 STUDIES ON NONFRUITING MYXOBACTERIA

I. CYTOPHAGA JOHNSONAE, N. SP., A CHITIN-DECOMPOSING MYXOBACTERIUM

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The concept of myxobacteria as a microbial assemblage characterized by a complex life cycle involving a unicellular phase of vegetative proliferation and a communal phase of fructification was clearly expressed by Thaxter (1892) in the first detailed publication dealing with the group. This concept remained unchallenged for forty years. Only during the past decade has the realization gradually arisen that "imperfect myxobacteria," to use the phrase of Imsenecki and Solntzeva (1945), which lack a fructificative stage but are recognizable by other means as members of the group, may also exist. The best-known nonfruiting myxobacteria are the cellulose-decomposing soil cytophagas, whose true systematic position, long obscure, has been gradually revealed by the discovery and recognition of their numerous myxobacterial properties: microcyst formation in some species (Krzemieniewska, 1930; Imsenecki and Solntzeva, 1936), creeping motility and flexing movements of the individual cells (Stapp and Bortels, 1934), and swarming on solid media (Stanier, 1940). The classical cellulose-decomposing soil cytophagas have a high degree of nutritional specialization, and although it has recently been discovered that some simpler carbohydrates can be metabolized in addition to cellulose (Fähræus, 1941; Stanier, 1942), they remain a sharply delimited group from the nutritional standpoint. However, a considerable number of nonfruiting myxobacteria with much broader nutrient requirements are now known. The first to be described were the marine agar-decomposing cytophagas, which, in contrast to the then known soil forms, could use peptides and a wide range of carbohydrates as energy sources, were not inhibited by heat-sterilized sugars, and required growth factors for their development (Stanier, 1940). During the past five years a considerable number of nonfruiting myxobacteria with varied nutrient requirements and morphological properties have been described by Fuller and Norman (1943), Imsenecki and Solntzeva (1945), Soriano (1945), Harmsen (1946), and Humm (1946). As Imsenecki and Solntzeva have pointed out, "imperfect" myxobacteria are not uncommon in nature, although for various reasons their recognition may at times be difficult.

A few years before the existence of nonfruiting myxobacteria was generally recognized, Johnson (1932) published a brief account of certain myxobacteria which attack chitin. Some of these organisms appear to have been typical Myxococcus spp., but others failed to form fruiting bodies while possessing the vegetative morphological characteristics of the fruiting types. Johnson's de-

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scription, although they suggested that these forms might be nonfruiting myxobacteria of the *Sporoeypthoga* and *Cytophaga* types, were not complete enough to permit a final decision without further investigation. The present work was accordingly started in an attempt to confirm and extend her findings.

**MATERIALS**

Chitin was prepared from lobster shells. After decalcification with dilute HCl, the shells were boiled for 12 hours in 10 per cent KOH, washed, treated with dilute KMnO₄, for 20 minutes at 60°C, washed again, suspended in a cold concentrated solution of sodium bisulfite until decolorized, and dried at 80°C, after a final thorough washing to remove the last traces of bisulfite. The leathery strips of material thus produced were used without further treatment in enrichment cultures. For incorporation in agar media, a very finely divided suspension of chitin was prepared by dissolving strips purified as above in cold 50 per cent H₂SO₄ and reprecipitating by rapid dilution with a 10- to 20-fold volume of water. The resulting product was freed from acid by repeated centrifugation and washing, and kept as a sterile aqueous suspension. Material prepared in this way is somewhat gelatinous and cannot be dried successfully, as it coheres during drying into a hard, horny mass impossible subsequently to re-suspend.

Enrichment cultures for chitin-decomposing bacteria were set up by adding strips of chitin to shallow layers of a dilute mineral base (K₂HPO₄, 0.1 per cent, MgSO₄, 0.05 per cent, NaCl 0.05 per cent, CaCl₂ 0.01 per cent, FeCl₃ 0.005 per cent, pH 7.0 to 7.2) in Erlenmeyer flasks, which were inoculated with soil or mud and incubated at 25 to 30°C. Some anaerobic enrichment cultures were also set up, using the same medium in completely filled glass-stoppered bottles, but no chitin decomposition was observed in them.

Mineral chitin agar was prepared by the addition of agar and chitin suspension to the foregoing mineral base in the requisite amounts. The same mineral base with 0.1 per cent NH₄Cl was employed for testing the utilization of carbon compounds, which were added in a concentration of 1.0 per cent. Utilization was determined by growth in comparison with that in an inoculated control not containing any carbon source. The utilization of nitrate as a hydrogen acceptor was tested by cultivation in glass-stoppered bottles filled with a medium consisting of 0.5 per cent tryptone and 0.5 per cent KNO₃. All other media were prepared in the customary manner.

**ENRICHMENT AND ISOLATION OF CHITIN-DECOMPOSING MYXOBACTERIA**

In aerobic enrichment cultures prepared as described above, the chitin undergoes slow decomposition at 25 to 30°C, accompanied by the development of an extremely varied microflora consisting only in part of chitin-decomposing microorganisms. Streaks from such enrichment cultures on 2 per cent agar plates containing the above-listed minerals, together with a finely divided suspension of chitin, usually reveal several eubacterial types as the most conspicuous chitin decomposers; they are for the most part pseudomonads,
which produce small white or pale yellow colonies surrounded by cleared zones in which the chitin has been dissolved. Occasionally chitin-decomposing *Cytophaga* *spp.* will be found. However, examination of such plates after 5 to 6 days also almost invariably reveals the presence of chitin-decomposing myxobacteria, although from their inconspicuous manner of growth they can easily be overlooked on cursory examination. Sometimes they are the only chitin decomposers to develop in an enrichment culture. Their colonies consist of thin, translucent, pale yellow, almost colorless swarms, which spread with great rapidity and soon cover large areas of the plate with a thin layer of cells. Because of their thinness, young swarms may be invisible by transmitted light, but when examined by reflected light their matt surface shows up in contrast to the surrounding agar. Except on old plates chitin decomposition is never very conspicuous, since the partial clearing which occurs beneath the swarm is masked by the overlying layer of cells, and because of the rapid extension of the swarm there is usually not a cleared zone beyond its periphery.

A number of strains were picked and purified for further study. At first, purification was attempted by cutting out small sections of agar at the periphery of the swarm and transferring them to fresh chitin agar plates; but after the discovery that abundant growth occurred on peptone or yeast extract agar, more expeditious and certain purification was achieved by streaking on plates of these media in the customary manner.

**Properties of the isolated strains**

All the strains isolated in the manner just described have proved to be nonfruiting and amicrocystogenous, thus conforming to the definition of the genus *Cytophaga*. Microcystogenous and fruiting myxobacteria were never encountered during this work, either in enrichment cultures or on plates, although the observations of Johnson (1932) suggest that chitin decomposers with these morphological properties also exist. The provenance of the strains studied in detail is shown below.

Strain 1. From enrichment culture inoculated with mud; Cambridge.
Strain 2. From enrichment culture inoculated with garden soil; Rothamsted.
Strain 3. Same source as 2.
Strain 4. From enrichment culture inoculated with field soil; Rothamsted.
Strain 5. Same source as strain 4.
Strain 6. From enrichment culture inoculated with compost; Rothamsted.

**Morphology.** The vegetative structures are thin, weakly refractile rods of even width, with rounded ends. In young cultures (less than 18 hours) on favorable media, there is usually a predominance of long rods (8 to 12 μ) showing the marked flexibility and active creeping movements so characteristic of myxobacteria. For the most part, such cells are not markedly curved, although a few may show this property, some having a slight hook at one extremity. Occasional branched, Y-shaped individuals like those observed in cultures of *Cytophaga columnaris* by Garnjobst (1945) can be found. However, in addition to these long rods very short, sometimes almost cocccoid elements, 1 to 3 μ in
length, are also evident. In strains 1, 2, 3, and 6 these short elements become predominant only in cultures several days old, or on unfavorable media; in strains 4 and 5, however, they are always present in appreciable numbers at the earliest stages of development and rapidly become the predominant or

![Vegetative Cells of Cytophaga Johnsonae from Cultures 14 Hours Old on 0.5 Per Cent Tryptone Agar](image)

Fixed with osmic acid and stained by Winogradsky’s method (1929). A. Strain 2. B. Strain 6 (var. denitrificans). Note the short elements in both photomicrographs.

even exclusive morphological form. Inspection of cultures with a predominance of short cells does not suggest that the organisms are myxobacteria: flexibility is not evident, and motility of the individual cells is hard to detect, the only indicative feature which remains being the weak refractility of the cells. The picture, both in wet mounts and in stained preparations, is strongly suggestive of a small, nonmotile, gram-negative true bacterium.
A careful search of cultures on many different media failed to reveal the presence of microcysts.

Growth on solid media. The manner of growth on mineral agar containing finely divided chitin has already been mentioned. The rapidity and extent of swarming on this medium can be controlled to some degree by varying the agar concentration. With 1.5 to 2.0 per cent agar, the whole surface of a streaked plate becomes covered with a thin layer of cells in 36 to 48 hours; however, when the concentration of agar is reduced to 1.0 per cent, surface spreading is greatly retarded and isolated colonies are formed (figure 2A). Such colonies are largely subsurface, the cells penetrating and creeping down through the medium. After a few days the colonies become surrounded by well-defined clear areas of chitin dissolution, as shown in figure 2A. The growth is sunken below the surface of the medium, each colony lying in a shallow crater (figure 2B). This is the "etching" phenomenon, previously described as occurring on plate cultures of cellulose-decomposing cytophasas by Stanier (1942) and Fuller and Norman (1943).

When chitin-decomposing myxobacteria are streaked on nutrient agar or yeast extract agar with the customary concentrations of proteinaceous materials, a radically different type of growth is obtained. The colonies are convex, smooth and glistening with an entire edge, and at least in the earlier stages of development there is absolutely no sign of swarming. As such plate cultures age, well-isolated colonies gradually increase in diameter to as much as 5 to 10 mm, but always remain unchanged in form. On the more crowded portions of a streaked plate, however, slight swarming may appear after 5 to 6 days; the originally compact growth becomes surrounded by a small, flat, swarming periphery which spreads 2 to 3 mm from the main mass. This delayed swarming is easily overlooked, in which case there is nothing to suggest the myxobacterial nature of the culture.

Further study of growth on peptone media showed that typically myxobacterial development could be obtained, but only if the nutrient concentration was kept low. At a peptone concentration of 1.0 per cent or over, the primary development is always compact and raised with an entire edge (figure 3A). As the peptone concentration is dropped below 1.0 per cent, swarming becomes more and more evident, 0.5 per cent peptone giving rise to colonies with slightly raised center and a broad, flat, actively motile periphery (figure 3B). Finally, at peptone levels of 0.25 per cent or less, completely flat, rapidly spreading, almost invisible swarms are produced (figure 3C). Thus colony structure in these organisms is not fixed, but can be changed at will by varying nutrient concentration from a compact, eubacterial type to a diffuse, swarming, myxobacterial manner of development.

Agar concentration also plays a part in conditioning colony form on peptone media. With 1.5 to 2.0 per cent agar, there is little penetration of the medium at any level of peptone concentration; but with 1.0 per cent agar, provided that the peptone concentration is 0.5 per cent or less, growth on streaked plates tends to be largely subsurface and sunk below the level of the surrounding
medium. With higher peptone concentrations, a reduction in the agar concentration produces no changes.

There appear to be two factors which operate to cause these variations in

![Image of Cytophaga Johnsonae, Strain 1](image)

**FIG. 2. CYTOPHAGA JOHNSONAE, STRAIN 1**

Streaked plates on mineral 1.0 per cent agar containing a suspension of chitin, incubated for 6 days at 25°C. A. Photographed by transmitted light to show colony structure and dissolution of chitin. B. Photographed by reflected light to show the shallow craters formed by the colonies.

the manner of growth. In the first place, it is evident that the characteristic creeping motility of these organisms can be inhibited by high concentrations of nutrients. This phenomenon can be corroborated by microscopic exami-
FIG. 3. CYTOPHAGA JOHNSONAE, STRAIN 1

Streaked plates on tryptone agar, incubated for 6 days at 25 C, showing the effect of nutrient concentration on colony form. A. Plate with 2.0 per cent tryptone. B. Plate with 0.5 per cent tryptone. C. Plate with 0.25 per cent tryptone.
nation: cells from nonswarming colonies show no signs of individual flexing or creeping movement. As a result of the inhibition of movement, colony growth becomes a passive phenomenon, as it is in most true bacteria, and a similar colony structure is produced. The second factor influencing colony form is the ability of the cells, provided that their movement is not inhibited by nutrient concentration, to penetrate a weak agar gel. Cells deposited on the surface of a 1.0 per cent agar gel can move freely through the medium as well as across it, and thus colonies approach in form a hemisphere buried in the medium with its plane surface at the surface of the agar. On the other hand, when the agar is sufficiently firm to offer a barrier to the penetration of the cells, organisms deposited on its surface can only move out in a flat swarm, and the

resulting colony approximates in form a plane of cells. The three possibilities are shown diagrammatically in figure 4.

Nutrient requirements and physiology. All strains can develop in a mineral medium containing the necessary salts together with ammonia nitrogen and a suitable carbon compound. The carbohydrates are the energy sources of preference. Heat sterilization does not render reducing sugars toxic. Development in such simple media is rather slow and never very abundant; it can be greatly accelerated by the addition of small amounts of peptone or yeast extract. It is possible, however, to maintain all strains indefinitely in a mineral-carbohydrate medium without such additions. A wide range of simple carbohydrates is attacked; of the polysaccharides, chitin and starch are utilized by all, cellulose and agar by none. Some strains, notably 4, develop
much better on disaccharides than on their constituent monosaccharides. In addition to ammonia, nitrate but not nitrite can be used as a nitrogen source.

Maximal development occurs on complex nitrogenous media, Difco tryptone being particularly favorable. Milk and peptone gelatin are rather poor substrates; this is apparently due to the high concentration of nutrients, since vigorous development can be obtained with diluted milk. In general, concentrations of peptone and other proteinaceous materials of 1.0 per cent or over are deleterious, and even if, as sometimes proves the case, development is abundant, viability turns out to be poor. Thus good growth will occur on nutrient agar or 1.0 per cent tryptone agar, but cultures on these media are often dead after 2 to 3 weeks, whereas cultures on 0.25 to 0.5 per cent tryptone agar will remain viable for 2 months or longer.

All strains are actively proteolytic, although this property is not evident on the customary media because of poor growth. On plates of plain agar containing 10 per cent milk, the growth is surrounded by wide zones of casein dissolution in 2 to 3 days. Bacteriolytic enzymes of the type found in many higher myxobacteria are absent; all strains proved without effect when tested against a strain of *Aerobacter aerogenes* readily lysed by members of the *Myxococcaceae*.

The metabolism is strictly oxidative. There are no signs of carbohydrate fermentation, and in the absence of nitrates development only occurs aerobically. In the presence of nitrates, two strains can grow anaerobically, using nitrate as a hydrogen acceptor in the place of free oxygen. One of these (strain 4) reduces nitrate only to nitrite, and the resulting anaerobic growth is somewhat sparse. The other (strain 6) is an active denitrifier, which grows abundantly with nitrates under anaerobic conditions and gives rise to considerable gas production.

**TAXONOMIC POSITION**

It is clear that in spite of the morphological and cultural anomalies mentioned above, the strains studied are myxobacteria which belong, on the absence of fruiting bodies and microcysts, in the family *Cytophagaceae*. For reasons which will be made clear in the subsequent discussion I prefer to place them in the genus *Cytophaga*, although on the basis of their morphology and nutrient requirements, respectively, they might be assigned to either of the two new genera of nonfruiting myxobacteria, *Promyzobacterium* (Imsenecki and Solntzeva, 1945) or *Flexibacter* (Soriano, 1945).

Although there are minor differences between strains in cell size, carbon requirements, and action on nitrates, none of the distinctions seem sufficiently important or well correlated to justify creation of more than one species. A possible exception is strain 6, with its pronounced denitrifying abilities. However, this strain is very similar in other respects to the remaining strains, and hence it seems wiser to regard it simply as a variety. Since all strains appear to correspond to the chitin decomposers described by Johnson (1932) as nonfruiting, amicrocystogenous myxococci, they may be appropriately grouped
under the designation *Cytophaga johnsonae* n. sp., with strain 6 as a special variety, var. *denitrificans*. The descriptions follow:

*Cytophaga johnsonae, n. sp.*

Morphology: Thin, weakly refractile rods of even width and very variable length, with rounded ends. Dimensions 0.2 to 0.4 by 1.5 to 15 μ. Long rods (8 to 10 μ) predominate in very young cultures on dilute media, but in most strains soon give place to shorter, sometimes almost coccoid, elements as cultures age. Branched cells occasionally occur in young cultures. Creeping motility on media of suitable nutrient concentrations. Gram-negative. Cells do not contain volutin or fat bodies, and stain evenly with Giemsa and Wigradsky’s stains.

Peptone agar plate: Growth smooth, glistening, translucent, butyrous, and bright yellow in color. The appearance of growth varies greatly with peptone concentration. With 0.5 per cent or less, a flat, thin, spreading, inconspicuous, pale yellow swarm of the characteristic myxobacterial type is formed. With higher concentrations, swarming decreases or completely disappears, and the colonies are raised, convex, and confined, with an entire edge. With agar concentrations of 1.0 per cent or less and a low nutrient concentration there is marked penetration of the medium with decreased lateral swarming.

Mineral chitin agar plate: Smooth, glistening, butyrous, translucent, pale yellow growth, accompanied by dissolution of the suspended chitin. Swarm extension is extremely rapid on 2 per cent agar, but on 1 per cent agar discrete colonies are formed owing to penetration of the medium and decrease of surface swarming. Development is not as profuse as on peptone agar.

Plain agar with 10 per cent milk: abundant, spreading growth with dissolution of the casein.

Peptone gelatin stab: Scanty growth with some strains followed by very slow liquefaction. Others fail to develop.

Liquid media (peptone water, yeast extract, mineral media with an oxidizable carbohydrate): Turbidity with pronounced silkiness. After several days a slimy coherent sediment is formed, and in the course of time the suspended cells tend to sink to the bottom, leaving the upper layers of the medium clear. Some strains produce a pellicle.

Milk: Very slow peptonization. One strain fails to develop.

Peptone water with 3 per cent NaCl: No growth.

Utilizable energy sources: Arabinose, xylose, glucose, galactose, mannose, lactose, maltose, sucrose, cellobiose, raffinose, starch, inulin, chitin, asparagine (all strains). Succinate is used by three strains out of five, fumarate and malate by one. Cellulose, mannitol, dulcitol, lactate, acetate, malonate, tartrate, citrate, and alanine are not attacked. Proteinaceous materials can also serve as the energy source in the absence of carbohydrates.

Utilizable nitrogen sources: Nitrate, ammonia, alanine, and peptones. Not nitrite or glycine.

Casein hydrolyzed by all strains, gelatin by most. Bacteriolytic enzymes not produced.
Catalase produced.
Indole not formed.
Strictly aerobic, except for one strain which can develop anaerobically to a slight extent in the presence of nitrate (reduced to nitrite).
Optimum temperature: 25 to 30 C.
Source: Soil and mud.
Habitat: Probably common in soil.

_Cytophaga johnsonae var. denitrificans_

Morphology: Similar to _C. johnsonae._
Peptone agar plate: Similar to _C. johnsonae._
Mineral chitin agar plate: Similar to _C. johnsonae_, but attack on chitin very weak.
Plain agar with 10 per cent milk: Similar to _C. johnsonae._
Peptone gelatin stab: Very slight growth, followed by slow liquefaction.
Liquid media: Similar to _C. johnsonae._
Milk: Slow peptonization.
Peptone water with 3 per cent NaCl: No growth.
Utilizable energy sources: Arabinose, xylose, glucose, galactose, mannose, maltose, sucrose (growth weak and delayed), starch, inulin (weak), chitin (weak), succinate, fumarate, and asparagine. Lactose, cellobiose, raffinose, cellulose, mannitol, dulcitol, sorbitol, lactate, acetate, malate, malonate, tartrate, citrate, and alanine not attacked.
Utilizable nitrogen sources: Same as _C. johnsonae._
Gelatin and casein hydrolyzed. Bacteriolytic enzymes not produced.
Catalase produced.
Indole not formed.
Strictly aerobic except in the presence of nitrate. Can develop abundantly under anaerobic conditions by employing denitrification as a mechanism for oxidative metabolism. Such growth is accompanied by vigorous gas production and a transient appearance of nitrite.
Optimum temperature: 25 to 30 C.
Source: Compost.
Habitat: Probably widely distributed in soil.

DIFFERENTIATION OF NONFRUITING MYXOBACTERIA FROM ROD-SHAPED TRUE BACTERIA

When the taxonomic recognition of nonfruiting myxobacteria was first proposed (Stanier, 1940), the differentiation of these organisms from rod-shaped true bacteria appeared fairly simple. This is still true of the microcystogenous forms (genus _Sporocytophaga_); microcyst formation is a highly distinctive developmental process, never found in eubacteria. On the other hand, it now appears from the work of Imsenecki and Solntzeva (1945) as well as from the present study that myxobacteria which exist only in the vegetative state (family _Cytophagaceae_) may well simulate true bacteria under certain circumstances. The two species described by Imsenecki and Solntzeva (1945) under the
names *Promyzobacterium flavum* and *P. lanceolatum* were for some time regarded by those authors as nonsporeforming true bacteria, on account of the shortness of the cells and the, for myxobacteria, uncharacteristic manner of growth. *Cytophaga johnsonae*, if examined solely from cultures on the customary concentrated media, would appear to be a short, gram-negative, nonmotile rod classifiable according to the system of Bergey et al. in the regrettable genus *Flavobacterium*; indeed, it seems might easily a classifiable possible that the concentrated media, would johnsonae, *Cytophaga* they are rods of even centered groups. Achromobacter, and supposedly nonsporeforming, nonflagellated rods—*Bacterium*, *Achromobacter*, and *Flavobacterium* of Bergey et al.—may be harboring unsuspected myxobacteria of the *Cytophaga* type which were studied under conditions inapt to reveal their true properties. Since the differentiation of myxobacteria from true bacteria is a matter of some practical as well as theoretical importance, it seems worth while to discuss, in the light of recent work, what is now known about the general properties of imperfect myxobacteria and to indicate features likely to be helpful in the recognition of these organisms.

**Morphology.** The earlier concept that the cells of the cytophaga group are slender, spindle-shaped rods with pointed ends must now be abandoned. Derived from the study of the classical cellulose-decomposing species in stained preparations, this concept is not properly speaking true even of these organisms, since, as I have previously remarked (1942), when examined in the living state they are rods of even width with slightly rounded ends. The same description applies to all other species so far studied. It is now also evident that the length of the cells may be extremely variable, not all species having the long, slender cells characteristic of the cellulose- and agar-decomposing groups. The two species described by Imsenecki and Solntzeva (1945) are almost coccoid under some conditions; in *Cytophaga columnaris*, of which Garnjobst (1945) has given an admirable morphological description, very short cells occur intermingled with longer elements; in *Cytophaga johnsonae* the cells may be long and slender or short and almost coccoid depending on the conditions of cultivation and the age of the culture. The possibility exists that in all cytophagas the basic cellular units are short, and that the length of the vegetative entities is an expression of the degree of chain formation. In any case, there is no clear distinction between these organisms and rod-shaped eubacteria on the basis of cell shape alone. Two properties of the cell are, however, very strong indications of membership in the cytophaga group. One is marked flexibility, as shown by the presence of irregularly curved, horseshoe-shaped, or ringed vegetative elements. This character will naturally not be evident if the cells are very short, and even in long-celled cultures it is not invariably conspicuous. The other feature is the relatively weak refractility of living cytophaga cells, due to

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3 The systematic position of this interesting species, an important pathogen of freshwater fishes, is not entirely clear. Ordal and Rucker (1944) have claimed discovery of fruiting body formation in strains which appear to be similar to those studied by Garnjobst.
the absence of a cell wall. Imsenecki and Solntzeva (1945) suggest that this is less marked in short-celled types, but it has proved a constant feature in all the species that I have examined. As far as staining reactions are concerned, it may be noted that all members of the cytophaga group are gram-negative. Some species have a tendency to stain rather poorly with basic aniline dyes, but this is not universally true. With the exception of the microcystogenous species during the process of microcyst formation (Krzemieniewska, 1930), the cytophagas do not show discrete chromatinic structures when stained by ordinary methods. This has been interpreted by Imsenecki and Solntzeva (1945) as indicating that the chromatinic material is diffuse, a feature which in their opinion serves as a link with the true bacteria, in supposed contrast to the higher myxobacteria which have discrete chromatinic structures. It may be remarked that in all eubacteria so far properly examined discrete chromatinic structures have been found, although they are rarely demonstrable by direct staining with basic aniline dyes because of the strongly basophilic nature of the cytoplasm (cf. Robinow, 1945, for a discussion). Since the cytology of the cytophaga group has not yet been investigated in detail from the standpoint of nuclear structure, it seems decidedly premature, in view of the above-mentioned findings with true bacteria, to describe the distribution of chromatin in them as diffuse.

**Motility.** The most decisive distinguishing property of nonfruited myxobacteria vis-à-vis true bacteria is their nonflagellar creeping movement. So far, permanently immotile members of the *Cytophagaceae* have not been described, nor is it likely that they would be recognizable as such. On the other hand, the present work demonstrates that creeping motility is not invariably evidenced by organisms potentially capable of it. It thus becomes necessary to define as far as possible the conditions favorable for bringing this important characteristic to light.

Of primary importance is the use of dilute media for cultivation of the organisms, a point mentioned by Soriano (1945). Motility can usually be discerned in wet mounts by examining cells adherent to the slide or cover slip. Since all known myxobacteria are strict aerobes, a fairly light suspension should be used for such examinations; otherwise movement will soon cease owing to depletion of the oxygen supply. Particularly if the vegetative units are small, a convincing demonstration of creeping movement in wet mounts may prove difficult. In such cases, a variant of the usual hanging block technique, first used to study swarming by the marine agar-decomposing cytophagas (Stanier, 1940), may be applied. A loopful of melted agar is spread on a sterile cover slip and inoculated, after solidification, at one or two spots with a mass of young cells from the tip of a needle. A dilute nutrient agar may be used, although for the chitin-decomposing strains equally satisfactory results can be obtained with nonnutrient agar. The inoculated cover slip is mounted over a moist chamber, incubated at a suitable temperature and examined at intervals. After a variable period, spearheads of cells begin to creep out from the smooth periphery of the artificial microcolony, and usually within 2 to 3 hours the whole edge breaks down into a
Fig. 5. C. JOHNSONAE, STRAIN 2

Swarming edge of an artificial microcolony on 2 per cent agar without nutrients, photographed at intervals. A. 11:50 A.M. B. 11:57 A.M. C. 12:05 P.M. D. 12:15 P.M.
series of actively moving columns, which spread out and interlace with one another over the surrounding agar. Provided that wet mounts have shown the absence of flagellar locomotion, this constitutes a decisive proof of creeping motility. A series of consecutive photomicrographs of swarming from such an artificial microcolony of \textit{C. johnsonae} are shown in figure 5.

\textit{Cultural characters.} Certain cultural characters offer hints to the observant for the identification of imperfect myxobacteria. The most suggestive features are flat, rapidly spreading growth on solid media, the penetration of weak agar gels by the cells, and the etching of the surface of agar media. These properties are not, however, invariably shown; they were apparently absent in the cultures studied by Imsenecki and Solntzeva (1945), and are not found in \textit{C. hutchinsoni} (Stanier, 1942) or in \textit{C. johnsonae} when grown on concentrated media.

\textit{Other properties.} There are no really distinctive nutritional, biochemical, or physiological characters common to all nonfruiting myxobacteria. Strict aero-biosis and a rather low temperature optimum (30 C or less) are common; but an adequate search for myxobacteria with other oxygen and temperature relationships has so far not been made, and these particular properties are widely found in the euabacterial groups most likely to be confused with the nonfruiting myxobacteria. Although there is a definite group tendency toward the decomposition of complex polysaccharides, this is by no means universal; \textit{C. columnaris} (Garnjobst, 1945) and the \textit{Promyxobacterium} spp. described by Imsenecki and Solntzeva (1945) lack this property. In addition, I have examined several strains of imperfect myxobacteria isolated by chance from soil which failed to attack any of the polysaccharides tested (agar, chitin, cellulose) except starch. The inability to use any source of carbon save carbohydrates is a highly distinctive feature of the classical cellulose-decomposing cytophagas, but is not found in the more recently described species. The members of the group are markedly heterogeneous in their relation to growth factors: some (viz., the soil cellulose-decomposing group, \textit{C. johnsonae} and the \textit{Promyxobacterium} spp. of Imsenecki and Solntzeva) are capable of satisfactory growth with ammonium salts and a suitable carbon source, but others definitely require growth factors, although in no case have the exact needs been worked out.

\textbf{RECENT PROPOSALS ON THE TAXONOMY OF THE NONFRUITING MYXOBACTERIA}

When the taxonomic recognition of the nonfruiting myxobacteria was first proposed (Stanier, 1940, 1942), the suggestion was made that the microcystogenous species should be included in the family \textit{Mycococcaceae} as a new genus, \textit{Sporocytophaga}, and that a new family, \textit{Cytophagaceae}, with a single genus \textit{Cytophaga} Winogradsky emend. should be adopted for the amicrocystogenous species. As originally defined by Winogradsky (1929), the genus \textit{Cytophaga} was characterized in part by obligate cellulose decomposition, a physiological property which proved no longer valid following the discovery that the species included could attack other carbohydrates. The family \textit{Cytophagaceae} and the genus \textit{Cytophaga} were then redefined (Stanier, 1942), on purely morphological grounds, as containing organisms with the characteristic features of myxobacterial vegeta-
tive cell structure, but incapable of forming either fruiting bodies or microcysts.

Recently two modifications in the taxonomy of this group have been proposed. Imsenecki and Solntzeva (1945) suggest a family *Promyxobacteriaceae*, defined as containing myxobacteria which fail to form fruiting bodies, and divided into three genera: *Promyxobacterium*, *Cytophaga*, and *Sporocytophaga*. The new genus *Promyxobacterium*, created for two species described by Imsenecki and Solntzeva, is stated to consist of organisms whose vegetative cells are rod-shaped with rounded ends, in supposed contradistinction to the other two genera in which the vegetative cells are stated to be long, thin, and slightly bent with pointed ends. The proposed family *Promyxobacteriaceae*, which would unite all nonfruiting myxobacteria and separate them from myxobacteria at a higher level of biological organization as expressed by the formation of discrete fruiting bodies, has much to recommend it. The possible merits of this arrangement have been assessed previously, and it has been pointed out that the decision between such a taxonomic scheme and one which places the genus *Sporocytophaga* in the *Mycococcaceae* must remain more or less a matter of arbitrary choice (Stanier, 1942).

On the other hand, the genus *Promyxobacterium* as it is defined by Imsenecki and Solntzeva would, I feel, prove completely unworkable, since the supposed morphological distinction between organisms of this type and *Cytophaga* species is not a clear-cut one. When examined in the living state, cells of the classical cellulose-decomposing cytophagas are of even width, with ends which are only slightly pointed, if at all. In the last analysis, the difference between *Promyxobacterium* and *Cytophaga* appears to be one of cell length, which is not really tenable. In *Cytophaga johnsonae* and *C. columnaris*, the cells may be short and *Promyxobacterium*-like or long and *Cytophaga*-like, and, depending on cultural conditions, one or the other type can predominate.

The taxonomic proposals of Soriano (1945) are more far-reaching, and comprise part of a scheme for rearranging the major groups of bacteria. Only the pertinent sections of his suggested changes will be discussed here. He proposes that the order *Myxobacteriales* should be kept as originally conceived for fruiting myxobacteria alone, and that a new order *Flexibacteriales* should be created to contain all bacteria with flexible, rod-shaped cells and creeping motility that are incapable of forming fruiting bodies. In this order he assembles three families: the *Cytophagaceae*, containing the genera *Cytophaga* and *Sporocytophaga*; the *Beggiaioaceae*, containing the genus *Beggiaioa*; and the *Flexibacteriaceae*, containing the genus *Flexibacter*. The latter family and genus were created by Soriano for several nonfruiting, amicrocystogenous myxobacteria discovered by him that develop on peptone and meat extract media and are unable to attack cellulose. The three families are defined and distinguished primarily on nutritional grounds as follows:

*Flexibacteriaceae*. Forms which do not contain granules of sulfur and are incapable of attacking cellulose.

*Cytophagaceae*. Flexible bacteria, spindle-shaped at some period of their development, which attack cellulose and may or may not produce cysts.

*Beggiaioaceae*. Flexible, filamentous forms, without photosynthetic pigments, containing sulfur granules within the cells.
Two principal criticisms can be made of the proposals of Soriano. The first, and less serious, is that so many lines of evidence link the nonfruiting myxobacteria with the higher myxobacteria—all the common features of vegetative cell structure, together with the mode of mycrocyt formation in the Sporocytophaga species—that an ordinal separation is too drastic. The second is that the order Flexibacteriales is not in itself a natural assemblage, since the families Flexibacteriaceae and Cytophagaceae differ radically in structure and organization from the Beggiatoaceae. As is now commonly recognized, the latter family comprises colorless counterparts of blue-green algae belonging to the family Oscillatoriaceae. Since the practice in other algal groups of placing colorless genera and families near morphologically related photosynthetic forms is well established (cf. Lwoff, 1943, and Pringsheim, 1941, for discussions of colorless flagellates), there is no justification except a false bacteriological tradition for not following the same procedure in the case of the Beggiatoaceae and removing them from the bacteria altogether. If this is done, Soriano’s order Flexibacteriales becomes a repository for nonfruiting myxobacteria, equivalent to Imsenecki and Solntzeva’s Promyzobacteriaceae, or to my family Cytophagaceae with the addition of the genus Sporocytophaga.

The problem of generic and familial segregations among the nonfruiting myxobacteria is a difficult one. If these organisms really comprise as large and varied a group as appears to be the case from recent work, a single genus and family may well prove insufficient to include them all; but before additional genera and families are accepted, careful thought should be given to the selection of significant, mutually exclusive differential characters. As already mentioned, the Promyzobacterium-Cytophaga division along morphological lines proposed by Imsenecki and Solntzeva (1945) is untenable. We are faced here with a taxonomic difficulty which has previously been encountered in the true bacteria; namely, that of finding foolproof physiological differential characters which can be used to split up a group of strict aerobes sharing common morphological properties. Viewed in this light, Soriano’s proposed Flexibacter-Cytophaga division on the basis of ability or inability to attack cellulose is simply a continuation of the policy expressed in the generic pairs Vibrio-Cellvibrio and Pseudomonas-Cellulomonas. In all these cases, the mutational loss of a single hydrolytic enzyme, an event not too difficult to envisage in the light of recent work on the biochemical genetics of microorganisms, would be sufficient to change the generic position of an organism. An additional obstacle to such generic separations, clearly evident in the Vibrio-Cellvibrio group, is provided by the intergrading species endowed with a general ability to attack complex polysaccharides, but lacking the specific enzyme cellulase (e.g., the agar-decomposing vibrios).

Until nonfruiting myxobacteria have been more extensively studied, the genus Cytophaga appears to me to provide an adequate taxonomic pigeonhole for all known amicrocytogenous species. Mention may be made here, however, of one significant physiological property which might be applied to give a generic segregation roughly equivalent to that suggested by Soriano; namely, the type of organic compound which can be used as a source of energy. The classical cellulose-decomposing cytophagas are highly restricted in this respect, being
unable to develop in the absence of carbohydrates. All other known species of nonfruiting myxobacteria can use peptides, and in certain cases other simpler organic compounds, to fulfill their energy requirements. A division into two genera, *Cytophaga* and *Flexibacter*, on this basis would, I believe, prove more satisfactory than one which rested solely on the ability or inability to hydrolyze cellulose.

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SUMMARY

A microcystogenous nonfruiting myxobacteria commonly occur as a component of the chitin-decomposing microflora in aerobic chitin enrichment cultures.

Several strains of these organisms have been isolated and studied. Although atypical in some respects from the morphological and cultural standpoints, they are all representatives of the genus *Cytophaga*. The nutrition is unspecialized, and good growth occurs on a wide variety of media. Only slight differences between strains were found, and they are consequently regarded as representatives of a single species, *Cytophaga johnsonae*, n. sp. One strain characterized by pronounced denitrifying abilities is separated as a variety, var. *denitrificans*.

The taxonomy of the nonfruiting myxobacteria and the problem of their differentiation from rod-shaped eubacteria are discussed in the light of recent work.

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