyses were determined by inoculating the surfaces of double-layered plates and looking for zones of clearing. The bottom layer consisted of MS medium (but with 1% agar) with 0.2% of powdered cellulose or chitin in place of the glucose; the thin upper layer contained 10% (wt/vol) of the substrate and 1% agar in distilled water. Cellulose decomposition was also examined by inoculating filter paper layered over the bottom medium. Esculin and tributyrin hydrolyses were detected by the methods described by Holding and Collee (10).

Production of specific enzymes or end products. The methyl red and Voges-Proskauer (VP) tests, the tests for the production of indole and H_2S , and the tests for β -galactosidase (ONPG), oxidase, catalase, phosphatase, urease, lecithinase, phenylalanine deaminase, lysine decarboxylase, ornithine decarboxylase, and the reduction of nitrate were performed by the methods described by Holding and Collee (10). In addition, tests for urease, lysine and ornithine decarboxylases, lysine deaminase, ONPG, oxidase, nitrate reduction, indole, H_2S production, VP, malonate utilization, and esculin hydrolysis were performed by the PathoTec Rapid I-D System (Warner-Lambert Co., Morris Plains, N. J.). Results were the same by either method unless specified otherwise.

Susceptibility to antibiotics. Except for actinomycin D, susceptibility to antibiotics and sulfa drugs was determined by placing antibiotic-impregnated disks on the surface of MS agar inoculated with the organisms. Susceptibility to actinomycin D was detected by the method of Dworkin (6).

Ability to grow on various media. The abilities of the organisms to grow on various media were investigated by making a single streak of the organism from a fresh slant over the surface of the medium and incubating at 25°C for a minimum of 3 weeks. The media tested were chocolate agar, blood agar,

eosin methylene blue agar, phenol red mannitol salt agar, phenyl ethyl alcohol agar, nutrient agar, nutrient agar + 5% sucrose (NAS), Trypticase soy agar (TSA), TSA + 3% sucrose, TSA + 3% glucose (TSAG), peptonized milk agar, MS agar, yeast extract acetate tryptone agar, McConkey agar, bis-muth sulfide agar, and *Salmonella-Shigella* agar.

Additional characters. Deoxyribonucleic acid (DNA) was purified by the method of Marmur (14), and the moles percent guanine plus cytosine (G+C) was calculated from the melting-point profile obtained with a Gilford model 2400 spectrophotometer equipped with a temperature-controlled cuvette compartment (13). Measurements of cell size were obtained with a Filar micrometer. A Gillet and Seibert microscope equipped with a Nikon model AFM camera was used for photomicrography.

RESULTS

The strains fell into three distinct groups, which are differentiated in Tables 1 to 7. In Table 1, it can be seen that *M. flavus* ATCC 23276, the *Spirosoma* strains, and strain 5 produce curved rods which tend to form long, undulating filaments and coiled spirals and which produce yellow colonies. The G+C contents of the DNAs of these four strains range from 51.0 to 52.9 mol%. *M. major* strain Gromov and strain 3 produce curved rods which form long, undulating filaments but which form coiled spirals only infrequently; both strains produce pink colonies. The G+C contents of the DNAs of these two strains range from 39.5 to 40.3 mol%. The cells of *M. aquaticus* strains Ørskov and 1C are smaller than those of the other strains and never form filaments or coils. The colonies of these strains are white. The G+C value of both strains is 67.3 mol%, which is much higher than the values of the strains of the other two groups. The clear differences in cellular morphology of the three groups are shown in Fig. 1.

Table 2 shows that all of the organisms were active in producing acid oxidatively from pentoses and hexoses. The *M. aquaticus* strains were less active than the strains of the other groups on glucosides, disaccharides, trisaccharides, and polysaccharides. After prolonged incubation, the tubes of carbohydrate which were covered with a mineral oil seal, and were presumably anaerobic, would often show acid production, contrary to previous reports (1, 19). Therefore the ability of each organism to grow anaerobically under a pyrogallol-KOH seal was tested, and all results were negative. It was concluded that the strains cannot grow anaerobically and that the delayed acid production in the mineral-oil-covered tubes was due to slow diffusion of oxygen. Acid production in the aerobic tubes always began at or near the sur-

TABLE 1. Selected properties of the strains studied

Strain	Gram reaction	Pigmentation	Fluorescence	Coiling tendency	Filament formation	Motility	G + C content of DNA (mol%)	Cell length (μm)	Cell diam (μm)	Ring diam (μm)	Filament length (μm)
<i>Microcyclopus flavus</i> ATCC 23276	-	Yellow	-	+	+	-	51.5	3.0-6.0	0.5-1.0	1.5-3.0	>50
<i>Spirosoma</i> sp. strain 1	-	Yellow	-	+	+	-	52.9	2.0-5.0	0.6-0.9	1.2-2.5	>50
<i>Spirosoma</i> sp. strain 2	-	Yellow	-	+	+	-	51.0	1.5-3.5	0.6-0.9	1.5-2.0	>50
Strain 5	-	Yellow	-	+	+	-	52.4	2.5-4.0	0.5-0.9	2.5-3.0	>50
<i>Microcyclopus major</i> strain Gromov	-	Pink	-	+	+	-	39.5	2.0-5.0	0.6-1.0	5.0-10.0	>50
Strain 3	-	Pink	-	+	+	-	40.3	1.5-4.5	0.6-1.0	5.0-10.0	>50
<i>Microcyclopus aquaticus</i> strain Ørskov	-	White	-	-	-	-	67.3	0.7-1.5	0.6-0.8	0.7-1.0	-
<i>M. aquaticus</i> strain 1C	-	White	-	-	-	-	67.3	0.7-1.6	0.6-0.8	0.7-1.0	-

face, indicating that a respiratory acid-producing mechanism was also functional.

In contrast to these results, the *M. aquaticus* strains were the only organisms to produce acid from alcohols.

The three groups were quite different in their abilities to produce specific enzymes or end products (Table 3). Strains of the "*M. major*" group and of the "*M. aquaticus*" group produced urease, whereas strains of the "*M. flavus*" group did not. Only the *M. aquaticus* strains produced ornithine decarboxylase or reduced nitrates to nitrites. Strains of the *M. major* group produced phosphatase, the *M. aquaticus* strains did not, and two yellow strains produced only slightly positive reactions.

The *M. aquaticus* strains were the only ones unable to hydrolyze gelatin, starch, casein, and esculin, and the three groups were quite distinctive in their reactions in litmus milk (see Table 4).

Differences in the abilities of the organisms to utilize single carbon sources may be seen in Table 5. The *M. aquaticus* strains were the most versatile, for not only did they utilize succinate and malate, but also they were the only organisms to utilize acetate, citrate, and formate. Strains of the *M. major* group were the only ones to utilize glycerol phosphate and tartrate; in addition, the members of this group utilized succinate and malonate.

In general, the *M. aquaticus* strains were able to grow on a wider variety of media than were the organisms in the other two groups (Table 6), and high concentrations of carbohydrates (3 to 5%), as in NAS and TSAG media, inhibited members of one or both of the other two groups. In addition, rich media such as chocolate and blood agars and TSA generally were not suitable for the strains of *M. flavus* and *M. major* groups but supported good growth of the *M. aquaticus* strains.

Only the *M. aquaticus* strains were resistant to penicillin, gentamycin, actinomycin D, sulfathiazole, and sulfamethoxazole/trimethoprim (Table 7).

DISCUSSION

In 1894, Migula (15) established the genus *Spirosoma* to contain the organism previously isolated and named *Vibrio lingualis* by Eisenberg (7). Migula described it as follows: "Cells are usually fairly wide, curved in the shape of a screw, nonmotile, without flagella, rigid, nonflexible, single, free, or in gelatinous clumps" (translated from the German).

In 1887 and 1888, Weibel (20, 21) isolated several curved organisms, which he described

TABLE 2. *Oxidative production of acid from carbohydrates and alcohols by the strains studied*

Substrate	Acid production ^a							
	<i>Microcyclus flavus</i> ATCC 23276	<i>Spirosoma</i> sp. strain 1	<i>Spirosoma</i> sp. strain 2	Strain 5	<i>Microcyclus major</i> strain Gromov	Strain 3	<i>Microcyclus aquaticus</i> strain Ørskov	<i>Microcyclus aquaticus</i> strain 1C
Pentoses								
Arabinose	A ₃	A	A ₃	A ₃	A	A	A	A
Ribose	A	A	A ₃	A	—	—	A	A
Xylose	A	A	A	A	A	A	A	A
Methyl pentose								
Rhamnose	A	A	A ₃	A	A	sl A	A ₃	A ₃
Hexoses								
Fructose	A	A	A ₃	A	A	A	A	A
Galactose	A	A	A	A	A	A	A	A
Glucose	A	A	A	A	A	A	A	A
Mannose	A	A	A	A	A	A	A ₃	A ₃
Sorbose	—	—	—	—	—	—	—	—
Glucosides								
α-Methyl-D glucoside	A	A	A	A	A	A	—	—
Salicin	A	A	A	A	A	A	—	—
Disaccharides								
Cellobiose	A	A	A	A	A	A	—	—
Lactose	A	A	A	A	A	A	—	—
Maltose	A	A	A	A	A	A	—	—
Melibiose	A	A	A	A	sl A	A	—	—
Sucrose	A	A	A	A	A	A	—	—
Trehalose	A	A	A	A	A	A	—	—
Trisaccharide								
Raffinose	A	A	A	A	A	sl A	—	—
Polysaccharides								
Dextrin	A	A	A ₃	A	A	A	—	—
Inulin	A	A	A	A	A	—	—	—
Alcohols								
Glycerol	—	—	—	—	—	—	A	A
Erythritol	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	A	A
Sorbitol	—	—	—	—	—	—	A	A

^a Symbols: A, positive result; A₃, positive result took at least 3 weeks to occur; slA, slightly positive result; —, no acid production.

as follows (translated from the German): "In morphological respects. . . shows a diversity and complication of structures. . . one might find long or short, straight or curved single rods. More often, however, one can see a variety of forms. Interlinked rods, more or less curved, result in the formation of wavy or winding threads of strongly twisted spirals. . . an immense possibility of structural variation arises from the fact that the individual links within the groups may show unequal curvature. In this way we find very different curvatures and torsions—a pattern of scrolls and runes." In 1900, Migula placed Weibel's organisms in the genus *Spirosoma*.

This genus was dropped from the 6th edition of *Bergey's Manual* (2) because cultures of spiro-somas were no longer available and because the descriptions of the species did not meet the taxonomic criteria of the day. The generic name *Spirosoma* was validly published and is legitimate (3).

From the above-mentioned descriptions by Weibel and Migula and from our observations of the morphology of the yellow-pigmented group of organisms (Fig. 1), there can be little doubt that *M. flavus* ATCC 23276, *Spirosoma* sp. strains 1 and 2, and our strain 5 are organisms similar to those originally placed in the genus *Spirosoma*. We therefore concur with

TABLE 3. Production of specific enzymes or reactions by the strains studied

Strain	Production of enzyme or reaction ^a														
	Urease	Lecithinase	Lysine decarboxylase	Ornithine decarboxylase	Phenylalanine deaminase	ONPG	Oxidase	Catalase	Phosphatase	Hemolysin	Indole	Methyl red	VP	NO ₃ reduction	H ₂ S
<i>Microcyclopus flavus</i> ATCC 23276	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
<i>Spirosoma</i> sp. strain 1	-	-	-	-	-	+	+	+	sl +	-	-	-	-	-	-
<i>Spirosoma</i> sp. strain 2	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Strain 5	-	-	-	-	-	+	+	+	sl +	-	-	-	-	-	-
<i>Microcyclopus major</i> strain Gromov	+	-	-	-	-	+	+	sl +	+	-	-	-	-	-	-
Strain 3	+	-	-	-	-	+	+	sl +	+	-	-	-	-	-	-
<i>Microcyclopus aquaticus</i> strain Ørskov	+	-	-	+	-	+	+	+	-	-	-	-	-	+	-
<i>M. aquaticus</i> strain 1C	+	-	-	+	-	+	+	+	-	-	-	-	-	+	-

^a Symbols: +, Positive result; sl +, slightly positive result; -, negative result.

Claus et al. (5), who studied the first three named strains and suggested that these organisms be placed in the genus *Spirosoma*. An emended description of the genus follows:

Genus *Spirosoma* Migula 1900 *emend.*

(*Spi. ro. som* a. Gr. n. *spira* a coil; Gr. n. *soma* body; M. L. neut. n. *Spirosoma* coiled body.)

Rods, 0.5 to 1.0 by 1.5 to 50.0 μ m with various degrees of curvature, often resulting in rings, coils, and undulating filaments. The outer diameters of the rings range from 1.5 to 3.0 μ m. Nonmotile. Resting stages are not known. Gram-negative. Colonies contain a pale yellow, non-water-soluble pigment.

Metabolism is respiratory; acids are produced aerobically from a variety of carbohydrates. Strictly aerobic. Chemoorganotrophic.

The G+C content of the DNA is 51.0 to 52.9 mol% (thermal denaturation).

Isolated from soil and fresh water.

The type species (monotype) is *S. linguale* (Eisenberg) Migula 1894.

The four yellow-pigmented strains described above (*M. flavus* ATCC 23276, *Spirosoma* sp. strains 1 and 2, and our strain 5) conform to Eisenberg's (7) original description of *V. linguale*, and Migula's (15, 16) later descriptions of *S. linguale*, and they should be considered as strains of that species. Since the type strain of *M. flavus* Raj 1970 is placed in the species *S. linguale* (Eisenberg 1891) Migula 1894, the name *M. flavus* is a later subjective synonym of *S. linguale*. In the absence of the type strain of *S. linguale*, *Spirosoma* sp. DSM 74 is here designated as the neotype strain for the species. An emended description of this species follows.

Spirosoma linguale (Eisenberg 1891) Migula 1894 (Basonym: *Vibrio linguale* Eisenberg 1891; synonym *Microcyclopus flavus* Raj 1970.)

(lin.guá le. L. adj. *linguale* of the tongue.)

Rods varying from short, nearly straight cells to longer, curved cells in which the degree of curvature varies giving rise to rings, coils, and serpentine, undulating filaments. The cell diameter varies from 0.5 to 1.0 μ m, and the length of the cells varies from 1.5 to 50.0 μ m. Rings and coils may be from 1.5 to 3.0 μ m in outer diameter. Gram-negative. The cells are nonmotile and nonflexible. Pigmentation on MS agar is from pale to medium yellow.

Metabolism is respiratory. No growth occurs under an anaerobic seal of pyrogallol-KOH. Acid is produced oxidatively from many carbohydrates but not from alcohols. Oxidase and catalase are produced. Gelatin, starch, and tributyrin are hydrolyzed, but cellulose and chitin are not. Glycerol phosphate, succinate, tar-

TABLE 4. *Hydrolytic reactions of the strain studied*

Strain	Hydrolytic reactions ^a							
	Cellulose ^b	Chitin	Gelatin	Starch	Casein	Esculin	Tributyrin	Litmus milk
<i>Microcycylus flavus</i> ATCC 23276	—	—	+	sl+	sl+ ₃	+	+	Soft curd, reduction, reoxidation
<i>Spirosoma</i> sp. strain 1	—	—	+	sl+	sl+ ₃	+	+	Soft curd, reduction, reoxidation
<i>Spirosoma</i> sp. strain 2	—	—	+	sl+	—	—	+	Soft curd, reduction, reoxidation
Strain 5	—	—	+	sl+	sl+ ₃	+	+	Slightly alkaline
<i>Microcycylus major</i> strain Gromov	—	—	sl+	Wide	—	+	+	No change
Strain 3	—	—	+	Wide	—	+	+	No change
<i>Microcycylus aquaticus</i> strain Ørskov	—	—	—	—	—	—	+	Very alkaline
<i>M. aquaticus</i> strain 1C	—	—	—	—	—	—	+	Very alkaline

^a Symbols: +, positive result; +₃, positive result after at least 3 weeks; sl+, slightly positive; —, negative result.

^b Substrate.

TABLE 5. *Utilization of single carbon sources by the strains studied*

Strain	Utilization of carbon source ^a										
	Acetate	Benzoate	Citrate	Formate	Glycerol phosphate	Methylamine	Propionate	Succinate	Tartrate	Malonate	Methanol
<i>Microcycylus flavus</i> ATCC 23276	—	—	—	—	+	—	—	+	+	+	—
<i>Spirosoma</i> sp. strain 1	—	—	—	—	+	—	—	+	+	+	—
<i>Spirosoma</i> sp. strain 2	—	—	—	—	+	—	—	+	+	+	—
Strain 5	—	—	—	—	+	—	—	+	+	+	—
<i>Microcycylus major</i> strain Gromov	—	—	—	—	—	—	—	+	—	—	—
Strain 3	—	—	—	—	—	—	—	+	—	—	—
<i>Microcycylus aquaticus</i> strain Ørskov	+	—	+	+	—	—	—	+	—	+	+
<i>M. aquaticus</i> strain 1C	+	—	+	+	—	—	—	+	—	+	+

^a Symbols: +, utilized; —, not utilized.

trate, and malonate may be used as sole sources of carbon, but acetate, benzoate, citrate, formate, methylamine, propionate, and methanol cannot. Urease, lecithinase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase are not produced. Indole, acetylmethylcarbinol, and H₂S are not produced. Nitrate is not reduced. The G+C content of the DNA ranges from 51.0 to 52.9 mol% (thermal denaturation).

The neotype strain is DSM 74 (*Spirosoma* sp. strain 1). A description of this strain is given in Tables 1 through 7.

M. major strain Gromov and our strain 3 are nearly identical but differ significantly from both *M. flavus* ATCC 23276 and *M. aquaticus* strain Ørskov. For the two former strains we suggest the establishment of a new genus, *Flectobacillus*, with the following description.

Flectobacillus gen. nov.

(*Flec.to.ba.cil'* lus. L. v. *flecto* to curve; L. n. *bacillus* a little staff, rod; M. L. mas. n. *Flectobacillus* curved rod.)

Straight to curved rods, the degree of curvature varying among individual cells within a

TABLE 6. Growth of the strains studied on various media

Strain	Growth on various media ^a															
	Choco- late ^b	Blood	EMB	NA	NAS	PRMS	PEA	TSA	TSAS	TSAG	PMA	MS	YEAT	McConkey	BS	SS
<i>Microcycylus flavus</i> ATCC 23276	++ ^b	++	—	+++	+++	—	—	+++	+	—	++	+++	+++	—	—	—
<i>Spirosoma</i> sp. strain 1	+	++	—	+++	+++	—	—	—	—	—	++	+++	+++	—	—	—
<i>Spirosoma</i> sp. strain 2	—	—	—	++	++	—	—	—	—	—	+	++	—	—	—	—
Strain 5	+	++	—	+++	+++	—	—	—	+	—	+++	+++	+++	—	—	—
<i>Microcycylus major</i> strain Gromov	++	++	—	+++	—	—	—	++	—	—	+++	+++	+++	—	—	—
Strain 3	++	++	—	+++	—	—	—	—	—	—	+++	+++	+++	—	—	—
<i>Microcycylus aquaticus</i> strain Ørskov	+++	+++	++	+++	+++	—	—	+++	—	+++	++	+++	+++	—	—	—
<i>M. aquaticus</i> strain 1C	+++	+++	++	+++	+++	—	—	+++	—	+++	++	+++	+++	—	—	—

^a Growth response is graded from + to ++++ indicating scant to good growth, respectively; a negative sign indicates no growth.^b Medium. Abbreviations: EMB, eosin methylene blue agar; NA, nutrient agar; NAS, Na + 5% sucrose; PRMS, phenol red mannitol salt agar; PEA, phenyl ethyl alcohol agar; TSA, Trypticase soy agar; TSAS, TSA + 3% sucrose; TSAG, TSA + 3% glucose; PMA, peptonized milk agar; MS, *Microcycylus-Spirosoma* agar; YEAT, yeast extract tryptone agar; BS, bismuth sulfide agar; SS, *Salmonella-Shigella* agar.

TABLE 7. Susceptibility of the strains studied on antimicrobial agents

Strain	Susceptibility to antimicrobial agent ^a																		
	Actino- mycin D (100 µg/ml)	Ampi- cillin (10 µg)	Aureo- mycin (15 µg)	Carbeni- cillin (50 µg)	Cephalo- thin (30 µg)	Coli- stin (10 µg)	Erythro- mycin (15 µg)	Fura- dantin/ macro- dantin (300 µg)	Genta: Kana- mycin (10 µg)	Neo- mycin (30 µg)	Mito- mycin C (1 µg/ ml)	Peni- cillin G (10 units)	Poly- myxin B (300 units)	Strepto- mycin (10 µg)	Sulfa- metho- xyzo/ trime- thoplerin (25 µg)	Sulfa- thia- zole (300 µg)	Tetra- ccline sulfa (30 µg)	Triple sulfa (1 mg)	
<i>Microcycylus flavus</i> ATCC 23276 <i>Spirosoma</i> sp. strain 1 <i>Spirosoma</i> sp. strain 2 Strain 5	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	
	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	
	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	
	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	-	
<i>Microcycylus major</i> strain Gromov Strain 3	+	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+	+	-	
	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	
<i>Microcycylus aquaticus</i> strain Ørskov <i>M. aquaticus</i> strain 1C	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	
	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	

^a Symbols: +, susceptible; -, resistant.

culture; the most abundantly occurring are cells in the shape of the letter C. The cells measure 0.6 to 1.0 by 1.5 to 5.0 μm . Long sinuous filaments up to 50 μm long are present. Rings 5 to 10 μm in outer diameter are formed by overlapping of the ends of a cell. Coils or helical spirals are infrequently formed. Gram-negative. Nonmotile. Not flexible. Resting stages are not known. Colonies on MS agar contain a pale pink or rose-colored, non-water-soluble pigment.

Metabolism is respiratory; acids are produced aerobically from a variety of carbohydrates. Strictly aerobic. Chemoorganotrophic.

Isolated from fresh water.

The G+C content of the DNA ranges from 39.5 to 40.3 mol% (thermal denaturation).

The type species is *F. major* (Gromov) comb. nov.

Flectobacillus major (Gromov 1963) comb. nov. (Basonym: *Microcyclus major* Gromov 1963.)

(ma' jor. L. adj. *major* larger.)

Rods varying from nearly straight to crescent shaped, to long, sinuous filaments. Rings with an outer diameter of 5 to 10 μm also occur. The cells vary in diameter from 0.6 to 1.0 μm , and the usual length is 1.5 to 5.0 μm . Coiled filaments are produced infrequently. Gram-negative. Nonmotile and nonflexible. Pigmentation on MS agar is a pale pink to rose color.

Metabolism is respiratory. No growth occurs under an anaerobic seal of pyrogallol-KOH. Acid is produced oxidatively from many carbohydrates but not from alcohols. Oxidase, catalase, and phosphatase are produced. Gelatin, starch, esculin, and tributyrin are hydrolyzed, but cellulose and chitin are not. Succinate, but not acetate, benzoate, citrate, formate, glycerol phosphate, methylamine, propionate, tartrate, malonate, or methanol, may be utilized as a sole source of carbon. Urease is produced, but lecithinase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase are not. Indole, acetylmethylcarbinol, and H_2S are not produced. Nitrate is not reduced. The G+C content of the DNA ranges from 39.5 to 40.3 mol% (thermal denaturation).

The type strain of *F. major* is, by monotypy, strain Gromov (= BKM 859 = DSM 103). A description of this strain is given in Tables 1 through 7.

The removal of *M. flavus* and *M. major* from

the genus *Microcyclus* necessitates that the definition of this genus be modified as follows.

Genus *Microcyclus* Ørskov *emend.*

Curved rods which measure 0.5 to 1.0 by 1.0 to 3.0 μm . Rings with an outer diameter of 0.7 to 3.0 μm are infrequently formed. Filaments and coils are not produced. Gram-negative. Nonmotile. Resting stages not known. Colonies on MS agar are nonpigmented to cream colored.

Metabolism is respiratory; acids are produced from several carbohydrates and alcohols. Strictly aerobic. Chemoorganotrophic.

Isolated from soil and fresh water.

The G+C content of the DNA ranges from 66.3 to 68.4 mol%.

The type species is *M. aquaticus*. The type strain (by monotypy) of *M. aquaticus* is strain Ørskov, a description of which is given in Tables 1 through 7.

It seems premature to attempt to place the genera *Microcyclus*, *Spirosoma*, and *Flectobacillus* into families until their relationships with other genera are better known.

The lack of cultures of these organisms for a long time is somewhat surprising when it is considered that Weibel succeeded in isolating several strains, and *Spirosoma* was the first genus in the family *Spirillaceae* in Migula's "System der Bakterien" (16). Moreover, the unusual morphology of these organisms makes it relatively easy to recognize them. In the course of isolating some of the strains used in this study, we repeatedly saw similar morphological forms in bodies of water. These sites included fill ponds, the Tangipahoa and Trinity rivers in Louisiana and Texas, respectively, a canal in New Orleans, the Ponchatoula Creek, and runoff from rice fields. Two strains which are morphologically similar to *F. major* have been recently isolated by us and are currently being studied.

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REPRINT REQUESTS

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FIG. 1. Phase micrographs of (a) *M. flavus* ATCC 23276, (b) *Spirosoma* sp. strain 1, (c) *Spirosoma* sp. strain 2, (d) strain 5, (e) *M. major* strain Gromov, (f) strain 3, (g) *M. aquaticus* strain Ørskov, and (h) *M. aquaticus* strain 1 C. All are magnified $\times 1,050$ except (g) and (h), which are $\times 2,640$.

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