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A NEW SPECIES - MICROCYCLUS FLAVUS

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ABSTRACT. A description is given of Microcycclus flavus sp. nov., found in fresh water from deep wells in Long Beach, California, U.S.A., in comparison with Microcycclus aquaticus (Ørskov).

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The genus Microcycclus (Ørskov 1928, 180) and its type species M. aquaticus are accepted in the Bergey's Manual of Determinative Bacteriology (1957, 253). "Microcycclus" (meaning literally "small circles") seems appropriate as a genus name for these Gram-negative bacteria because they have in common formation of closed rings under suitable cultural conditions. However, other morphological variations of the basic ring curvature occur frequently. Several workers (Weibull 1887, 465; Migula 1900, 955; Sturges 1923, 11; Hallock 1960, 404; Gromov 1963, 733; Claus 1967, 254) have reported organisms mostly from soil and water that form ring structures characteristic of the genus Microcycclus. In 1964, during my studies of bacterial ecology of fresh water from deep wells (Water Department Report No. WD-546, City of Long Beach, California) an organism was isolated whose morphology indicated assignment to the genus Microcycclus (Ørskov). Since then, radio-respirometric and electron microscopic studies of this isolate have been made (Raj and Wang 1965, 78; Raj and Kluss 1967, 172; Raj 1968, 44). The studies reported here concern the taxonomic status of this organism believed to represent a new species.

MATERIALS AND METHODS

For morphological and cultural characteristics, the organisms were grown in tryptone glucose extract agar plus 0.1% yeast extract (TGEY) medium at room temperature. Also, various commercially available media, such as brain heart infusion (BHI), trypticase soy broth without glucose

(T-soy), Lowenstein-Jensen (LJ) agar, triple sugar iron (TSI) agar and eosin methylene blue (EMB) agar were used.

Microcycylus aquaticus and *M. flavus* were simultaneously compared for various biochemical and physiological properties. All inoculated tubes and plates were incubated at room temperature for as long as 60 days before a test was declared negative. For optimum temperature of growth, inoculated TGEY broth tubes were incubated at 7°C, 22°C, and 37°C. For growth in presence of salt, TGEY broth tubes containing various levels of NaCl (0, 3.5 and 10%) were inoculated. Methods to establish biochemical and physiological peoperties (Tables 2, 3, 4) have been described elsewhere (Manual of Microbiological Methods 1957; Raj *et al.* 1961, 295). The tests for lipase and oxidase were carried out as described by Gillespie (1952, 187) and by Kovacs (1956, 703). The utilization of 22 different carbohydrates (Table 4) was tested at 1% substrate concentration in three different media: phenol red broth base (Difco), purple broth base (Difco) and Hugh and Leifson (1933, 24) medium which were used in duplicate tubes for both oxidative and fermentative reactions. Oxidation or utilization of various carbon compounds (Table 3) was examined in a basal medium having the composition of Simmons citrate agar medium (Difco) without citrate and agar.

Antibiotic and Sulfonamide sensitivity tests were done using Bacto-sensitivity disks (Difco) on TGEY medium (Table 2).

Electron microscopy of whole cells and thin sections was done with preparations on 200 mesh copper screen grids on a Hitachi H56 electron microscope. Procedures for fixation, embedding, sectioning, staining (500-600Å sections made on Porter-Blum microtone using glass knives) were the same as described by Kellenberger *et al.* (1958, 671). This was followed by post-staining as described by Millonig (1961, 736).

RESULTS AND DISCUSSION

An isolate forming yellow colonies on a TGEY agar plate after three days' incubation at room temperature was obtained originally from fresh water from a deep well in the city of Long Beach, California. It has been given the name *Microcycylus flavus* (Latin adj. *flavus* yellow) because of its typical morphology as implied literally by the genus name and because of its consistently yellow pigment. In order to establish its taxonomic status, it has been compared morpho-

TABLE 1

Morphological and Cultural Characteristics

Characteristics	<u>M. flavus</u>	<u>M. aquaticus</u>
<u>Morphological</u> (TGEY medium at RT):		
1. Unicellular cells	+	+
2. Curved rods (vibrio-like)	-	+
3. Curved rods (horseshoe-like)	+	+
4. Closed ring-like cells	+	+
5. Coils or spiral forms	+	-
6. Filamentous forms	+	-
7. Pleomorphic forms	-	+
8. Cells with rounded ends	+	+
9. Cells with pointed ends	-	-
10. Cells mostly occur singly	+	+
11. Some cells occur in pairs or chains	+	+
12. Branching cells	-	-
13. Sheathed cells	-	-
14. Flagellated cells	-	-
15. Non-motile cells	+	+
16. Common capsule for aggregated cells	+	+
17. Cells individually capsulated	+	+
18. Cyst or zoogloae	-	-
19. Gram negative cells	+	+
20. Gram variable cells	-	-
21. Cells stain with giemsa	+	+
<u>Cultural</u>		
22. mucoid	+	+
23. dull	-	+
24. Colony yellow	+	-
25. on off-white	-	+
26. TGEY entire	+	+
27. agar circular	+	+
28. at opaque	+	+
29. RT smooth	+	-
30. contoured	-	+
31. medium size (2-3mm)	+	+
32. Growth even turbidity	+	+
33. in pellicle	-	+
34. TGEY broth ring	-	-
35. at RT even sediment (shaken)	+	-
36. flaky sediment (shaken)	-	+
37. Growth in brain heart infusion broth	-	+
38. Growth in trypticase soy broth (without glucose)	+	+

Number of total similarities = 24

Number of positive characters shared = 16

Number of dissimilarities = 14.

TGEY = tryptone glucose extract (Difco) medium + 0.1% yeast extract

** The most common feature

* Seen under certain cultural conditions only

TABLE 2

Physiological Characteristics

Characteristics		<u>M. flavus</u>	<u>M. aquaticus</u>
39.	Some growth at 7C	+	+
40.	Good growth at 22-25C	+	+
41.	Growth at 37C	-	-
42.	More growth on shaking	+	+
43.	Growth in fluid thioglycollate medium	-	-
44.	Growth in 0% NaCl broth	+	+
45.	Growth in 3.5% NaCl broth	-	+
46.	Growth in 10% NaCl broth	-	-
47.	Growth on Lowenstein-Jensen agar	-	+
48.	Growth on Eosin methylene blue agar	-	+
49.	Reaction on TSI agar (Slant/stub)	yellow/yellow	pink/no change
50.	Chloromycetin (30 µg)	4.0 cm*	R
51.	Tetracycline (30 µg)	4.0 cm	4.0 cm
52.	Erythromycin (15 µg)	3.0 cm	R
53.	Novobiocin (30 µg)	2.5 cm	2.5 cm
54.	Penicillin G (10 units)	2.0 cm	R
55.	Streptomycin (10 µg)	2.0 cm	2.5 cm
56.	Kanamycin (30 µg)	1.5 cm	2.5 cm
57.	Neomycin (30 µg)	R	1.5 cm
58.	Sulfathiazole (50 µg)	4.0 cm*	R
59.	Triple Sulfa (50 µg)	3.0 cm	R
60.	Gantrisin (50 µg)	3.0 cm	R
61.	Thioasulfil (50 µg)	2.0 cm	R
62.	Elkoin (50 µg)	1.0 cm	R
63.	Sulfadiazine (50 µg)	1.0 cm	R
64.	Sulfamerazine (50 µg)	1.0 cm	R
65.	Sulfamethoxy-Pyridazine (50 µg)	1.0 cm	R
66.	Guanine + cytosine (moles %) of DNA base	51.0	67.3

Number of total similarities = 11; Number of positive characters shared = 8;
 Number of dissimilarities = 17

* Sensitivity tests were done on tryptone glucose agar (Difco) plus 0.1 yeast extract without blood. The results were read after 7 days incubation at room temperature and expressed as diameter of zone inhibition. R = resistant

TABLE 3

Biochemical Characteristics

Characteristic	<u>M. flavus</u>	<u>M. aquaticus</u>
67. Agar digestion	-	-
68. Chitin digestion	-	-
69. Cellulose digestion	-	-
70. Gelatin liquefaction	-	-
71. Starch hydrolysis	-	-
72. Litmus milk reduction	-	+
73. Litmus milk acid	-	-
74. Litmus milk alkaline	-	+
75. Litmus milk hard curd	-	-
76. Litmus milk soft curd	-	+
77. Litmus milk peptonization	-	+
78. Nitrate	-	+
79. Reduction { Nitrite	-	+
80. { Ammonia	-	-
81. { Nitrogen	+	+
82. NH ₃ from peptone water	-	-
83. Indole production	-	-
84. Methyl red reaction	-	-
85. Voges Proskauer reaction	-	-
86. H ₂ S (from thiosulfate or cysteine)	-	-
87. Catalase	+	+
88. Oxidase	+	+
89. Reductase	-	-
90. Urease	-	+
91. Lipase	-	-
91. Hemolysins	-	-
<u>Utilization of Various Substrates as Carbon Sources*</u>		
92. Acetate	-	+
93. Benzoate	-	-
94. Citrate	-	+
95. Formate	-	+
96. Fumarate	-	-
97. Gluconate	-	-
98. α-Ketoglutarate	-	-
99. Lactate	-	+
100. Malate	-	-
101. Malonate	-	+
102. Oxaloacetate	-	+
103. Pyruvate	-	+
104. Succinate	-	+
105. Tartarate	-	-

Number of total similarities = 24; Number of positive characters shared = 3;
 Number of dissimilarities = 15.

*Broth base medium used was same as Simmons citrate (Difco) medium but without citrate and agar. Fully positive results showing deep blue color (= pH > 7.6) of the indicator were obtained between 20 - 40 days of incubation at room temperature. Negative (-) results were recorded after 60 days' incubation.

TABLE 4

Utilization of Carbohydrates when Tested in Three Different Basal Media

Carbohydrate	<i>Microcycylus flavus</i>				<i>Microcycylus aquaticus</i>			
	Purple broth base	Phenol red broth base	H & L base* (OX)	(F)	Purple broth base	Phenol red broth base	H & L base* (OX)	(F)
106. Adonitol	-	-	-	-	-	-	-	-
107. Arabinose	a (25) [▲]	A (25)	A (12)	-	a (25)	A (25)	A (12)	-
108. Dulcitol	-	-	-	-	-	-	-	-
109. Fructose	-	A (25)	A (17)	-	-	a (25)	A (17)	-
110. Galactose	-	A (25)	A (17)	-	-	-	A (17)	-
111. Glucose	-	A (25)	A (12)	-	-	-	A (12)	-
112. Glycerol	-	-	-	-	-	-	-	-
113. Inulin	-	a (12)	A (12)	-	-	-	A (12)	-
114. Inositol	-	-	-	-	-	-	-	-
115. Lactose	-	-	A (12)	-	-	-	-	-
116. Maltose	-	-	A (12)	-	-	-	-	-
117. Mannitol	-	-	-	-	-	-	A (17)	-
118. Mannose	-	A (25)	A (12)	-	-	-	A (17)	-
119. Melezitose	-	-	-, V	-	-	-	-	-
120. Melibiose	-	-	A (17)	-	-	-	A (17)	-
121. Raffinose	-	-	A (12)	-	-	-	-	-
122. Rhamnose	-	-	-	-	-	-	-	-
123. Salicin	-	-	-, V	-	-	-	A (17)	-
124. Sorbitol	-	-	-	-	-	-	A (17)	-
125. Sucrose	-	-	A (12)	-	-	-	A (17)	-
126. Trehlose	-	-	A (12), V	-	-	-	-, V	-
127. Xylose	A (25)	A (25)	A (12)	-	A (25)	A (25)	A (12)	-

Number of total similarities = 16; Number of positive features shared = 10;

Number of dissimilarities = 6

A = definite acid production; a = very slight acid produced which remained consistent up to 60 days; - = no reaction even after 60 days; V = variable reactions obtained from time to time.

▲ The numbers in () refer to the number of days taken before the designated reaction was obtained.
 ● Hugh and Leifson's medium was used in duplicate tubes; one for oxidative (OX) reaction and the other overlaid with mineral oil for fermentative (F) reaction.

logically, culturally, biochemically and physiologically with the type species M. aquaticus (Ørskov strain) of the genus Microcycclus.

Cultural characteristics: As shown in Table 1 and Figure 1, M. flavus forms yellow, circular, convex, smooth, mucoid, viscid colonies of medium size (2-3 mm diameter) with an entire edge and a colorless, narrow peripheral zone (Figure 1B), whereas M. aquaticus forms off-white, circular raised, opaque, butyrous, contoured colonies of medium size on TGEY agar medium (Figure 1A). The "fried egg" appearance of contoured colonies of M. aquaticus is shown (Figure 1C) in contrast to the smooth colonies of M. flavus (Figure 1D). M. flavus yields good growth in TGEY broth mostly as a yellow sediment which on shaking results in even turbidity. In the same medium, M. aquaticus produces a pellicle and some sediment which becomes a flaky suspension on shaking. The yellow pigment produced by M. flavus is insoluble in water but soluble in acetone-ethanol mixture and is possibly carotenoid in nature. In contrast to M. aquaticus which is not fastidious in its growth requirements, M. flavus grew not at all or slightly in BHI and T-soy broths. However, neither organism grew in fluid thioglycollate medium (Difco).

Morphological characteristics: A young culture of M. flavus grown on TGEY agar slant shows consistently Gram-negative organisms forming horseshoes (0.5-0.8 μ wide and 3 μ long), closed rings (1.5-2.5 μ outer diameter) with hollow centers (0.4-1.0 μ inner diameter) and other formations resembling letters C and S and the number 8 (Figure 2A). Spiral forms (Figure 2B) or coils of as many as 10 or more cells are also quite commonly found with this organism. Electron micrographs of these various shapes are shown in Figures 3 and 4. Contrary to Ørskov's observations of M. aquaticus, septa between the cells in a coil have been noted in M. flavus (Figures 3C and 3E). Occasionally a few stretched rods (5-10 μ long) with or without any bending are seen occurring singly. Such forms are found in pure cultures and are not contaminants. Similar observations were also made by Weibel (1887, 465) on his vibrio culture later named Spirosoma nasale by Migula (1900, 955). Perhaps the most characteristic form of this organism are the rings which may cover the whole microscopic field (Figure 2A). Electron micrograph of a ring (Figure 3B) shows clearly that the ends of such a cell are positioned one over the other as if it truly represents a closed ring. Figures 4B and 4C show ring cells

Figure 1. Colonies grown on TGEY agar at room temperature: (A) Colonies of *M. aquaticus* compared to those of (B) *M. flavus* (both X4). Also, "fried egg" appearance of contoured colonies of (C) *M. aquaticus* compared with the smooth colonies of (D) *M. flavus*, under transmitted light (both X24).

Figure 2. Photomicrographs of normal morphology of M. flavus grown on TGEY agar at room temperature.

Figure 3. Electron micrographs of various forms of *M. flavus* seen in Figure 2: (A) a coil. (B) a ring and a coil. (C) a spiral; (D) a short coil. (E) two connected horseshoes.

dividing into two horseshoes. However, it remains conjectural whether the mode of binary fission in this organism follows common cell wall-membrane constriction or transverse septational division known in some, if not most, Gram-negative bacteria (Steed and Murray 1966, 263). The examination of the ultra thin sections of M. flavus (Figure 4D) shows that its cell wall has the characteristics of Gram-negative bacteria. It seems to consist of possibly three layers which are quite sinuous and not parallel to its more nearly planar cytoplasmic membrane.

Stained smears of the organism grown in different media showed the same morphological features as described above in practically all instances. However, under certain cultural conditions, M. flavus showed some morphological variations; for example, greater variations in individual cell volume were seen (Figure 5A) when the organism was grown in TGEY broth at 7°C than at room temperature. Also when it was grown in MR-VP medium, gelatin medium or Hugh and Leifson (H & L) medium with glucose, it showed very long spaghetti-like strings (Figures 5B-D).

Microscopic examination of a Gram-stained smear from a young culture of M. aquaticus grown on TGEY agar slant showed very small, slightly curved, Gram-negative rods ($0.7 \times 1.2 \mu$) (Figure 6A). An electron micrograph of these cells is shown in Figure 6B. However, when the organism is old (15 days) or grown at 7°C in TGEY broth or in H & L medium containing any oxidizable carbohydrate, it shows great morphological variations (Figures 7A, B, C, E) such as pleomorphism, swollen cells, ring-like structures and horseshoe shapes, as compared to its normal morphology of a slightly curved rod (Figure 7D). From a morphological point of view, there is little resemblance between the two organisms. M. aquaticus normally looks more like a vibrio and very much similar to Hallock's Vibrio alternans, whereas M. flavus seems similar in morphology to Spirosoma sp. strains 1 and 2 of Claus et al. (1968, 26). The only feature common to both is the formation of half and full circles. Unlike M. aquaticus, M. flavus has never been seen to form very small rods (1 μ long) or highly pleomorphic forms. On the other hand, unlike M. flavus, M. aquaticus does not commonly form rings; in fact, Claus (1967, 254) never succeeded in seeing such ring-like structures in this organism. In my experience, it has never been seen to form spirals or coils.

Figure 4. Electron micrographs of dividing cells of *M. flavus*: (A) a ring and a curved cell; (b) rings and a dividing cell; (C) a number 8 formation and a dividing cell; (D) a thin section of a ring cell.

Figure 5. Morphological variations seen in M. flavus:
(A) Light micrograph showing cell size variations when grown in TGEY broth at 7°C; (B) Light micrograph showing filamentous forms when grown in MR-VP medium at RT; (C) and (D) Electron micrographs of the filamentous cells seen in (B).

Figure 6. Normal morphology of *M. aquaticus* grown on TGEY agar at room temperature: (A) Photomicrograph; (B) electron micrograph.

Figure 7. Morphological variations seen in *M. aquaticus*: (A) Pleomorphic cells when 15 days old in TGEY broth at room temperature; (B) Doughnut cells when grown in H & L medium containing arabinose; (C) Swollen cells (E) and Horseshoe shapes when grown in H & L medium containing xylose as compared to the normal morphology (D) of a slightly curved rod.

Both the species are nonmotile in motility test agar medium (Difco) as well as in hanging drop preparations. Neither stained preparations for flagella (Leifson's method) nor electron micrographs of these organisms show flagella (Figures 3-7). Capsule staining (Tyler's method) showed both the organisms to be encapsulated when grown in TGEY agar medium. Also, some electron micrographs of M. flavus show the presence of capsular material around the aggregated cells (Figures 4C and 4D).

Though in the literature some other organisms such as Cytophaga annulus (Stapp and Bortels 1934, 28), Eubacterium sp. (Bladen et al. 1964, 763), Myconostoc gregarium and Ochrobium tectum are reported to show certain morphological resemblance to Microcycilus, they seem to bear no close relationship to this genus (Bergey's Manual of Determinative Bacteriology 1957, 260 and 225).

Physiological characteristics: As shown in Table 2, the optimum temperature for growth of both the organisms is room temperature (22-25°C). However, both failed to grow at 37°C but formed slight sediments at 7°C in TGEY broth after prolonged incubation. On shaking the sediments, M. flavus showed viscid growth compared to the flaky growth of M. aquaticus. When tested for growth in the presence of various levels of NaCl in TGEY broth, both the organisms did not grow in the presence of 10% NaCl; while M. flavus failed to grow in 3.5% NaCl broth, M. aquaticus grew fairly well producing viscid growth in this medium. Also, when these organisms were cultivated on some of the selective media, M. flavus failed to grow on LJ and EMB media but grew on TSI agar and slowly turned both the slant and the stub yellow only after 17-25 days. Compared to this, M. aquaticus grew on all the three selective media within a week and produced pink color on the TSI slant with no color change in its stub even after 25 days. Table 2 presents sensitivities of these organisms to various antibiotics and sulfonamides. While M. flavus was sensitive to all the eight sulfonamides tested, M. aquaticus was quite resistant to all of them. Both the organisms were quite sensitive, in the increasing order, to kanamycin, streptomycin, novobiocin and tetracycline. However, while M. flavus was sensitive, in the increasing order, to penicillin G, erythromycin and chloromycetin, M. aquaticus was resistant to them. Of all the antibiotics tested, M. flavus was resistant to neomycin but M. aquaticus was somewhat sensitive to it.

Biochemical characteristics: As shown in Table 3, both the organisms did not hydrolyze any of the polysaccharides tested and failed to produce indole, methyl red reaction, acetyl methyl carbinol, gelatin liquefaction, and H_2S from both thiosulfate and cysteine media. However, both produced NH_3 from peptone water and possessed catalase and cytochrome oxidase but lacked reductase, urease and hemolysins. M. aquaticus showed lipase activity, reduced the nitrates to NO_2 and NH_3 and caused blue coloration and peptonization of the litmus milk, but M. flavus gave completely negative reactions in all these tests. While M. flavus failed to oxidize any of the compounds listed in Table 3, M. aquaticus utilized acetate, citrate, formate, lactate, malonate, pyruvate, oxaloacetate and succinate. However, radiorespirometric studies of M. flavus using various ^{14}C -labeled substrates in the TGEY medium, showed that it utilized acetate and gluconate but not glycine, α -ketoglutarate, methionine and pyruvate. Similar studies made of M. aquaticus indicated that ^{14}C -labeled gluconate was utilized by this organism contrary to the results shown here. Furthermore, these studies revealed that M. flavus metabolizes glucose mainly via the Embden-Meyerhof (EM) pathway and gluconate via the Entner-Doudoroff (ED) pathway, both in conjunction with the tricarboxylic acid (TCA) cycle and with some concurrent participation of the pentose phosphate (PP) pathway. M. aquaticus, however, shows no evidence of EM pathway but metabolizes both glucose and gluconate via the ED-TCA and PP pathways (Lehner and Raj 1969, 36).

The acid production from 22 carbohydrates was tested in three different basal media as shown in Table 4. Both the organisms showed acid reaction from only two carbohydrates (arabinose and xylose) in purple broth base, compared to positive results from three to seven carbohydrates in the phenol red broth base and as many as twelve to fourteen in the H & L medium. It is thus obvious that H & L medium is most satisfactory whereas purple broth base is least satisfactory for these tests. Ørskov (1928, 180) had reported that M. aquaticus could utilize only two carbohydrates (arabinose and xylose) out of the twelve tested. Since there is no specific information in the literature as to the basal medium he used, it seems very likely that a medium similar to the purple broth base was perhaps used which may account for the negative results he reported for many carbohydrates with this organism. Considering only those reactions which were obtained in the H & L medium, it seems very likely

that both the organisms can utilize arabinose, fructose, galactose, glucose, inulin, mannose, melibiose, salicin, sucrose and xylose but not adonitol, dulcitol, glycerol, inositol and rhamnose. In addition, M. flavus utilizes lactose, maltose and raffinose but not mannitol and sorbitol which may help to differentiate it from M. aquaticus. It may be noted here that with certain carbohydrates (Table 4) variable reactions were obtained with both the organisms when tested from time to time. Both the organisms oxidized carbohydrates in any of the three basal media rather slowly, producing acid but no gas only after prolonged incubation of 20 to 40 days. However, both failed to ferment any of the 22 carbohydrates in tubes of H and L medium which were overlaid with sterile mineral oil for fermentative reactions.

Characteristics numbering 127 have been recorded for each organism (Tables 1-4); these include 38 (i. e. 30%) morphological and cultural features, and 89 (i. e. 70%) biochemical and physiological features. Of these, 77 (36 positive and 41 negative) characteristics are shared which at best account for only 60% cross-relation between the two organisms. Applying the Adansonian principles of numerical taxonomy and using Sneath's equation (1957, 201) for the similarity index (S value) on the basis of only positive features shared, the S value for these organisms is calculated to be about 42%. Since, according to Liston et al. (1963, 1061), this value is far below that expected of strains within the same species, it is clear that M. flavus and M. aquaticus do not belong to the same species and that 42% S value seems significant enough to justify a separate species status for each organism within the same genus.

Recently Claus and his associates (1968, 26) observed, mostly on the basis of deoxyribonucleic acid (DNA) base composition, that the above two organisms represent two different genera. Though the guanine + cytosine (GC) content in the DNA base of M. flavus is 16.3% less than that of M. aquaticus (Table 2), such a variation is not uncommon among species within the same genus, particularly in the genus Flavobacterium, Pseudomonas, Spirillum, or Vibrio (Hills 1966, 419). If gross morphology and DNA base composition were the only criteria for bacterial classification, then both M. aquaticus and M. flavus could easily belong to the genus Spirillum or Vibrio (DeLey 1964, 17). However, both the Microcycilus species are separated from the above

genera because they are nonmotile, without flagella and oxidative in their biochemical capabilities besides having characteristic morphological features. If, however, the DNA base composition is emphasized as the only criterion and the characteristic morphology is overlooked, then these organisms may very well be included in the genus Flavobacterium or Pseudomonas (DeLey 1964, 17; Redfearn et al. 1966, 293). Obviously, the DNA base composition cannot be used as a sole determinant in deriving taxonomy of bacteria.

It seems from the reports in the literature, that M. flavus and two other isolates, Spirosoma sp. strains 1 and 2 reported by Claus et al. (1968, 26) possibly belong to the same species. These three organisms are reported to have the same morphological features and the same GC content of 51%. Also, these authors have vaguely suggested that Hallock's Vibrio alternans is possibly related to M. aquaticus. On the other hand, the same authors feel that Gromov's M. major, because of its lower GC content (39.5%) and peculiar morphology of slightly bent rods with pointed ends, represents a different genus related neither to M. aquaticus nor M. flavus. Obviously a detailed comparative study such as presented here is needed of all such organisms before their taxonomic status could be ascertained.

Cultures of the type strain of Microcycylus flavus have been deposited with the Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia as CM No. 2041 and with the American Type Culture Collection, Washington, D.C., as ATCC No. 23276. Also, recently another organism tentatively named as Microcycylus marinus was isolated from the ocean and has been deposited as ATCC No. 25205. M. aquaticus has been deposited as ATCC No. 25396.

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