STUDIES ON THE CYTOPHAGAS

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INTRODUCTION

The morphology and taxonomy of the cytophagas have been the subject of much controversy during the last decade. The difficulties of these problems may be judged from the fact that these organisms have been placed in no less than three orders by different workers; the *Spirochaetales* (Hutchinson and Clayton), the *Actinomycetales* (Bokor) and the *Myxobacteriales* (Krzemieniewska).

Van Iterson (1904), the first to demonstrate the aerobic decomposition of cellulose by bacteria, undoubtedly obtained representatives of the cytophagas in his enrichment cultures. In addition to *Bacillus ferrugineus*, which he considered to be the active organism in cellulose decomposition, a large coccus often occurred. This is figured in his paper as covering the cellulose fibers in a manner highly characteristic of the microcysts of *Cytophaga myxococcoides*. Van Iterson's attempts to isolate the different organisms found in enrichment cultures were unsuccessful, and as a result he failed to appreciate the true importance of the "coccus," regarding it as favoring, but not playing an active role in, cellulose decomposition.

In 1919, Hutchinson and Clayton isolated from soil an aerobic cellulose decomposer, characterized by its strict adaptation to cellulose (no other energy source could be used) and by its peculiar morphology. Two morphological types always occurred in their cultures; a long, thin, flexible, pointed rod and a large coccus. In young cultures the rod form predominated, while in older cultures it was replaced by the coccus. All attempts at separating these two forms having failed, Hutchinson and Clayton concluded that they were stages in the developmental cycle of a single species, a contention which was supported by the occurrence of cells which could be regarded as transitional types. The organism was placed in the genus *Spirochaeta* with the comment that its peculiar developmental cycle made it impossible to regard it as a representative of the true bacteria, and that some of its characteristics seemed to favor a closer relationship with the spirochaetes. The authors emphasized that:

> . . . the organism under consideration exhibits a number of features which have not hitherto been observed in the spirochaetes, features which however appear to indicate a more complex development than that of the true bacteria.

To the coccus form they gave the designation of "sporoid."

Winogradsky (1929), in an extensive investigation of aerobic cellulose decomposition in the soil, isolated and studied a number of organisms which resembled morphologically the vegetative cells of *Spirochaeta cytophaga*. In certain of his crude cultures Winogradsky observed coccus forms but in others, which were otherwise apparently identical, they were not present. Consequently, Winogradsky regarded the cocci as contaminants and always chose for further work strains in which they did not appear. All the organisms studied by Winogradsky were physiologically similar to *Spirochaeta cytophaga*, being strictly adapted to cellulose and preferring nitrates or ammonia as a source of nitrogen. To the whole group he gave the generic name *Cytophaga*, rightly concluding that their morphology did not justify their inclusion in the genus *Spirochaeta*. On the basis of pigmentation and cell size five species were differentiated, of which the type species, *Cytophaga hutchinsoni*, was considered to be identical with *Spirochaeta cytophaga*.

Bokor (1930), claimed to have isolated a pure culture of *Spirochaeta cytophaga* on which he made morphological studies. As a result of these, he considered its cycle of development to be similar to that of the *Actinomycetes*, with which he placed it under the new name of *Mycococcus cytophagus*. The disparity between

Bokor's findings and those of all other workers in the field, as well as the illustrations in his paper, make it apparent that he was dealing with a mixed culture of *Spirochaeta cytophaga* and an *Actinomyces*.

In 1930 and 1933, Krzemieniewska subjected the previous work on the morphology of the cytophagas to a critical examination. She was able to show that Hutchinson and Clayton's *Spirochaeta cytophaga* and the organism described by Winogradsky as *Cytophaga hutchinsoni* were not identical, although very similar in vegetative morphology and pigmentation. In *Spirochaeta cytophaga* the coccus actually represented a stage in the developmental cycle, as had been claimed by Hutchinson and Clayton, whereas in *Cytophaga hutchinsoni* no such stage occurred. Her studies were facilitated by the discovery that the organisms could be grown on cellophane, and using this as a substrate she followed in detail the developmental cycle of *Spirochaeta cytophaga*, demonstrating beyond doubt the transition from rod to coccus and the subsequent germination of the coccus with the formation once more of the characteristic rod form. The whole process showed a remarkable similarity to the development of the myxobacteria belonging to the genus *Myxococcus*. In view of the lack of such a cycle in other cytophagas, Krzemieniewska was reluctant to place the whole group without reservation in the *Myxobacteriales*, and merely drew attention to the evidence in favor of a close relationship (1930):

> Les recherches sur les espèces désignées par le nom de *Cytophaga*, sans aucun doute très proches à *Sp. cytophaga*, ne peuvent pas être considérés comme finis, il est donc possible qu'après quelque temps on pourra placer tout le groupe *Cytophaga*, y compris *Sp. cytophaga*, à côté des *Myxobacteriaceae* ou bien le compter parmi elles.

To the sporoids she gave the designation of microcysts, thus emphasizing their difference from the endospores of the *Eubacteriales*. *Spirochaeta cytophaga* she renamed *Cytophaga myxococcoides*.

In 1934, Stapp and Bortels described a number of new representatives of the genus which differed in pigmentation and temperature optimum from the previously known species. One of these, *Cytophaga globulosa,* had a developmental cycle similar to that of *C. myxococcoides,* while the others did not form microcysts. It is possible that at least some of these forms are identical with previously described species.

An important discovery made by Stapp and Bortels was the existence of motility in the cytophagas. Although *a priori* this had seemed likely in view of the extreme regularity with which the organisms invested cellulose fibers, it had not been previously shown. It is true that Hutchinson and Clayton had observed rotatory and flexing movements in hanging drops, but Winogradsky could not confirm this. Winogradsky was also unable to demonstrate flagella, and suggested that movement might be brought about by very small haustoria. Stapp and Bortels observed a slow creeping movement in the direction of the long axis of the cell, quite different from the flagellar motility of the *Eubacteriales,* but were unable to substantiate Winogradsky's suggestion of the existence of haustoria. In addition to this they noticed flexing movements ("Krümmungsbewegungen"), but considered them to be pathological in nature.

In concluding this review, it is perhaps as well to point out some of the difficulties which have made work with the cytophagas so confused and contradictory. Due to their extreme physiological specificity, it is impossible to grow them in the absence of cellulose, which makes it extremely difficult to obtain pure cultures. Hutchinson and Clayton claim to have succeeded in this by the arduous and uncertain dilution method, but neither Winogradsky nor Krzemieniewska, the two principal workers in this field, had any of their forms in pure culture. Bokor's claims may be considered invalidated by his morphological findings. Stapp and Bortels were likewise unsuccessful except with their microcyst-forming species, *Cytophaga globulosa.* Their contention that the non-microcyst-forming species cannot be grown except in symbiosis with other forms has so far not been confirmed. A further obstacle, of particular gravity where morphological work is concerned, is the impossibility of growing the organisms in the absence of cellulose. This was partially obviated by Krzemieniewska's discovery that cellophane could be used as a substrate, but even on this medium, observation of the organisms in the living state is not easy, due to the fineness of the cells.

THE DISCOVERY OF TWO NEW CYTOPHAGAS

The above-mentioned difficulties do not exist in the case of some morphologically closely related bacteria which are, however, physiologically less specialized. They were encountered during the course of an investigation on marine agar-decomposing bacteria, and were easily obtained in pure culture. Two species have been differentiated: *Cytophaga krzemieniewskii*, n. sp.[1] and *Cytophaga diffluens* n. sp. Since the primary purpose of this paper is to draw attention to the morphology and type of motility of these forms, only their salient physiological and cultural characteristics will be mentioned here. Complete descriptions will be published later.

Both are strictly aerobic and grow well on a wide variety of media. Peptone and yeast extract are the only suitable nitrogen sources found, the organisms being unable to grow with inorganic nitrogen salts or any of a number of amino-acids in the presence of a suitable source of carbon and energy. Agar, cellulose, and some simpler carbohydrates, are decomposed, but starch and chitin are not attacked. Both strains liquefy gelatin and reduce nitrates, but do not produce indole or H_2S. Both are weakly catalase-positive. Growth on solid media is characteristically rapidly spreading; isolated colonies can never be obtained, the whole plate being covered with a thin layer of organisms in 2 to 3 days. *Cytophaga diffluens* produces a salmon pink pigment which slowly becomes orange on most media; *Cytophaga krzemieniewskii* produces a very pale pink pigment which is later masked by the production of a brown or black pigment.

The remarkable morphology of these organisms attracted attention from their first isolation. Both are large, gram-negative, non-sporeforming rods, occurring always in a variety of forms;

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[1] Named after Mme. H. Krzemieniewska, whose work on the soil cytophagas first indicated their true systematic position.

straight, arcuate, U-shaped, S-shaped, and even sometimes looped around into a full circle.

In young cultures, the cells of *C. diffluens* are 4 to 10 μ long with an average of about 7 μ, and 0.5 to 1.0 μ wide at the center. When examined in the living state, the ends are slightly pointed and the whole cell appears spindle-shaped, but in stained preparations these characters tend to disappear. As cultures become older the morphology changes; long threads twisted into bizarre shapes and often attaining a length of 20 to 30 μ occur. Star-shaped aggregates of cells are often formed in liquid media; this appears to be a process similar to the "Sternbildung" described by Stapp and Bortels for *C. globulosa.* No evidence of the production of spores or other resting stages has been found.

The cells of *C. krzemieniewskii* in young cultures are somewhat larger than those of *C. diffluens;* the length varies from 5 to 20 μ with an average of about 12 μ, and the width from 0.5 to 1.5 μ. Markedly spindle-shaped, pointed cells are rare; even in unstained preparations the majority are of more or less even thickness with slightly rounded ends. Star-shaped aggregates are commonly produced in liquid media. In older cultures the cells become swollen and refractile, but thread forms are never seen. Spores or other resting stages have never been found.

It is clear from the above account of the morphology of these two forms that they do not belong to any of the more common groups of rod-shaped bacteria. The only known simple rod-shaped bacteria which display these characteristics of extreme flexibility and spindle-shaped pointed cells are the cytophagas. Consequently, we were led to consider these two forms as representatives of this genus in spite of the fact that physiologically they differ from the previously known species.

MOTILITY OF THE CYTOPHAGAS

At first, all attempts to demonstrate motility failed. When the organisms were examined in wet mounts or hanging drops, no positive evidence of it could be observed, although occasionally rotatory movements or slight displacements of the cells were noted. Although the organisms are strict aerobes, aerotaxis never occurred. Attempts to demonstrate flagella with *C. diffluens* failed, but in view of the difficulty of making satisfactory stained preparations of marine bacteria, this could not be regarded as conclusive.. On the other hand, the extreme rapidity with which the organisms spread on solid media, only to be compared to the swarming of *Proteus vulgaris*, pointed to the existence of some method of locomotion. Since it seemed possible that movement might be restricted to a solid surface, another method of examination was adopted. A loopful of seawater-peptone-agar was streaked on a coverslip, allowed to solidify, and inoculated in several places with small masses of bacteria from the tip of a needle. The coverslip was then placed in position as the lid of a moist chamber. After 15 to 60 minutes it was observed that bacteria had concentrated in small, pointed swellings at certain points along the peripheries of the "microcolonies" (fig. 1*a*). These presently began to move out across the agar (pl. 1, fig. 1). The moving masses, consisting of from 25 to more than a thousand cells, travel, each as a body, the majority of the individual cells being strictly oriented with their long axis in the direction of the movement. In the early stages, the mass is quite sharply pointed, a few cells at the front end acting as a spearhead. However, as movement continues, the shape may change in various ways. The head of the mass often loops back to join the main body of moving cells, forming a blunt, finger-shaped projection (fig. 1, *a–f*). Sometimes the tip turns around and rejoins the main colony, leaving a sterile island in the agar

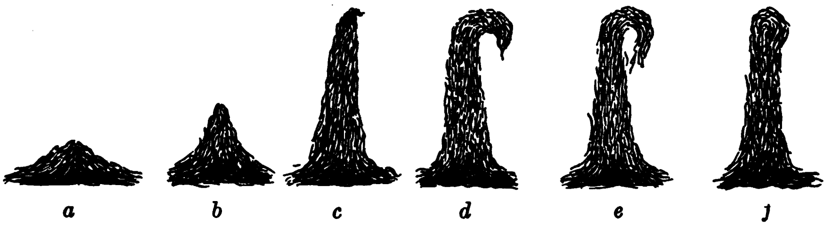

FIG. 1. CYTOPHAGA KRZEMIENIEWSKII. A TYPICAL MOVING MASS OF CELLS
a, concentration of cells at the periphery of the microcolony. *b–f*, subsequent stages in the formation and movement of the migratory mass. ×200.

surrounded by bacteria. More often, the migratory masses will meet, fuse, branch, and produce further outgrowths, ultimately leading to the formation of irregularly spaced fields of bacteria such as are shown in plate 1, figure 2.

The type of movement is completely different from the flagellar motility of the *Eubacteriales*. It consists of a slow, even gliding in one direction with none of the side-to-side movements or rapid directional changes associated with the motility of flagellated organisms. The rate of movement varies considerably, and is conditioned by at present unexplainable factors. Groups of cells in vigorous movement will suddenly slow up and stop, while others, previously immotile, will as suddenly start creeping across the agar. The movement of *C. diffluens* is in general much less rapid than that of *C. krzemieniewskii*. For the latter species, speeds of as much as 15 μ a minute have been noted, but the average is probably not much more than 5–6 μ a minute.

Particularly among moving cells, rapid flexing movements ("Krümmungsbewegungen") can be observed. These are sometimes caused by collision with some visible obstruction (such as a neighboring non-motile cell), but in other cases they seem to be purely spontaneous. They are evidently not causally related to the forward movement, since only a small minority of the cells in a moving mass exhibit them. As they occur in young, healthy cells under normal conditions of growth, it is unlikely that they are pathological in nature as was suggested by Stapp and Bortels (1934).

An interesting point is the apparent coordination between the different cells in a moving mass. While no physical connection between the cells is evident, the direction and speed of movement of a few cells at the apex control the activities of a large number of following cells. This is most clearly shown where an outgrowth consisting of perhaps a hundred cells has broken away from the microcolony and continued its movement across the agar in perfect order and coherence and at an undiminished rate. Single, isolated cells are very rarely motile, although occasionally they can be found separated by a distance of 10 μ or so from the nearest mass in a position which implies that independent movement must have occurred. The unit of effective movement seems to consist of 20 to 30 cells.

SYSTEMATIC POSITION AND TAXONOMY

As already mentioned, the morphological similarities between the two species described here and the soil cytophagas are such that, in spite of the physiological differences which exist between them, we have been led to the conclusion that they are closely related. The demonstration of creeping motility and flexing movements in *C. globulosa*, a typical member of the soil group (Stapp and Bortels, 1934), is another evidence of the nearness of this relationship.

If we examine the physiological differences in more detail, it becomes clear that they are not as profound as would appear at first glance. Both *C. krzemieniewskii* and *C. diffluens* are able to decompose cellulose, although neither so rapidly nor so completely as the soil forms. The specialization of the latter group with regard to carbon and energy source as contrasted with the wider assimilatory powers of the former is an essentially secondary difference, and is found in many other groups of closely related bacteria, as for example the cellulose-decomposing vibrios. The differences in nitrogen requirements may also be more apparent than real. It is quite probable that *C. krzemieniewskii* and *C. diffluens* require certain growth factors, and that with these supplied they can use inorganic nitrogen just as well as peptone. There is an indication that even among the soil cytophagas special growth factor requirements may exist. The assertion of Stapp and Bortels that the non-microcyst-forming cytophagas studied by them were unable to grow except in symbiosis with other organisms can be explained logically by the assumption that these cytophagas are unable to synthesize certain growth factors which are supplied them by the symbionts.

The whole problem of the creation of genera on the basis of physiological specialization alone requires very careful consideration. For example, the genus *Cellvibrio*, created by Winogradsky for cellulose-decomposing vibrios growing "feebly" on "ordinary" media, presents considerable difficulties to the taxonomist. Should organisms like *Vibrio agar-liquefaciens* and certain of the cellulose-decomposing vibrios described by Kalnins (1930) which may grow well on "ordinary" media even to the extent of preferring peptone to inorganic nitrogen, and which may utilize other energy sources much more rapidly and extensively than cellulose, be included in this genus? Where is the line to be drawn? For that matter, on the basis of the reasoning which led to the creation of the genus *Cellvibrio* a similar genus *Agarvibrio* might well be proposed, yet the only result would be further confusion.

More extensive research on the known cytophagas, and the discovery of new species may show the desirability of creating new genera within the group, but in the present highly incomplete state of our knowledge concerning them, Winogradsky's genus *Cytophaga* (although with a modified definition) may be used to cover all species with the exception of the two known to form microcysts. These clearly call for a separate position.

In considering the relationship of the cytophagas to the higher myxobacteria, we have two lines of evidence, which, taken in conjunction, make such a relationship certain. The first is the close analogy between the developmental cycles of the microcyst-forming cytophagas and of the myxobacteria belonging to the genus *Myxococcus*. Since this was conclusively demonstrated by Krzemieniewska (1930, 1933) no further discussion of it will be given here. The second is the similarity in the structure of the vegetative cell and the highly characteristic method of locomotion, which is exemplified most clearly for the cytophagas by *C. krzemieniewskii* and *C. diffluens*. The remarkable phenomenon of the production of fruiting bodies by the higher myxobacteria has tended to obscure the other less obvious, but even more important, characteristics of the group. An admirable description of these characteristics coupled with a recognition of their significance occurs in the following passage from Benecke's "Bau und Leben der Bakterien" (1912, p. 89):

> Sind nun *alle* Bakterien in diesem weiteren Sinn derart gebaut, dass sie eine besondere Zellhaut besitzen? Dies scheint nicht der Fall zu sein. Man hat bei manchen Formen eine typische Zellhaut, wie sie der

> echten Pflanzenzelle zu eigen ist, trotz grosser Sorgfalt nicht nachweisen können. Statt ihrer ist nur jene dichtere und festere Aussenlage des Protoplasmas nachweisbar, von den Zoologen meistenteils als Pellicula bezeichnet.... Es handelt sich hier hauptsächlich um die Zellen der sog. Schleimbakterien, Myxobakterien, Formen die auch sonst z. B. in ihrer Bewegungsweise, von Bakterien im engeren Sinn wesentlich abweichen..., ferner zumal auch dadurch dass ihre Zellen "flexil" sind, d.h. die normalerweise gerade, stäbchenförmige Myxobakterienzelle kann sich kreisförmig biegen oder auch zusammenknicken, wobei es zweifelhaft bleibt ob das Folge einer aktiven Krümmungsbewegung ist.

These three characteristics of the vegetative myxobacterial cell, namely, the absence of a rigid cell wall, the occurrence of flexing movements and the peculiar method of locomotion, which, as Benecke points out, are not found in the true bacteria, all occur in the cytophagas. Even the rate of movement of 5 to 6 μ a minute established for *C. krzemieniewskii* compares closely to that of 10 μ a minute given by Baur (1904) for the vegetative myxobacterial cell. Furthermore, the pointed, spindlelike cell shape so characteristic of the cytophagas is also found in many of the myxobacteria (Jahn, 1924).

In view of this evidence, the absence of fruiting bodies can hardly be considered a sufficient ground for the exclusion of the cytophagas from the *Myxobacteriales*. Indeed, it would seem more rational to characterize the order on the basis of the structure of the vegetative cell and the manner of locomotion, which are sufficiently distinctive to exclude forms belonging to any other order of bacteria, rather than on the basis of the production of fruiting bodies and cysts. The cytophagas can then be included as the simplest representatives of the order.

Once this is done, the further classification of the cytophagas presents no difficulties. The microcyst-forming cytophagas can be placed most conveniently in the family *Myxococcaceae*. The absence of organized fruiting bodies prevents their being placed in the genus *Myxococcus*, and calls for the formation of a new genus. The following name and definition are proposed:

Genus **Sporocytophaga.** Spherical spores (microcysts) formed loosely among the vegetative cells. Fruiting bodies absent. The type species is *Sporocytophaga myxococcoides*, syn. *Spirochaeta cytophaga* Hutchinson and Clayton and *Cytophaga myxococcoides* Krzemieniewska. *Not* synonymous with *Cytophaga hutchinsoni* Winogradsky.

It is further proposed to create a new family in the *Myxobacteriales* to include all those forms in which microcysts (spores) and fruiting bodies are absent, with the following name and definition:

Family **Cytophagaceae**. Long, flexible rods, often pointed and spindle-shaped. Creeping motility. No spores or fruiting bodies formed.

One genus, *Cytophaga* Winogradsky emend. Description as for family. The type species is *Cytophaga hutchinsoni* Winogradsky.

It is quite possible that simple myxobacteria of the *Sporocytophaga* and *Cytophaga* types have been observed and described in the past by workers who failed to realize their true taxonomic position. Such a failure would be understandable in the case of organisms not exhibiting the strict physiological adaptation to cellulose which has hitherto been considered so highly characteristic of the cytophagas, and which has been so strongly stressed, particularly by Winogradsky. Although a careful search of the literature has not been made, two papers have been encountered which are highly suggestive in this connection.

Johnson (1932) described briefly a series of chitin-decomposing bacteria, of which some were typical representatives of the genus *Myxococcus* while others failed to form fruiting bodies but resembled the myxococci in their vegetative morphology. Of the non-fruiting group some formed spores while others did not. Johnson considered it possible that the absence of fruiting bodies was due to the fact that a suitable medium for their production had not been used. However, in the light of our present knowledge, an equally logical explanation would be that these strains were actually representatives of the genera *Sporocytophaga* and *Cytophaga*. This is a point which can be settled only by long-continued examination of the organisms in question on a variety of media.

Benton (1935) has also described certain chitin-decomposing bacteria which might conceivably belong to the cytophagas.

Unfortunately, cultures of the organisms obtained by these workers are no longer available, so that for the present the question of their position must remain an open one. Nevertheless, it does not seem unlikely that the simple myxobacteria are quite a widespread group. Henrici, in a private communication, has also suggested this:

> I suspect that there is a large group of bacteria similar to the *Myxobacteria* but failing to produce definite fruiting bodies. This, however, may be due to a failure to find an appropriate medium for fruiting.

PHYLOGENY OF THE MYXOBACTERIALES

In view of the above outlined thesis that the cytophagas are primitive representatives of the *Myxobacteriales*, it becomes of interest to speculate on the phylogeny of this order.

In the first place, no support can be provided for the hypothesis that a close relationship exists between the *Myxobacteriales* and the *Eubacteriales*. The fact that the vegetative cells of these two orders are analogous and not homologous in structure is well brought out by the cytophagas, although it was already quite evident from a consideration of the higher myxobacteria alone (Benecke, 1912, Jahn, 1924).

A much more plausible hypothesis in the light of present knowledge is the derivation of the myxobacteria from the *Myxophyceae*, which was suggested by Jahn in 1924. Among the unicellular blue-green algae there are several little-known genera whose morphological resemblances to the cytophagas are striking. This is particularly true of certain species of the genera *Rhabdoderma* and *Dactylococcopsis*, in which spindle-shaped, pointed cells, often arcuate or S-shaped, are found. Furthermore, the highly characteristic creeping motility of the myxobacteria also occurs in the blue-green algae. While commonly associated with the more complex representatives of this class, such as *Oscillatoria*, it has been shown by Jahn (1924) to exist also among the unicellular forms. The production of slime, probably connected with the mechanism of movement (see Jahn, 1924), is another common characteristic of the two groups.

Two other hypotheses concerning the relationships of the myxobacteria have been proposed, but there is no substantial evidence in support of either. The possibility of a relationship to the myxomycetes, tentatively suggested by Thaxter (1904) and strongly upheld by Vahle (1910) has been adequately dealt with by Jahn (1924). Thaxter's (1904) suggestion of a relationship with the sulfur bacteria can also be discounted. It was based on the hypothesis that the pink color often shown by myxobacterial cells in the mass was due to the presence of a pigment similar to bacteriopurpurin. This would imply the existence of a photosynthetic mechanism in the myxobacteria, for which there is no evidence. Furthermore, the purple bacteria resemble the *Eubacteriales* very closely morphologically, so that the objections outlined above to a connection of the myxobacteria with the true bacteria hold equally for the purple bacteria. Buchanan (1939), in his general discussion of the myxobacteria which was prepared for the fifth edition of Bergey's Manual, makes the statement:

> The *Myxobacteriales* may be regarded as a well differentiated order of the *Schizomycetes* resembling the true bacteria on the one hand and the *Myxophyceae* (*Cyanophyceae*) and *Thiobacteriales* on the other.

However, it is probable that Buchanan considers primarily the family of the *Beggiatoaceae* among the *Thiobacteriales* as relatives of the *Myxobacteriales*. The *Beggiatoaceae* are, however, not related to the purple sulfur bacteria and the *Athiorhodaceae* (although still placed in the same order in Bergey's Manual), but may be considered to be colorless representatives of the filamentous blue-green algae. In view of this it is not surprising that they show certain resemblances to the myxobacteria, if we consider the latter group as also having developed from the blue-green algae. However, the *Beggiatoaceae* and the myxobacteria probably had their origins in widely separated groups of the *Myxophyceae*; the similarities between them are very slight.

Summing up on the basis of the existing evidence, the most plausible hypothesis is that the *Myxobacteriales* are an end group in evolution, derived originally from unicellular *Myxophyceae*. They have diverged from these through the loss of the photosynthetic mechanism (with consequent adaptation to a saprophytic existence) and the gradual development of highly organized fruiting bodies.

SUMMARY

Two new representatives of the cytophagas have been isolated which differ in certain respects from the previously known members of this group. A detailed study of their morphology and method of locomotion has been made. On the basis of this and previous work, it is proposed that the cytophagas be included in the *Myxobacteriales* as the simplest representatives of this order. It is proposed to create a new genus *Sporocytophaga* in the family *Myxococcaceae* for those cytophagas which produce spores (microcysts), and a new family *Cytophagaceae* with one genus, *Cytophaga* Winogradsky emend., for the non-spore-producing forms. The phylogeny of the *Myxobacteriales* is discussed in the light of these new concepts.

ADDENDUM

While the manuscript of this paper was in press, the writer's attention was drawn to two publications by Imsenecki and Solntzeva (Bull. Acad. Sci. URSS, 1936, pp. 1115–1172; Mikrobiologia, 1937, v. 6, pp. 3–15) in which essentially the same ideas on the systematic position of the cytophagas have been advanced.

In conclusion, the writer wishes to express his gratitude to Prof. C. B. van Niel for much criticism and advice.

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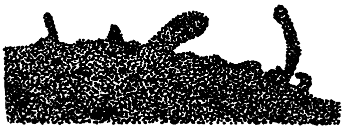
PLATE 1

Fig. 1. *Cytophaga krzemieniewskii.* Edge of a microcolony, showing several masses of cells in the early stages of movement. ×75.

Fig. 2. *Cytophaga krzemieniewskii.* Edge of a microcolony several hours older than that shown in figure 1. The irregularly scattered fields of bacteria are formed by the fusion of separate masses, followed by further migration. ×75.

Figs. 3–5. *Cytophaga krzemieniewskii.* Successive stages in the development of a moving mass, taken during a period of 10 mins. ×340.

All figures drawn from photomicrographs.



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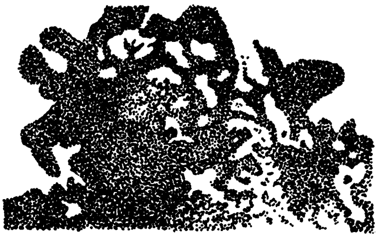

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(R. Y. Stanier: Studies on the Cytophagas)