A Classification of Flexibacteria

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SUMMARY

About 90 strains of gliding microbes (flexibacteria) have been considered. Data obtained by Lewin & Lounsbery (1969) and Mandel & Lewin (1969) have been used by Fager (1969) in a computer programme designed to indicate affinities and thereby possible relationships. Largely on the basis of Fager's analysis, a classification of these organisms is here proposed.

Including a few species described elsewhere, 27 species and varieties are distinguished, most of them apparently new. A simplified diagnostic key is presented for their identification. They are assigned to six genera, three (*Saprospira*, *Flexithrix* and *Herpetosiphon*) are recognized primarily on morphological grounds; three others (*Cytophaga*, *Flexibacter* and *Microscilla*), here somewhat re-defined, are based largely on other convenient characteristics.

INTRODUCTION

When these studies began, some years ago, the taxonomy of gliding bacteria was in a state of confusion. The Myxobacterales, having recognizably distinct fruiting bodies, were known to a few specialists, though until recently few myxobacteria had been cultured in the laboratory. The gliding microbes which do not form spores were even less well known. *Saprospira* was erroneously included among the spirochaetes. One or two sheathed representatives were associated with the Chlamydo bacteriales. Gliding organisms which attack cellulose, and similar organisms capable of digesting a variety of other polysaccharides, were assigned to the genus *Cytophaga*, the delimitation of which has become more and more diffuse in recent years. Other similar microbes were usually unrecognized when material collected from nature was examined directly under the microscope, and tended to be overlooked in bacteriological studies of natural waters since most of the culture media routinely used for such purposes contain concentrations of organic nutrients inhibitory to their growth. Those which did grow, being generally yellow, were usually classed along with yellow eubacteria in the ill-defined genus *Flavobacterium*, and were rarely classified further. Many of these and related taxonomic problems were discussed by Soriano & Lewin (1965). In the present paper we shall first review the descriptions of the genera to which such microbes might be assigned, and then indicate some specific and generic distinctions which we consider to be useful.

A few species, more or less regularly helical, have been set apart in the genus *Saprospira*, comprising *S. grandis* (Lewin, 1962), *S. albida*, *S. flammula* and *S. thermalis* (Lewin, 1965*a, b*). (The nutrition of *S. albida* and *S. flammula* presented special problems, and these species were therefore excluded from consideration in this paper.)

Others are distinguished by the presence of a sheath, as in the new genera *Herpetosiphon* (Holt & Lewin, 1968) and *Flexithrix* (Lewin, 1969*a*). In the present article, attention is concentrated chiefly on free-living, aerobic, non-helical species. Some of these form filaments of indefinite length (*Flexibacter* spp. and *Microscilla* spp.); others have either shorter filaments or more or less fusiform cells only a few μm . long. Several of the latter forms have been previously recognized as species of *Cytophaga* (Winogradsky, 1929, as amended by Stanier, 1940), but this genus is clearly in need of further revision. *Cytophaga* and certain other genera have accordingly been somewhat re-defined. (Revision of the genus *Flavobacterium*, which may include similar forms, is even more necessary; Quadling, Cook & Colwell, 1964; Hendrie, Mitchell & Shewan, 1968), but until the nature and necessity of this genus are clarified we prefer to avoid using it.) We have based generic distinctions primarily on morphology, and specific distinctions largely on nutritional and other biochemical characteristics of laboratory cultures. This may not be phylogenetically sound, but it has been commonly accepted practice among bacteriologists. Our recommendations are based largely on the analysis of recurrent groups by Fager (1969), somewhat weighted (or biased!) by considerations of GC values (Mandel & Lewin 1969). Formal descriptions of the new species are presented in Table 1 of this paper.

Genera and families of flexibactera reviewed and revised

Before considering the various specific groupings indicated by computer analysis we should first define and describe the several genera to which they may be assigned. These are presented, with some historical annotations, in the following review.

Among our most serious problems was that of distinguishing among the genera *Cytophaga*, *Flexibacter* and *Microscilla* (see Soriano & Lewin, 1965). We first considered the practicability of assigning all of these forms, or at least the majority—those with shorter cells or filaments—to the genus *Cytophaga*. As indicated by Imshenetsky & Solntseva (1936), Stanier (1947) and Soriano & Lewin (1965), this genus is in need of a stricter formal definition. The following list of names and references summarizes its history:

1929. *Cytophaga hutchinsonii* Winogradsky. (Also 3 to 4 other spp.) Winogradsky, though he did not obtain pure cultures, recognized that the cells were quite unlike those of eubacteria or spirochaetes. He created the genus on the ability of the cells to digest cellulose, a faculty which he considered to be obligate.
1934. *Cytophaga* spp. Stapp & Bortels. From soil. Gliding ability noted.
1940. *Cytophaga* (em. Stanier, 1940) based on *C. hutchinsonii* Winogradsky. This is the type species of the genus *Cytophaga* as first redefined by Stanier, comprising aerobic, facultatively cellulolytic gliding microbes that do not form microcysts. *C. krzemieniewskae* (note corrected spelling) Stanier and *C. diffluens* Stanier, two new species capable of digesting agar as well as cellulose, were also described.
1942. *Cytophaga* (em. Stanier, 1942) based on *C. hutchinsonii* Winogradsky. The genus was extended in scope to include other aerobic gliding forms recognized 'on purely morphological grounds'; not necessarily able to attack cellulose, but generally capable of digesting other polysaccharides, such as agar. Four species were recognized from soil, and two from marine mud.

1945. *C. columnaris* Garnjobst. A fish pathogen, capable of growth on media containing protein hydrolysates; not cellulolytic, and not requiring any specific polysaccharide for growth.
1946. *C. sensitiva* Humm. Digests agar, apparently used as major carbon source.
1947. *C. johnsonae* Stanier. Digests chitin or cellulose, but can otherwise be grown in protein hydrolysate media. Facultatively anaerobic.
1948. *C. psychrophila* Borg. A fish pathogen; cf. *C. columnaris* Garnjobst, 1945.
1955. *C. fermentans* Bachmann. Utilizes a variety of soluble carbon sources, but not cellulose, agar or chitin. Facultatively anaerobic.
1961. *C. succinicans* Anderson & Ordal. Can grow on protein hydrolysate media; cellulose 'not fermented'. Facultatively anaerobic.

Excludenda (? = *Sporocytophaga myxococcoides* Stanier, 1940).

1919. *Spirochaeta cytophaga* Hutchinson & Clayton.

1930. *Cytophaga myxococcoides* Krzemieniewska.

1934. *Cytophaga globulosa* Stapp & Bortels.

1936. Some *Cytophaga* spp. Imshenetsky & Solntseva.

It may be noted that at least *C. psychrophila*, *C. fermentans* and *C. succinicans* can grow anaerobically as well as aerobically. Though certain obligate anaerobes, such as the gliding species of *Fusobacterium*, could with justification be reassigned to the genus *Cytophaga*, as defined by Stanier in 1942, we feel that this would extend the bounds of the latter genus beyond convenient limits. We consider that it is now necessary to delimit *Cytophaga* more precisely, and propose the following redefinition. (Additional characters, not strictly part of the definition, are added in parentheses.)

Cytophaga Winogradsky (1929) emend. Non-photosynthetic (normally with yellow, orange or red carotenoid pigments); non-flagellate but capable of gliding (and, if sufficiently long, of flexing) on solid substrata; short or elongate rods or filaments (usually 5 to 50 μm . long, 0.3 to 0.7 μm . wide, with rounded or tapered ends); unbranched, unsheathed, not helical; not forming distinct fruiting bodies (though the cells may aggregate in pustular assemblages); not forming either endospores or microcysts (though inflated, more or less spherical cells are commonly formed in some cultures); Gram-negative (generally with a lower refractility than most eubacteria); obligately or facultatively aerobic; obligately heterotrophic; usually capable of digesting (or depolymerizing) several insoluble or macromolecular colloidal polysaccharides such as cellulose (or carboxymethyl-cellulose), chitin, agar, alginates, etc. The last characteristics, in particular, help to distinguish *Cytophaga* (as redefined here) from *Flexibacter* and *Microscilla* (both redefined below), which have generally more limited extracellular polysaccharase activity.

The ability of certain strains of *Cytophaga* to dissolve and digest cellulose is known to be a labile feature, which tends to be 'lost' in the course of cultivation when the organism is regularly maintained on more readily assimilable organic nutrients. Probably the same is true for the faculty of certain strains to digest other polysaccharides such as agar.

Promyxobacterium, a genus of 'imperfect myxobacteria', was distinguished by Imshenetsky & Solntseva (1936) mainly by the tapered ends of the cells or filaments. However, as these authors indicated, and as Stanier (1947) and we ourselves have confirmed in several clones, this feature is inconstant: the ends may be blunt or tapered according to the state of nutrition and the age of the culture. Stanier (1947) wrote: 'until nonfruiting myxobacteria have been more extensively studied, the genus *Cytophaga* appears to me to provide an adequate taxonomic pigeonhole for all known amicrocystogenous species.' As explained above, we can no longer agree with Stanier completely on this matter; but we, nevertheless, accept his reservations about the genus *Promyxobacterium*. This name may perhaps be conveniently reserved for gliding forms which have sufficiently high GC values to indicate a phylogenetic relationship with the fruiting Myxobacterales. Since cultures were unavailable, we were unable to compare our strains with those non-fruiting myxobacteria described as *Cytophaga* species by Imshenetsky & Solntseva (1936), Fuller & Norman (1943), Humm (1946), Starr (1953) and others referred to in *Bergey's Manual* (1957).

Many bacteriologists have assigned all kinds of yellow, Gram-negative, rod-shaped bacteria to the genus *Flavobacterium*, without regard to other criteria such as flagellation. Some of these can glide, including the generic neotype *F. flavescens* (Taylor's strain B. 2157). Although almost all of the gliding microbes under discussion are yellow or orange (or, more rarely, some shade of red), we cannot yet envisage any causal connection between carotenoid formation and gliding motility. Limitations of time and resources hindered us from any serious attempt to sort out the problems of this 'regrettable genus' (Stanier, 1947). Floodgate & Hayes (1963) and Hayes (1963) have attempted to bring some measure of order to this genus, but we did not compare our isolates with those in other collections by direct examination of the organisms themselves or by consideration of their described features. A comparison on the basis of the published literature would be difficult and relatively profitless, since we had chosen to study a set of characteristics somewhat different from those selected by Hayes and others.

The genus *Flexibacter* was described by Soriano (1945) with the following features (translated from Spanish): 'flexible rods capable of gliding movement, without intracellular sulphur granules, unable to attack cellulose, not forming fruiting bodies on substrate (as do Myxobacterales), and not spiral in form.' Since his type species, *F. flexilis*, has cells 0.5 to 0.7 μm . wide, 10 to 20 μm . long, and his *F. giganteus* has filaments 100 μm . or more in length, it is clear that Soriano's use of the word 'rods' (= 'bastoncitos') is to be interpreted broadly.

On the basis of this description it is clear that *Flexibacter* cannot be distinguished from *Microscilla* (Pringsheim, 1951; see below), as was pointed out by Soriano & Lewin (1965). Rather than dismiss the later generic name as invalid, we have chosen to redefine both genera as follows, and to use them for our two largest groups of species.

Flexibacter Soriano (1945) emend. (a) Characters in common with *Microscilla* (below) include the following: flexible but not helical rods or filaments, usually 1 μm . or less in width; crosswalls not apparent (at magnifications of about $\times 1000$); not branched or sheathed; without flagella; capable of gliding on solid substrata; Gram-negative; without photosynthetic pigments or intracellular sulphur granules; unable to attack cellulose; reproduction by simple fragmentation; not forming fruiting bodies, spores or microcysts; (b) filaments generally

5 to 50 μm . long; colour (seen only in packed masses) pink, red, orange or yellow; liquefying gelatin but not agar or alginate. Habitats: mostly along freshwater banks, hot springs, etc. (However, two of our species were marine).

Microscilla was described by Pringsheim (1951) as one of the new genera of his new family Vitreoscillaceae which he defined as 'colourless, filamentous, gliding micro-organisms, differing from Myxophyceae in lacking assimilatory pigments, and from Myxobacteria by a more pronounced trichome formation and the absence of microcysts'. His brief definition of *Microscilla* was: 'trichomes narrow without perceptible septation. Gliding movements active. Reproduction by division into relatively long daughter trichomes. Type species *M. marina* (trichomes 0.5 to 0.6 μm . wide, up to 100 μm . long. . .)'

As thus defined, *Microscilla* is not clearly distinguishable from *Flexibacter* (Soriano, 1951; see above). Partially to clarify this distinction, the following amended description is proposed:

Microscilla Pringsheim (1951) emend. (a) Characters in common with *Flexibacter* as above; (b) filaments usually 20 to 100 μm . or longer; colour (seen only in packed masses) yellow or orange; do not digest cellulose or agar, although some species liquefy alginate and gelatin. Habitat: marine shores.

Vitreoscilla spp. (Pringsheim, 1951) are distinguished by their apparent lack of pigments and by the torulose nature of their filaments, which exhibit marked constrictions at the intercellular nodes that may betray a wholly distinct mode of cell division. They seem less closely related to the other gliding microbes described above and may have closer affinities with the blue-green algae.

The following three flexibacterial genera are characterized by morphological features (helical filaments, simple or branched sheaths) which normally distinguish them from the genera described above.

Saprospira Gross (1911) emend. Lewin (1962). Unbranched, helical, multicellular, filamentous, usually 50 to 500 μm . long. (All of our strains of *S. grandis*, *S. tovisformis*, and *S. thermalis* liquefy gelatin but not agar, grow on media containing 5.0 g. tyrosine/l. without clearing (i.e. dissolution of the insoluble excess) or discoloration, and are inhibited by 0.1 g. sodium lauryl sulphate/l.; but we do not consider these necessary prerequisite features for species in this genus.)

Herpetosiphon Holt & Lewin (1968). Unbranched, multicellular, filamentous, sheathed, usually 50 to 500 μm . long. (Our marine strains all failed to hydrolyze starch, agar, alginate or carboxymethyl cellulose, produced neither catalase nor H_2S , tolerated 5.0 g. tyrosine/l., grew on medium containing 0.1 g. DOPA/l. without clearing or discoloration, and are inhibited by 0.1 g. sodium lauryl sulphate/l.; but we do not consider these necessary prerequisite features for species in this genus.)

Flexithrix Lewin (1969a). Falsely branched (under certain conditions), multicellular, filamentous, up to 500 μm . or more long. The only strain we have isolated was marine, contained the yellow carotenoid zeaxanthin, and in many other respects resembled *Microscilla aggregans* (described below). In the initial survey we observed at least one other dissimilar strain, which was not isolated or studied further. No meaningful generalization can be made at this time about the physiology and biochemistry of species in this genus.

In the light of new information presented in this paper, and the proposed definitions and redefinitions of the genera, it is also appropriate to re-examine here the problems of assigning the genera to higher taxa. This matter was left unresolved in the earlier paper by Soriano & Lewin (1965), in which the literature was reviewed and the arguments discussed. At present, the following modified classification seems to us as convenient as any:

1. Beggiatoaceae	Cylindrical; with sulphur granules
2. Leucotrichaceae	Tapering
3. Simonsiellaceae	Flattened
4. Vitreoscillaceae (excluding <i>Microscilla</i>)	Filaments constricted at nodes
5. Cytophagaceae (or Flexibacteraceae)	Cylindrical; with carotenoids
<i>Saprospira</i>	Helical
<i>Flexithrix</i>	Branched
<i>Herpetosiphon</i>	Sheathed
<i>Sporocytophaga</i>	Microcysts
‘ <i>Sphaerocytophaga</i> ’ (<i>Fusobacterium</i> p.p.)	Anaerobic (see Dworkin, 1966)
<i>Cytophaga</i>	Mostly < 20 μ m. polysaccharolytic
<i>Flexibacter</i>	Lengths various: mostly from fresh-water or mud
<i>Microscilla</i>	Mostly > 20 μ m. marine
(Moraxella should no longer be considered in this order; see Lautrop, 1965–67).	

It is possible that all prokaryotic microbes capable of gliding are phylogenetically related, and that such apochlorotic forms as we have studied here originated from photosynthetic cyanophytes. This possibility was reviewed briefly by Soriano & Lewin (1965). However, it is now the opinion of the present author that, although members of Beggiatoaceae, Leucotrichaceae, Simonsiellaceae and Vitreoscillaceae could have evolved in this way, the Cytophagaceae, with which this paper is primarily concerned, may have had different origins. New light will be shed on this problem when more biochemical information becomes available.

Preliminary considerations for a revised and expanded classification of flexibacteria

‘A taxonomy has to be used; groups need to be named and to be capable of recognition... Even the best of numerical classifications must be regarded as an approximation, or a guide to judgement, not a judgement in itself... There is an inalienable region of judgement into which numerical taxonomy cannot enter... The taxonomist... must have the courage to reallocate in the light of his own experience.’ (Selected sentences from Williams, 1967.)

We have confined our attention almost exclusively to our own collection of cultures, which we isolated from nature, under aerobic conditions at room temperature, on agar media containing low concentrations of organic nutrients (Lewin & Lounsbery, 1969). We accepted or rejected them in the first place solely on the basis of a single criterion, the ability to glide, although it is not known to what extent this feature is

phylogenetically fundamental (see Lautrop, 1965-67). Perhaps because of our choice of sources, or our choice of media or physical conditions, we isolated no colourless filamentous types (such as might be recognized as *Vitreoscilla* or *Leucothrix* spp.); only one apparently colourless, spore-forming myxobacterium (which has been excluded from further consideration in this paper), and no fruiting myxobacteria. In attempting to classify these microbial strains, we have tried to reconcile three more or less independent, although interrelated, approaches:

1. *Orthodox or classical taxonomy*, in which daily experience with the organisms and their reactions under a variety of experimental conditions in the laboratory leads to the subjective formulation of groupings based on cultural similarities.

2. *Numerical taxonomy*, in which a number of characters (selected on the subjective bases of experience and expediency) are recorded for each strain, essentially on a yes-or-no basis, and assigned unit values unweighted by prejudice. The data were then processed by a computer programme designed to extract more objectively defined groupings of organisms.

3. *Molecular genetics*, based on the generally accepted theory that, since the biochemical basis of heredity is nucleic acid, organisms genetically similar cannot exhibit very wide divergences in the overall chemical compositions of their nucleic acids. In other words, wide differences indicate an absence of close relationship. For example, all but one of the 23 organisms originally identified on morphological and physiological grounds as *Saprospira grandis* have GC values of $47.7 \pm 1.1\%$, which suggests that this is a reliable distinguishing characteristic. The close morphological, physiological and biochemical resemblances among the various strains identified with this species endorse the soundness of the argument for supporting taxonomy by GC values. The finding of a single aberrant strain, A-I, with a GC value of 38%, throws the argument into question; but we have now detected sufficient minor physiological and cultural characteristics of this strain to warrant distinguishing it as a separate species, to be called *Saprospira toviformis* (Lewin & Mandel, 1969).

It could hardly be expected that we would be able to reconcile perfectly the groupings suggested by these three approaches, but we have tried to eliminate the major points of disagreement; actually, there were few. In such cases, we have chosen to 'split', rather than to 'lump', since this expedient is less likely to be a source of confusion in the future when more relevant information comes to light, but to certain strains we have assigned names of only varietal rank (see below).

We have used for primary generic distinctions such few morphological features as are readily apparent. The four genera or genus complexes under consideration are:

Saprospira (not sheathed; helical): Gross, 1911.

Herpetosiphon (sheathed; unbranched): Holt & Lewin, 1968.

Flexithrix (sheathed; with false branches): Lewin, 1969 a.

The *Cytophaga*+*Microscilla*+*Flexibacter* complex (not sheathed; not helical).

Although the genera have been distinguished as above we admit that the features we have used are not necessarily constant and invariable. As noted elsewhere (Lewin, 1962), many strains of *Saprospira grandis* tend to lose their helical nature in culture; and we have even picked up in culture a spontaneous bacillary variant, which in the course of laboratory subculture, has now reverted to its original filamentous form. Such changes must occur in nature, too, as the following indicates. From ponds

and ditches in Costa Rica we isolated various pink filamentous forms, of which we called the helical ones *Saprospira* and the non-helical ones *Flexibacter*; yet in almost all other respects certain of these strains were indistinguishable. *Flexithrix* is another debatable case; it may be regarded as a 'form genus'. Though under some circumstances it forms sheathed, falsely branched filaments which clearly distinguish it from other bacteria, it can be grown in liquid as single naked cells which, in their pigmentation and other biochemical and nutritional features, are otherwise indistinguishable from a species of *Microscilla* as defined below. *Flexithrix*, thereby, presents taxonomic problems which are discussed elsewhere (Lewin, 1969*a*).

The classification of the remaining organisms, which constituted the large majority, presented a major problem, and we are especially grateful to Dr E. W. Fager for aid in its solution. Details of his computer analysis are given in a separate paper (Fager, 1969); here we discuss only our treatment of his 19 groups.

Our objective was to sort the organisms of our collection into named species or subspecies. Since we have still insufficient information on the ranges of various characters within our proposed taxa, we considered designating similar strains merely by a code number, rather than a formal binomial, to indicate the provisional nature of our taxa; but we rejected this device since (a) it would not conform with the currently accepted International Code of Bacteriological Nomenclature, and (b) numbers are less convenient for the human memory than are names. We therefore decided to take a more orthodox taxonomic approach, since we feel that for the gliding bacteria, most of which have at present no formal identity at all, even provisional names are better than none. We are not attempting to impose an immutable nomenclature on these organisms, fully recognizing that the validity of any taxon lasts only as long as it is useful and until something better is proposed. But a start must be made somewhere. We have tried to arrive at a workable classification. Similar strains have been grouped, the groups have been called species, and the species have been assigned names for mnemonic and logistic convenience. Likewise, similar species have been grouped, and each resulting assemblage has been called a genus (this seems the only reasonable definition of a genus), and the genera have been assigned names for the same reasons of convenience.

We have tried to be conservative in the creation of new taxa. Whenever possible we have tried to select existing generic names, with a minimum violation of their original definitions, as indicated in an earlier section of this paper. Thus we have assigned almost all of our isolates to one of the four established genera *Cytophaga*, *Flexibacter*, *Microscilla* and *Saprospira*, though in each case it has been found necessary to modify the original descriptions of these genera. At the specific level, we have delimited as species only those in which distinctions were clearly indicated on the basis of such stable characters as GC value, pigment type, experimentally established requirements for particular growth factors, etc. In several cases we have combined more than one of Fager's groups into single species, which we have then subdivided into two or more varieties. Future research or other considerations may indicate the advisability of either submerging these varieties, or of raising them to specific rank.

Proposed classification

Fager (1969) sorted our strains into groups which for the most part seem acceptable to us as useful taxonomic units; and we therefore regard most of them as equivalent to species or varieties, after making certain changes. Each of Fager's groups is discussed in turn below, with proposed changes by which we have tried to make them conform as genera and species in the customary sense. The specific names which we have assigned to the final specific groupings, after the modifications have been made, are formally presented in Table 1, in which their essential distinguishing features are summarized in tabular form.

We have somewhat rearranged the 85 strains, which by Fager's analysis fell into 19 groups, into 24 species (comprising 31 varieties) as follows:

Group 1 (19 strains of *S. grandis*) has been accepted unchanged. An associated member of this group, strain A-1, is recognized as a separate species, *S. tovisformis* (Lewin & Mandel, 1969).

Group 2 (N-3, HJ-1, NN-3, DD-1, DUB-4, B-1, O-2, LIM-1) has been considerably modified in our re-evaluation.

Strain HJ-1 has been removed from this group and recognized as a separate species, since it has a GC value (32.5%) lower than that of other group 2 members (35 to 42%) and, unlike them, cannot digest agar or gelatin. We propose for it the new name *Microscilla arenaria*.

Two strains (GOL-12 and Y-1), constituting Fager's group 14, and two others, JL-4 from the group 15 and its associate ST-1, have been included. These changes are justified on the following bases: affinities, as expressed by computer, GC values, pigmentation, agar digestion and other enzymic activities. The resulting 11 strains have then been divided into 2 varieties.

One (DUB-4, JL-4 and ST-1), with a GC value of 35 to 37%, unites the only agar-digesters which tolerate lauryl sulphate; for these we propose the new varietal name *Cytophaga diffluens* var. *aprica*.

The remaining eight strains differ in these features, but are very similar among themselves. Since they digest CMC, we consider that they may be assigned to the species *Cytophaga diffluens* Stanier emend., although we could not demonstrate the digestion of cellulose (cigarette paper) by any of these strains. (*Cytophaga diffluens* Stanier was originally cellulolytic; Stanier, 1942.) Our strains evolve hydrogen sulphide. According to Breed *et al.* (1957), *Cytophaga diffluens* Stanier does not. However, I find no reference to this feature in the original descriptions of this species (Stanier, 1940, 1942).

Groups 3 (QQ-1, QQ-3, Q-3, QQ-11, JL-13, NN-13). Strain QQ-3, which under certain conditions forms sheathed and sometimes branching filaments, has been removed and assigned to a new genus and species, *Flexithrix dorotheae* (Lewin, 1969a).

The remaining five strains form a very homogenous group, for which we propose the new specific name *Microscilla aggregans*.

The single strain HI-3, an associate member of group 3, is regarded as a separate variety, since it has complex nitrogen requirements and is catalase positive. For this latter reason we propose to call it *Microscilla aggregans* var. *catalatica*.

Group 4 (CR-103, CR-104, CR-124, CR-141, CR-155). All of these five strains can grow in freshwater media. The first three form pigment of type II and have a GC value of

32 %, whereas strains CR-141 and CR-155 are characterized by pigment of type I and GC values of 35.5 and 38 % respectively. Strains CR-141 and CR-155 tolerate sea water or even double-strength sea water, whereas the first three strains listed in this group

Table 1. *Conspectus of the major genera and species of flexibacteria, with their chief diagnostic characteristics*

The species or subspecies are listed in order of increasing GC value.

Genus and species	Number of strains	Molar % GC in DNA	Helical	Branched	Sheathed	Length in μm .	Pigment type	Marine/freshwater	Digests CMC	Digests starch	Digests agar	Digests alginate	Digests gelatine	Catalase	H ₂ S evolved
<i>Cytophaga</i>															
<i>C. latercula</i>	1	34	—	—	—	5	6	M	+	—	+	+	+	—	+
<i>C. lytica</i>	5	33.5–34.5	—	—	—	10–20	4	M	+	+	+	+	+	V	V
<i>C. diffluens</i> (?)	1	35 ⁶	—	—	—	20	3	M	+	+	+	+	+	—	+
<i>C. diffluens</i> var. <i>aprica</i>	3	35.5–37	—	—	—	10–50	3	M	+	+	+	+	+	—	+
<i>C. diffluens</i> var. <i>carnea</i>	1	38	—	—	—	30	3	M	+	—	+	+	+	—	—
<i>C. diffluens</i>	7	40–43	—	—	—	5–30	3	M	+	+	+	+	+	—	V
<i>Flexibacter</i>															
<i>F. litoralis</i>	1	30	—	—	—	50	1	M	—	—	—	—	+	—	+
<i>F. aurantiacus</i> ¹	2	31	—	—	—	5–20	5	F	+	V	—	—	+	+	—
<i>F. aurantiacus</i> var. <i>copepodarum</i>	1	31	—	—	—	5	4	M	—	—	—	—	+	—	—
<i>F. giganteus</i> ²	3	31	—	—	—	50	2	F	—	+	—	—	+	—	V
<i>F. aurantiacus</i> var. <i>excathedrus</i>	1	34	—	—	—	10	4	F	—	+	—	—	+	—	—
<i>F. roseolus</i>	2	34.5–38	—	—	—	50	1	F	—	—	—	—	+	—	—
<i>F. ruber</i>	1	36	—	—	—	50	1	F	—	+	—	—	+	—	—
<i>F. flexilis</i> var. <i>pelliculosus</i>	1	39	—	—	—	10–20	3	F	+	+	—	—	+	—	+
<i>F. flexilis</i>	4	40.5–43	—	—	—	10–50	3	F	—	V	—	—	+	—	+
<i>F. flexilis</i> var. <i>iolanthae</i>	1	41	—	—	—	10–30	3	F	—	—	—	—	+	—	—
<i>F. sancti</i>	3	46–47	—	—	—	5–50	4	F	+	V	—	—	+	—	V
<i>F. elegans</i>	1	47	—	—	—	50	3	F	—	—	—	—	+	—	—
<i>Flexithrix</i>															
<i>F. dorotheae</i> ³	1	37	—	+	+		4	M	+	—	—	—	—	—	—
<i>Herpetosiphon</i>															
<i>H. cohaerens</i>	1	44	—	—	+	60–150	3	M	—	—	—	—	+	—	—
<i>H. geysericolus</i> ⁸	1	48	—	—	+	10–150	3	F	+	+	—	—	+	+	—
<i>H. persicus</i>	1	52.5	—	—	+	30–150	3	M	—	—	—	—	+	—	—
<i>H. nigricans</i>	1	53	—	—	+	30–50	4	M	—	—	—	—	+	—	—
<i>Microscilla</i>															
<i>M. arenaria</i>	1	32	—	—	—	20	3	M	+	+	—	+	—	—	+
<i>M. aggregans</i> var. <i>catalatica</i>	1	34	—	—	—	20	4	M	—	—	—	—	—	+	+
<i>M. aggregans</i> ⁴	5	36.5–41.5	—	—	—	30	4	M	V	—	—	—	—	—	—
<i>M. tractuosa</i>	8	34.5–39.5	—	—	—	10–50	3	V	—	V	—	—	V	—	V
<i>M. sericea</i>	2	38–39	—	—	—	30–100	3	M	—	+	—	+	—	—	—
<i>M. marina</i>	1	42	—	—	—	50	3	M	—	—	—	—	+	—	—
<i>M. furvescens</i>	1	44	—	—	—	5–100	3	M	+	+	—	+	+	—	+
<i>Saprospira</i>															
<i>S. thermalis</i> ⁵	3	34.5–37.2	+	—	—	50	2	F	—	+	—	—	+	—	+
<i>S. toviformis</i>	1	38	+	—	—	10–50	3 ⁷	M	+	—	—	—	+	—	—
<i>S. albida</i> ⁸	3	41.5–46.5	—	—	—	50	3	F	V	—	—	—	+	—	—
<i>S. grandis</i>	19	46–48	+	—	—	50	3	M	—	—	—	—	+	—	—
<i>S. flammula</i> ⁸	1	48	+	—	—	30	3	F/M	—	—	—	+	—	+	—

For footnotes see foot of facing page

will not grow even in half-strength sea water. These two subgroups also differ in their amino-acid requirements.

The characters of CR-103, CR-104 and CR-124 agree with those of *Flexibacter giganteus* Soriano.

CR-141 and CR-155 are separated from those above, and we propose that they represent a new species, *Flexibacter roseolus*.

We have regarded two associate members of this group, SIO-4 and GEY, as two separate species, on the basis of pigment and other features. For strain SIO-4 we propose the new specific name *Flexibacter litoralis*, mentioned provisionally by Fox & Lewin (1963).

We propose GEY as the type strain of the new species *Flexibacter ruber*, also mentioned provisionally by Fox & Lewin (1963), erroneously as '*F. rubrum*'.

Group 5 (T-13, EE-13, EG-13, H-43, JK-11) has been accepted essentially unchanged; but we consider it convenient to combine with it Fager's group 13 and its associate (i.e. GH-1, GH-2 and HI-15). For all of these strains we propose the new specific name *Microscilla tractuosa*.

Group 6 (CR-63, CR-81, A-52, WAR-5) has been accepted unchanged; its characters agree with those of *Flexibacter flexilis* Soriano.

Group 7 (B-9, BON, WFB-21, ENS). Strain LIM-21 from group 15 has been added, since it is clearly similar in most respects. For strains of this group we propose the new specific name *Cytophaga lytica*.

Group 8 (BA-3, BA-23, FLE, MIC). Strain FLE has been removed and established as a separate variety, which we propose to call *Flexibacter flexilis* var. *pelliculosus*. It is distinguished by its orange pigment (type III) and GC value, 39.5%. The others are yellow (pigment type IV) and have a GC value of 47 to 48%.

For the remaining three strains we propose the new specific name *Flexibacter sancti*. In most respects these strains exhibit characteristics similar to those described for *Cytophaga johnsonae* (Stanier, 1947); but since none of them could be shown to digest chitin we have decided not to identify them with the latter species.

Group 9 (BEG, CR-123, CR-125) has been accepted unchanged. BEG has already been established as the type strain of *Saprospira thermalis* Lewin (1965b).

Group 10 (DWO, PSY) has been accepted unchanged. Although sent to us under different names, (*Cytophaga aurantiaca* and *Cytophaga psychrophila*), these two strains differ in no major respect as far as our experience goes, and we therefore consider

Footnote to Table 1

¹ Supplied as *Cytophaga aurantiaca* and *C. psychrophila*, although in our hands neither strain digests cellulose.

² Cf. *Saprospira thermalis*.

³ *Flexithrix dorotheae*, cf. *M. aggregans*.

⁴ Cf. *Flexithrix dorotheae*.

⁵ Cf. *F. giganteus*.

⁶ GC value lower than that of other strains of this species.

⁷ The intact, living cells of A-1 are yellow, whereas those of all *S. grandis* strains are peach coloured. The absorption curve of the pigment extracted from A-1 does not exactly correspond with that of saproxanthin (Aasen & Jensen, 1966; Lewin & Mandel, 1969).

⁸ Not in Fager's analysis.

+ Indicates that all strains tested reacted positively.

— Indicates that all strains tested reacted negatively.

V Indicates variability of reaction among the strains tested.

them conspecific. We note that the latter name is listed by Buchanan, Holt & Lessel (1966) as illegitimate. In our tests neither strain digested cellulose, though they did digest CMC. For this species we propose the new combination *Flexibacter aurantiacus*. This species may be identical or close to an organism described as *Sphaeromyxa xanthochlora* by Bauer (1962).

Group 11 (T-3, II-2), with the addition of its associate ss-2, has been accepted otherwise unchanged. These forms, being sheathed, have been set apart in the genus *Herpetosiphon* (Holt & Lewin, 1968). (The type species of this genus, *H. aurantiacus*, was obtained too late for inclusion in this survey.) We propose to distinguish them as three separate species: *H. cohaerens* (II-2), *H. nigricans* (ss-2) and *H. persicus* (T-3) (Lewin, 1969*b*.)

Group 12 (SIO-7, SIO-9) has been accepted unchanged. We propose for these strains the new specific name *Microscilla sericea*.

Group 13 (GH-2, HI-15), with its associate GH-1, has been combined with group 5 under the new specific name *Microscilla tractuosa* (see above).

Group 14 (GOL-12, Y-1) has been combined with group 2, as explained above, in the species *Cytophaga diffluens* Stanier emend.

Its associate member, Q-1, is regarded as sole representative of a separate variety distinguished in part by its inability to digest starch or to liquefy agar. We propose to call it *Cytophaga diffluens* var. *carnea*.

Group 15 (LIM-21, JL-4) and its associate ST-1 have been distributed between *Cytophaga diffluens* (group 2) and *C. lytica* (group 7).

Group 16 (COP, CR-134) has been separated into two varieties, differing distinctly in their salt tolerances; one is marine, the other is from a freshwater habitat. We propose to call them, respectively, *Flexibacter aurantiacus* var. *copepodarus* and *Flexibacter aurantiacus* var. *excathedrus*.

An associate of this group, strain SIO-1, which digests CMC, agar and alginate, is regarded as sufficiently distinct to warrant its separation and description under a new specific name, *Cytophaga latercula*.

Group 17 (BA-24, NZ-1) has been divided into two separate species on the basis of their considerably different GC values, respectively 41 and 47.5%. For NZ-1, it seems appropriate to use the name *Flexibacter elegans* Soriano. For strain BA-24 we propose the new varietal name *Flexibacter flexilis* var. *iolanthae*.

Group 18 (SIO-8) has been accepted unchanged and assigned to the species *Microscilla marina* Pringsheim (1951), the type species for this genus (Though now lost, the strain which Fox & Lewin (1963) referred to as *Flexibacter marinum* nov. comb. was essentially identical with this species.)

Group 19 (TV-2) has been accepted unchanged, though we had less information on this strain than on any other at the time of Fager's computer analysis. We are naming it *Microscilla furvescens*.

Similarly, we have accepted Fager's seven main assemblages (FA-FG) as bases for genera, again subject to certain changes as explained below:

1. We have combined all helical forms in the genus *Saprospira*, comprising *S. grandis* (assemblage FD, group 1) *S. toviformis* (assemblage FD, associate of group 1) *S. thermalis* (assemblage FA, group 9) and *S. albida*.

2. We have created a separate genus, *Flexithrix* (Lewin, 1969*a*), for branched, sheathed forms, represented here by the single strain QQ-3, which therefore has been

taken out of assemblage FB, group 3. We recognize that this is a matter of convenience, which obscures close relationships between *Flexithrix dorotheae* and some of the yellow, rod-like forms of assemblage FB.

3. We have adopted another separate genus, *Herpetosiphon* (Holt & Lewin, 1968), for unbranched, sheathed forms; and we have, therefore, removed group 11, comprising strains T-3, II-2 and the associate SS-2, from assemblage FB. The new species are described by Lewin (1969*b*.)

4. Assemblage FA has been accepted almost unchanged as the basis for a genus, comprising almost all freshwater species studied in this survey. (We have excluded only group 9, for reasons given in paragraph 1 above, and strain SIO-1, which by reason of its various carbohydrase activities is better assigned to the genus *Cytophaga*.) For most members of this assemblage we propose to use the generic name *Flexibacter* (Soriano, 1945), as redescribed above.

In separating organisms of group 9 from the rest of assemblage FA, we recognize that, on the basis of a single structural feature, helicity, we are separating *Saprospira thermalis* from organisms with which it shares a number of common physiological and biochemical features, and associating it with those of assemblage FD (chiefly *S. grandis*) with which it has fewer common characteristics. This, nevertheless, seems to us to be justifiable, as we have already explained, because it is generally convenient to distinguish genera by readily recognizable features.

5. Assemblage FB has been accepted almost unchanged as the basis for a genus, comprising marine species which do not digest agar and digest few or no other polysaccharides. We have excluded strain QQ-3 (from group 3) and strains T-3, II-2, and SS-2 (i.e. group 11 and its associate) for reasons given in paragraphs 2 and 3 above. We propose to use the generic name *Microscilla* (Pringsheim, 1951), as redescribed above. (The type species, *M. marina*, is not in this assemblage; however, see 7 below.)

In dissociating organisms of group 11 from the rest of assemblage FB, we recognize that on the basis of a single structural feature, sheaths, we are separating *Herpetosiphon* spp. from organisms which they resemble in several physiological and biochemical features. This, too, seems justifiable, because the presence of a sheath is a readily recognizable feature.

6. Assemblage FC has been accepted unchanged as the basis for a genus, comprising marine species which digest cellulose or CMC, agar and alginate. We propose to use for these forms the generic name *Cytophaga* as redescribed above.

7. In the interest of nomenclatural economy, the three smallest groups, which by Fager's analyses appear unrelated to any of his four major assemblages, have nevertheless been assigned, as separate species, to the nearest appropriate genus of those defined above, which for each of these strains seems to be *Microscilla*. Strains in group 12 (SIO-7, SIO-9) have been named *M. sericea* nov. sp.; group 18 (SIO-8) has been identified with *M. marina* Pringsheim (the type species); and group 19 (TV-2) has been designated by the new name *M. furvescens*.

All of the above changes are summarized schematically in Fig. 1, for comparison with Fig. 1 in the paper by Fager (1969).

Diagnostic keys

Having allocated the organisms to such specific groupings, we then wished to make our classification useful and usable by other workers handling different but possibly

some, such as pigmentation of packed living cells or filaments, not of medium or extract, halophily, and starch digestion are similar to those used by Stanier (1957) in his key to *Cytophaga* (*sensu lato*).

The use of 'marine', in the following keys, may require some justification. In our experience, almost all flexibacteria from marine sources grow in media based on sea water or a saline equivalent, but not in freshwater media; whereas those strains which we isolated from freshwater sources did not tolerate sea-water media. Origin is, therefore, a good indication of the osmotic or ionic tolerance range of a strain (*cf.* MacLeod, 1965).

Provisional diagnostic key to the genera of flexibacteria which we examined

1 a At least partly sheathed under some conditions		2
1 b Sheaths never present; all cells capable of gliding		3
2 a Filaments with false branches	<i>Flexithrix</i>	
2 b Filaments unbranched	<i>Herpetosiphon</i>	H. 1
3 a More or less regularly helical	<i>Saprospira</i>	S. 1
3 b Not regularly helical		4
4 a Digests cellulose or CMC, agar and alginate	<i>Cytophaga</i>	C. 1
4 b Does not digest these carbohydrates		5
5 a Marine (key includes also 2 <i>Flexibacter</i> species and one <i>Cytophaga</i> species)	<i>Microscilla</i>	M. 1
5 b Freshwater or from soil (key includes also 3 marine species)	<i>Flexibacter</i>	F. 1

Provisional diagnostic key to the species of Herpetosiphon

H. 1 a Freshwater or from soil		H. 2
H. 1 b Marine		H. 3
H. 2 a From soil	<i>H. aurantiacus</i> †	
H. 2 b From hot spring	<i>H. geysericolus</i> *	
H. 3 a Darkens Tryptone agar	<i>H. nigricans</i> *	
H. 3 b Does not darken Tryptone agar		H. 4
H. 4 a Colonies on agar coherent	<i>H. cohaerens</i> *	
H. 4 b Colonies on agar not coherent	<i>H. persicus</i> *	

† Holt & Lewin, 1968.

* Lewin, 1969b.

Provisional diagnostic key to the species of Saprospira which we examined

S. 1 a Marine		S. 2
S. 1 b Freshwater or from soil		S. 3
S. 2 a Peach-coloured; gliding about 1 μ m. sec.	<i>S. grandis</i>	
S. 2 b Yellow; gliding about 0.2 μ m./sec. or imperceptibly	<i>S. tovisformis</i> §	
S. 3 a Bright orange	<i>S. flammula</i>	
S. 3 b Pink		S. 4
S. 4 a Abundant growth in synthetic media containing leu isoleu val. as sole essential amino acids	<i>S. thermalis</i> †	
S. 4 b Slow and slight growth even in complex nutrient media	<i>S. albida</i> †	

§ Lewin & Mandel, 1969.

† Lewin, 1965a.

Provisional diagnostic key to the species of Cytophaga which we examined

C. 1 a Red	<i>C. latercula</i>	
C. 1 b Yellow or orange		C. 2
C. 2 a Yellow; softens agar; catalase +	<i>C. lytica</i>	
C. 2 b Orange; catalase -		C. 3
C. 3 a Pale orange; softens agar	<i>C. diffluens</i> var. <i>carnea</i>	
C. 3 b Bright orange; liquefies agar		C. 4
C. 4 a Grows on 0.01 % sodium lauryl sulphate	<i>C. diffluens</i> var. <i>aprica</i>	
C. 4 b Does not grow on 0.01 % sodium lauryl sulphate	<i>C. diffluens</i>	

Provisional diagnostic key to the species of Microscilla (plus 3 other species from marine sources) which we examined

M. 1a Digests alginate		M. 2
M. 1b Does not digest alginate		M. 4
M. 2a Red (digests agar)	<i>C. latercula</i>	
M. 2b Orange		M. 3
M. 3a Digests CMC	<i>M. arenaria</i>	
M. 3b Does not digest CMC	<i>M. sericea</i>	
M. 4a Yellow		M. 5
M. 4b Orange, red or pink		M. 7
M. 5a Catalase+	<i>M. aggregans</i> var. <i>catalatica</i>	
M. 5b Catalase—		M. 6
M. 6a On 5 g. tyrosine/l., growth with 'clearing'	<i>F. aurantiacus</i> var. <i>copepodarum</i>	
M. 6b On 5 g. tyrosine/l., growth without 'clearing'	<i>M. aggregans</i>	
M. 7a On 5 g. tyrosine/l., growth without discoloration or 'clearing'	<i>M. tractuosa</i>	
M. 7b On 5 g. tyrosine/l., growth with ring and/or 'clearing'		M. 8
M. 8a On 5 g. tyrosine/l., growth with red ring and 'clearing'		M. 9
M. 8b Growth with grey ring	<i>M. furvescens</i>	
M. 9a Pink	<i>F. litoralis</i>	
M. 9b Orange	<i>M. marina</i>	

Provisional diagnostic key to the species of Flexibacter (including also Cytophaga latercula) which we examined

F. 1a Yellow		F. 2
F. 1b Pink, orange or red		F. 5
F. 2a Catalase+	<i>F. aurantiacus</i>	
F. 2b Catalase—		F. 3
F. 3a Digests CMC	<i>F. sancti</i>	
F. 3b Does not digest CMC		F. 4
F. 4a Marine	<i>F. aurantiacus</i> var. <i>copepodarus</i>	
F. 4b Freshwater or from soil	<i>F. aurantiacus</i> var. <i>excathedrus</i>	
F. 5a Digests CMC		F. 6
F. 5b Does not digest CMC		F. 7
F. 6a Marine	<i>C. latercula</i>	
F. 6b Freshwater or from soil	<i>F. flexilis</i> var. <i>pelliculosus</i>	
F. 7a Mucilaginous		F. 8
F. 7b Not mucilaginous		F. 9
F. 8a Marine	<i>F. litoralis</i>	
F. 8b Freshwater or from soil	<i>F. flexilis</i>	
F. 9a Can also grow on SW media		F. 10
F. 9b Cannot grow on SW media		F. 11
F. 10a Red	<i>F. roseolus</i>	
F. 10b Orange	<i>F. elegans</i>	
F. 11a Red	<i>F. ruber</i>	
F. 11b Pink or orange		F. 12
F. 12a Pink	<i>F. giganteus</i>	
F. 12b Peach or orange	<i>F. flexilis</i> var. <i>iolanthae</i>	

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