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CHAPTER 3.4.3

The Myxobacteria

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The myxobacteria are Gram-negative, unicellular bacteria with rod-shaped vegetative cells (Fig. 1). Because of their gliding movement, colonies develop as thin, spreading swarms, particularly on media low in organic constituents (Fig. 2). Under starvation conditions, the myxobacteria undergo an impressive process of cooperative morphogenesis: the vegetative cells aggregate into large mounds and then form a fruiting body by directed cell movement (Fig. 3). Myxobacterial fruiting bodies show various degrees of complexity. They typically measure between 50 and 500 μm and can be seen with the naked eye. Within the maturing fruiting body the vegetative cells convert into short, optically refractile myxospores (Figs. 1 and 3). The myxospores are desiccation resistant and allow the organism to survive unfavorable environmental conditions. Many features of the life cycle are richly illustrated in a series of film clips created by Hans Reichenbach and assembled by Martin Dworkin (see the online version of *The Prokaryotes*). The clips are presented with the permission of the Institut für den Wissenschaftlichen Film.

Commentary and References for Time-lapse Film.

This time lapse film is from a series of films created by Dr. Hans Reichenbach. It was assembled by Dr. Martin Dworkin and is presented with the permission of the Institut fur den Wissenschaftlichen Film, Göttingen, Germany.*

1. Movement of cells of *Archangium violaceum* on agar. Note that movement ceases while the cells are dividing. [2 frames/min. over approximately 8 hr.; Kühlwein, H. and H. Reichenbach, 1968a]

2. Movement of cells of *Myxococcus xanthus* on agar. [4 frames/min over approximately 1.5 hr.; Kühlwein, H. and H. Reichenbach, 1968b]

3. Movement of cells of *Myxococcus virescens* over agar. [8 frames/min. over approximately 1 hr.; Kühlwein, H. and H. Reichenbach, 1968c]

4. Spiral movement characteristic of masses of cells of *Chondromyces apiculatus* on an agar surface. [1 frame/min. over approximately 16 hr.; Kühlwein, H. and H. Reichenbach, 1968d]

5. Aggregation of *Chondromyces apiculatus* as a prelude to fruiting body formation. Note the streams of cells moving into the aggregation center. [1 frame/min. over approximately 13 hr.; Kühlwein, H. and H. Reichenbach, 1968e]

6–8. Side views of the development of the fruiting body of *Chondromyces apiculatus* on a cellophane membrane overlaid on agar. The aggregated spherical mass of cells excretes a slime stalk, which propels the mass of cells upward. The cells within the knob at the top of the stalk differentiate into individual sporangioles containing the myxospores. [4, 8, and 4 frames/min. over 3, 3, and 5 hr.; Kühlwein, H. and H. Reichenbach, 1968f]

9–10. Germination of sporangioles of *Chondromyces apiculatus*. The myxospores of *C. apiculatus* are contained in sporangioles arrayed around the head of the fruiting body. Prior to germination the sporangioles fall off the fruiting body. The sporangiole swells and movement of the cells within the sporangiole becomes discernable. The sporangiole lyses at one end and then at the other, whereupon the cells emerge and constitute a small swarm, leaving the empty hull behind. Note that as the swarm moves, individual cells, which can move faster than the entire swarm, never escape from the swarm. Opposing swarms move toward each other and eventually fuse. The attractive mechanism is unknown. [2 frames/min. over 23.5 hr.; Kühlwein, H. and H. Reichenbach, 1968g]

11. Germination of myxospores of *Myxococcus xanthus*. Unlike the resting cells of *Chondromyces*, which are contained in sporangioles, the myxospores of *Myxococcus* germinate as individual cells. The cells lose their optical refractility and break out of their spore coat, leaving the empty hull behind. The cells elongate, reacquire their characteristic gliding motility and move off.

* The real time of each segment can be calculated from the fact that the film was projected at a speed of 24 frames/second and that the rate at which the frames were taken will be presented for each segment.

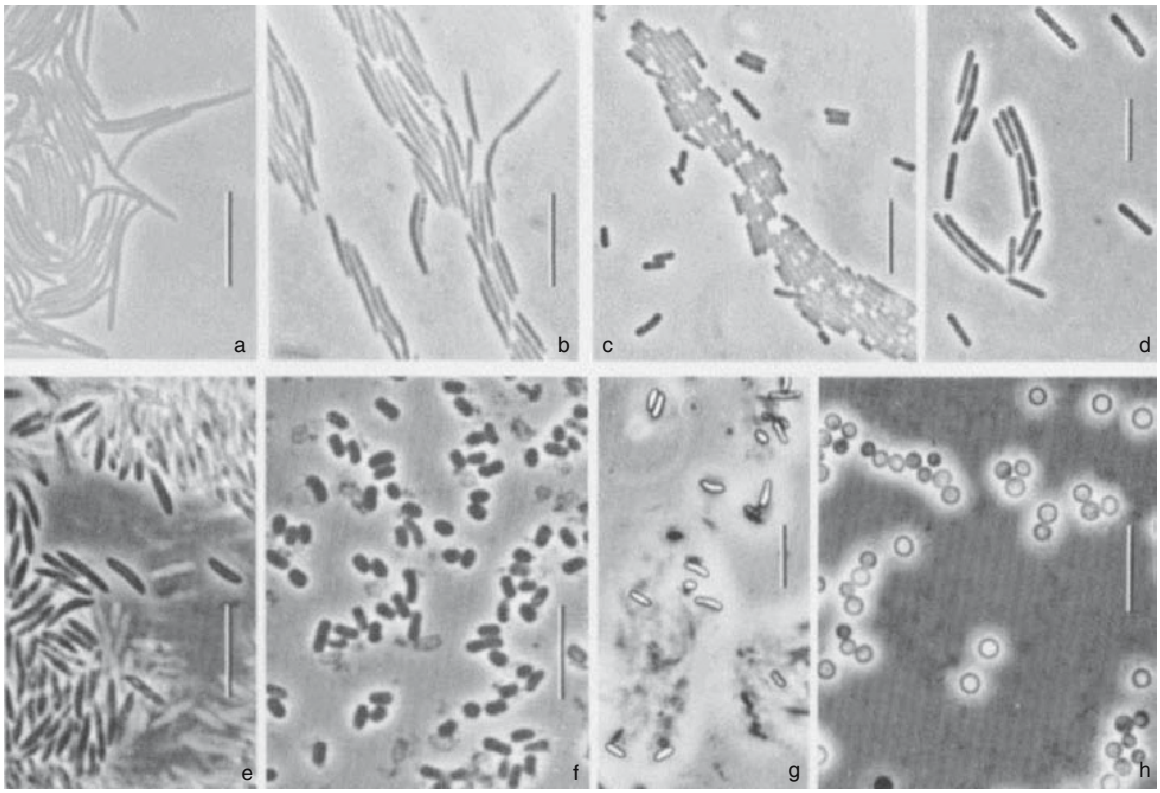


Fig. 1. Various types of myxobacterial cells. (a–d) Vegetative cells. (a and b) The Cystobacterineae type. a) *Cystobacter ferrugineus*, cells from a liquid culture. b) *Stigmatella aurantiaca*, cells in situ on agar surface in a chamber culture. (c and d) The Sorangineae type. c) *Chondromyces crocatus* in a chamber culture. d) *Sorangium compositum* in a chamber culture. Cells of the Nannocystineae resemble those of the Sorangineae. (e–h) Myxospores of members of the Cystobacterineae. e) Myxospores of *Cystobacter ferrugineus* from a crushed, degenerated, *Archangium*-like fruiting body; under oil immersion, the high optical refractility of the myxospores is not apparent. f) Myxospores of *Cystobacter velatus* from a crushed fruiting body sporangiole; oil immersion. g) Experimentally induced myxospores of *Stigmatella aurantiaca* on the agar surface in a chamber culture; under the 40X dry objective, the high optical refractility of the myxospores becomes very conspicuous. And h) myxospores from a *Myxococcus xanthus* fruiting body; the optical refractility of these spherical myxospores is so high that it is recognizable even under oil immersion. All photographs are in phase contrast. Bars = 10 μm.

Several cells can be seen dividing. [2 frames/min. over an approximately 9 hr.; Reichenbach, H. 1966]

12–13. Slime trails of *Chondromyces apiculatus*. The gliding cells leave a visible trail on the agar surface. Cells move slowly when laying the trail, but more rapidly if they move onto a previously laid trail. Cells may join on a trail and move together as a group. [5 frames/min. over a 1 and 2 hr period for *C. apiculatus*; Kühlwein, H. and H. Reichenbach, 1968h]

14–15. Rippling. Cells of the Myxococcaceae characteristically undergo what Reichenbach originally referred to as "rhythmic oscillations." Starting at the center of a swarm of *Chondrococcus coralloides* (now *Corallococcus coralloides*), rhythmically pulsating ripples travel outward. The oscillations periodically stop and start. The phenomenon is shown at a lower and higher magnification. [1 frame/min. for approximately 3 hr and, at a two-fold higher magnification, 8 frames/min. over about 0.5 hr.; Kühlwein, H. and H. Reichenbach, 1968i]

16. Edge of a swarm of *Archangium violaceum*. The cells move in a pack. If a cell occasionally moves away from the pack, it quickly returns. [8 frames/min. over 1.5 hr.; Kühlwein, H. and H. Reichenbach, 1968j]

17. Lysis of bacteria. Living cells of *Sarcina flava* are lysed by *Archangium violaceum*. Note that the cells are lysed at a distance, as evidenced by their loss of phase darkness. Note also that the cells of Archangium seem to head directly for the Sarcina, as if they can detect them at a distance. [4 frames/min. over 3.5 hr.; Reichenbach, H., 1968]

The phylogenetic position of the myxobacteria has been elucidated by a comparison of 16S rRNA (Ludwig et al., 1983; Oyaizu and Woese, 1985) and 16S rRNA gene sequences (Shimkets and Woese, 1992; Sproer et al., 1999). According

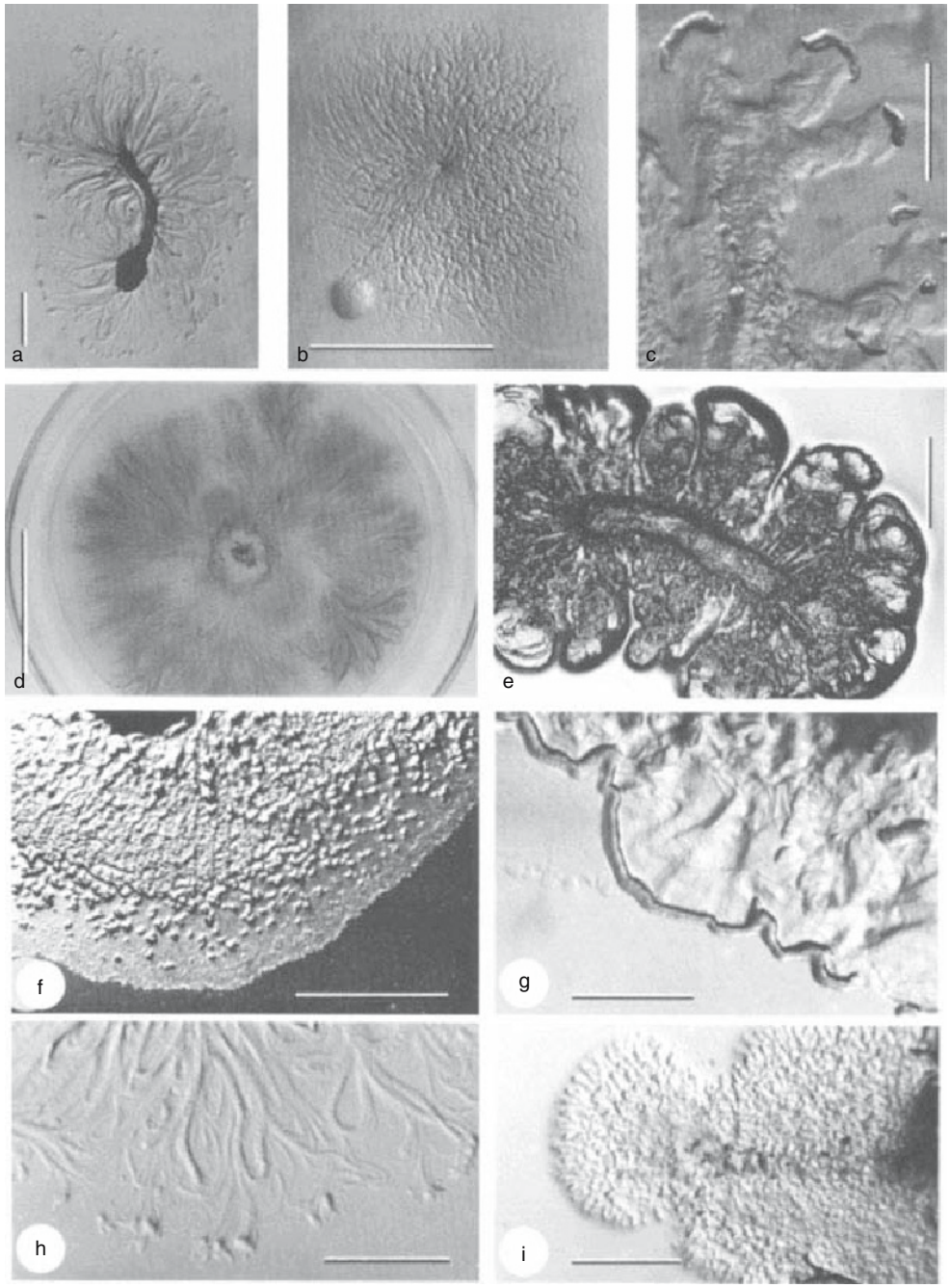


Fig. 2. Swarm colonies of various myxobacteria. (a, b, d, f and h) Swarms of members of the Cystobacterineae. (c, e and g) Swarms of members of the Sorangineae. i) Swarms of members of the Nannocystineae. a) *Stigmatella erecta* on CY agar; dissecting microscope, oblique illumination. Bar = 1 mm. b) *Myxococcus xanthus*, very young colony on Casitone agar; dissecting microscope, oblique illumination. Bar = 0.5 mm. c) *Polyangium* sp. on water agar, swarm edge; the spreading colony falls apart into cell clumps, which separate, thereby etching trenches into the agar surface; dissecting microscope, oblique illumination. Bar = 1 mm. d) *Cystobacter violaceus* (formerly *Archangium violaceum*) on VY/2 agar; from the inoculum in the center, the swarm has spread over the whole agar plate. Bar = 30 mm. e) *Nannocystis exedens* on Casitone agar; the swarm developed from the ridge in the center, and the agar plate has become deeply corroded; Leitz Aristophot. Bar = 5 mm. f) *Corallococcus coralloides* (Reichenbach, 2005) on Casitone agar; the central part of the swarm is covered with rudimentary fruiting bodies; Leitz Aristophot; Bar = 5 mm. g) *Polyangium* sp. on a streak of *Escherichia coli* (which is still recognizable at the left) on water agar; the swarm edge is a compact, ridge-shaped mass of vegetative cells, behind which the agar surface is deeply corroded; dissecting microscope, oblique illumination. Bar = 1 mm. h) *Stigmatella erecta* on Casitone agar, swarm edge with flame-like protrusions; dissecting microscope, oblique illumination. Bar = 1 mm. And i) *Nannocystis exedens* on a streak of *Micrococcus luteus* on water agar; the entire swarm has sunk into the agar, which is deeply corroded; dissecting microscope, oblique illumination. Bar = 2 μm.

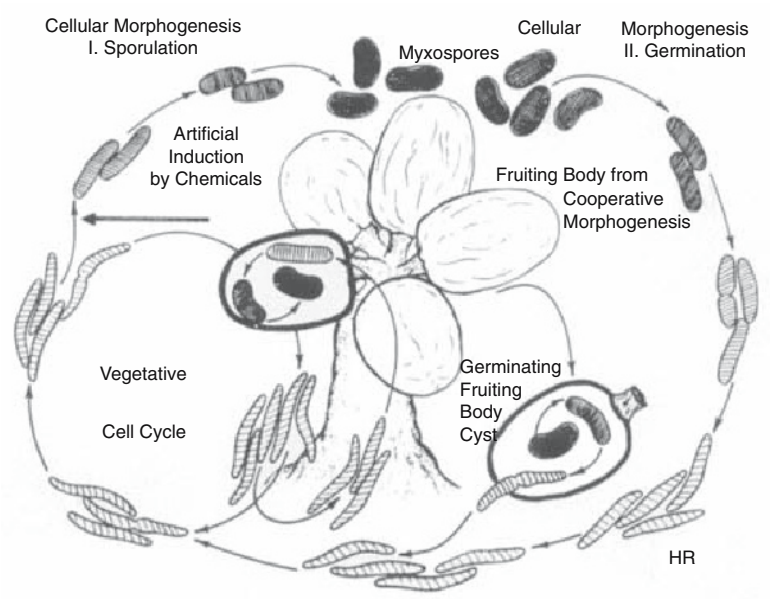


Fig. 3. Cellular morphogenesis in myxobacteria shown with *Stigmatella aurantiaca* as an example. Fruiting body and cells are not drawn to scale. From Gerth and Reichenbach (1978).

to these data, the myxobacteria belong to the Deltaproteobacteria, which also contains the genera *Bdellovibrio*, *Geobacter*, *Pelobacter* (Lonergan et al., 1996), *Anaeromyxobacter* (Sanford et al., 2002) and certain sulfate reducers such as *Desulfovibrio* and *Desulfonema* (Stackebrandt et al., 1988). About 50 species of myxobacteria have been assigned to seventeen different genera (Reichenbach, 2005; see the section Taxonomy of Myxobacteria in this Chapter).

While myxobacterial fruiting bodies had been observed by botanists early in the nineteenth century, they continued to be mistaken for fungi for almost a century. The first report in the scientific literature appears to be by Heinrich Friedrich Link (Link, 1809), who described a little "gasteromycete," *Polyangium vitellinum*, a taxon that is still valid. The description was accompanied by an exact and beautiful illustration (Link, 1809; Ditmar, 1814; see Fig. 14 below). Two more species, *Stigmatella aurantiaca* and *Chondromyces crocatus*, were defined by the British mycologist, M. J. Berkeley, who classified them as hyphomycetes (fungi imperfecti) (Berkeley, 1857). Again, both taxa are still valid. *Stigmatella aurantiaca* was described two more times as a new fungus under different names and with different systematic positions before it was finally recognized as a myxobacterium by the United States botanist Roland Thaxter (1892). Thaxter was the first to elucidate the astonishing and unexpected life cycle of the myxobacteria, which he published, together with descriptions of a substantial number of new species, in a series of brilliant articles (Thaxter, 1892; Thaxter, 1893; T. Thaxter, 1897; Thaxter, 1904). However, the reception by the scientific community was less than enthusiastic, and it took at least 20 years before Thaxter's work became widely accepted. In the first decade of the twentieth century, however, four voluminous studies on myxobacteria were published (Baur, 1905; Quehl, 1906; Vahle, 1910; Kofler, 1913) which deal with many problems that later became important topics of research. One of those four authors later became a very influential geneticist involved in breeding research (Erwin Baur). In the following decades, research was confined mainly to taxonomic, ecological and morphological questions (Jahn, 1911; Jahn, 1924; Krzemieniewska and Krzemieniewski, 1926; 1927a, 1927b; 1930). During that period, new species were discovered and the wide geographical distribution of myxobacteria was recognized. Furthermore, two large subgroups that could be distinguished by the shape of their vegetative cells were recognized (Krzemieniewska and Krzemieniewski, 1928), and some myxobacteria degrade cellulose and could be cultivated on a medium containing only cellulose and a few mineral salts (Imshenetski and Solntseva, 1936; Krzemieniewska and Krzemieniewski, 1937a, 1937b). For some time, cellulose decomposition by myxobacteria was a principal focus of interest, mainly in Russia, where it was studied in the laboratory of A. A. Imschenezki (for a review, see Imshenetski, 1959).

Footnote: In the remainder of the chapter the following abbreviations are used for genera of myxobacteria: *Ar*., *Archangium*; *Cb*., *Cystobacter*; *Cm*., *Chondromyces*; *Cl*., *Corallococcus*; *Ha*., *Haploangium*; *Ko*., *Kofleria*; *Me*., *Melittangium*; *Mx*., *Myxococcus*; *Na*., *Nannocystis*; *Pl*., *Polyangium*; *Sg*., *Stigmatella*; and *So*., *Sorangium*.

Studies utilizing pure cultures of myxobacteria did not become common practice until the middle of the twentieth century because myxobacteria were generally believed to be incapable of growth suspended in liquid media. Until then, physiological studies were tedious and only moderately conclusive, because the myxobacteria grew only as a film along the glass wall of the container and the surface of the liquid. Even under these experimental restrictions some insights were gained into the nutritional requirements of myxobacteria. Norén (1955), Loebeck and Klein (1956), and Mason and Powelson (1958) reported that at least some strains of *Myxococcus* grew well as suspension cultures. Subsequently, many more examples of myxobacteria growing in a dispersed manner have been described. Ironically, it later turned out (Dworkin, 1984) that dispersed growth of *Myxococcus* (*Mx.*) *xanthus* strains had been observed as early as 1948, but this information had never been published (Woods, 1948).

Beginning in the 1950s, the emphasis of myxobacterial research shifted to problems in developmental biology, with *Mx. xanthus* as the preferred subject. A major breakthrough was the discovery by Dworkin and Gibson (1964a) that vegetative cells of *Mx. xanthus* in suspension cultures could be induced to convert into myxospores by the addition of certain chemicals, such as 0.5 M glycerol. This was the first clearly defined experimental system for the investigation of a morphogenetic process in a myxobacterium, and it led to a long series of studies on the morphological, physiological and biochemical events that take place during induced myxospore formation.

Genetic analysis began with the development of systems for the transfer of plasmids, transposons and genes into and between strains of *Mx. xanthus*, based on the use of coliphage P1 (Kaiser and Dworkin, 1975; O'Connor and Zusman, 1983; Shimkets et al., 1983) and *Mx. xanthus* phages Mx4 and Mx8 (Campos et al., 1978; Martin et al., 1978). The availability of these genetic techniques made *Mx. xanthus* accessible as a model system for the study of morphogenetic processes and gliding motility. While these methods are not easily adaptable to other myxobacteria, a more versatile system that uses conjugation with *Escherichia coli* for the transfer of plasmids and transposons has been successfully applied to other myxobacterial genera (Breton et al., 1985). Progress in myxobacterial research has gained considerable momentum. The discovery that many myxobacterial strains produce secondary metabolites, most of which are novel compounds, has stimulated an even broader interest in myxobacterial biochemistry, physiology and taxonomy (for reviews, see Reichenbach and Höfle [1993] and Reichenbach [2001]).

Our knowledge of the myxobacteria has been summarized repeatedly in recent years. Apart from the articles mentioned above, the following reviews should be consulted for further details: 1) for books, Rosenberg (1984), Dworkin and Kaiser (1993), and Brun and Shimkets (1999); 2) for taxonomy, Reichenbach (2004); 3) for developmental biology, Shimkets (1990b), Shimkets (1992), Shimkets (1999), Zusman (1991), Kaiser (1993), Kaiser (1996), Kaiser (1998), Kaiser (1999), Kaiser (2001), Dworkin (1996), Crawford and Shimkets (1999), and Bonner and Shimkets (2001); 4) for genomics, Shimkets (1997a), Shimkets (1997b), and Ryding and Shimkets (2000); 5) for motility, Shi and Zusman (1993a), Hartzell and Youderian (1995), Youderian (1998), Ward and Zusman (1999), Kaiser (2000a), and Kearns and Shimkets (2001a). In addition, several movies are available from the Institut für den Wissenschaftlichen Film, Göttingen, Germany, that illustrate cell behavior, colony formation, and developmental processes in various species of myxobacteria (Reichenbach et al., 1965d; Reichenbach et al., 1965e; Reichenbach et al., 1965f; Reichenbach et al., 1965g; Reichenbach et al., 1980; Grimm et al., 1971; Kühlwein et al., 1971a; Kühlwein et al., 1971b).

Myxobacterial Ecology

The essential questions with regard to the ecology of any particular group of organisms are: Where are they? What is there? What do they do? How do their characteristic biochemical, physiological and developmental properties relate to their behavior in nature? With what do they interact?

Habitats

With regard to the first two questions—Where are they? and What is there?, a great deal of anecdotal and subjective information has been compiled on the habitats of the myxobacteria (e.g., Dawid, 2000; Reichenbach and Dworkin, 1992) since their recognition as bacteria by Roland Thaxter in 1892. Until recently (Dawid, 2000), however, these data have not been subjected to any sort of systematic analysis.

In addition, a number of other factors have made it difficult to draw useful quantitative descriptions. In view of the multicellular nature of the fruiting body, which may contain 10^4–10^6 resting cells, it is difficult, if not impossible, to determine the number of myxobacteria in a natural sample. Does a sample that contains one fruiting body represent 1 or 10^4–10^6 organisms?

Table 1. Convenient and preferential sources for the isolation of different myxobacteria.[a]

Substrate	Myxobacterial species typically found[b]
Soil	*Nannocystis exedens*,[5] *Sorangium cellulosum*,[4] *Archangium serpens*,[4] *Corallococcus coralloides*,[4] *Polyangium* spp.,[3] *Cystobacter* spp.,[3] *Melittangium* spp.,[3] *Myxococcus fulvus*,[2] *Mx. virescens*,[2] and *Mx. stipitatus*[2]
Dung of herbivores	*Myxococcus fulvus*,[5] *Corallococcus coralloides*,[5] *Mx. virescens*,[4] *Cystobacter fuscus*,[4] *Cb. ferrugineus*,[4] *Archangium serpens*,[4] *Nannocystis exedens*,[3] *Cb. violaceus*,[3] *Polyangium* spp.,[3] *Stigmatella erecta*,[2] *Mx. xanthus*,[2] *Melittangium* spp.,[2] and *Cb. velatus*[1]
Bark and rotting wood	*Stigmatella aurantiaca*,[4] *Chondromyces apiculatus*,[4] *Sorangium cellulosum*,[4] *Corallococcus coralloides*,[4] *Myxococcus fulvus*,[3] *Cm. pediculatus*,[2] and *Haploangium* spp.[2]

[a]The frequency of myxobacteria on a specific substrate may vary substantially in different environments. Furthermore, some species may be underestimated from a particular source because the isolation technique usually applied may not result in the isolation of a particular organism.

[b]Frequency of the encountered species: [5], ubiquitous; [4], very frequent; [3], moderately frequent; [2], relatively rare; and [1], rare.

The principal habitats of myxobacteria are soil, dung, decaying plant material, and the bark of living and dead trees. Nevertheless, myxobacteria have been isolated from marine environments as well as from a variety of climatic zones (Dawid, 2000). However, since the myxospores may tolerate considerable environmental extremes, and since most isolation techniques involve the cultivation of samples that have been extensively dried, it has not been possible to determine, in many cases, whether the myxobacteria were there adventitiously and simply survived as spores or were growing and thriving in that environment (Reichenbach, 1999). In a few cases, the isolated organisms were tested for their ability to grow under conditions similar to those in which they were isolated. Occasionally, they were able to (Dawid et al., 1988) but more often were not (Rückert, 1983). Iizuka et al. (1998) have isolated two myxobacteria from marine environments, which have been subsequently characterized and assigned to the new genus *Haliangium* (Fudou et al., 2002). The organisms were reported to require 2% NaCl for their growth. However, since this measurement was made on the basis of colony swarm expansion rather than growth per se, the claim must be viewed with caution.

A survey of common sources for different myxobacteria is given in Table 1. Most myxobacteria appear to be primarily soil organisms. However, almost all are "micropredators" (Singh, 1947) and are attracted by habitats with rich microbial communities, which they may colonize as secondary substrates. Even if certain myxobacteria can be isolated more easily from sources other than soil, their presence in the soil of the same environment can often be demonstrated. The preferred substrate of a particular species may vary under different climatic and edaphic conditions. Thus, in the central United States, *Chondromyces* species are common inhabitants of bark and rotting wood but are rare in soil (Nellis and Garner, 1964; H. Reichenbach, unpublished observations). In India, they are found regularly in soil, in the rhizosphere of plants, and on rotting wood, bark, and dung (Agnihothrudu et al., 1959; Singh and Singh, 1971). In Europe, in spite of apparently similar climatic conditions to those found in the United States, they appear to be extremely rare organisms and have been found almost exclusively on dung, rotting wood, and bark (Zukal, 1896; Quehl, 1906; Jahn, 1924; Krzemieniewska and Krzemieniewski, 1946). Dawid (1979) tested several thousand samples from the Siebengebirge, a mountain ridge near Bonn, Germany, and obtained only one isolate of *Chondromyces*, a strain of *Cm. apiculatus*, from a piece of rotting wood. Similar results have been obtained by Krzemieniewska and Krzemieniewski (1946) and by one of us (H. Reichenbach) who isolated myxobacteria over decades from all kinds of samples collected at many different sites in Europe.

Myxobacteria are very common in soils of neutral or slightly alkaline pH (Krzemieniewska and Krzemieniewski, 1927b; Norén, 1950; Norén, 1952; Sabados-Saric, 1957; Kühlwein, 1960; Brockman and Boyd, 1963; Peterson, 1965; Peterson and Norén, 1967; Michoustine, 1968; McCurdy, 1969b; Rückert, 1975b; Rückert, 1976; Rückert, 1979; Brockman, 1976; Hook, 1977; Rückert and Heym, 1977; Dawid, 1978; Dawid, 1979). They have been isolated from samples collected in tropical rain forests and in the arctic tundra, in steppes, deserts, and in bogs, at sea level as well as at high altitudes. Warm and seasonally dry areas, such as the southwestern United States, Mexico, northern India, the Mediterranean countries, and the Canary Islands, have been found to be especially rich in different myxobacteria. The same is true for areas with underground limestone in temperate climates. Still, a myxobacterial species may show an unequal distribution even within the same climatic zone. The case of *Chondromyces* has just been mentioned. Another example is *Sg. auran-*

tiaca, which is common in the middle of the North American continent (Nellis and Garner, 1964; McCurdy, 1969b; Reichenbach and Dworkin, 1969a), but very rare in Europe (Krzemieniewska and Krzemieniewski [1946] isolated myxobacteria in Europe 15 years before they found their first strain of *Sg. aurantiaca*). The decisive factor that controls the distribution may be the warm, humid American summers, which are usually in contrast to those in Europe. For unknown reasons, *Sg. aurantiaca* seems to be restricted to rotting wood and bark on both continents. Locations with acid soils and raw humus underground, such as the *Rhododendron* forests of West Virginia (H. Reichenbach, unpublished observations), can be totally devoid of myxobacteria. But under otherwise favorable conditions such as sufficiently high humidity and temperature, myxobacteria have also been isolated from soils with a bulk pH of below 5 (Rückert, 1975b; Rückert, 1979). In cool, humid environments, such as in Finland, northern Minnesota, and mountain ranges, myxobacteria may be abundant, albeit limited to a few species. Tropical rain forests are not necessarily rich in myxobacteria, perhaps owing to the nature of the underground material. Myxobacteria have also been isolated from Antarctic soil samples but at least in one case, the organisms had a temperature optimum around 30°C, which raises some doubts as to whether they were really indigenous (Rückert, 1985). In another instance, however, the isolated bacteria were true psychrophiles that developed, very slowly indeed, at temperatures between 4 and 9°C but did not grow at 18°C (Dawid et al., 1988). While the vegetative stages of the latter organisms closely resembled those of myxobacteria of the *Polyangium* and *Nannocystis* type, their identity could not be established with certainty since they did not produce fruiting bodies. No mesophilic myxobacteria were found in the Antarctic samples examined by Dawid et al. (1988).

Dung of various animals, especially of herbivores such as rabbits, hares, deer, moose, sheep and goats, is an excellent substrate for myxobacteria. Rabbit dung was the preferred source of the early myxobacteria investigators and appears to serve as natural bait. Apparently, however, myxobacteria can also pass unscathed through the digestive tract of animals (Kühlwein, 1950). Also, the observation of myxobacteria on dung pellets collected on the surface of deep snow layers suggests that, occasionally, they were deposited with the dung (Rückert, 1975b; H. Reichenbach, unpublished observations). However, the organisms primarily appear to arise in the surrounding soil after the dung has been dropped. This conclusion is based on the fact that aged dung is a better source of myxobacteria than is fresh dung, that dung collected on soil rich in myxobacteria results in a greater yield of strains than one from poor soil, and that the same species found on dung can also be found in the surrounding soil. A number of myxobacteria are regularly found on dung, such as *Mx. fulvus*, *Mx. virescens*, *Cl. coralloides*, *Cb. fuscus* or *Sg. erecta*, but no myxobacterium depends on dung-derived growth factors, as was thought for some time, and all strains isolated from dung can be cultivated on relatively simple media. Of course, occasional stimulation by substances such as vitamins found in dung extracts is conceivable.

Bark and rotting wood are good sources of certain myxobacteria. This includes the cellulose degraders of the genus *Sorangium*, but several noncellulolytic species are also regularly found in those habitats. Results vary with different species of trees: In Europe, relatively good yields have been reported with bark from living elder (*Sambus racemosa*), beech (*Fagus silvatia*), and black locust (*Robinia pseudacacia*; Dawid, 1979). In Minnesota and other locations in the United States, bark and rotting wood are reliable sources for *Stigmatella aurantiaca* and *Chondromyces apiculatus*. The latter has also been found regularly on wood samples from southeast Asia and on dry, rotten "leaves" of *Opuntia* cacti from the Canary Islands (H. Reichenbach, unpublished observations). The difference from the situation in Europe has already been pointed out. Clearly, other less obvious factors, perhaps climatic ones, also play a role in the distribution of myxobacteria. However, probably no myxobacteria have an absolute dependence on bark and wood. The cellulose degraders are also common in soil and dung, and because the other species are bacteriolytic, they often grow well on dung and in culture on media that contain peptone or protein. In fact, why in nature they grow preferentially on wood and bark is not understood. Even when they are found on dung, they may be there mainly because the dung of herbivores is always abundant in plant residues. However, *Haploangium* has only been found on bark and wood so far. It has never been cultivated, so its nutritional requirements are unknown.

Although myxobacteria have repeatedly been isolated from freshwater habitats (Geitler, 1925; Jeffers, 1964; Brauss et al., 1967; Brauss et al., 1968; Gräf, 1975; Hook, 1977; Trzilová et al., 1980; Trzilová et al., 1981), they probably are merely washed in from the soil. Myxobacteria have been used to classify rivers for the type of pollution present (Gräf, 1975; Trzilová et al., 1980; Trzilová et al., 1981). While myxobacteria appear not to be typical aquatic organisms, our experience with cultivated strains suggests that they can survive and thrive in suitable aquatic

niches. A clear indication of this is the case of a myxobacterium that parasitizes the green alga *Cladophora*, with fruiting body formation inside the emptied-out algal cells (Geitler, 1925). Another example may be the occurrence of structures resembling myxobacterial fruiting bodies on eggs of the water beetle *Dytiscus marginalis* (Jackson, 1959).

Dawid (2000) has compiled data on myxobacteria isolated from 1398 samples collected over an 18-year period from all continents. Representatives of 30 species and 11 of the 12 known genera of myxobacteria were isolated (only *Haploangium* was not). Approximately 20,000 strains representing 17 species were isolated from a limited area in Germany alone. Among the 55 nations and 9 states of the United States, myxobacteria were ubiquitous. The genera most frequently encountered were *Myxococcus*, *Corallococcus* (Reichenbach, 2005) and *Archangium*. Whether this reflects their true predominance or the bias of the isolation procedure is unclear.

Myxobacteria were found most commonly in environments with pH 6–8.

The search for new organisms able to produce novel and useful bioactive compounds has stimulated attempts to isolate bona fide marine myxobacteria. While isolation of myxobacteria from marine environments has been reported (Brockman, 1967; Rückert, 1975c; Dawid, 1979), in no case have these authors claimed that the organisms require or even can grow in the presence of the concentration of NaCl found in seawater. Several recent reports show strains with moderate halotolerance (Iizuka et al., 1998; Iizuka et al., 2003a; Iizuka et al., 2003b; Li et al., 2002).

A most unusual myxobacterium, *Anaeromyxobacter dehalogenans*, which is outside the bounds of what is usually defined as a myxobacterium, has recently been described (Sanford et al., 2002). This organism is an anaerobe that uses acetate as an electron donor and a variety of chlorinated phenols as electron acceptors. While it has not been shown to produce fruiting bodies, it is a characteristically pigmented, Gram-negative rod that moves by means of gliding motility. The result of 16S rRNA/DNA analysis places it within the Myxococcales as a separate genus. Clearly, the taxonomic and phylogenetic boundaries of the myxobacteria have not yet been reached or even defined.

Ecologically Relevant Characteristics of Myxobacteria

The features that are characteristic of the myxobacteria are gliding motility, a complex life cycle culminating in the formation of fruiting bodies and myxospores, the ability to degrade insoluble macromolecules, and the production of an extensive battery of bioactive compounds. Each of these properties plays an important role in establishing the particular ecological function of the myxobacteria.

Degradation of Insoluble Macromolecules

In the grand scheme of molecular turnover in nature, bacteria play the major role. And myxobacteria have long been known to play an important part in the degradation of such insoluble macromolecules as protein, cellulose, peptidoglycan, lipids and nucleic acids as well as intact cells, both eukaryotic and prokaryotic, and their subcellular fragments (see the section Lysis of Other Bacteria in this Chapter). They do so by excreting a powerful battery of hydrolytic enzymes. As such, however, they are at the mercy of the diluting effect of diffusion of their enzymes away from the cell and the effect of hydrolyzed products diffusing back toward the cell. Thus, the suggestion on theoretical grounds that *Mx. xanthus* when feeding on macromolecules behaves as a bacterial "wolf pack" (Dworkin, 1973a), seeking at all times to optimize its feeding by maintaining a high cell density, has been experimentally demonstrated: *Mx. xanthus* can feed on hydrolyzed protein quite efficiently at a low cell density but can only utilize the equivalent high molecular weight protein when at a high cell density (Rosenberg et al., 1977). The characteristic swarms of migrating cells accomplish this, and the fruiting body may be a mechanism for maintaining the resting myxospores at a high cell density in preparation for future germination and feeding.

The Function of Gliding Motility

Myxobacteria exist in nature on surfaces—soil particles, leaf surfaces, bark and other detritus. Thus, flagellar motility through an aqueous milieu is likely to be of little value. The ability to crawl over a surface, on the other hand, seems well suited for the myxobacterial life style. Thus, their gliding motility is consistent with their ecological niche.

Developmental Aspects of Myxobacterial Ecology

The myxobacteria, with the exception of the taxonomically problematic *Anaeromyxobacter dehalogenans*, are aerobes and are found on surfaces or close to the surface of soil. As such, they are exposed to the vicissitudes of desiccation, irradiation and temperature extremes. Myxospores

while do not possess the extraordinary resistance of endospores, but they are sufficiently resistant (Sudo and Dworkin, 1969) to withstand the kinds of extremes they are likely to encounter in normal environmental situations. The fruiting body can be assumed to maintain the resting cells packed closely enough together so that upon germination they can begin feeding at the optimally high cell density. The sporangioles, whether multiple on a stalk (as in *Stigmatella* or *Chondromyces*), or single on a stalk (as in *Haploangium* or *Melittangium*), or lying on the substrate (as in *Cystobacter*, *Polyangium*, *Sorangium* or *Nannocystis*), may be simply an optimal packaging mechanism of the resting myxospores.

Myxobacterial Bioactive Compounds

The myxobacteria produce an astounding variety of bioactive compounds (Reichenbach, 1989; Reichenbach, 1993; Reichenbach, 2001; Reichenbach and Höfle, 1998). Among over 2000 bacteriolytic strains of myxobacteria, 55% produce bioactive compounds; among over 700 cellulolytic myxobacteria, 95% produce bioactive compounds (Dawid, 2000). These include inhibitors of protein synthesis as well as compounds interfering with electron transport, nucleic acid synthesis, and actin and tubulin synthesis. Their structures include polyketides, linear and cyclic peptides and heterocyclic molecules. Clearly, when the group is fully characterized, the spectrum and number of bioactive compounds produced will rival those produced by the Actinomycetes.

The biological function of antibiotics is equivocal. While antibiotics might assist a soil organism in its struggle for nutritional "lebensraum," the matter has not been settled. How then do the myxobacteria interact with other myxobacteria and other bacteria in nature?

Lytic Activities

The ability of the myxobacteria to kill and lyse a wide variety of Gram-positive and Gram-negative bacteria, yeast, fungi, algae and protozoa was noticed early and often and came to be considered as a characteristic property of the group (see the section Lysis of Other Bacteria in this Chapter). Thus they might reasonably play some sort of role in nature, either to control other microbial populations or to clear the soil of the carcasses of other dead or dying cells. In fact, Callao et al. (1966) demonstrated that after *Mx. xanthus* and *Azotobacter* were simultaneously inoculated into soil, the number of *Azotobacter* decreased substantially.

Symbiosis

Myxobacteria have been shown to exist in a close symbiotic association with other bacteria. Early on, Pinoy (1913) demonstrated that a culture of *Cm. crocatus*, which constructs the most complex of all the myxobacterial fruiting bodies, existed in a close association with a non-myxobacterial companion. This matter was more carefully examined and defined by Jacobi et al. (1997), who showed that the growth of almost all strains of *Cm. crocatus* examined depended on a small rod-shaped bacterium tentatively identified as belonging to the genus *Sphingobacterium* present in the sporangioles of the fruiting body.

Myxobacterial Interactions and Territoriality

When observing the multicellular behavior of the myxobacteria, an obvious question is how able or inclined they are to interact with other myxobacteria. Will related species form mixed fruiting bodies? Will their migrating swarms fuse? And if not, what is the basis upon which the myxobacterial cells distinguish between self and non-self? Do the myxobacteria establish territories? These questions were examined by Smith and Dworkin, who observed the interactions between feeding and developing swarms of *Mx. xanthus* and *Mx. virescens* (Smith and Dworkin, 1994).

A common observation in natural samples of myxobacterial fruiting bodies is that the localized areas in which they occur contain only one species of fruiting body, i.e., the myxobacteria do indeed seem to establish an exclusive territory. When cultures of *Mx. xanthus* and *Mx. virescens* were mixed and placed under fruiting conditions, the cells sorted themselves out and established separate fruiting body territories. And the contents of the individual fruiting bodies were limited to a single species. Furthermore, *Mx. virescens* was able to produce an extracellular material that, when applied to *Mx. xanthus*, caused spheroplasting and loss of viability. The toxic material had no effect on other bacteria and its properties were consistent with those of bacteriocins, which have previously been described for the myxobacteria (McCurdy, 1974). Thus, the myxobacteria do establish territoriality, the cells of different species do not mix, and one mechanism of exclusion may be myxobacterial bacteriocins (Smith and Dworkin, 1994).

Isolation

Although myxobacteria are common in many types of soil (a teaspoonful of soil is usually sufficient to isolate four or five species), they are

rarely mentioned in articles on soil microbiology. The reason is that the usual dilution and plating techniques used for the isolation of soil microorganisms are unlikely to reveal the presence of myxobacteria. Owing to the slime matrix they produce, myxobacterial cells do not disperse easily when the soil sample is shaken in water, so that the organisms are highly underrepresented in number when the suspension is plated. Even when other, more suitable methods are used, the estimated numbers probably are only approximate ones; depending on the type of soil, they are in the range of 10^3 to 4.5×10^5/g and thus under favorable conditions seem to be a rather substantial component (Singh, 1947; Sabados-Saric, 1957; McCurdy, 1969b). Another problem is that on lean media the myxobacterial colonies develop as easily overlooked delicate, spreading swarms, while on rich media they remain compact and are not recognized as myxobacteria. In addition, they grow relatively slowly so they are often overgrown by other soil microorganisms, particularly fungi. The fruiting bodies, however, are conspicuous and have probably been frequently observed by soil biologists. The use of available nucleotide probes and fluorescent in situ hybridization techniques should make it possible now to arrive at a more realistic appraisal of myxobacterial numbers.

The isolation of myxobacteria can start from fruiting bodies that have developed on natural substrates or from swarms growing in agar cultures. These isolations can also be preceded by enrichment and baiting techniques. The methods have frequently been reviewed (Kühlwein and Reichenbach, 1965; McCurdy, 1969b; Peterson, 1969).

Collection and Treatment of Samples

Soil to be used for the isolation of myxobacteria should be taken from the upper few centimeters of the soil profile. Soils rich in higher organisms, and samples collected from between plant roots and near the base of stems, give the best yields. If the material cannot be processed soon after collection, it should be air-dried as quickly as possible because otherwise the sample may become moldy, creating problems later during isolation. A few cubic centimeters of soil are sufficient for most isolation techniques. In general, it is better to have several small samples from different localities than one large batch from a single spot. Dry material can be stored for long periods at room temperature. Apparently, in the natural substrate, myxobacteria are much more stable than, for example, fruiting bodies dried on filter paper. Thus, we have found essentially the same species over a 12-year period of sampling of stored soil samples, and we are regularly able to isolate myxobacteria from samples stored 10–15 years (H. Reichenbach and M. Dworkin, unpublished observations).

With dung, better results are usually obtained if the samples are neither very fresh nor very old, but are completely free of low-molecular weight components. It is very important to dry the samples if they have to be stored for more than one day, and such dry dung will yield myxobacteria even after months and years of storage. Rabbit dung to be used for baiting (see below) should be taken from wild animals; pellets from laboratory or domestic rabbits are usually unsuitable, perhaps because they become soaked with urine in the cage.

Bark and wood for the isolation of myxobacteria must be taken from tree species that are low in resins and tannin compounds, and specimens have to be quickly dried if they are not to be processed immediately. Successful isolations are often obtained with wood that has already been decomposed to a rather soft state and with bark from the base of the tree and from fallen trees.

Direct Isolation from Natural Substrates

Isolation can often be achieved directly from the natural substrate. Bark of living or dead trees, rotting wood, or dung pellets are kept in large Petri dishes lined with two to three layers of filter paper. The samples are soaked in distilled water containing cycloheximide (up to 0.08 mg/ml) for a few hours to suppress the growth of molds. The water is then decanted and appropriate amounts of water are added at intervals during cultivation. If the culture is kept too dry, it may soon be covered with molds; if the substrates are inundated, no myxobacterial fruiting bodies will develop. The air in the incubator should be kept sufficiently humid so that the crude cultures do not dry out too quickly. The initially saturated system is allowed to dry out gradually over 14–21 days; in this way it will eventually pass through the optimum for the development of myxobacterial fruiting bodies. Such crude cultures are likely to contain a variety of fungi, mites, springtails, nematodes, and other organisms that may contaminate other cultures. The danger of a contamination with mites is especially serious and can quickly spoil a culture collection. The cultures should therefore be kept in tightly closed plastic bags or, more conveniently, in a separate incubator, which should frequently be sterilized.

The cultures can be incubated at room temperature (around 20°C) or at 30°C. We have never observed unequivocal differences in the yield of myxobacteria when parallel crude cultures were kept at different temperatures, but we have found that at room temperature, the development

of myxobacteria proceeds more slowly and molds tend to spread more vigorously. Thus, we keep such cultures at 30°C (see also Krzemieniewska and Krzemieniewski, 1927b). However, samples from mountains at high altitudes or from arctic environments may contain myxobacteria that are adapted to lower temperatures (see the section Habitats in this Chapter), and even in forest soils in Central Europe, strains are occasionally found that grow reasonably well at 30°C but produce fruiting bodies only at lower temperatures.

Initially, the crude cultures should be inspected for fruiting bodies every day, beginning with the third or fourth day of incubation, because fruiting bodies often spring up and then rapidly collapse and become very inconspicuous. Also, the fruiting bodies may soon become overgrown by other organisms. Most fruiting bodies will appear during the first 10 days, either on the substrate itself or on the filter paper lining; only rarely will anything of interest develop later than 18–20 days. Sometimes, fruiting bodies appear very early on the substrate, within hours after moistening; probably, in such cases, desiccated structures have simply been rehydrated and thus have become recognizable, while the fruiting bodies that appear later are produced de novo. In the later phases of culture development, spherical or ridge-shaped masses of vegetative cells, which are usually bright yellow, orange, or red, can often be observed on the filter paper and can be used for isolation; in this way one may obtain species whose fruiting bodies are too small and inconspicuous to be recognized directly on the substrate. These organisms are almost always members of the Sorangineae, usually *Polyangium* and *Nannocystis* strains, which produce swarms with a massive ridge at the edge. Sometimes, cellulose decomposers may develop on the filter paper lining itself.

Isolation from Soil by Baiting

A baiting technique can be used to isolate myxobacteria from soil (Krzemieniewska and Krzemieniewski, 1926). A large Petri dish is filled with soil, which is moistened with distilled water up to its water-holding capacity. Autoclaved dung pellets from wild rabbits are partly buried in the soil. The culture is then incubated and examined as described above. Vegetative cells will migrate to the dung pellets, colonize them, and in time develop the characteristic fruiting bodies. Here, as with the technique described above, the discovery of the myxobacterial fruiting bodies is greatly facilitated by their tendency to appear locally in large numbers and by their brightly colored and glistening appearance. With some experience, they can be located quickly with the unaided eye, despite their relatively small size (50–500 µm). For a more careful examination of the culture, a dissecting microscope with incident illumination and a magnification between 10 and 40X is perfectly adequate (see Fig. 2).

Isolation from Swarms

The bacteriolytic properties of myxobacteria can be used for their enrichment from natural substrates, particularly from soil and plant debris. Streaks of living food organisms (e.g., *E. coli*) on water agar (WAT agar, see below), in the form of a cross-streak, three parallel streaks, or a number of circular patches, are inoculated with a small quantity (approximately the size of a lentil) of the material to be analyzed (Singh, 1947). The cross and the patches are inoculated in the center, and the parallel streaks at the ends. Care should be taken not to scatter the inoculum over the plate. To facilitate application of the sample, the soil may first be moistened with sterile water and then applied with sterile swabs.

To restrict the development of fungi, the addition of the antibiotic cycloheximide (25–100 µg/ml) to the enrichment medium has been recommended (WCX agar, see below; Brockman and Boyd, 1963). Results are indeed superior when cycloheximide is included, and the cultures can be maintained for a longer period. The antibiotic does not control the growth of amoebae, a major nuisance with this isolation technique. Also, it does not result in the selection of particular myxobacteria or in the suppression of any other gliding bacteria, such as *Cytophaga*-like bacteria, flexibacters, *Taxeobacter*, or *Herpetosiphon*, which can also be isolated with this method.

WAT Agar and WCX Agar

$CaCl_2 \cdot 2H_2O$	0.1% (w/v)
Agar	1.5% (w/v)
HEPES	20 mM

Prepare media in distilled water and adjust pH to 7.2. Autoclave the medium. Then, add 25 mg of cycloheximide per ml from a filter-sterilized stock solution, yielding WCX Agar. As the pH is very difficult to adjust in this completely unbuffered medium, addition of, e.g., 20 µM *N*-2-(hydroxy-ethyl)piperazine-*N*′-2-ethane-sulfonic acid (HEPES), is advisable.

Many bacteria and yeasts can be used as food organisms by the myxobacteria. Food organisms that are readily recognized and can be easily eliminated later are, of course, preferred. Selection for myxobacteria works better if the prey microbes used are alive rather than dead. In the first study, an *Aerobacter* strain was used as the food organism (Singh, 1947). We have found the following bacteria to be useful: 1) *Micrococcus luteus* is easy to distinguish, is nonmotile, and is relatively large, so that it is not readily spread

around; while it is not well utilized by soil amoebae, not all strains of myxobacteria, specifically *Nannocystis* strains, grow on it. 2) *Escherichia coli* is a favorable substrate for myxobacteria, including *Nannocystis*, which can be subcultured indefinitely on it; but soil amoebae also use it well, and this sometimes makes *E. coli* inconvenient. 3) Autoclaved yeast, *Saccharomyces cerevisiae*, can be cheaply prepared from commercial bakers' yeast; the pH of the suspension must be adjusted to 7.0, since the yeast cake is usually acidic; unfortunately, the autoclaved yeast is also a good substrate for many contaminants. Generally, living *E. coli* is our preferred food organism.

The cultures are incubated at 30°C and checked under a dissecting microscope for the appearance of myxobacterial swarms and fruiting bodies, at first daily, beginning on the second or third day, and later at longer intervals (Fig. 2). After about 3 weeks, no additional myxobacteria are likely to appear, and the cultures can be discarded. Most myxobacteria appear within 8–14 days. The myxobacteria grow first on the lawn of the food organism, but later often spread over the agar surface between the smears. A fast-spreading organism such as *Cystobacter* may reach the end of a streak within 1–2 days. If two streaks are made in a crossed pattern, different myxobacteria may develop on each of the four arms. Different organisms also may follow one another on the same streak. As a rule, species of the Cystobacterineae are seen first, while the Sorangineae develop later. Between two and five different myxobacteria can be expected on one plate. Three parallel plates are usually sufficient to secure most of the myxobacterial types that are readily isolated from a sample, with the exception of the cellulose degraders. Fruiting bodies are often produced within the swarms after a couple of days. Fruiting bodies may also appear on the soil crumbs or other particles of the inoculum. To detect the delicate, film-like, swarms of myxobacteria, one must use a dissecting microscope with a tilting mirror so that light can be applied at a shallow angle to the surface of the plate.

The sooner a swarm is recognized, the more easily a myxobacterium can be isolated, as at the beginning, the contaminants may still be confined to the site of the inoculum. Transfers are then made from the swarm edge, which is usually the purest part of the colony.

A disadvantage of isolating myxobacteria from the swarm stage is that most species cannot be identified by their swarm morphology. With some experience, however, at least certain genera or types can be differentiated (Fig. 2). The ubiquitous *Nannocystis*, for example, produces a network of trails outside the streak of the food organism. These trails are deeply etched into the agar and end with a small cluster of cells. On the streak, there is often a heavy, meandering ridge. *Polyangium* shows a similar pattern, but with coarser structures. Swarms with an edge that consists of a massive, often brightly colored ridge are in general typical for the suborder Sorangineae. *Cystobacter* and *Archangium* swarms are usually tough slime sheets with a pattern of delicate, but conspicuous, branched, radiating veins. The swarms of the Myxococcaceae tend to be smooth, soft-slimy sheets, often with dense fields of tiny waves or ripples. If there are radial veins, they typically are wavy and meandering. Swarms of *Mx. stipitatus* show a bright yellow fluorescence in ultraviolet (UV) light of 366-nm wavelength (Lampky and Brockman, 1977). The shape of the vegetative cells under the microscope may also provide a clue to the identification of the swarm observed.

Isolation by Direct Plating of Heated and Dried Soil on Antibiotic-containing Media

Karwowski et al. (1996) have described a method for the isolation of *Myxococcus* from soil by plating treated soil samples directly on a medium containing antibiotics.

Soil was air-dried at room temperature overnight, diluted in sterile distilled water, heated at 56°C for 10 min and then plated on CY-C10 medium. (CY-C10 medium is CY medium with 1% Casitone instead of 0.3% and with 50 μg of cycloheximide/ml.) Vancomycin, ristocetin or tiacumycin B at 10 μg/ml was also included. The combination of heat, desiccation and antibiotics killed or inhibited the growth of many non-myxobacterial cells, and the higher concentration of Casitone inhibited excessive swarming of the myxobacteria, while still allowing their colonies to be recognized. Colonies were picked and plated on media allowing fruiting body formation, and from these plates the final purification and identification were achieved. This approach was effective for isolating *Myxococcus*.

Isolation of Cellulose Decomposers

For the isolation of cellulose-degrading myxobacteria of the genus *Sorangium*, a mineral agar with cellulose as the only carbon source is used. While NH_4^+ is an excellent nitrogen source for *Sorangium*, much better results are obtained when NO_3^- is used in the isolation medium, as in ST21 agar. The cellulose is applied in the form of sterile filter paper, which is placed on top of the agar surface. Since the cultures must be incubated for a longer time before the cellulose decomposers can be recognized, inclusion of cycloheximide in the medium is essential.

ST21 Agar and ST21CX Agar

Solution A:

K_2HPO_4	0.1% (w/v)
Yeast extract (Difco)	0.002% (w/v)
Agar	1% (w/v)

Make up in about two-thirds of the water volume in distilled water.

Solution B:

KNO_3	0.1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
$CaCl_2 \cdot 2H_2O$	0.1% (w/v)
$FeCl_3$	0.02% (w/v)
$MnSO_4 \cdot 7H_2O$	0.01% (w/v)

Make up in the remaining water volume. Autoclave separately. Combine solutions A and B and add 1 ml of trace element solution (see below) per liter of medium. For crude and enrichment cultures, add 25 mg of cycloheximide per ml from a filter-sterilized stock solution, yielding ST21CX agar.

Trace Element Solution (10× Original Formulation; Drews, 1974)

$MnCl_2 \cdot 4H_2O$	100 mg
$CoCl_2$	20 mg
$CuSO_4$	10 mg
$Na_2MoO_4 \cdot 2H_2O$	10 mg
$ZnCl_2$	20 mg
LiCl	5 mg
$SnCl_2 \cdot 2H_2O$	5 mg
H_3BO_3	10 mg
KBr	20 mg
KI	20 mg
EDTA, Na-Fe^{+3} salt (trihydrate)	8 g

Dissolve in 1 liter of distilled water and filter sterilize. This solution is stable for months at room temperature. Use the mixture at 1 ml/liter of medium.

The filter paper is inoculated in the center with a pea-sized amount of soil or plant debris, either in one spot or in the form of a streak. The cultures are incubated at 30°C. Unequivocal cellulose decomposition cannot be expected before 8–10 days of incubation, and most *Sorangium* strains are seen only after 10–20 days. They are recognized as bright, more or less translucent patches, which are yellow, orange, brown, or even black, often several cm across, and consisting of macerated cellulose, slime, and usually masses of densely packed fruiting bodies. The latter are responsible for most of the color, but also the vegetative parts of the swarms may be tinged yellow and orange, and at the edges, dense, orange ridges often develop. Besides the sorangia, other cellulolytic bacteria may also appear. The lysed areas always contain complex mixtures of a variety of bacteria, protozoa, and nematodes. In such cultures, particularly at later stages, noncellulolytic myxobacteria can also develop that live off the cellulose degraders (Brockman and Boyd, 1963). They appear in the form of long, heavy, orange ridges, which are the edges of expanding swarms, and are usually strains of *Nannocystis*, *Polyangium* or *Chondromyces*. Often fruiting bodies are also produced, not only in the macerated areas, but also on the edges of the intact filter paper, on the inoculum, and on the agar just beyond. Of course, these latter myxobacteria, not being cellulolytic, have to be subcultured on streaks of food bacteria and not again on filter paper. As the fruiting bodies observed in the lysis zones are not necessarily those of the cellulose degraders, in case of doubt, subcultures are best started both from fruiting bodies and from the swarm edge, using both types of substrates.

Isolation from Fresh Water

Sediments and plant and animal debris collected in lakes, ponds, rivers, etc. may be processed in the same way as described above. If free-floating myxobacteria are to be isolated, between 10 and 1000 ml of water is passed through a sterile membrane filter of small pore size (0.15–0.45 µm). The filter is then placed right side up on the surface of rabbit dung agar (another lean medium, e.g., VY/2 agar with a reduced yeast content or CY agar with a lowered peptone concentration, would also do). The cultures are incubated at a suitable temperature, e.g., 34°C. After 4–5 days, myxobacterial swarms and fruiting bodies may be observed (Gräf, 1975).

Rabbit Dung Agar

Boil 20 g of dry rabbit dung (wild rabbits) in 1 liter of distilled water for 20 min. Make up the filtrate to 1 liter again, and add 1.5% agar. Adjust the pH to 7.2, and autoclave the medium.

In summary, the various direct isolation techniques all have certain limitations, and each may be more or less appropriate for the isolation of a specific organism. For the cellulose degraders there is little choice. However, with some experience, they may also occasionally be found on dung pellets. Conversely, the cellulose plates are also useful for the isolation of *Chondromyces*, *Stigmatella*, *Polyangium* and *Nannocystis*. While *Myxococcus* is most easily obtained on dung pellets; *Cb. fuscus*, *Sg. erecta*, *Archangium*, *Corallococcus*, *Chondromyces* and *Melittangium* are also often found with this technique. The most generally effective technique is the use of bacterial smears; under these conditions, myxobacteria with small and inconspicuous fruiting bodies as well as strains that do not form fruiting bodies at all under the culture conditions are reliably isolated. Bark and rotting wood incubated in a moist chamber are in some cases excellent sources for fast and reliable isolation of *Stigmatella aurantiaca* and *Chondromyces* species.

Purification Methods

In the simplest case, pure cultures of myxobacteria can be obtained in one step from fruiting bodies, which usually contain an uncontaminated population of myxospores, at least as long as they are young. By carefully transferring material from fruiting bodies to a suitable agar medium, such as CY or VY/2 agar (see below), pure swarms can be obtained. An excellent tool for such delicate work is a glass rod drawn out into a fine tip. More convenient for a swift operation is the sharp point of a disposable, 1-ml, injection syringe. Direct isolation from fruiting bodies is possible with *Myxococcus* species because they produce soft-slimy fruiting bodies, and enough myxospores to start a culture can be obtained just by touching the fruiting body at the top. The fruiting bodies of *Chondromyces* and *Stigmatella* consist of sporangioles that are borne on a relatively long stalk that lifts them above the heavily contaminated substrate surface. The sporangioles can be plucked off and used as an inoculum. With all other myxobacteria, the chance of a selective transfer from fruiting bodies is low because they are too intimately interwoven with the substrate and the slime layer of the swarm.

VY/2 Agar

Bakers' yeast (commercial yeast cake)	0.5% (w/v)
$CaCl_2 \cdot 2H_2O$	0.1% (w/v)
Cyanocobalamin	0.5 mg/µl
Agar	1.5% (w/v)

Adjust pH to 7.2. Autoclave the medium. The yeast may be stored as an autoclaved stock suspension for several weeks. To obtain a uniform suspension of the yeast cells in the agar medium, add the yeast to the molten medium.

CY Agar

Casitone (Difco)	0.3% (w/v)
Yeast extract (Difco)	0.1% (w/v)
$CaCl_2 \cdot 2H_2O$	0.1% (w/v)
Agar	1.5% (w/v)

Adjust the pH to 7.2. Autoclave the medium.

ENRICHMENT TECHNIQUES. If direct isolation is not possible, enrichment techniques must be applied. Fruiting bodies or vegetative cells are transferred to streaks of a food organism on WCX agar. Three parallel streaks can be made on one plate, and each streak can be inoculated at both ends. As the swarm is often firmly attached to the substrate or forms a very tough slime sheet, from which tiny bits cannot easily be removed, or because the myxobacteria migrate within the agar, the inoculum often with a small piece of agar must be cut out using the tools mentioned above. An inoculation loop would be much too coarse and would risk carrying over too many contaminants. The bacterial smears are best inoculated at their edge so that the developing swarm also has a chance to spread over the free agar surface, which sometimes gives a better separation from the contaminants. New transfers can be made from the purest-looking sections of the developing swarms, preferentially from the swarm edge, until swarms are obtained that seem pure enough to justify a transfer to VY/2 or CY agar. A transfer to smears of autoclaved *E. coli* on WCX agar may be necessary as an intermediary step, particularly if the myxobacterium grows only within the streaks of the living *E. coli*. Much time can be saved later if the cultures are initially transferred within short intervals (1–2 days) before the contaminants have multiplied and spread. While it is advisable to make several transfers from the crude culture—by inoculating all three streaks of the enrichment plate, if possible from different sites—to enrich for the organism and to have a choice of contaminants to deal with, transfers can also be made one at a time, so that the three streaks of a plate can be used consecutively.

Sometimes transferred fruiting bodies do not germinate. This may happen, for example, if fruiting bodies found on a dung pellet have erroneously been identified as a bacteriolytic species (which is indeed the case with almost all dung organisms) and therefore were transferred to a bacterial smear, while in reality the fruiting bodies had been produced by a cellulose degrader. The latter will readily develop if inoculated to filter paper on ST21 agar (see the section Isolation of Cellulose Decomposers in this Chapter). However, some bacteriolytic myxobacteria do not always germinate on bacterial streaks, e.g., *Cb. fuscus*. With these strains, transfer of fruiting bodies directly to VY/2 agar sometimes helps. After germination, the swarms should be inoculated as soon as possible on bacterial streaks, because many contaminants can grow vigorously on VY/2 agar. If a sufficient quantity of fruiting bodies can be obtained from the crude culture, some of them should first be heated in water (see the section Purification by Heating in this Chapter) before they are inoculated to the growth medium. Note at least one myxobacterium (*Haploangium*) will not germinate on any known medium and has not been cultivated so far.

If the myxobacteria are left growing on the streaks for a longer time, they usually will produce fruiting bodies. Fruiting bodies can be very useful as starting material for further, more sophisticated purification steps. Also, fruiting bodies are important for the identification of the isolated strains.

The main contaminants in the enrichment cultures are other bacteria. Occasionally, other gliding organisms, more often small Gram-negative rods, become trapped in the slime of the swarm and are carried around by the moving myxobac-

teria. These two types of contaminants are often difficult to eliminate. Nematodes, fungi, and especially soil amoebae may also become a serious problem.

The first step in purification is to eliminate all animals from the culture because they move very actively and thus quickly spread bacterial contaminants over the plate. The transfer of nematodes can usually be avoided if some caution is observed, because they are large enough to be easily recognized and eliminated. Occasionally, however, a culture is heavily infested with nematodes, or the worms cannot be seen because an opaque substrate is used, e.g., cellulose plates. In these cases, the plate can be frozen by simply storing it in an –80°C deep-freeze for one or two days, which seems to kill all nematodes. After thawing the plate, it is important to make a transfer to a fresh plate immediately after the surface of the plate has softened sufficiently, because the thawed agar will be very runny and soft (Reichenbach, 1983). Exposing the plate to ammonia vapors (M. Aschner, personal communication) can also kill the amoebae, which sometimes multiply extremely fast. We use a 5% ammonia solution in a Petri dish and place the culture plate upside down over the ammonia dish for 1–2 min. The plate is then left with a closed lid for another 2–5 min, and then the myxobacteria are transferred to a fresh plate because the old one is now strongly alkaline. The myxobacteria usually survive the treatment very well, even when no fruiting bodies are present. A further transfer should be made as soon as possible from the fresh culture because a few amoebae, probably encysted stages, sometimes survive. Soil amoebae do not seem to be inhibited by cycloheximide, nystatin, or freezing.

Occasionally, cycloheximide-resistant fungi are present, but dusting the inoculum with nystatin powder can almost always inhibit them. The myxobacteria grow between the nystatin particles and can then be transferred to another plate.

The remaining contaminants are bacteria. By transferring pieces from the fast-spreading swarm edge, most of the larger and less motile organisms are quickly eliminated, in particular *Bacillus* species and their spores, but the slime matrix of the swarm may still shelter many small, mainly Gram-negative bacteria. Apart from the slime, the relatively slow growth of myxobacteria compared with that of typical contaminants is a major factor that makes purification time-consuming and difficult. Usually it takes 3–6 weeks before an isolated strain is pure; only rarely can it be accomplished in 1–2 days (by direct transfer of fruiting bodies). Fortunately, those isolations that may take 1–2 years (e.g., of a cellulose degrader) are equally rare.

Often spreading growth under selective nutritional conditions is not sufficient to shake off contaminants, or the procedure becomes too time-consuming. In such cases, a number of more specific purification steps may be tried.

PURIFICATION BY PLATING. Plating of diluted cell suspensions, the technique of choice for the isolation of most soil bacteria, is only partly useful with myxobacteria. The first difficulty is that the slime makes it difficult to suspend the cells homogeneously in water. We have sometimes had success using a small (1 ml) sterilized tissue homogenizer to overcome this problem. If fruiting bodies are available, treatment with ultrasound for 1–2 min may result in a suspension of myxospores. (The use of ultrasound to kill contaminants selectively, as suggested by Sutherland [1976b], has not appeared very promising in our hands.) Cell suspensions can also be produced in a mixer with or without glass beads (McCurdy, 1963). The cell suspensions can be diluted without problems in sterile distilled water, although a special dilution medium (DM) has also been used.

Dilution Medium (DM; McCurdy, 1963)

Soluble starch	0.5% (w/v)
$MgSO_4 \cdot 7H_2O$	0.05% (w/v)
K_2HPO_4	0.025% (w/v)

The second difficulty is that quite a few myxobacteria, particularly members of the suborder Sorangineae, do not readily produce swarms from single cells. Often a long and tedious optimization and adaptation procedure is required before single-cell colonies are obtained, even from pure strains. Nevertheless, with many myxobacteria, a reasonably high proportion of the cell population will grow to form colonies, so that plating may be successful. However, in our experience, plating has a chance only if the myxobacterium is already nearly pure, which may be due to their relatively low plating efficiency and relatively slow growth rate. CY agar is often an appropriate medium for plating; sometimes the yield is improved if the peptone concentration is increased to 0.8–1.2% or if 0.5% glucose is added. ECM agar and SP agar have also been recommended. While VY/2 agar is a good growth medium, its turbidity reduces its utility and, in addition, myxospores sometimes do not germinate on this medium.

ECM Agar (McCurdy, 1963)

> Suspend washed cells of *E. coli* (100 mg of dry mass per 100 ml) in a medium containing: $MgSO_4 \cdot 7H_2O$, 0.05% (w/v); NaCl, 0.6% (w/v); and agar, 1.5% (w/v). Adjust the pH to 7.2 and autoclave the medium. On this medium, myxobacterial colonies are surrounded by lysis zones.

SP Agar (McDonald and Peterson, 1962; McCurdy, 1963)

Raffinose	0.1% (w/v)
Sucrose	0.1% (w/v)
Galactose	0.1% (w/v)
Soluble starch	0.5% (w/v)
Casitone (Difco)	0.25% (w/v)
$MgSO_4 \cdot 7H_2O$	0.05% (w/v)
K_2HPO_4	0.025% (w/v)
Agar	1.5% (w/v)
Vitamin solution	2.5 ml/liter

Vitamin Solution (McDonald and Peterson, 1962)

Thiamine	100 mg
Riboflavin	75 mg
Pyridoxine	75 mg
Ca pantothenate	200 mg
p-Aminobenzoic acid	5 mg
Nicotinamide	75 mg
Choline HCl	200 mg
Folic acid	1 mg
Inositol	1000 mg
Biotin	0.05 mg
Cyanocobalamin	0.05 mg

Dissolve these ingredients in 1 liter of 100% ethanol. Add 2.5 ml per liter of medium.

Whether the diluted cell suspensions are spread on the agar surface or included in pour plates does not seem to make much difference. In the latter case, the agar content of the medium should be reduced to about 1% and temperatures above 40°C of the molten agar should be avoided. Myxobacterial colonies may be recognized by their swarming behavior, which they also tend to show when embedded in agar. On rich media, swarming may be considerably reduced, and the colonies may become compact and look like those of ordinary bacteria. If they are dense enough, however, they may be brightly colored in shades of yellow, orange, red, or purple.

PURIFICATION BY HEATING. This procedure can be used if fruiting bodies with mature myxospores are present. The fruiting bodies are suspended in 1 ml of sterile water and incubated in a water bath. After 10, 20 and 40 min, samples are transferred to CY or VY/2 agar and carefully spread all over the plate. The useful temperature—too high for the contaminant, but still withstood by the myxobacterium—has to be found by trial and error. We usually work at 58°C, a temperature most myxospores can survive for at least 10 min. This temperature is often sufficient to kill contaminating bacteria, fungi, and amoebae. Only rarely do myxobacteria tolerate higher temperatures, and some do not withstand 58°C, in which case 56°C or less may still lead to success. However, this method should be tried in the later part of the purification process, because then the chances are higher that thermoresistant contaminants, e.g., *Bacillus* spores, will no longer be present. It may take a week or longer at 30°C before swarms can be seen on the plates, as a substantial part of the myxospore population may also have been killed. However, often swarms are visible within 1–2 days.

PURIFICATION WITH ANTIBIOTICS. This procedure may also be useful if mature myxospores are present. Fruiting bodies are suspended in 1 ml of a rich growth medium, e.g., EBS medium (see below). To this is added 0.1 ml of a concentrated mixture of potent antibiotics, and the suspension is shaken overnight at 30°C (Reichenbach, 1983). The myxospores do not germinate in the rich medium, but the contaminants will grow and be killed. If a contaminant is resistant to the inhibitors, the culture will be turbid the next morning, and the procedure must be repeated with a different mixture of antibiotics. (Occasionally the liquid becomes turbid owing to suspended dead cells. In this case, the culture fluid can be replaced by fresh medium and the culture incubated a few hours more.) We use three increasingly aggressive combinations of antibiotics, AB-1, -2, and -3 solutions (see below), which we apply consecutively when necessary. If the supernatant is still clear on the next morning, it is replaced by sterile distilled water, and the fruiting bodies are washed by shaking for an additional 4–6 h. They are then transferred to VY/2 or CY agar or to streaks of autoclaved *E. coli* on WCX agar. Usually swarms develop after 1–5 days. Sometimes an individual swarm is still contaminated, although no foreign colonies can be seen on the plate. Therefore, several swarms should always be isolated and tested separately for purity. Again, the procedure is less effective during the early stages of purification, before the majority of the contaminants have been removed. Note that the vegetative growth of myxobacteria is, in general, as sensitive to inhibition by antibiotics as is the growth of other Gram-negative bacteria. However, the metabolically quiescent myxospores within the fruiting bodies are resistant as long as their germination is prevented.

EBS Medium

Peptone from casein (Merck, Darmstadt, Germany) 0.5% (w/v)	
Proteose peptone (Difco)	0.5% (w/v)
Peptone from meat (Merck)	0.1% (w/v)
Yeast extract (Difco)	0.1% (w/v)

The peptone from casein is a tryptic digest. Adjust the pH to 7.0 and autoclave the medium.

AB-1 Solution

Chloramphenicol	20 mg
Streptomycin sulfate	30 mg
Tetracycline HCl	25 mg
Na cephalotin	20 mg

Dissolve the above ingredients in 50 ml of distilled water and filter sterilize. The solution remains stable for several weeks at 4°C.

AB-2 Solution

Chloramphenicol	20 mg
Streptomycin sulfate	30 mg
Tetracycline HCl	25 mg
Kanamycin sulfate	35 mg
Erythromycin	25 mg
Polymyxin B sulfate	20 mg

Dissolve the above in 50 ml of distilled water and filter sterilize. The solution remains stable for several weeks at 4°C. Use only as a last resort since it is often inhibitory to myxobacteria.

AB-3 Solution

Chloramphenicol	20 mg
Tetracycline HCl	30 mg
Neomycin sulfate	20 mg
Gentamycin sulfate	30 mg
Collistin methane sulfonate	30 mg

Dissove the above in 50 ml of distilled water and filter sterilize. The solution remains stable for several weeks at 4°C. This mixture is specifically designed to eliminate pseudomonads. It usually serves that purpose but is less well tolerated by the myxobacteria than is AB-1 solution.

The inclusion of inhibitors, e.g., neomycin and sulfanilamide, directly in the isolation medium has been suggested (McDonald, 1967; McCurdy, 1969b). However, while some myxobacteria can be isolated in this way, we do not recommend this approach. (Cycloheximide is of course an exception, as it does not inhibit prokaryotes.) It is remarkable how many soil bacteria can grow in the presence of high doses of powerful antibiotics, and there is always the danger of a counterselection of some myxobacteria. Addition of antibiotics to purification media is a different story, however, as some groups of myxobacteria are naturally resistant to certain potent antibiotics (see later).

PURIFICATION OF CELLULOSE DECOMPOSERS. The purification of *Sorangium* strains is especially tedious and time-consuming because the organisms only grow slowly on the selective substrate, giving the contaminants ample time to multiply. Furthermore, within the water-soaked filter pad, many contaminants spread rapidly over the whole area, complicating the problem of separating the myxobacteria from the other organisms.

The first transfer from the crude culture should be made again to filter paper on ST21CX agar (see the section Isolation of Cellulose Decomposers in this Chapter) to retain the selective conditions. For the preparation of subcultures, filter paper is cut into small rectangles, about 1.5 × 3 cm, three or four of which are placed at some distance from each other on the agar surface. The filter pads are inoculated from different parts of the original swarm. In this way, an initial reduction of the contaminants may be achieved. The next step is to remove nematodes and amoebae using the techniques recommended above. To eliminate bacterial contaminants, transfers can be made to streaks of autoclaved *E. coli* on WCX agar to which 250 mg of kanamycin sulfate has been added per liter medium (after autoclaving, from a filter-sterilized stock solution: KAN4 agar). All *Sorangium* strains tested so far are completely resistant to kanamycin as well as to neomycin and gentamycin (H. Reichenbach, unpublished observations). Most sorangia grow reasonably well on autoclaved (but not on living) *E. coli*, often forming large, delicate swarms (another advantage of this purification step). Pieces from the swarm edge that appear to be uncontaminated can be transferred to VY/2 agar. The pure strain is reinoculated to filter paper to make sure that the desired organism has been isolated. We have never observed a *Sorangium* strain that lost its ability to degrade cellulose during isolation. Addition of kanamycin initially to the crude culture would prevent the appearance of many other myxobacteria and, at the outset, select for kanamycin-resistant contaminants.

If the method just described fails, heating of the fruiting bodies or treatment with the antibiotic solution may lead to success. If the *Sorangium* isolate is already relatively pure, it may also be transferred to chitin agar (CT7 agar; see below) or to cellulose overlay agar (CEL3 agar, see below). Most but not all sorangia also decompose chitin and grow relatively quickly, producing large swarms on chitin agar with chitin as the only carbon, nitrogen, and energy source. Large swarms also arise on cellulose overlay agar, but development is delayed so that the contaminants have a better chance to predominate. On both media, the sorangia penetrate the agar, so that sometimes a pure inoculum can still be obtained from the deeper layers of the plate even though the surface is contaminated.

CEL3 Agar

Cellulose powder	0.5% (w/v)
KNO_3	0.1% (w/v)
Agar	1.0% (w/v)

Cellulose powder MN 300 from Macherey and Nagel (Düren, Germany) works well. Adjust the pH to 7.2. Autoclave the medium. Autoclave the KNO_3 separately and add when the medium has cooled. Pour as a thin layer on top of ST21 agar plates.

Another strategy often helpful in the purification of sorangia is incubation at 38°C, e.g., on KAN4 agar + autoclaved *E. coli*. Most but not all strains are able to grow at this temperature, sometimes vigorously so, while growth of the contaminants is often prevented.

TESTING FOR PURITY. The final task in pure culture isolation is to check for purity of the culture. Some swarm material may be streaked

on nutrient agar, on which myxobacteria, in contrast to most contaminants, grow only slowly or not at all. Other media useful for purity control are CY and MYX agar. Although most myxobacteria grow on those media, they are usually easily distinguished from nonmyxobacterial colonies.

MYX Agar

Glutamate Na	0.5% (w/v)
Yeast extract (Difco)	0.1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
Glucose	0.2% (w/v)
Agar	1.5% (w/v)

Autoclave glucose separately. Adjust the pH to 7.2. Autoclave the medium. Finally, add the autoclaved glucose to the autoclaved medium.

In addition, some material may be inoculated into a liquid medium, e.g., nutrient broth or CAS medium, and incubated with shaking for one day at 30°C. Under such conditions, myxobacteria usually grow in clumps, flakes, or as a ring around the glass wall, if at all. Dispersed growth at that stage is almost always an indication of contamination.

Myxobacteria can often be differentiated easily from other bacteria by their size, cell shape, and their inability to swim actively. Thus microscopic examination will also often quickly reveal contaminants, although this is not always reliable and requires some experience.

Counting Myxobacteria in Soil

The quantitative determination of myxobacteria in soil is not likely to give accurate results because, in the swarm and in the fruiting bodies, the cells are always linked together by slime, and in the fruiting bodies, cells are often surrounded by the tough walls of the sporangioles. Still, estimates are possible and have been obtained in the following way: Small circular smears (about 2 cm in diameter) of a food organism are placed on a water agar or a silica gel plate, and each circle is inoculated with a small quantity of soil which has been carefully homogenized and weighed (Singh, 1947). Alternatively, the weighed and homogenized soil is made into a paste with sterile water, and small drops are applied to the ends and centers of cross-streaks of a food organism on water agar or, for counting cellulose decomposers, to filter paper disks on mineral agar (Brockman, 1976). After incubation, the number of myxobacteria is calculated from the number of swarms and fields of fruiting bodies that have developed. Another approach is to add soil dilutions to antibiotic cell extract (ACE) agar (McCurdy, 1969b). This method has severe limitations because the medium allows a rapid development of contaminants, and only *Myxococcus* and *Corallococcus* cells can be counted. Nevertheless, the numbers that have been obtained are sometimes quite impressive.

ACE Agar (Antibiotic Cell Extract Agar; McCurdy, 1969b)

SP medium	
Yeast extract	0.1% (w/v)
Yeast (or *E. coli*) cells	0.5% (dry w/v)
Neomycin	10 mg/ml
Sulfanilamide	10 mg/ml
Cycloheximide	100 mg/ml

Add the remaining ingredients to the SP medium (see the section Purification by Plating in this Chapter).

The myxobacteria are detected by their swarm morphology and surrounding lysis zones.

Cultivation

Contrary to a belief that still seems widely held, myxobacteria are not particularly fastidious organisms. With the exception of *Haploangium*, all species have been cultivated, and almost all can be grown in axenic, pure culture. Of course, as with all other organisms, myxobacteria have their peculiarities, but no unusual requirements, and not even particularly complex ones, have been discovered to date. Exotic substrates such as the rabbit dung media that were so popular for a long time are not necessary. Although they were not always easy to develop, fully defined, synthetic media have been developed for some myxobacteria. In connection with possible commercial antibiotic production, quite a few myxobacteria have been cultivated in large-scale fermentors (5– 50 m^3 and greater), often on technical media, such as corn steep powder and soy meal.

Plate Cultures

Myxobacterial cultures are often incubated for extended periods (1–4 weeks) when dehydration may occur, so that plates must be poured thicker than for the usual bacterial work. Also, a high humidity in the incubator is helpful.

All myxobacteria, including the cellulose degraders, seem to grow well on yeast agar (VY/2 agar). The yeast cells are usually decomposed, but not always completely and sometimes not at all. Vitamin B_{12} is occasionally required (e.g., by many *Polyangium* strains) so cyanocobalamin is included in this standard medium. Typically, on VY/2 agar, gliding motility is considerably stimulated, and the swarm colonies tend to become very large. Fruiting bodies are often formed even after several transfers on VY/2 agar, although sooner or later most strains cease fruiting on this medium as well as on most other growth media. Cultures on VY/2 agar are usually

rather stable, and most strains need not be transferred more often than every 2–3 weeks (30°C). The reason is probably that VY/2 agar is relatively lean, nutrients are only gradually solubilized, and the pH does not change very much. The medium therefore is also well suited for stock cultures.

Another useful medium is CY agar (see the section Purification Methods in this Chapter). On this medium, growth often is more vigorous, but the swarms spread less and may become somewhat slimy and often the cells die earlier (after 1–2 weeks) because of copious ammonia production. The choice of the peptone for media of this type is critical. In general, the most suitable products appear to be pancreatic and tryptic digests of casein, e.g., Casitone (Difco), tryptone (Difco), or peptic digests (Marcor, Merck). But other peptones such as soybean peptones can also be used. Meat peptones, on the other hand, are usually not useful, perhaps because their phosphate content is too high. In any case, the suitability of a peptone for cultivation of the myxobacterial strain must first be tested. How critical the quality of the peptone can be is seen from the fact that even the specific batch of a particular peptone brand may make a difference. Thus, for example, not every batch of Difco Casitone supported successful induction of myxospore formation with *Stigmatella aurantiaca* (Gerth and Reichenbach, 1978); similar observations have also been made with glycerol induction of myxospores of *Mx. xanthus*. Replacement of peptone by a total protein hydrolysate, e.g., casamino acids (Difco), is often not possible. But casamino acids are a perfect N source for *Cystobacter* and *Sorangium* strains.

Watson and Dworkin (1968) showed that *Mx. xanthus* lacked hexokinase and was unable to take up, metabolize, or grow on sugars. This, however, cannot be generalized for the myxobacteria, despite earlier impressions that this might be the case. Many myxobacteria are known that efficiently metabolize sugars, e.g., *Stigmatella* and *Cystobacter*. In fact, it seems that only the *Myxococcus* species, *Nannocystis*, and perhaps some *Corallococcus* strains are unable to use carbohydrates. In some cases, e.g., certain *Corallococcus* strains, polysaccharides such as starch can be utilized, although no mono- and disaccharides can be used. Apparently the starch is degraded to the trisaccharide, which can be metabolized (Irschik and Reichenbach, 1985a). Thus, it may sometimes be useful to supplement a peptone medium with starch or glucose. Another beneficial effect of this may be that the pH rises more slowly, so that the culture can be maintained for a longer time. We have found the following formulations useful for plate cultivation:

CYG2 Agar

Casitone (Difco)	0.3% (w/v)
Yeast extract (Difco)	0.1% (w/v)
$CaCl_2 \cdot 2H_2O$	0.1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
Agar	1.5% (w/v)

Adjust the pH to 7.2. Autoclave, then add 0.3% (w/v) glucose from an autoclaved stock solution.

STK2 Agar

Base agar:

Casitone (Difco)	0.1% (w/v)
Yeast extract (Difco)	0.05% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
Agar	1.2% (w/v)

Adjust the pH to 7.2. Autoclave the medium and pour into Petri dishes. After the agar has solidified, add the following top agar.

Top Agar

Use the same base agar ingredients, but increase the agar concentration to 1.5% (w/v), and prepare the medium in about 85% of the final total water volume. In the remaining water, dissolve (as a percentage of the total volume of the top agar) soluble starch (0.2% [w/v]; see below) and K_2HPO_4 (0.02% [w/v]). Heat the starch solution in a water bath with stirring and then autoclave to prevent clumping of the starch. Combine the two solutions (the base agar solution and the starch/K_2HPO_4 solution) after autoclaving.

In the form just described, STK2 agar can be used for checking starch degradation. If it is only to be used for cultivation, just the top agar (but with 1.2% agar) is required.

The cellulose decomposers utilize NO_3^- or NH_4^+ as the only nitrogen (N) source and can be cultivated on simple synthetic media like CK6 and CA2 agar (see below). They respond favorably to the addition of an organic N source, such as peptone (0.1–0.2%), but usually they will not grow in the absence of a suitable carbohydrate, like glucose or starch. *Sorangium* strains thus can also be grown on VY/2, CEL3, and STK2 agar, and many strains will grow as well on CT7 agar, but for the reasons just mentioned, relatively few will grow on CY agar.

CK6 Agar (Modified from Couke and Voets, 1967)

Solution A:

$MgSO_4 \cdot 7H_2O$	0.15% (w/v)
Fe^{+3} citrate	0.002% (w/v)
Agar	1.5% (w/v)

Prepare this solution in 80% of the final water volume.

Solution B:

KNO_3	0.2% (w/v)
K_2HPO_4	0.025% (w/v)

Prepare this solution in 10% of the final water volume.

Solution C:

Glucose	0.5% (w/v)
$CaCl_2 \cdot 2H_2O$	0.15% (w/v)

Prepare this solution in 10% of the final water volume. After autoclaving, combine the three solutions.

CA2 Agar (Mullings and Parish, 1984)

Base agar:
Agar 1.5% (w/v)

Stock solution A:

KNO_3 7.5 g
K_2HPO_4 7.5 g

Dissolve these ingredients to give 100 ml.

Stock solution B:

$MgSO_4 \cdot 7H_2O$ 1.5 g

Dissolve to give 100 ml.

Stock solution C:

$CaCl_2 \cdot 2H_2O$ 0.27 g
$FeCl_2$ 0.15 g

Dissolve these to give 100 ml. Autoclave. Supplement the base agar with 1% (v/v) of each of the three stock solutions, 1% (w/v) of glucose (from an autoclaved 20% stock solution) and trace elements.

Since myxobacterial swarms tend to spread quickly, cultures are more conveniently kept in Petri dishes than on agar slants in tubes. In the latter, the swarm edge soon reaches the glass surface and the cells dry out and die; also transfer of sticky, tenacious swarms is easier from plates. The plates are inoculated best at one spot at the side to give the swarm as much space as possible. In large swarms, living cells are often found only in a rather narrow band along the edge. Therefore, the inoculum should always be taken from the edge, at least as long as there are no fruiting bodies in the interior zone. Many myxobacteria of the suborder Sorangineae tend to penetrate deeply into the agar, in which case an agar strip has to be cut out and used as an inoculum.

Viable Counting

Accurate viable counts are possible only with strains that grow in a dispersed fashion in liquid media. Plating efficiency has to be tested for every strain. Given below are some media that allow plating efficiencies close to 100%: CT and CTT agar for *Mx. xanthus* FB; PT agar for *Mx. fulvus* Mx f2; and SG agar for *Sg. aurantiaca* Sg a1. CF (clone-fruiting) agar supports the formation of colonies from single cells of *Mx. xanthus*, with the subsequent production of fruiting bodies (Hagen et al., 1978). If a plating medium is not satisfactory for a specific strain, usually a modification of the peptone and ion concentrations or the addition of some yeast extract will make it suitable.

CT Agar (Dworkin, 1962)

Casitone (Difco) 2% (w/v)
$MgSO_4 \cdot 7H_2O$ 8 mM
Potassium phosphate buffer, pH 7.2 0.01 M
Agar 2% (w/v)

Autoclave $MgSO_4 \cdot 7H_2O$ separately.

A variation of the pH sometimes improves the results; a pH of 7.6 is closer to the optimum than is 7.2 (H. Reichenbach, unpublished observations).

CTT Agar (Bretscher and Kaiser, 1978)

Casitone (Difco) 1% (w/v)
$MgSO_4 \cdot 7H_2O$ 8 mM
Potassium phosphate buffer, pH 7.6 1 mM
Tris buffer, pH 7.6 10 mM
Agar 1.5% (w/v)

For semisolid media, agar is at 0.6% (w/v). Autoclave the medium.

PT Agar (for *Mx. fulvus* Mx f2)

Casitone (Difco) 0.4% (w/v)
Yeast extract (Difco) 0.4% (w/v)
$MgSO_4 \cdot 7H_2O$ 0.2% (w/v)
$CaCl_2 \cdot 2H_2O$ 0.1% (w/v)
Agar 1.5% (w/v)

Adjust the pH to 7.2. Autoclave the medium.

SG Agar (Gerth, 1975)

Casitone (Difco) 1.5% (w/v)
$MgSO_4 \cdot 7H_2O$ 0.1% (w/v)
$CaCl_2 \cdot 2H_2O$ 0.2% (w/v)
Agar 1.5% (w/v)

Adjust the pH to 7.0. Autoclave the medium.

CF Agar (Hagen et al., 1978)

Casitone (Difco) 0.015% (w/v)
Na pyruvate 0.1% (w/v)
Na citrate 0.2% (w/v)
$(NH_4)_2SO_4$ 0.02% (w/v)
$MgSO_4 \cdot 7H_2O$ 8 mM
Potassium phosphate buffer, pH 7.6 1 mM
Tris buffer, pH 7.6 10 mM
Agar 1.5% (w/v)

Autoclave the medium.

Excellent viable counts, e.g., for survival curves after treatment with mutagens, have been obtained by us with *Mx. fulvus* Mx f2 and *Sg. aurantiaca* Sg a1 by adapting a miniaturized counting technique recommended by Sharpe and Kilsby (1971). One of the plating media mentioned above is prepared with a reduced agar concentration of 0.6% (w/v). The bacteria are diluted directly in this soft agar at 40°C, and 0.1-ml drops of appropriate dilutions are put into Petri dishes, covered with a drop of sterile agar of the same composition, and incubated at 30°C. Note that vegetative cells of *Mx. xanthus*, and perhaps other myxobacteria as well, are sensitive to temperatures higher than 43°C. Microcolonies are counted under a dissecting microscope. Up

to 150 colonies per drop can easily be counted (H. Reichenbach and M. Dworkin, unpublished observations). The method has several advantages. Without difficulty, 40 drops can be placed into one Petri dish, using the bottom and the lid. Besides enormous economy in materials and incubator space, results are obtained earlier (after 3–4 days instead of 5–8 days) and with superior accuracy (more replicates, e.g., 5 instead of 3); less divergence between replicates; and a better chance to hit the optimum dilution step, because a wide interval of dilution steps can conveniently be plated. Plating efficiency can be 100%. The peripheral drops often show a reduced colony count, probably because they dry more easily. Therefore a ring of sterile agar drops should be placed around the edge of the plate.

Production of Fruiting Bodies in Culture

Usually, fruiting bodies are not produced on media that allow good growth. High nutrient concentrations seem to repress the fruiting process. As already mentioned, on VY/2 agar, fruiting bodies are often produced during several transfers, but a vegetatively propagated strain normally stops fruiting soon after isolation and often cannot be made to fruit again. The reason for this is not really understood; perhaps there is a selection for nonfruiting variants. In several cases, induction conditions for fruiting have been found for certain individual strains. Such strains reliably form fruiting bodies even after many transfers of purely vegetative growth, but other strains of the same species usually do not respond to the same conditions. With freshly isolated strains, fruiting can often be induced by transferring swarm material from a lean growth medium, like VY/2 agar, to plain water agar (WAT agar) or to sterile filter paper pads on water agar. Also, fruiting may continue for some time on streaks of living *E. coli* cells on water agar. The nutrients carried over from the growth medium and the low nutrient level provided by the lysing *E. coli* cells, respectively, allow reasonable growth for some time, leading to sufficiently high cell densities; the subsequent more-or-less abrupt depletion of nutrients then somehow triggers development. The need for a high population density for fruiting has been established for several myxobacteria, e.g., *Mx. xanthus* (Wireman and Dworkin, 1975) and *Cm. apiculatus* (H. Reichenbach, unpublished observations).

Using some simple tricks, myxobacterial strains can sometimes be maintained in the fruiting state for a long period. We were able to do so with *Sg. aurantiaca* over a period of years by storing the strain in the form of fruiting bodies on WAT agar at room temperature (20°C). After 2–3 months, when the culture began to dry out, the fruiting bodies were inoculated to VY/2 agar and incubated at 30°C. The resulting swarms were immediately reinoculated to WAT agar, incubated for a few days at 30°C, during which time the organism began to fruit again, and then stored at room temperature as before. One or two more transfers to VY/2 agar were possible, but after that the strain failed to produce fruiting bodies. This procedure of cycling the culture between rich media that support growth and lean media that induce fruiting body formation will probably work with many different myxobacteria.

With several *Chondromyces* species (*Cm. apiculatus*, *Cm. crocatus*, *Cm. pediculatus* and *Cm. lanuginosus*), we have obtained fruiting bodies when the organism was kept in a mixed culture with cellulose degrading bacteria (not myxobacteria) on filter pads placed on ST21 agar plates. The cultures are incubated in the light (on the bench top in the laboratory) at room temperature (20–24°C). Transfers are made every 3–4 weeks by inoculating macerated cellulose and *Chondromyces* fruiting bodies together onto fresh filter pads on ST21 agar. Within 4–5 days, new fruiting bodies appear. Using this procedure, several of our strains have continued to produce dense populations of fruiting bodies over a period of years (H. Reichenbach, unpublished observations). The method can also be used with pure cultures of *Chondromyces*. The swarm material is simply inoculated into a developing culture of a cellulose degrader. While *Polyangium* strains often form fruiting bodies with this procedure, myxobacteria of the suborder Cystobacterineae rarely do so. In general, a slightly reduced temperature (24–28°C) seems to favor fruiting. Also, in two cases, a requirement of light for fruiting body differentiation has been demonstrated—with *Cm. apiculatus* (Reichenbach, 1974a, b) and with *Sg. aurantiaca* (Qualls et al., 1978).

Chamber Cultures

For the study of the social behavior of myxobacteria and of morphogenetic events, chamber cultures that can be observed and followed under the microscope are extremely useful. Figure 4 illustrates one procedure for making such cultures.

Liquid Cultures

If a freshly isolated myxobacterial strain is cultured for the first time in a liquid medium in a shake flask, the bacteria almost always grow in the form of flakes and nodules and as a film along the glass wall. In static cultures, they grow as a sheet on the glass in the upper parts of the vessel

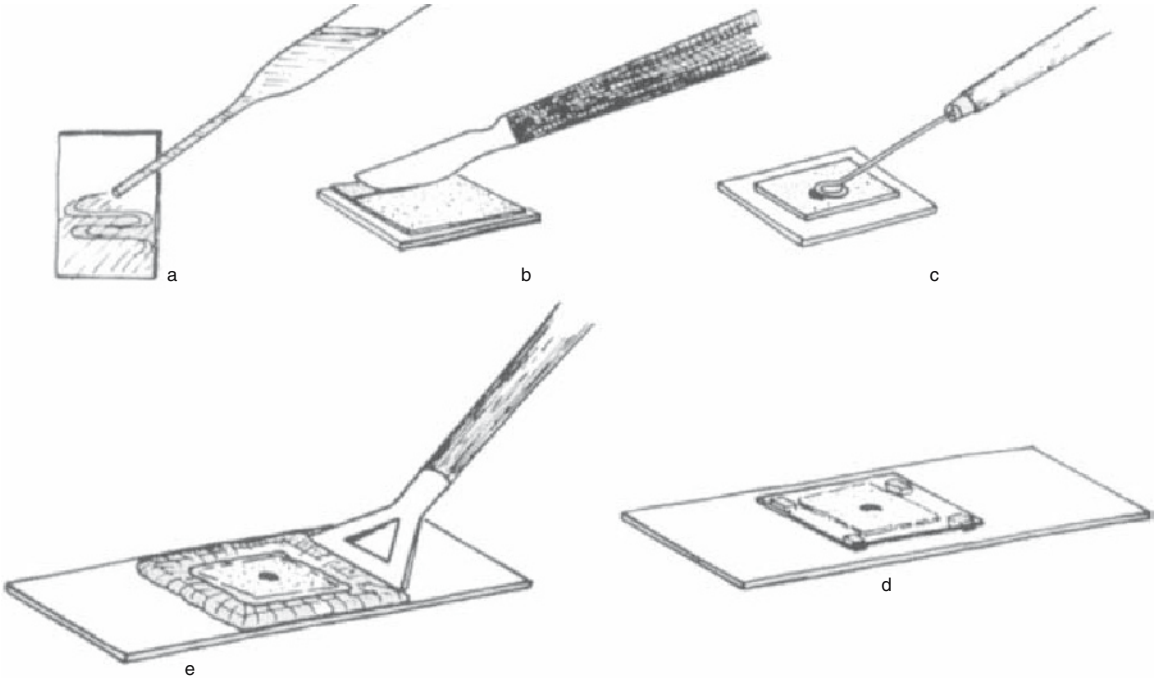


Fig. 4. Method for making a chamber culture: a) Coat a sterile cover slip (e.g., 40 × 22 mm) with a thin agar layer. The molten agar is applied with a Pasteur pipette; the hotter the agar, the thinner the resulting layer. b) Using a sterile scalpel, remove the agar layer along the edges of the cover glass. c) Inoculate the agar film. d) Mount the cover slip with the agar layer downward on a sterile slide; tiny pieces of glass from a broken microscopic slide are used as supports. e) Seal the chamber carefully with a molten mixture of paraffin and Vaseline (2 : 1) and incubate at a suitable temperature. From Heunert (1973).

and as a pellicle along the surface of the liquid. However, if repeated transfers are made from shake cultures, preferentially using the supernatant as an inoculum, strains that grow in a dispersed fashion may be obtained. While with many strains only a few such transfers are required, other strains may resist dispersed growth completely. Thus, for example, while most strains of *Myxococcus* can eventually be grown in dispersed culture, we once transferred a strain of *Mx. fulvus* for months in a variety of liquid media without ever observing the slightest inclination to dispersed growth (H. Reichenbach, unpublished observations). Cells of most members of the suborder Sorangineae show a strong tendency to stick together, and in spite of many attempts, we have never been able to get *Chondromyces*, *Polyangium* and *Nannocystis* to grow homogeneously in liquid cultures. The best that could be achieved was growth as tiny flakes with *Nannocystis* and *Polyangium*. With *Sorangium*, however, we have been able to select many completely dispersed strains. The list of myxobacteria that have been grown in a dispersed fashion is very long by now and, with the exception of the last-mentioned organisms, comprises virtually all taxa. However, even the recalcitrant species, which do not grow in a completely dispersed fashion, can at least be cultivated in suspension in liquid medium.

The main factor that determines whether *Mx. xanthus* cells will grow in clumps is the ability to produce an extracellular matrix composed of fibrils (Arnold and Shimkets, 1988a; Dworkin, 1999). Loss of type IV pili because of a *pilA* mutation, which encodes the major structural pilin subunit, also eliminates clumping (Wu et al., 1997), but this may be due to the fact that pilus mutants are often severely restricted in fibril synthesis. Fibril formation is also required for divalent cation-dependent agglutination (Shimkets, 1986), which is thought to mimic clumping in culture. The Sorangineae, which persistently fail to grow in a dispersed state, produce a slime that is chemically different from that of the Cystobacterineae (as shown by Congo red adsorption to the slime of the latter, but none to the slime of the former; McCurdy, 1969b). Thus, in the case of this group of the myxobacteria, the nature of the excreted slime may play a role in determining their mode of growth.

A homogeneously growing strain may start to clump again if growth conditions are changed, e.g., in a medium of different composition. Thus, cells of *Sg. aurantiaca* Sg a1, a strain that grows in a dispersed fashion, form tiny nodules if $CaCl_2$

is added to the medium. Freshly isolated *Cystobacter fuscus* cultures grow homogeneously when inoculated into a casamino acids medium, but produce nodules and flakes for many transfers in Casitone medium. Cells of *Sorangium cellulosum* strain So ce14 yield a homogeneous suspension when shaken overnight in Casitone medium (in which they cannot grow, however); when reinoculated into AMB growth medium, they produce nodules again. In this connection, although the myxobacteria are the classical "slime bacteria" (*myxo* is a combining form meaning "slime"), they do not produce copious amounts of slime, either on plates or in liquid media, which rarely become recognizably viscous. Only on certain agar media is a larger quantity of slime sometimes seen, e.g., with *So. cellulosum* on CA2 agar.

To facilitate the transition to dispersed growth, the addition of 0.1% agar to the liquid medium has been suggested (Schürmann, 1967). The agar forms tiny flakes that float in the liquid and are colonized by the myxobacteria. However, the selection of truly dispersed strains is not noticeably speeded up by this procedure. Sometimes replacing the usual rotary shaker with a reciprocating shaker is helpful, since the slime sheets are broken up more efficiently on the latter, and the transition to dispersed growth may be faster, although patience is still required (K. Gerth, personal communication).

Liquid media used for cultivation of myxobacteria are of the same general composition as the agar media mentioned above (see the sections Direct Isolation and Purification in this Chapter), but the nutrient requirements stand out more clearly in liquid media, and the organisms respond almost immediately to inadequacies of the medium. The prototype for the bacteriolytic myxobacteria is CT liquid medium, which was designed to provide the most rapid growth (3.5-h doubling time), maximum cell yield ($4–5 \times 10^9$ cells/ml), and uniform cell suspension of *Mx. xanthus* FB (Dworkin, 1962; the medium has the same composition as the CT agar described in the section Viable Counting, only without the agar). However, most other myxobacteria grow better when the peptone concentration is reduced, as in CAS medium and in MD1 medium (see below). The latter medium was originally developed for *Nannocystis*, but it is also often useful for *Archangium*, *Melittangium* and *Cystobacter*. Also, in many cases it is superior for starting liquid cultures with newly isolated strains.

CAS Medium

Casitone (Difco)	1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)

Do not adjust the pH (which is 6.8) if Difco Casitone is used. Autoclave the medium.

MD1 Medium (Behrens et al., 1976)

Casitone (Difco)	0.3% (w/v)
$CaCl_2 \cdot 2H_2O$	0.07% (w/v)
$MgSO_4 \cdot 7H_2O$	0.2% (w/v)
Cyanocobalamin	0.5 mg/ml
Trace elements	(see the section Isolation of Cellulose Decomposers in this Chapter)

Do not add vitamin solution for most strains. Autoclave the medium.

What has been said about the choice of peptone in agar media holds also for liquid media. For many myxobacteria, the addition of glucose (0.2–0.5%), starch, or another useful sugar to the peptone medium may be favorable. In general, the organisms do not grow faster with the carbohydrate, but they grow for a longer time, and they reach a higher cell density; also, the rise of the pH is delayed, so that the culture becomes more stable.

As already mentioned, some myxobacteria, e.g., *Cystobacter* strains, grow well on casamino acids, e.g., in CAC medium.

CAC Medium

Casamino acids, vitamin-free (Difco)	0.2% (w/v)
Sodium glutamate	0.3% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
Cyanocobalamin	0.5 mg/ml
Trace elements	

After autoclaving, complement the medium with 0.5% (w/v) glucose and 0.5 mM phosphate buffer (pH 6.5) from autoclaved stocks.

Stigmatella aurantiaca Sg a15 can be cultivated in the following defined medium:

STG Medium (Kunze et al., 1984)

Casamino acids, vitamin-free (Difco)	0.1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)
$(NH_4)_2SO_4$	0.1% (w/v)
$CaCl_2 \cdot 2H_2O$	0.05% (w/v)
Phosphate buffer, pH 7.0	1 mM
Trace elements	
Vitamins (see earlier)	

Autoclave. Complement the medium with 0.5% (w/v) glucose from an autoclaved stock solution. Alternatively, fructose, maltose, starch, or dextrin 10 could also be used.

The cost of the medium is of considerable importance when large-scale culture is necessary. Many myxobacteria have been cultivated on various technical media, like corn steep powder, maize gluten, soy meal, skim milk, or yeast cells (Gerth et al., 1983; Kunze et al., 1984; Kunze et al., 1985; Kunze et al., 1987). Zein medium is an example of a production medium.

Zein Medium (Kunze et al., 1987)

Zein (maize gluten, Maizena, Hamburg, Germany)	1% (w/v)
Peptone from casein (Merck, Darmstadt, Germany)	0.1% (w/v)
$MgSO_4 \cdot 7H_2O$	0.1% (w/v)

The casein peptone is tryptic digested. Adjust the pH to 7.3. Autoclave the medium.

All myxobacteria of the bacteriolytic type rely totally, or to a large extent, on peptides and amino acids for nitrogen, carbon and energy. As a consequence, relatively large quantities of ammonia are released, which (by regulatory mechanisms and by making the medium alkaline) seriously limits growth and productivity. Thus, in cultures of *Mx. virescens* Mx v48, concentrations of 35–42 mM NH_4^+ and more were measured in the culture supernatant and were 80–140 mM within the cells (Gerth and Reichenbach, 1986). On-line extraction of ammonia from a laboratory fermentor via hydrophobic-membrane hollow fibers maintained the NH_4^+ concentration in the medium between 3 and 7.5 mM with dramatic effects: the generation time was lowered from about 4 to 2 h, the cell density increased by 233%, and the yield of the antibiotic myxovirescin rose from 8 to 115 mg/liter (K. Gerth, personal communication).

For a few strains of bacteriolytic myxobacteria, fully defined, synthetic media have been developed. Examples are given in Table 2. Gen-

Table 2. Composition of five fully defined, synthetic media for bacteriolytic myxobacteria.[a]

Mx. xanthus FB		*Ar. violaceum*		*Cb. fuscus/Cb. ferrugineus*	
Component	S medium[b]	Ml medium[c]	Al medium[d]	medium[e]	Hpl6 medium[f]
L-Ala	50	1000	—	50	—
L-Arg	100	100	—	100	—
L-Asn	500	500	100	100	—
L-Asp·K	—	—	5000	—	—
L-Cys	—	100	—	—	—
L-Glu·Na	—	—	—	1000	—
L-Gln·Na	—	—	—	—	1000
L-Gly	50	100	—	50	—
L-His	50	100	—	50	—
L-Ile	500	1000	100	300	40
L-Leu	1000	2000	50	500	—
L-Lys	250	500	—	150	—
L-Met	50	500	10	50	—
L-Phe	—	1000	100	—	40
L-Pro	500	1000	—	250	—
L-Ser	100	200	—	100	—
L-Thr	100	100	—	100	—
L-Trp	50	1000	—	—	—
L-Tyr	600	400	—	500	—
L-Val	100	200	100	100	—
Djekolic acid	100	—	—	—	—
Glycogen	3000	—	—	—	—
D-Glucose	—	—	—	—	5000
Na pyruvate	—	—	5000	—	—
Spermidine·3HCl	—	—	125	—	—
Cyanocobalamin	—	—	1	—	—
$(NH_4)_2SO_4$	—	—	5000	—	—
$MgSO_4 \cdot 7H_2O$	2000	200	2000	200	1000
$Ca_3(PO_4)_2$	—	—	—	20	—
$CaCl_2$	—	2	1.1	—	—
KCl	—	—	—	20	—
NaCl	—	200	—	—	—
$FeCl_3 \cdot 6H_2O$	—	2	2.7	—	—
KH_2PO_4	—	140	—	—	—
Phosphate buffer	A	—	B	—	C
Tris buffer	—	A	A	D	—
Trace elements	—	—	—	—	1 ml/liter

Abbreviations: —, 0 mg/ml; A, 10 mM, pH 7.6; B, 1 mM, pH 7.6; C, 0.5 mM, pH 6.5; and D, 20 mM, pH 7.5.

[a] In mg/ml.

[b] From Dworkin (1962).

[c] From Witkin and Rosenberg (1970).

[d] Minimal medium: from Bretscher and Kaiser (1978).

[e] From Mayer (1967). The organism is now called "*Cystobacter violaceus*."

[f] Reichenbach (1984a) and H. Reichenbach (unpublished observations). Some strains also require thiamine and/or biotin when grown in this minimal medium.

eration times increase substantially in such media. Thus, with *Mx. xanthus* FB, the doubling times were 3.5 h in CT medium, 8 h in S medium, and 6.5 h in M1 medium, 6.5 h in M1 medium without lysine, 9.5 h in M1 medium without threonine, and 11.5 h in M1 medium without lysine and threonine (Filer et al., 1973).

The cellulose degraders can be cultivated on very simple media, such as CK1 or CK6 medium (see below). The addition of a small quantity of peptone, e.g., 0.05% (w/v) peptone from a tryptic digest of casein (Merck, Darmstadt, Germany), usually improves growth and keeps the cells in the dispersed state, whereas without peptone they sometimes form cell clusters or tiny clumps (Irschik et al., 1987). Media with peptone as the only N source usually also allow good growth, e.g., AMB medium.

CK1 Medium (Kleinig et al., 1971; modified from Couke and Voets, 1967)

$MgSO_4 \cdot 7H_2O$	0.3% (w/v)
$CaCl_2 \cdot 2H_2O$	0.18% (w/v)
Fe^{+3}-citrate	20 mg/liter
KNO_3	0.2% (w/v)
K_2HPO_4	0.025% (w/v)
Glucose	1% (w/v)

Autoclave nitrate and phosphate together and $CaCl_2$ and glucose together and separate from the other ingredients Then add the three solutions when cooled.

CK6 Medium (Irschik et al., 1987; modified from Couke and Voets, 1967)

$MgSO_4 \cdot 7H_2O$	0.15% (w/v)
$CaCl_2 \cdot 2H_2O$	0.15% (w/v)
$FeCl_2 \cdot 7H_2O$	8 mg/liter
KNO_3	0.2% (w/v)
K_2HPO_4	0.025% (w/v)
Glucose	0.5% (w/v)

Autoclave nitrate and phosphate together and $CaCl_2$ and glucose together and separate from the other ingredients. Then add the three solutions when cooled.

AMB Medium (Ringel et al., 1977)

Soluble starch	0.5% (w/v)
Casitone (Difco)	0.25% (w/v)
$MgSO_4 \cdot 7H_2O$	0.05% (w/v)
K_2HPO_4	0.025% (w/v)

Autoclave the medium.

MD1 medium (see above) can also be used for cellulose degraders if it is supplemented with 0.1% (w/v) glucose or soluble starch (Irschik et al., 1987). Some strains can grow in liquid media, like CB medium containing Casitone as the only C, N, and energy source (Sarao et al., 1985), but this must be exceptional because none of the many *Sorangium* strains we have tested would do so.

CB Medium (Casitone Broth; Sarao et al., 1985)

Casitone (Difco)	0.25% (w/v)
$MgSO_4 \cdot 7H_2O$	0.05% (w/v)
K_2HPO_4	0.0025% (w/v)

For liquid cultures of myxobacteria, ordinary Erlenmeyer flasks are perfectly satisfactory as culture vessels. For agitation, a rotary shaker at about 150 rpm is sufficient. As the oxygen demand of myxobacteria is rather moderate, flasks with baffles give little or no improvement in growth and actually are unfavorable with peptone-containing media because they produce too much foam. Several types of antifoam can be used with myxobacteria, but the type of antifoam acceptable in a certain situation and tolerated by a specific strain must always be tested first. We have found that with small culture volumes and limited foaming, a few drops of a 0.5% (w/v) cholesterol solution in acetone works well. In fermentors, as a rule, more efficient antifoams must be applied (Mizrahi et al., 1977; Ringel et al., 1977). We have had good results in many different myxobacterial fermentations with polyoxypropylene-based antifoams at 0.02–0.03% (w/v), for instance, of antifoam agents LB625 and M115 (both from Brenntag, Mülheim/Ruhr, Germany; Gerth et al., 1980; Irschik et al., 1983b), or with silicone antifoam agent at 0.005–0.02% (w/v; Merck, Darmstadt, Germany; these sometimes require further additions later during fermentation; Irschik et al., 1983a; Kunze et al., 1984; Kunze et al., 1985). Many, but not all, myxobacteria can be cultivated in the presence of an Amberlite adsorber resin, e.g., with 1% XAD-1180 (Röhm and Haas, Darmstadt, Germany; e.g., Gerth et al., 1983; Kunze et al., 1985). This strategy often is extremely useful for the production and recovery of secondary metabolites that are excreted by the cells.

Mass cultivation of myxobacteria in fermentors has not presented any serious problems. Mechanical stress seems not to be a limiting factor. If no continuous control and regulation of culture parameters is required, cultivation in large flasks (10–15 liters) also gives excellent results. The flasks are aerated with three air outlets of sintered glass at the ends of silicone tubing, which are lowered to close to the bottom of the vessel. The aeration produces enough turbulence in the liquid so that additional stirring is unnecessary. The appropriate temperature is achieved by placing the flask in an incubation room or by connecting a length of silicone tubing coiling within the culture broth to a thermostat with a pump. With such an inexpensive system, yields on the order of 8–12 g cells/liter (wet weight, corresponds to 2–3 g of dry weight) can be obtained.

Cultivation in commercial fermentors permits the continuous monitoring of various culture parameters and allows them to be balanced and controlled. Although the optimum conditions depend on the type of fermentor used, the strain, and the medium, some generalizations are possi-

ble: In fermentations on the scale of 50–5000 liters, typical aeration rates are between 0.01 and 0.2 liter air per liter of medium per min, which, with stirring rates between 150 and 650 rpm, result in oxygen levels between 90 and 100% saturation (Mizrahi et al., 1977; Gerth et al., 1980; Gerth et al., 1982; Gerth et al., 1983; Irschik et al., 1983b; Irschik et al., 1983a; Irschik et al., 1987; Kunze et al., 1984; Kunze et al., 1985, Kunze et al., 1987). In a special study on myxovirescin production with *Mx. virescens* Mx v48 with fed-batch fermentations in a peptone medium, a cell yield of 0.5 g (dry weight) per g oxygen consumed and of 0.31 g per g peptone was obtained (Nigam et al., 1984). The oxygen consumption was 6.25 g of O_2 per liter of culture in 55 h. Respiration quotients between 0.3 and 1.3 mol of CO_2 per mol of O_2 were calculated under different culture conditions, with values of 0.8–1.0 during logarithmic growth. The maximum dry weight yield was 3.1 g/liter. The specific growth rate, m, varied between 0.05 and 0.12/h during exponential growth, depending on the feeding rate.

Other types of special fermentations have also been performed with myxobacteria; for instance, continuous cultures of *Mx. virescens* Mx v48 have been achieved in a chemostat with on-line extraction of myxovirescin in a vortex chamber (Hecht et al., 1987), and fermentations have been established with alginate-immobilized cells (Younes et al., 1984; Younes et al., 1987; Vuillemard et al., 1988). Cells of *So. cellulosum* So ce12 immobilized in 1-mm alginate beads are extremely stable and could be maintained in fermentors under continuous exchange of the medium for up to 75 days before the cells degenerated (Becker, 1990; K. Becker, personal communication). The system allowed the production conditions for the antibiotic sorangicin to be defined more clearly, and under optimal conditions, volume/time yields are five times higher than those measured with suspended cells in batch cultures.

When fermentations are started with an inoculum of 5–10% (v/v), the results are almost always satisfactory. Sometimes the age of the inoculum is a critical factor, and beyond a certain, relatively early stage but still within the logarithmic phase, the cells will start to grow only after a long and erratic lag period or not at all. This has been observed with *Polyangium* and *Sorangium* strains (H. Reichenbach, unpublished observations).

Under optimal growth conditions the doubling times of myxobacteria are between 3.5 and 15 h, with the members of the suborder Sorangineae comprising the most slowly growing myxobacteria. Cell densities of up to 10^{10}/ml can be reached, for example, with *So. cellulosum*. Cell mass yields are in the range of 4–12 g of wet weight per liter (e.g., Irschik et al., 1983b; Irschik et al., 1983a; Irschik et al., 1985b). The dry mass is 20–25% of the wet weight.

The pH range for growth is rather narrow, approximately 6.5–8.5; in general, there is no good growth below a pH of 6.6 or above pH 8.0. While some of the cellulose decomposers will grow at a pH down to about 5.0 (Krzemieniewska and Krzemieniewski, 1937a; Couke and Voets, 1967), below pH 5.8, growth is marginal (H. Reichenbach, unpublished observations). As with other bacteria, the pH in myxobacterial cultures can be stabilized by adding a buffer. HEPES and 3-[*N*-morpholino]propanesulfonic acid (MOPS) buffers (50 mM) are well tolerated by many myxobacteria, and Tris may also be used. (Note that many of the media formulations do not contain phosphate; the low amount of phosphate contained in the peptones—in a 0.2% [w/v] solution of peptone from casein, 0.5 mM phosphate was present [Becker, 1990]—is usually sufficient to satisfy the phosphate requirement of myxobacteria.)

The temperature optimum for growth is usually between 32 and 36°C, and there is a sharp maximum around 38°C. The minimum is less clear. *Myxococcus xanthus* FB grew exponentially with a generation time of 14 h at 14°C, the lowest temperature tested (Janssen et al., 1977); the lower limit for cellulose decomposition by two *Sorangium* strains was found to be 11°C (Krzemieniewska and Krzemieniewski, 1937a). *Myxococcus fulvus* Mx f2 grows, albeit slowly, at 6°C. However, other myxobacteria obviously have a different temperature range: The psychrophilic myxobacteria from Antarctica have already been mentioned (Dawid et al., 1988), and at the other end of the scale, strains of *Archangium*, *Polyangium* and *Sorangium* that are able to grow at 40°C have been known for some time (McCurdy, 1969b). About 70% of the *Sorangium* strains grow at 40°C and about 80% at 38°C. A variety of other myxobacteria can grow at these somewhat elevated temperatures, e.g., most *Polyangium*, many *Archangium*, *Cystobacter*, *Chondromyces* and some *Mx. virescens* strains, but usually not *Mx. fulvus* and *Corallococcus*.

Most myxobacteria do not require vitamins when growing in the usual complex media, but, as already mentioned, a vitamin dependence, usually for thiamine or biotin or both, may develop when the organism is transferred to a minimal medium. *Stigmatella aurantiaca* Sg a15 needs both thiamine and B_{12} (Kunze et al., 1984); B_{12} dependence is not unusual and has also been observed with many *Polyangium* (but not with *Sorangium*), some *Nannocystis*, and one strain of *Corallococcus macrosporus*.

The mineral requirements of myxobacteria are similar to those of other bacteria. Myxobacteria seem to need relatively high Mg^{+2} concentrations (the optimum is often around 5–10 mM, equivalent to 0.075–0.15% $MgSO_4 \cdot 7H_2O$). *Sorangium* strains require Ca^{+2} as well and grow better on media with Ca^{+2} than with Mg^{+2} (McCurdy, 1969b). The addition of Co^{+2} sometimes has a stimulating effect (Ringel et al., 1977; Gerth et al., 1982). *Myxococcus xanthus* is sensitive to elevated concentrations of monovalent cations (Mason and Powelson, 1958), and the salt tolerance of myxobacteria is generally low. An exception is *Mx. virescens*, which seems to form fruiting bodies better when 0.5% (w/v) NaCl is added to the medium (Rückert, 1978). This organism can still grow on media containing 1% NaCl, although at a clearly reduced rate, and, in fact, it can be selectively isolated on streaks of *E. coli* on WCX agar at that salt concentration.

Usually, myxobacterial cultures are kept in the dark or, in an incubation room, under continuous illumination. As already mentioned, some myxobacteria produce fruiting bodies only when the cultures are illuminated. Also, a phototactic behavior has been reported for some myxobacteria (Aschner and Chorin-Kirsch, 1970). Illuminated cultures often are much more deeply colored than those grown in the dark because carotenoid synthesis is induced by light (Burchard and Dworkin, 1966b). As color is sometimes a taxonomically relevant characteristic, cultures used for taxonomic work should at least be intermittently illuminated.

Isolation of Mutants

Mutant strains of myxobacteria can be isolated without difficulty provided strains capable of dispersed growth and procedures for obtaining a high plating efficiency are available. *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) is often (but not always) an excellent mutagen for myxobacteria. The useful concentration range is 50–150 µg NTG/ml when the cultures are treated for 1 h. Also UV irradiation has successfully been used to obtain mutants. However, in contrast to NTG, UV was ineffective in generating carotenoid mutants in *Mx. fulvus* Mx f2. Further details on the application of different mutagens and a comparison of their effects on a variety of myxobacteria can be found in the literature (e.g., Burchard and Parish, 1975; Hodgkin and Kaiser, 1977; Grimm, 1978).

In recent years, transposon insertion mutagenesis has enjoyed particular favor because of the ease in identifying the gene containing the mutation. Transposon insertions have been generated with Tn*5* (Kuner and Kaiser, 1981), Tn*5 lac* containing a *lacZ* reporter (Kroos and Kaiser, 1984), Tn*V* containing an origin of replication (Furuichi et al., 1985), and magellan-4 (Youderian et al., 2003). Transposon mutagensis typically employs a suicide vector that is introduced by specialized transduction with coliphage P1 (Shimkets et al., 1983), conjugation with RP4 (Breton et al., 1985), or electroporation (Kashefi and Hartzell, 1995).

One typically mutagenizes cells and then screens for a particular phenotype of interest. One can also take the opposite approach and eliminate specific genes whose sequences are known. Mutations can be introduced in a directed way by homologous insertion of a nonreplicating circular DNA molecule containing a small portion of a gene lacking both 5′ and 3′ coding sequences (Shimkets and Asher, 1988). Integration of a truncated gene inactivates that gene and any genes following it in the same transcriptional unit. Such systematic mutagenesis has already been applied to a large cosmid (Lancero et al., 2002). The genome sequences of *Mx. xanthus* and *So. cellulosum* are now complete. This will facilitate the directed mutagenesis approach.

Preservation Methods

Vegetative cultures of myxobacteria on agar plates kept at room temperature or at 30°C will usually remain viable for about 2–3 weeks. Storage of such cultures at 4°C leads to a rapid loss of viability.

If fruiting bodies are produced, the cultures remain viable for a much longer time. One has to be certain, however, that the fruiting bodies do indeed contain myxospores, since under some conditions the fruiting bodies are empty or contain only vegetative cells.

Storage in the Form of Desiccated Fruiting Bodies

Pieces of agar that contain fruiting bodies can be placed on sterile filter paper in an empty Petri dish, dried in an evacuated desiccator, and stored as such for years. This method is especially useful with myxobacteria such as *Nannocystis*, whose fruiting bodies are mainly located within the agar.

Alternatively, vegetative cells can be transferred from a suitable growth medium, preferably VY/2 agar, to small rectangles (about 1.5 × 3 cm) of sterile filter paper on water (WAT) agar, and the cultures are incubated at 28°C. Usually, large numbers of fruiting bodies develop within 2–3 days. These fruiting bodies are allowed to mature for about 8 days. The filter pads are then placed into suitable containers, such as screw-cap

tubes or small medicine bottles, and dried in an evacuated desiccator over silica gel for a few days. The containers are then tightly closed and stored at room temperature or at 6°C (it is not known which temperature is better). The dried fruiting bodies survive for about 5–15 years. This method has the advantage of being easy and cheap, and it conserves the ability of the strain to form fruiting bodies. It works, however, only if the strain has not been transferred too many times since its isolation. The method should therefore be applied as soon as a strain is pure and, if fruiting is a desired feature, even earlier. Sometimes myxospores are still produced on the filter although fruiting bodies are no longer formed. In that case, the filter can still be dried and a viable preparation produced. The growth medium taken from the vegetative cells must not be too rich. Thus, for example, fruiting bodies usually do not appear after transfer from CY agar. Sometimes the organism must be grown directly on the filter paper to obtain fruiting bodies. This may be done on filter pads on water agar after streaking autoclaved yeast or food bacteria onto the filter. In this way, *Chondromyces* strains are sometimes induced to fruit. The cellulose decomposers almost always produce many fruiting bodies when growing on filter paper, which has been placed on ST21 agar. As the fabric of the filter paper is destroyed during growth, the paper can no longer be removed, but the macerated mass may be transferred to a fresh filter pad, which is then treated as described above.

To start a culture from dried fruiting bodies, the filter pad is placed upside down on CY agar, VY/2 agar, or WAT agar with streaks of autoclaved *E. coli*. After 24 h, the filter strip should be shifted to another place on the plate, because reduced oxygen access may prevent germination under the wet paper. When the paper is removed, many of the soaked and softened fruiting bodies will stick to the agar surface.

Alternatively, if a sufficient quantity of fruiting bodies is obtainable, e.g., after growth on VY/2 agar, after having been transferred to water agar or, in the case of the cellulose degraders, on filter paper on ST21 agar, they may be dried in skim milk. The fruiting bodies are suspended in skim milk, and a few drops of the suspension are applied to a freeze-dried milk plug in an ampoule. The ampoules are kept at 4°C for at least 10 min, and the plug is then dried again for about 5 h (this time at room temperature) in a desiccator connected to an efficient pump. After that, the ampoules are filled with nitrogen gas and sealed. Strains thus preserved also retain their ability to form fruiting bodies and may remain viable for a longer time than on filter paper. While drying in skim milk also works well with myxospores, including those that were experimentally induced (Dworkin and Gibson, 1964a), results with vegetative cells are highly erratic. Vegetative cells invariably die during drying, and only those cultures that contain myxospores survive. Experiments with true lyophilization and with protective substances other than skim milk did not give better results.

An excellent and convenient method for the preservation of myxobacteria is freezing at –80°C. Samples of 1 ml are transferred from exponentially growing liquid cultures to screw-cap tubes and placed directly into the deep freeze. Alternatively, the bacteria may be scraped from the surface of a young agar culture, transferred to 1 ml of CAS medium, and frozen. All of the myxobacteria that we have tested so far (26 species) have survived in the deep freeze for 8–10 years, the longest period over which data have been collected. Storage at ultra-low temperature is the best way to preserve strains growing in the dispersed state, nonfruiting, nonspore-forming strains, and mutants. It is also a fast and reliable method for the storage of all kinds of strains of a culture collection. However, if the temperature rises above –30 to –25°C, the cells will die unless they are transferred to a growth medium within 5–10 h.

For safe long-term storage, freezing in liquid nitrogen is the method of choice. We use the same cell suspension as described above, but add 5% dimethyl sulfoxide (DMSO) to the suspension medium, which is usually CAS medium. We fill 0.5-ml amounts of the suspension into glass ampoules, seal them, and immerse them in liquid nitrogen without further precautions. The DMSO may not be required since all strains stored without DMSO have survived thus far for 2 years.

Storage of frozen cell suspensions at –25°C is not recommended because most myxobacteria will die within several days or, at the most, a few weeks.

To reactivate frozen cultures, the tubes or ampoules are immersed in cold water to speed up thawing. The cells must be transferred to fresh medium immediately after they have thawed because if the cells remain in the original medium after thawing, they will lyse within 15–30 min. We generally use VY/2 agar for the reactivation of the culture, usually with good results, but CY agar, CAS medium, and MD1 medium can also be used. If experience is lacking with a certain strain, the thawed suspension should always be inoculated into several different media, including a liquid medium. Sometimes, albeit rarely, recovery will occur only on a specific medium, although the other media normally support growth. For example, we have observed that some strains can be reactivated only in a liquid medium or only on agar plates. If a liquid

medium is used, care should be taken not to dilute the organisms too much, e.g., by inoculating a few drops into 100 ml. In some cases, for example with particularly fragile mutants, reactivating the cells by embedding them in a growth medium containing 0.6% (w/v) agar is useful. The soft agar appears to provide a matrix that protects cells that have been slightly damaged by freezing and thawing (D. Zusman, personal communication).

At least some myxobacteria can be stored in a 1% (w/v) NaCl solution at room temperature. The cell suspension must not be too dense: the liquid should be just visibly turbid; otherwise the cells will lyse overnight. For one strain of *Mx. xanthus*, a suspension of vegetative cells has been kept alive for 5 months in this way. In another case, we suspended vegetative cells and glycerol-induced myxospores of *Mx. xanthus* FB in 1% (w/v) NaCl solution and kept the suspensions in sealed ampoules for 7 years at room temperature. After that time, a culture could be started from the myxospores, but not from the vegetative cells. While the original strain was very sensitive to NaCl, the revived strain grew on CY agar with 1% (w/v) NaCl. This clearly indicates that in such cases the bacteria are not completely dormant and that a selection for certain characteristics may have taken place.

Characterization

Nutrition and Energy Metabolism

All myxobacteria decompose macromolecules, but there are two sharply separated metabolic groups. Group 1 myxobacteria decompose cellulose and are classified in two genera, *Sorangium* and *Byssophaga*. The few strains that have been studied in detail are all able to grow on inorganic nitrogen (NH_4^+ or NO_3^-) as the only nitrogen source, although their growth is stimulated if a small quantity (0.05–0.1% [w/v]) of peptone is added. They also grow on organic nitrogen compounds as the only nitrogen source, provided a carbohydrate is also present. A few strains appear even to grow on peptone alone (Sarao et al., 1985). These organisms utilize cellulose and sugars such as glucose equally well, but cellulase production is repressed in the presence of sugar (Krzemieniewska and Krzemieniewski, 1937a; Krzemieniewska and Krzemieniewski, 1937b; Pronina, 1962; Couke and Voets, 1967).

Group 2 myxobacteria, by far the majority of species, depend on an amino acid-containing medium such as peptone for growth. They obtain oligopeptides by hydrolyzing proteins. Their dependence on peptides may be such that developing defined amino acid media for them is difficult or impossible. In other cases, the organisms grow on remarkably simple media with glucose, glutamate, and two essential amino acids (see the section Cultivation in this Chapter). In nature, these organisms seem to subsist mainly on other organisms, which they degrade by means of a variety of hydrolytic exoenzymes. Proteases, nucleases, lipases, glycanases, and cell wall lytic enzymes have been demonstrated. These myxobacteria are particularly efficient in the destruction of other bacteria and of yeast cells and therefore have been called "micropredators" (Singh, 1947). However, they do not depend on living organisms and could as well be regarded as scavengers. Their association with animal dung is probably due to its rich mixture of many types of living and dead cells and to its content of organic matter at every level of decomposition.

Almost all known myxobacteria (with the possible exception of *Anaeromyxobacter dehalogenans*) are strictly aerobic organotrophs. As a prerequisite for aerobic energy generation, *Myxococcus* possesses *a*-, *b*-, and *c*-type cytochromes as well as reduced β-nicotinamide adenine dinucleotide (NADH)-oxidase (Dworkin and Niederpruem, 1964b; Kleinig, 1972) and a complete tricarboxylic acid cycle (Watson and Dworkin, 1968; McCurdy and Khouw, 1969c; Sarao et al., 1985). The genus *Myxococcus* cannot utilize mono- and disaccharides (Watson and Dworkin, 1968) and depends on amino acids (or pyruvate) as a carbon and energy source (Bretscher and Kaiser, 1978). Recently it was discovered that methyl oleate dramatically increases the growth yield of *Mx. xanthus*, suggesting the presence of a fatty acid β oxidation pathway (Lau et al., 2002). Some myxobacteria can take up and metabolize oligosaccharides, which they produce from polysaccharides such as starch through the action of special hydrolytic enzymes (Irschik and Reichenbach, 1985a). This ability may be more common than presently believed, for virtually all *Myxococcus* and *Corallococcus* strains are able to degrade one or more polysaccharides, e.g., yeast cell wall β-glucan, xylan, and starch.

An overview of the metabolism of myxobacteria has been given by Shimkets (1984). Apparently, myxobacterial metabolism is similar to that of other bacteria, and unique biochemical pathways have not been discovered. Of course, myxobacterial metabolism has often been studied mainly in relation to biochemical reorganization during morphogenesis, and many enzymes have been described that are turned on or off at certain stages of development. These data will be found among the reviews cited earlier.

The glycolytic pathway seems to play a major role in the glucose metabolism of *So. cellulosum* (Sarao et al., 1985; D. Hofmann, personal com-

munication). In *Mx. xanthus*, glucose is not metabolized, presumably because hexokinase (ATP-dependent) and pyruvate kinase are absent (Watson and Dworkin, 1968). Nevertheless, an intact phosphofructokinase and a fructose-1,6-diphosphatase are present (Watson and Dworkin, 1968), implying that glycolysis and gluconeogenesis are both possible. The explanation for this apparent contradiction is not obvious. In *Cl. coralloides* strain Cc c127, fructose-1,6-diphosphate aldolase activity is very weak, and intracellular hexose seems to be metabolized via the pentose phosphate pathway (Irschik and Reichenbach, 1985a). The latter pathway is present in other myxobacteria, e.g., *So. cellulosum* (D. Hofmann, unpublished observations). In fact, this pathway must be present in all myxobacteria because all are able to synthesize the pentose required in anabolic reactions. In *Mx. xanthus*, the initial enzyme, phosphoenolpyruvate carboxylase, has been shown to be present (Watson and Dworkin, 1968). The conversion of fructose-6-phosphate into cell wall precursors has also been demonstrated (Filer et al., 1977). In *So. cellulosum*, gluconeogenesis appears to be blocked, which may explain the dependence of this organism on external carbohydrates (D. Hofmann, unpublished observations).

Morphology and Fine Structure

The vegetative cells of the myxobacteria are rod-shaped, typically 3–6 μm long and 0.7–1.0 μm wide. They are found in two morphological types: 1) slender, flexible rods with more-or-less tapering ends and 2) cylindrical, somewhat rigid rods with bluntly rounded ends (Krzemieniewska and Krzemieniewski, 1928; Fig. 1a–d). These different cell types correlate with a number of other basic characteristics and represent suborders of the Myxobacterales, the Cystobacterineae and the Sorangineae/Nannocystineae. The cell shape makes it possible to assign a strain at once to one of the two groups.

Most electron microscopic studies on myxobacteria are rather old now, and a thorough reinvestigation would be desirable. In addition to *Mx. xanthus*, several other myxobacteria have been studied, including *Mx. fulvus*, *Mx. virescens*, *Cystobacter*, *Stigmatella*, *Sorangium* and *Chondromyces* species (Voelz and Dworkin, 1962; Voelz, 1965; Voelz, 1966a; Voelz, 1966b; Voelz, 1967; Abadie, 1967; Abadie, 1968; Abadie, 1971a; Abadie, 1971b; Schmidt-Lorenz and Kühlwein, 1968; McCurdy, 1969a; Reichenbach et al., 1969b; MacRae and McCurdy, 1975; Lampky, 1976; Galván et al., 1986). The myxobacteria are typical Gram-negative bacteria. Both the tapered and the cylindrical cell types appear to be identical in fine structure, with ultrathin sections revealing a typical triple-layered outer membrane and a thin peptidoglycan sheet. The cells divide by septum formation (Abadie, 1971b; MacRae and McCurdy, 1975). Within the cells, mesosomes and mesosome-like membrane bodies are often seen (Voelz, 1965; Abadie, 1967; Abadie, 1968; Abadie, 1971b; MacRae and McCurdy, 1975; Lampky, 1976; Galván et al., 1986). The visualization of ribosomes was initially a problem, because they were difficult to fix properly (Voelz, 1967), but it later turned out that they are of the usual type. At low phosphate levels, the formation of polysomes seems to be induced in *Mx. xanthus* (Voelz, 1966a). Polysomes were also observed in cells of *Cm. crocatus*, and they were often attached to the cytoplasmic membrane and arranged in a helical fashion (Abadie, 1971b). Unusual disk-shaped, membrane-associated structures have been described from *Cm. crocatus* (MacRae and McCurdy, 1975). In cross sections they appear as two double tracks, each 7.5–11 nm wide and between 55 and 130 nm long, connected to the interior of the cytoplasmic membrane by 11- to 15-nm-long, fibrillar extensions. Several classes of intracellular granules, or inclusion bodies, have been found. Black granules, apparently produced by all myxobacteria, have been identified as polyphosphate (Voelz et al., 1966c). Electron-transparent granules are of at least two types: 1) granules that are pale gray, containing material that does not contrast well; these have been suggested to be polysaccharide (Reichenbach et al., 1969b), "slime vacuoles" (Schmidt-Lorenz and Kühlwein, 1969; Abadie, 1971b), or glycogen (Voelz et al., 1966c). This has been corroborated by light microscopy after performing the periodic-acid Schiff reaction (Schmidt-Lorenz and Kühlwein, 1969). 2) Completely empty, large, circular areas, which are sudanophilic under the light microscope, have been seen in the cells of *So. cellulosum* (Lampky, 1976) and may be lipid material. Also, poly-β-hydroxybutyric acid has been demonstrated chemically in *So. cellulosum* (H. Jansen, personal communication). In cells of *Mx. xanthus* grown under suboptimal conditions, large parts of the cytoplasm sometimes appear to have a crystalloid pattern (Voelz, 1966a; Voelz, 1967; Voelz, 1968).

In many myxobacteria, intracellular microtubules and fibrils may be seen (Schmidt-Lorenz and Kühlwein, 1968; Abadie, 1971b; MacRae and McCurdy, 1975; A. C. Burchard et al., 1977). The diameter of the microtubules is 10–16 nm (with *Cm. crocatus* 15–19 nm), and that of the fibrils is 4–5 nm (with *Cm. crocatus* 7.5–10 nm). Once it was thought that microtubules and fibrils were just two different aspects of the same structure depending on the plane of sectioning (Schmidt-Lorenz and Kühlwein, 1968), but this is not the

case. The fibrils are usually arranged in bundles that may be 40 nm wide and 3.5 μm long. The bundles usually lie close to the cytoplasmic membrane and often follow the long axis of the cell, sometimes to the cell poles, but they also may cross the cell from one side to the other. A herringbone-like periodicity with a 12-nm spacing has occasionally been seen (A. C. Burchard et al., 1977). Also, transverse bands with a very regular, crystal-like pattern may occur (A. C. Burchard et al., 1977). Originally, fibrils were found only in cells from swarming agar cultures (Schmidt-Lorenz and Kühlwein, 1968), but they were subsequently found in cells from liquid cultures as well (A. C. Burchard et al., 1977; H. Reichenbach, unpublished observations). These systems of fibrils and microtubules have been interpreted as contractile elements responsible for gliding motility by producing contraction waves (Schmidt-Lorenz and Kühlwein, 1968; A. C. Burchard et al., 1977), but there is no evidence that those structures can really contract, and they could have some other function or even be artifacts. In *Cm. crocatus*, those structures were assumed to be composed of ribosomes (paracrystalline system; Abadie, 1971b).

An extremely complex structure has been discovered in the surface layers of *Mx. fulvus*, just below the outer membrane (Lünsdorf and Reichenbach, 1989). One or several helical belts appear to be wrapped around the periphery of the cell, beginning close to the cell pole. From isolated material, it was deduced that the unit element consists of a long series of tiny rings at a regular, periodic distance, connected to each other by two longitudinal structures, perhaps two fibrils. Several of these unit elements, or strands, form a ribbon, and it could be shown that the rings in those ribbons can change their conformation, thereby producing a reduction of the transverse dimension of the ribbon by 40%. The belt is composed of about seven ribbons, and conceivably a coordination of the conformational changes occurring in the ribbons could produce a traveling wave in the belt and concomitantly in the cell surface, which would propel the cell. Nothing is known yet about the biochemistry of the system, and not even the structural details are fully understood. However, the components of the apparatus have been seen also in other myxobacteria, including *Mx. xanthus*.

Chemical Composition

While much has been learned about the chemical composition of the myxobacterial cell, the precise nature of the extracellular polysaccharides remains undetermined, as it is usually accompanied by a variety of other large and small excreted molecules (Sudo and Dworkin, 1972; Hanson and Dworkin, 1974). The excreted slime appears usually to be a heteropolysaccharide containing, among others, *N*-acetyl aminosugars (Sutherland and Thomson, 1975; Sutherland, 1979). Slime from fruiting bodies resembles in its overall composition that produced in vegetative cultures. A large extracellular protein-polysaccharide-lipid complex with proteolytic activity has been isolated from culture supernatants of *Mx. virescens* (Gnosspelius, 1978b). A glycopeptide with a heat-stable blood anticoagulant activity, named "myxaline," has been obtained from the culture broth of *Mx. xanthus* (El Akoum et al., 1987; Masson and Guespin-Michel, 1988). The glycan part contains *N*-acetyl aminosugars, and the peptide is rich in glutamic acid and serine. The excreted slime must be chemically different in the suborders of myxobacteria, since Congo red (0.01% [w/v] aqueous solution) stains the slime of the Cystobacterineae but not that of the Sorangineae/Nannocystineae (McCurdy, 1969b).

LIPOPOLYSACHARIDES AND CELL WALLS. There are several studies on the chemistry of the lipopolysaccharides (LPS) of various myxobacteria (Weckesser et al., 1971; Rosenfelder et al., 1974; Sutherland and Thomson, 1975; Sutherland, 1979). Contrary to the statement of Ruiz et al. (1987), myxobacterial LPS, including lipid A, can be readily and completely extracted from the cells by the usual phenol procedure (e.g., Sutherland and Smith, 1973; Rosenfelder et al., 1974; Sutherland and Thomson, 1975; Panasenko, 1985). Typical LPS yields are 0.5–1.0% (up to 1.9%) of the cell dry weight. Chemically, the LPS of the myxobacteria resembles that of the enterobacteria. The LPS of *Mx. fulvus* contains mannose, galactose, glucose, rhamnose, arabinose, glucosamine, and 3-*O*-methyl-D-xylose, a sugar only very rarely seen in bacterial LPS (Weckesser et al., 1971; Rosenfelder et al., 1974). The LPS of other myxobacteria may differ somewhat in sugar composition, but 2-keto-3-deoxyoctonic acid is always present, and heptose is never present. The 3-*O*-methyl-D-xylose may or may not be found, and in some cases, similar nonpolar but thus far unidentified sugars have been observed (Sutherland and Smith, 1973; Sutherland and Thomson, 1975; Sutherland, 1979). While the scant data do not really allow generalizations, a complete lack of rhamnose seems to be characteristic for *Sorangium* and may be another distinguishing characteristic of that suborder. The lipid A moiety contains glucosamine and the usual 3-hydroxy fatty acids, mostly *iso*-3-OH-C15 and *iso*-3-OH-C17 (Rosenfelder et al., 1974).

Man (1998) has carefully analyzed the LPS of *Mx. xanthus* and has found that the O-antigen portion consists of a small, repeating disaccha-

ride comprising → 4) -α -D-Gal*N*Ac*p* - (1 → 6)-α -D-Glcp - (1 →, where about 40% of the Gal*N*Ac*p* residues are methylated at their 6-positions. The "*p*" denotes pyranoside. The core LPS was found to consist of glucose, mannose, 2-acetamido-2-deoxy-galactose, and 2-acetamido-2-deoxy-6-*O*-methyl-galactose, in relative molar ratios of 1.00 : 0.41 : 0.40 : 0.32, respectively. Also present in the core LPS were small amounts of rhamnose, arabinose, xylose, galactose, and 2-acetamido-2-deoxyglucose.

Several mutations have been described that eliminate LPS O-antigen biosynthesis in *Mx. xanthus*. Fink et al. discovered that mutants resistant to phage Mx8 lack O-antigen and fail to react with monoclonal antibodies specific to O-antigen (Fink et al., 1989c). The genes containing these mutations have not been identified. The *wzm* and *wzt* gene products are predicted to encode an ATP-binding cassette (ABC) transporter involved in export of the O-antigen to the periplasm (Bowden and Kaplan, 1998). The *wbgA* and *wbgB* genes share similarity with bacterial glycosyltransferases and are suggested to be required for O-antigen synthesis (Bowden and Kaplan, 1998; Yang et al., 2000a). In all of these reports the authors noted deficiencies in motility, but there is some discrepancy as to whether the deficiencies are in A motility, S motility, or both. The consensus appears to be that both A and S motility are greatly reduced (Yang et al., 2000a). Man (1998) examined the LPS of two O-antigen mutants of *Mx. xanthus* (Fink and Zissler, 1989b) and found that both lacked the O-antigen repeating polysaccharide present in the wild-type parental strain. Man speculated that the corresponding reduction in cell surface hydrophilicity may be responsible for the motility defect.

During sporulation of *Mx. xanthus*, the LPS, or at least its polysaccharide part, is completely lost (Sutherland, 1976a), and a capsule composed of an α-1,3-glucan is produced (Sutherland and Mackenzie, 1977). That capsule is ruptured and discarded during germination rather than enzymatically solubilized (Voelz, 1966b). Monoclonal antibodies have also been obtained against O-antigens and against a core antigen of *Mx. xanthus* LPS (Gill and Dworkin, 1988; Fink and Zissler, 1989a). Several O-antigen mutants were defective in the A system but not in the S system of gliding. They were still able to form normal fruiting bodies (Fink and Zissler, 1989b). Endotoxin-like effects (anticomplement test and Shwartzman skin reaction) could be produced with whole cells and myxospores of *Mx. xanthus* (Ruiz et al., 1987).

Myxococcus xanthus, the only myxobacterium for which the organization of the cell wall has been investigated, has a thin peptidoglycan layer that seems to be organized in patches rather than in a homogeneous sacculus (White et al., 1968). The composition of the peptidoglycan resembles that of *E. coli*, with diaminopimelic acid at the cross-links. Apparently during sporulation, the degree of cross-linking increases. At the same time, galactosamine and glycine accumulate in the wall layer. The peptidoglycan content of vegetative cells and myxospores is, however, the same (0.6% of the dry weight).

LIPIDS. The fatty acid composition of the myxobacteria has not been thoroughly examined. However the data that are available suggest characteristic differences among the suborders (Fautz et al., 1981). While all myxobacteria contain substantial quantities of iso-branched fatty acids, mainly saturated C15:0 and C17:0, the fatty acid composition is dominated by them only in the suborder Cystobacterineae (usually between 50 and 70% total fatty acid; Schröder and Reichenbach, 1970; Ware and Dworkin, 1973; Fautz et al., 1979; Fautz et al., 1981; Yamanaka et al., 1988). *Nannocystis exedens* (suborder Nannocystineae) is the only myxobacterium so far in which larger amounts (about 30%) of *iso*-C15:1 and *iso*-C17:1 are found (Fautz et al., 1981). *Sorangium cellulosum*, suborder Sorangineae, has an intermediate phenotype with a more balanced representation of the saturated and unsaturated members of the iso odd family (Fautz et al., 1981).

The distribution of 2-hydroxy(OH) fatty acids also follows a trend, being high in the Cystobacterineae and virtually absent in the Nannocystineae and Sorangineae (Fautz et al., 1981). *Iso*-2-OH C17:0 was first seen as a contaminant from phospholipid in LPS preparations of *Mx. fulvus* and *Cb. ferrugineus* (Rosenfelder et al., 1974). Later, proof was presented for the chemical structure of the 2- and 3-OH fatty acids of *Mx. fulvus* and *C. ferrugineus*. In total cell hydrolysates, 2-OH fatty acids, mainly *iso*-2-OH C17:0, constitute 10–12% of the total fatty acids, while 3-OH fatty acids only play a minor role (2.5– 4.5%; Fautz et al., 1979). In members of the Cystobacterineae, two types of phosphatidylethanolamine have been demonstrated, one of which contains exclusively nonhydroxy fatty acids and the other 50–70% 2-OH fatty acids (Yamanaka et al., 1988).

Another major component of the fatty acid pattern of the Cystobacterineae is C16:1 (15–39%), which is greatly reduced in the Nannocystineae and Sorangineae. This fatty acid was later identified as 16:1 ω5c (which is extremely rare in nature; Kearns et al., 2001b). Anteiso fatty acids have also been reported in *Mx. xanthus* (Ruiz et al., 1985; Toal et al., 1995; Kearns et al., 2001b). In another study, a clear difference was seen between the fatty acid patterns of *Cor-*

allococcus and those of *Myxococcus* species (Monteoliva-Sanchez et al., 1987): branched and unbranched C17:0 fatty acids were present in large quantities in the latter (27–38%) but completely absent in the former. While such a clear difference would be useful for taxonomic reasons, it is not supported by other investigations (Yamanaka et al., 1988).

Myxococcus fulvus Mx f2 contains phosphatidylethanolamine as the main component (72%), with phosphatidylglycerol (9%), phosphatidylinositol (7%), and an unknown, ninhydrin-positive phospholipid (8%) as minor components (Kleinig, 1972). A small proportion of the phospholipids were alk-I-enyl-acyl (1–15%) and alkyl-acyl compounds; however, they comprised 22% of the phosphatidylinositol compounds. The phospholipid content of the isolated membranes was 25% (dry weight). The occurrence of two species of phosphatidylethanolamine in myxobacteria has already been mentioned (Yamanaka et al., 1988). The outer membrane of *Mx.* xanthus MD-2, an S motility mutant, showed an unusually low buoyant density (1.221 g/ml) which was probably due to an exceptionally high phospholipid content (Orndorff and Dworkin, 1980). The low buoyant density of the outer membrane was recently confirmed in the fully motile derivative DK1622 (Simunovic et al., 2003). The main phospholipid in both membranes was phosphatidylethanolamine (60–70% of total phospholipid). The other major phospholipids were phosphatidylglycerol and two unknown phospholipids. Phosphatidylserine and cardiolipin were found only in the cytoplasmic membrane. Substantial amounts of lysophospholipids were found in both membranes, although somewhat more in the outer membrane. They obviously indicate lipase activities, which seem not to be restricted to the outer membrane.

In *Sg. aurantiaca* the main phospholipid was phosphatidylethanolamine (50%), followed by phosphatidylinositol (20%) and phosphatidylglycerol (12%; Caillon et al., 1983). Very high amounts of lysophosphatidylethanolamine were also found. Each phospholipid had a different fatty acid composition. Alkyl ether linkages were common; phosphatidylinositol occurred only as the dialkyl compound. The structural details of an alk-I-enyl-acyl-phosphatidylethanolamine, i.e., a plasmalogen, from *Mx. stipitatus* have been elucidated (Stein and Budzikiewicz, 1987).

From the amount of phosphorus in the lipid fraction of *Sg. aurantiaca*, it has been estimated that only about 40% of the fatty acids are present in phospholipids (Schröder and Reichenbach, 1970). Ceramides and cerebrosides have been isolated from *Cystobacter fuscus* and chemically characterized (Eckau et al., 1984; Dill et al., 1985). Ceramides are long-chain, 1,3-dihydroxy-2-amino bases (sphinganine) with an amide-bound fatty acid. Cerebrosides are sphingolipids consisting of a ceramide with a C-1-sugar. The myxobacterial ceramides all contain 2-hydroxy fatty acids. A novel, long-chain base, 17-methylsphinga-4E,8E-dienine, was found in two of them. The cerebrosides are all galactosides. Cerebrosides are common in eukaryotes, where they play a role in cell-cell recognition and cell adhesion. They have also have been found to be fruiting-body inducers in basidiomycetes.

The capnoids, discovered as a characteristic constituent of the lipids of another group of gliding bacteria, the *Cytophaga*/*Flavobacterium* group, have not been found in myxobacteria (Godchaux and Leadbetter, 1983). The capnoids are C-1 sulfonic acids of sphinganine bases and their ceramides.

With one exception, *Methylococcus capsulatus* (Bird et al., 1971), myxobacteria are the only prokaryotes that are known to synthesize true steroids. *Nannocystis* strains produce cholestenols (Kohl et al., 1983; Zeggel, 1993); *Polyangium* (but not *Sorangium*) and *Cb. minus* produce lanosterol; and *Cb. violaceus*, *Sg. erecta* and *Sg. aurantiaca* contain cycloartenol (Bode et al., 2003). Squalene has been found in many myxobacteria. The cholestenol and squalene content of *Na. exedens* may be up to 0.4% of the dry weight. In contrast to most secondary metabolites, steroids are found in all strains of a species. Recently the key enzyme 2,3(S)-oxidosqualene oxidase has been demonstrated in *Sg. aurantiaca* (Bode et al., 2003).

PIGMENTS AND RESPIRATORY COMPOUNDS. Carotenoids are the main pigments of most myxobacteria. They have been extensively characterized by Kleinig et al. (1970) and especially in *Mx. fulvus* by Reichenbach and Kleinig (1971), who reported the presence of 50–60 different carotenoids and were able to identify 24 of these. Among the Cystobacterineae, the main compounds are always monocyclic carotenoid glycosides containing glucose with a fatty acid attached to the sugar via an ester bond (Kleinig et al., 1970; Kleinig et al., 1971; Reichenbach and Kleinig, 1971). While the Cystobacterineae have glycosides with keto but not with hydroxyl groups, members of the suborder Sorangineae produce glycosides with hydroxyl but not keto functions on the chromophore. *Sorangium* has also been shown to produce a nonesterified carotenoid rhamnoside (Kleinig et al., 1971). Four new carotenoids were recently identified in *Polyangium fumosum* (Jansen et al., 1995). *Nannocystis* contains mainly aromatic carotenoids and no glycosides. Thus apparently certain pigment types are characteristic for taxonomic subgroups (for a review, see Reichenbach and Kleinig, 1984b).

Carotenoid pigments forming esterified glycosides are rather unusual. The main pigment of many myxobacteria, including *Mx. xanthus*, is the deep-red myxobacton, which comprises 70% (by weight) of the total carotenoids of *Mx. fulvus*; it consists of 1′,2′-dihydro-1′-hydroxy-4-keto-torulene glucoside, esterified via glucose to a variety of unbranched, straight-chain fatty acids. This structure has been reinvestigated and corroborated (Eckau et al., 1984). An additional 10% of the total carotenoids consists of myxobacton esters, which contain 3,4-dehydro torulene rather than 4-keto torulene. In *Myxococcus*, complex mixtures of carotenoids are found, but only monocyclic and acyclic compounds occur. The carotenoid content of late log-phase cultures of *Mx. fulvus* Mx f2 has been determined. Acetone extracted carotenoids of such cells typically represent 0.003% of the dry weight of dark-grown cells and 0.03–0.06% of light-grown cells (Reichenbach and Kleinig, 1971). This difference between light- and dark-grown cells increases to 20-fold as the cells enter stationary phase. The carotenoids are exclusively located in the cytoplasmic membrane fraction, where they constitute up to 0.14% of the dry weight (Kleinig, 1972).

The effect of various inhibitors of the cyclization reaction of carotenoid synthesis has also been studied (Reichenbach and Kleinig, 1971; Kleinig, 1974a; Kleinig, 1975). In laboratory cultures, there is virtually no turnover or degradation of carotenoids. A system for the in vitro synthesis of C15 to C60 polyprenols from isopentenylpyrophosphate with a crude enzyme preparation from *Mx. fulvus* has been developed (Beyer and Kleinig, 1985).

The only demonstrated role of carotenoids in myxobacteria is to provide protection against photooxidation (Burchard and Dworkin, 1966b). Dark-grown cultures, which produce only small amounts of carotenoid, lyse quickly if illuminated upon entry to stationary phase of growth. If, however, the cells are exposed to light during exponential growth, carotenogenesis is photoinduced and the cells become resistant to photokilling. In *Mx. xanthus*, protoporphyrin IX was shown to be the photosensitizing pigment (Burchard and Dworkin, 1966b). Photokilling has also been demonstrated with carotenoid-free mutants of *Mx. fulvus* Mx f2. The killing effect is seen only if the mutants are illuminated with daylight intensities (about 40,000 lux; H. Reichenbach, unpublished observations). However, whether all myxobacteria accumulate protoporphyrin IX as a photosensitizer is unclear; protoporphyrin IX could not be detected, for example, in *Mx. fulvus* (H. Reichenbach, unpublished).

The carotenoid synthesis of myxobacteria is induced by light (Burchard and Dworkin, 1966b; Burchard and Hendricks, 1969; Reichenbach and Kleinig, 1971), and its control in *Mx. xanthus* involves a complex regulatory circuit (for reviews, see Hodgson and Murillo [1993] and Hodgson and Berry [1998]). Many carotenoid biosynthetic genes (*crt* genes) are clustered in the *crtEBDC* and *carB* operons, which are repressed in the dark by CarA. In outline, the reception of a blue light signal by CarF results eventually in the inactivation CarA. The light receptor, CarF, is remarkably similar to Kua, a family of proteins of unknown function that is widely distributed among eukaryotes (Fontes et al., 2003). But little is known about its interaction with protoporphyrin IX. Central to this response is the activation of the light-inducible promoter, *PcarQRS*, and the transcription of three downstream genes, *carQ*, *carR* and *carS* (Gorham, et al., 1996). CarQ is a member of the ECF (extracytoplasmic function) subfamily of RNA polymerase σ factors that is responsible for initiation at *PcarQRS*. CarR is an anti-σ factor that sequesters CarQ in a transcriptionally inactive complex. CarR was found to be unstable in illuminated stationary phase cells, providing a possible mechanism by which the CarR-CarQ complex is disrupted (Browning et al., 2003). In the light, CarS is synthesized and activates the *crtEBDC* operon by preventing CarA from binding to its operator (Whitworth and Hodgson, 2001). CarS is therefore an antirepressor. CarA is a novel protein consisting of a DNA-binding domain of the MerR family of transcriptional regulators, directly joined to a cobalamin-binding domain. The presence of vitamin B_{12} or some other cobalamin derivatives is absolutely required for activation of the *carB* promoter by light (Cervantes and Murillo, 2002).

Pigments other than carotenoids are also produced by myxobacteria. A pale-yellow pigment is found in *Mx. xanthus* (Gerth et al., 1983). On certain media, the colonies of *Cb. violaceus* (= *Ar. violaceum*) develop a deep purple-violet color, probably due to melanoid pigments (Kuhlwein and Gallwitz, 1958; Reichenbach, 1965c). A red pigment of *Cb. violaceus* is probably a dopachrome (Mayer, 1967). Melanoid pigments are also relatively often seen with individual strains of many other species, especially on peptone-containing media, e.g., with *Cystobacter*, *Archangium*, *Corallococcus* and *Stigmatella*. Liquid cultures of *Sg. aurantiaca* in peptone medium turn a deep black color within 1–2 h after reaching the stationary phase (Reichenbach and Dworkin, 1969a). The slimy swarms of some strains of *So. cellulosum* on CA2 agar become deep violet to black after 2–4 weeks. *Myxococcus virescens* and *Kofleria flava* are bright greenish-yellow, especially on protein-containing media. Several myxococci, especially

Mx. virescens, excrete a greenish-yellow diffusing pigment that appears to be myxochromid (Trowitzsch-Kienast et al., 1993). Plate cultures of *Mx. stipitatus* show under the UV lamp at 366 nm a bright yellow fluorescence (Lampky and Brockman, 1977) that is caused by phenylamides, or stipiamides (Kim et al., 1991; Trowitzsch-Kienast et al., 1992). Often the various shades observed with different strains and species are only due to differences in the quantitative proportions of the same pigments. Fruiting bodies, and especially the walls of the sporangioles, are often very intensely colored, but the chemical nature of these pigments is not known.

The respiratory quinones of the myxobacteria are exclusively menaquinones (Kleinig, 1972; Kleinig et al., 1974b). A survey of 11 genera and 20 species of both suborders showed that the menaquinone pattern of the myxobacteria is completely uniform and comprises virtually only MK-8 (Yamanaka et al., 1987; M.D. Collins, personal communication). Dworkin and Niederpruem (1964b) have characterized the terminal respiratory pigments of *Mx. xanthus* and have shown them to include cytochromes *a*, *b* and *c* in addition to presenting tentative evidence for the presence of flavin-linked enzymes.

MEMBRANES AND MEMBRANE PROTEINS. The membranes of *Mx. fulvus* consist of about 64% protein and 29% lipid, the latter comprising mainly (25%) phospholipid (Kleinig, 1972). The inner and outer membranes of *Mx. xanthus* strain DK1622 were fractionated (Simunovic et al., 2003). Membranes were enriched from spheroplasts of vegetative cells and then separated into three peaks on a three-step sucrose gradient. The high density fraction corresponded to inner membrane (IM), the medium density fraction corresponded to hybrid membrane (HM), and the low density fraction corresponded to outer membrane (OM). Each fraction was subjected to further separation on discontinuous sucrose gradients, resulting in the emergence of discrete protein peaks within each major fraction. The purity and origin of each peak were assessed using succinate dehydrogenase (SDH) activity as the IM marker and reactivity to lipopolysaccharide core and O-antigen monoclonal antibodies as the OM markers. As previously reported (Orndorff and Dworkin, 1980), the OM markers localized to the low density membrane fractions, while SDH localized to high density fractions (Simunovic et al., 2003). Immunoblotting was used to localize important motility and signaling proteins within the protein peaks. CsgA (the developmental C-signal producing protein) and FibA (a fibril-associated zinc metalloprotease) were localized in the IM. The motility lipoproteins Tgl and Cgl were localized in the OM. FrzCD (a methyl-accepting chemotaxis protein) was predominantly located in the IM, although lesser amounts were found in the OM. PilA (the major subunit of type IV pili) was distributed in all the fractions. Two-dimensional polyacrylamide gel electrophoresis illustrated the presence of proteins that were unique to the inner and outer membranes. Characterization of proteins within an unusually low density (ρ = 1.072–1.094 $g \cdot cm^{-3}$) membrane peak showed the presence of Ta-1 polyketide synthetase, which synthesizes the antibiotic myxovirescin (Simunovic et al., 2003).

Six of the membrane polypeptides changed substantially during aggregation (Orndorff and Dworkin, 1982): during the early stages of fruiting, protein T disappeared from the soluble fraction of *Mx. xanthus*, when proteins S and U first appeared, and then increased dramatically. Protein S (Inouye et al., 1979b) has been particularly well studied. In vegetative cells it amounts to less than 1% of the soluble protein, but during fruiting body formation, its rate of synthesis rises to 15% of total protein synthesis. In the presence of Ca^{+2}, protein S assembles on the surface of the maturing myxospores (Inouye et al., 1979a). Certain domains in the amino acid sequence of protein S show homologies to the Ca^{+2}-binding sites of calmodulin. The functional correspondence of those areas was demonstrated by site-specific mutations, which eliminated the Ca-binding property of protein S together with its ability to accumulate on the myxospores. Protein S also has some homology with the bovine-lens protein, α-crystallin (Inouye et al., 1983a; Teintze et al., 1988; Wistow, 1990).

Another development-specific protein is a myxobacterial hemagglutinin (MbhA), a lectin that is induced during the developmental aggregation of *Mx. xanthus* (Cumsky and Zusman, 1981). It appears to be localized in the periplasmic space or loosely bound to the cell surface and to recognize a galactose-containing, complex receptor. Its distribution over the cell surface is uneven, as it is found in patches mainly at the cell poles (Nelson et al., 1981). The 28-kDa protein contains four highly conserved domains of 67 amino acids each, which are reflected in four strong internal homologies in its gene (Romeo et al., 1986). MbhA is produced primarily by the peripheral rods, which never enter fruiting bodies or sporulate (O'Connor and Zusman, 1991a; O'Connor and Zusman, 1991b; O'Connor and Zusman, 1991c).

Using an iodination technique, substantial changes in the pattern of accessible surface proteins of *Mx. xanthus* were shown to take place during development (Maeba, 1983). A glycoprotein, probably a peripheral membrane protein that is present only in vegetative cells, was further characterized (Maeba, 1986). The 74-kDa

protein contained about 15% carbohydrate, mainly neutral sugars, but also some hexosamines and uronic acids. It made up 1% of the total cell protein and might play a role in cell-cell interactions.

A different approach to the study of the dynamics of the cell surface during development has been the use of monoclonal antibodies against cell-surface antigens (CSAs) of vegetative and developing cells (Gill et al., 1985). In this way, many different CSAs of *Mx. xanthus* have been identified (e.g., Gill and Dworkin, 1986; Gill and Dworkin, 1988). While most of the antibodies are directed against cell-surface proteins, some are directed against LPS or, in the case of CSA 1604, against a large, 150-kDa complex consisting of at least two protein subunits and LPS (Jarvis and Dworkin, 1989a; Jarvis and Dworkin, 1989b). Other monoclonal antibodies are directed against cell-surface proteins that appear uniquely during development (Gill and Dworkin, 1986).

Transfer and expression of myxobacterial genes in *E. coli* have identified some proteins. An 18.7-kDa basic protein coded by the *vegA* gene of *Mx. xanthus* turned out to have amino acid homologies with certain histones and may function as a DNA-binding protein. It is essential for vegetative growth (Komano et al., 1987). Also, the *Mx. xanthus* gene *fprA* was shown to code for a protein that binds flavin mononucleotide. Overexpression of the gene in *E. coli* leads to a spectacular increase in flavin biosynthesis, but the FprA protein seems to be an enzyme not in the flavin biosynthetic pathway (Shimkets, 1990b) but rather in vitamin B_6 biosynthesis (Lam and Winkler, 1992).

NUCLEIC ACIDS. The G+C content of myxobacterial DNA is between 67 and 72 mol% (as measured by buoyant density, thermal melting, and high pressure liquid chromatography [HPLC]; Mandel and Leadbetter, 1965; McCurdy and Wolf, 1967; Behrens et al., 1976; Mesbah et al., 1989).

As a group, the myxobacteria have among the largest bacterial genomes. The genome size of *Mx. xanthus* FB is 9450 kilobase pairs (kbp; Chen et al., 1990), double that of *E. coli* (4700 kbp). The genome size of *So. cellulosum* So ce56 is about 12.2 Mb (Pradella et al., 2002). Genome sequencing of these two species is in progress. The genome size of *Sg. aurantiaca* DW 4/3.1 is 9350 kbp (Neumann et al., 1992). Genome sizes of a variety of *Sg. aurantiaca* and *Sg. erecta* isolates were examined and ranged from 9.2 to 10.01 Mb. Thus the genome size of this group appears to be uniformly large. In the two species that have been examined, the genome is arranged in a single circular chromosome (Chen et al., 1990; Chen et al., 1991; Neumann et al., 1993).

The DNA of *Mx. xanthus* strain FB is methylated at cytosine sites (Yee and Inouye, 1982). In rapidly growing cells, the DNA is undermethylated, and the degree of methylation increases when growth slows. *Myxococcus xanthus* RNA polymerase shows the usual subunit composition, but two slightly different σ factors have been obtained from vegetative cells (Rudd and Zusman, 1982).

A most unusual kind of nucleic acid, msDNA, which was first discovered in *Mx. xanthus*, has raised many intriguing questions (Yee et al., 1984). Found in nearly all myxobacteria (Dhundale et al., 1985), msDNA is a single-stranded DNA and 500–700 copies of it are present per genome. The DNA is 162 bases long, and its 5′-end is linked via a 5′ to 2′-phosphodiester bond with a guanidine residue in an RNA (msdRNA), which is 77 bases long. The RNA has an extremely stable stem-and-loop structure. The coding regions for msDNA and msdRNA are found side by side at one specific site in the *Myxococcus* genome, but they are oriented in opposite directions and overlap with 8 bases at their 3′-ends (Dhundale et al., 1987). A very similar msDNA (81% sequence homology) with a corresponding genome structure has been found in *Sg. aurantiaca* (Furuichi et al., 1987a; Furuichi et al., 1987b). A second species of such DNA (mrDNA) was later discovered in *Mx. xanthus* (Dhundale et al., 1988b). However, mrDNA is much shorter, and it has a completely different DNA and RNA base sequence, but the RNA still has a similar secondary structure to that of msRNA. Also, there seem to be only 100 copies of mrDNA per genome.

The msDNA is synthesized by a reverse transcriptase with an associated ribonuclease H activity. The enzyme uses a folded RNA precursor as a primer and a template (Lampson et al., 1989). The gene of the reverse transcriptase has been found immediately downstream from the msdRNA region and codes for 485 amino acids, which show sequence homology with retroviral reverse transcriptases (Inouye et al., 1989). In fact, *Mx. xanthus* actually contains two reverse transcriptases (Inouye et al., 1990b). The wide distribution of msDNA in myxobacteria, as well as a comparison of codon usage in the reverse transcriptase gene and in other *Myxococcus* genes, suggests that the system is very old and was not acquired recently (in contrast to a similar system in certain *E. coli* strains). By deletion mutation, msDNA synthesis could be eliminated, but this had no significant effects on growth, motility and morphogenesis compared to the wild-type strain (Dhundale et al., 1988a).

No evidence has been found for the presence of a poly(dT-dG) · poly(dC-dA) (TG) element in *Mx. xanthus* (Morris et al., 1986).

While the half-life of mRNA is about 3.5 min in vegetative cells of *Mx. xanthus*, 5–10 species of mRNA with a much longer half-life (13–30 min) could be demonstrated during fruiting body formation (Smith and Dworkin, 1981; Nelson and Zusman, 1983). One of the long-lived mRNAs is for protein S. The stable mRNA can amount up to 30–40% of the total mRNA.

Although no clear evidence has been presented for free, self-replicating plasmids in myxobacteria, several early reports suggest that extrachromosomal DNA may be present. Thus, after chloramphenicol resistance was induced in *Mx. xanthus* RB, a burst of synthesis of circular, covalently linked, extrachromosomal DNA was observed (Brown and Parish, 1976a). The resistance was due to chloramphenicol acetylation (Burchard and Parish, 1975). Also, it was possible to transfer resistance to several different antibiotics from R factor-containing strains of *E. coli* to several *Myxococcus* species by conjugation (Parish, 1975). In some cases, extrachromosomal DNA could be demonstrated in the resistant *Myxococcus* strains, and the resistance could be transferred to other *Myxococcus* strains by conjugation.

PHAGES AND BACTERIOCINS. All myxophages that have been found thus far contain double-stranded DNA of around 50 kbp. The first myxophage to be isolated was *Mx. xanthus* phage MX-1. It came from a sample of cow dung and is a virulent phage resembling coli phage T2 with an icosahedral head and a contractile tail sheath. It has a latent period of 2–2.5 h (30°C), a 2-h rise, and a burst size of about 100 (Burchard and Dworkin, 1966a). MX-1 attacks *Mx. xanthus* as well as the related species *Mx. virescens* and *Mx. fulvus* (Tsopanakis and Parish, 1976). It has a genome size of 130–150 $\times$ 10^6 Da and a G+C content of 56 mol% (using buoyant density; a reinvestigation with a chemical method gave 50–52 mol%; Brown et al., 1976c).

MX-1 shows the classical pattern of infection and propagation known for *E. coli* phages (Voelz and Burchard, 1971). However, if cells are infected with MX-1 during glycerol induction of myxospores (see the section Functions of Myxospores and Fruiting Bodies in this Chapter), there is a brief period during which the phage may be taken up without interfering with myxospore induction. The phage is trapped in the myxospore and is released upon germination (Burchard and Dworkin, 1966a).

Defective phage particles, originally described as rhapidosomes (Reichenbach, 1965a), have been seen in several myxobacteria (Reichenbach, 1967; McCurdy and McRae, 1974; Brown et al., 1976b). When the ultracentrifugal pellets of culture supernatants of six strains of *Mx. xanthus*, *Mx. virescens* and *Mx. fulvus* were examined under the electron microscope, all six strains produced small quantities of phage particles (Brown et al., 1976b). An endogenous bacteriophage was detected in a derivative of *Mx. xanthus* FB through the use of transposon tagging (Starich et al., 1985). Transposon mutagenesis with Tn*5* revealed a class of bacterial mutants that transduced the transposon through the culture supernatant. Virus-like particles, 30–35 nm in diameter, co-purified with the transducing activity. A physical map of this region of the genome comprised multiple units of about 80 kbp each (Starich and Zissler, 1989). All but one of these has been deleted in *Mx. xanthus* DK1622, which was subjected to UV irradiation, resulting in a genome that is 220 kbp smaller than that of FB (Chen et al., 1990).

Restriction of phage MX-1 has been shown directly with a strain of *Mx. virescens* (Morris and Parish, 1976). The strain became fully sensitive after curing by UV irradiation. Also, two restriction endonucleases have been demonstrated in the restrictive strain. Such enzymes have been found in many different myxobacteria (Mayer and Reichenbach, 1978). Mx8 expresses a nonessential DNA methylase, Mox, which modifies adenine residues in *Xho*I and *Pst*I recognition sites, CTCGAG and CTGCAG, respectively, on both phage DNA and the host chromosome (Magrini et al., 1997). Mox may protect Mx8 phage DNA against restriction upon infection of some *Mx. xanthus* hosts.

A strategy was applied to isolate transducing phages from a collection of *Mx. xanthus* strains using certain sensitive strains as indicators, such as strain FB. Several *Mx. xanthus* phages have been isolated (Brown et al., 1976b; Campos et al., 1978; Geisselsoder et al., 1978; Martin et al., 1978), including morphological types either with very short tails, e.g., MX-4, or with long tails, e.g., MX-8 and MX-9. Also isolated have been λ-like myxophages (Rodriguez et al., 1980). MX-8, which has been particularly well characterized (e.g., Orndorff et al., 1983; Stellwag et al., 1985), is a generalized transducing phage with a linear, double-stranded DNA (56 kbp). The genome has a terminal redundancy of 8% and is circularly permuted over at least 40% of its length. The MX-8 prophage is integrated into the bacterial genome by a site-specific recombination between the *attB* and *attP* sites that has been extensively examined (Tojo et al., 1996; Salmi et al., 1998; Magrini et al., 1999a; Magrini et al., 1999b; Tojo and Komano, 2003). Another well-studied transducing phage is MX-4 (Campos et al., 1978; Geisselsoder et al., 1978).

Myxobacteria have been shown to produce bacteriocins, termed "myxocins." The first, xanthacin, was discovered in *Mx. xanthus* (McCurdy and McRae, 1974). This myxocin is produced

after mitomycin C induction, is active exclusively on a few myxobacteria, and consists of spherical bodies of somewhat variable size. It resembles membrane vesicles and may in fact have been a defective phage. Fulvocin C, one of three substances with bacteriocin activity from *Mx. fulvus* Mx f16 (Hirsch, 1977), turned out to be a protein consisting of 45 amino acid residues (molecular weight 4672 Da) with no lipid or carbohydrate attached. Disulfide bridges (probably four) make it a very compact molecule (Tsai and Hirsch, 1981). It was the first bacteriocin for which a complete structure could be presented. Fulvocin C, at a minimal inhibitory concentration of 0.1–0.25 μM, killed 16 out of 17 tested *Mx. fulvus* strains, the exception being the producer, and appeared to exert its effect on the cell membranes (Hirsch et al., 1978).

Gliding Motility

Gliding is the translocation of cells on surfaces without the use of flagella (McBride, 2001). The speed of gliding cells varies, depending on the substrate and the temperature; typically it is between 3 and 13 μm/min (*Mx. virescens*, 32°C, on peptone agar, measured in movie frames; Reichenbach, 1966). Gliding cells always deposit slime trails (Fig. 5). *Myxococcus xanthus* cells glide on solid surfaces in the direction of the long cell axis at rates of 1–2 μm/min (Kaiser and Crosby, 1983) and reverse their direction of movement every 6.8 min on average (Blackhart and Zusman, 1985).

Genetic analysis of *Mx. xanthus* motility has revealed the presence of two nearly separate motility systems enabling cell translocation over solid surfaces (Hodgkin and Kaiser [1979a] and Hodgkin and Kaiser [1979b]; for reviews, see Shimkets [1990b], Shi and Zusman [1993a], Hartzell and Youderian [1995], Youderian [1998], Ward and Zusman [1999], Kaiser [2000a], and Kearns and Shimkets [2001a]). The "A" system (for adventurous) enables cells to move as individuals without direct contact with other cells. In nature this system may allow cell dispersal for foraging. The A system also contributes to the movement of cells within groups. Thus far, 33 genes known to be essential for A motility have been identified and sequenced. From the frequency of mutagenesis and the target sizes of the genes it has been estimated that about half of the A motility genes have been discovered (Youderian et al., 2003). Six of these genes encode different homologues of the TolR, TolB and TolQ transport proteins, suggesting that A motility is dependent on biopolymer transport (Youderian et al., 2003).

The mechanism of A motility is unknown. One hypothesis is that A motility may involve extrusion of a propellant that swells as it hydrates, pushing the cell forward (Wolgemuth et al., 2002). Another hypothesis is suggested by the presence of a complex cell surface apparatus, revealed by electron microscopy in *Mx. xanthus*, *Mx. fulvus* and implied in *Sg. aurantiaca*. It has been suggested that the apparatus produces traveling waves along the surface of the cell which in turn may propel the cell (Freese et al., 1997; Lünsdorf and Reichenbach, 1989; Lünsdorf and Schairer, 2001). To this point, the A motility mutants have not been studied carefully enough to provide support for any particular model.

The "S" system (for social) enables cell movement only if cells are within one cell length of one another (Kaiser and Crosby, 1983). In nature this system plays an important role in multicellular behaviors like fruiting body formation. S motility is genetically and functionally similar to twitching motility of many Gram-negative bacteria including *Pseudomonas* and *Neisseria* (Kaiser, 2000a). Twitching and S motile cells

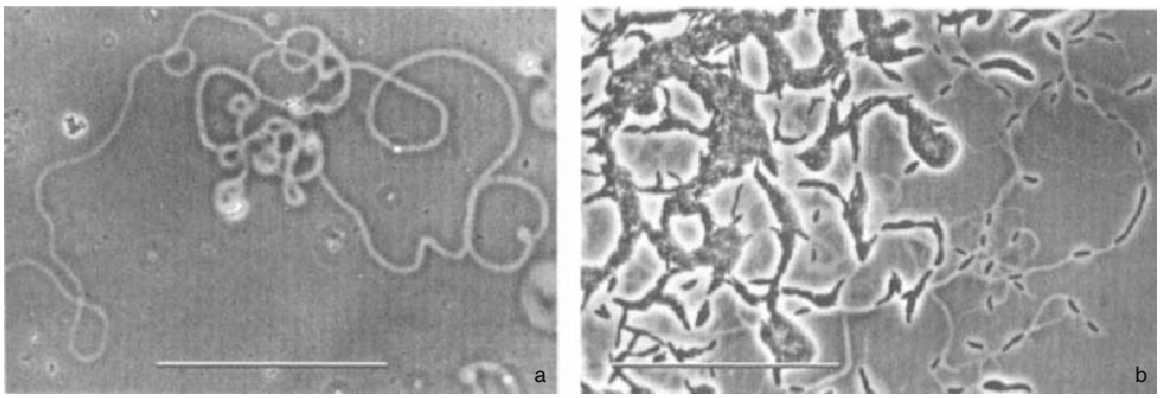


Fig. 5. Slime tracks as seen on thin agar layers in chamber cultures. a) *Nannocystis exedens*. b) *Myxococcus fulvus*; the spots on the left are cell clusters, not single cells. Bar = 100 μm. Phase contrast.

display tufts of type IV pili (also called "fimbriae") on the leading cell pole (Kaiser, 1979a; Henrichsen, 1983). Pili are 5–8 nm wide and may exceed the length of a cell (MacRae and McCurdy, 1975; MacRae and McCurdy, 1976; MacRae et al., 1977; Dobson and McCurdy, 1979; Arnold and Shimkets, 1988a). Fimbriae have been observed with many different species of both suborders; the only negative result was with *Nannocystis*, but this may have been due to technical problems. The fimbriae may be restricted to one of the two cell poles, and up to six have been seen per cell (Kaiser, 1979a). Tiny holes in the outer membrane, 6.5–8.5 nm wide and surrounded by a collar of 2.6 nm, which sometimes shows a ring of 12 spikes, have been proposed to be the insertion sites of the fimbriae (MacRae et al., 1977). Movement appears to result from pilus extension, pilus attachment, and pilus retraction to pull the cell forward like a grappling hook (Merz et al., 2000; Sun et al., 2000; Skerker and Berg, 2001). The pilus retraction motor, PilT, is the most powerful molecular motor yet examined. The force exerted by a single retracting *Neisseria* pilus was estimated to exceed 100 pN (pico Newtons; Maier et al., 2002). When a cell reverses direction, the pili on one cell pole are replaced by pili on the opposite cell pole (Sun et al., 2000). It has been suggested that the pili from one cell attach to cell surface appendages known as fibrils from another cell to mediate group movement (Li et al., 2003). There is an excellent correspondence between genetics and physiology with regard to S motility. The majority of the known S motility genes are involved in the production of type IV pili or the pilus receptor on the cell surface (Wu and Kaiser, 1995; Wu et al., 1997; Wu et al., 1998; Lancero et al., 2002; Li et al., 2003).

A mutant becomes nonmotile if it is A^-S^-, which normally requires two mutations. A single mutation in one gene, *mglA*, has the same effect, suggesting that it is shared between the two systems. The *mglA* gene encodes a 22-kDa GTPase that is thought to act as a switch between the two motility systems (Hartzell and Kaiser, 1991). MglA interacts with MasK, a protein of the STY kinase family (Thomasson et al., 2002). MasK, expressed in *E. coli*, was shown to phosphorylate tyrosine residue(s). The gain-of-function *masK815* mutant is restored in S motility and displayed increased production of cell surface fibrils. Attempts to eliminate the *masK* gene have been unsuccessful, suggesting that it is essential for growth. The A motility system also utilizes a tyrosine kinase, AgmO, although the role of this protein has not yet been determined (Youderian et al., 2003).

Myxococcus xanthus cells show directed movements towards glass and latex beads and clumps of prey bacteria (Dworkin, 1983a). The mechanism(s) of such long range directed movement remain a mystery. Elasticotaxis, a phenomenon first described by Roger Stanier, results from delicate structural disturbances in the substrate surface, such as are produced in agar plates by mechanical stress. Cells orient their movement perpendicular to stress forces within the agar (Stanier, 1942a). Elasticotaxis is mediated by the A motility system (Fontes and Kaiser, 1999). Cells also direct their movement to certain chemicals. Chemotaxis has been observed to complex chemical mixtures (Shi et al., 1993b) and to certain isomers of the major membrane lipid phosphatidylethanolamine (PE; Kearns and Shimkets, 1998).

Myxococcus xanthus exhibits the hallmarks of chemotactic behavior, excitation and adaptation in response to PE. Certain molecular PE species increase the reversal period as much as sixfold (excitation; Kearns and Shimkets, 1998). Cells return to the pre-stimulus reversal frequency in about an hour (adaptation; Kearns and Shimkets, 1998). Dilauroyl PE (PE-12:0/12:0), dioleoyl PE (PE-18:1ω9c/18:1ω9c), and PE with 16:1ω5c at both the *sn*-1 and *sn*-2 positions (PE-16:1ω5c/16:1ω5c) cause excitation, whereas many other species do not (Kearns and Shimkets, 1998). These results begin to bring into focus properties of the as yet unknown PE binding protein that serves as the receptor (hereafter called "PE receptor"). A major unresolved point is whether the PE receptor recognizes the *sn*-1 fatty acid, the *sn*-2 fatty acid, or both.

Chemosensory systems analogous to those found in the enteric bacteria appear to play roles in the response to PE. Excitation to PE appears to be mediated by the Dif signal transduction pathway. The *difA* gene encodes a methyl-accepting chemotaxis protein (MCP), *difC* encodes a CheW homolog, *difD* encodes a CheY homolog, and *difE* encodes a CheA homolog. Excitation to both PE-12:0/12:0 and PE-16:1ω5c/16:1ω5c requires DifA (Kearns et al., 2000). The DifA ligand is unknown, but binding of PE to DifA directly is unlikely. The periplasmic face of DifA is approximately 15 amino acids, much smaller than that of Tar (Kearns and Shimkets, 2001a). Thus understanding the nature of the stimulus for DifA is important.

Another set of chemosensory genes, the "*frizzy*" (*frz*) genes, also regulates the frequency with which the cells change the direction of their movement (Blackhart and Zusman, 1985) and the response to PE (Kearns and Shimkets, 1998). While wild type cells reverse their direction of movement every 6–7 min, some Frz mutants do so every 2 h or every 2 min. The genes and their gene products have been analyzed in considerable detail (for review, see Ward and Zusman,

1997). The FrzCD protein encodes the MCP (McBride et al., 1989b) and the FrzE protein is a fused CheA-CheY homolog (McCleary and Zusman, 1990a; McCleary and Zusman, 1990b). The *frzCD* and *frzE* mutants exhibit excitation to PE but had aberrant adaptation (Kearns and Shimkets, 1998). As in the enteric bacteria, the direction of *Mx. xanthus* movement is controlled by methylation and demethylation reactions in the Frz system (McCleary et al., 1990c).

Extracellular Matrix

An extracellular matrix composed of polysaccharide and protein connects bacteria living in biofilms. The extracellular matrix of *Mx. xanthus* appears to be extruded as long thin fibrils, about 30 nm in diameter, that emanate from many points on the cell surface. Fibrils have been visualized using a wide variety of fixation techniques and microscopy methods (Arnold and Shimkets, 1988b; Behmlander and Dworkin, 1991; Behmlander and Dworkin, 1994; Merroun et al., 2003), most notably transmission electron microscopy of freeze-substituted cell thin sections (Kim et al., 1999), which is the most advanced approach for examining surface layers (Beveridge, 1999). Fibril synthesis is induced by starvation (Arnold and Shimkets, 1988b), stimulated by cell contact (Behmlander and Dworkin, 1991) and Ca^{+2} (Kim et al., 1999), and repressed by the *stk* locus (Dana and Shimkets, 1993).

The extracellular matrix is composed of a polysaccharide core with noncovalently associated protein (Behmlander and Dworkin, 1994). Mutants lacking the ability to secrete the extracellular matrix polysaccharide fail to bind Congo red or calcofluor white (Arnold and Shimkets, 1988a; Arnold and Shimkets, 1988b; Dana and Shimkets, 1993; Ramaswamy et al., 1997; Kim et al., 1999). The most carefully studied of these mutants has been isolated twice; the first was based on loss of S motility, where it was called *dsp* (Shimkets, 1986), and the second based on loss of development, where it was called *dif* (Yang et al., 1998). Now clearly these two sets of mutants are defective in the same genes (Lancero et al., 2002). The phenotype of the *dsp*/*dif* mutants provides one level of insight into the many functions of the extracellular matrix. These mutants are defective in adhesion to surfaces, cohesion to other *Mx. xanthus* cells, S motility (also known as twitching in other Gram-negative bacteria), fruiting body morphogenesis, sporulation, developmental gene expression, and chemotaxis to phosphatidylethanolamine (PE). The *dsp*/*dif* mutants are blocked about 8 h into the developmental program and fail to express roughly one-third of the developmentally regulated genes (Li and Shimkets, 1993). The *dsp*/*dif* locus encodes components of a chemotaxis pathway with homology to the Che proteins of enteric bacteria (Yang et al., 1998).

Fibrils are extracted from cells with 0.5% sodium dodecylsulfate (SDS) and sedimented by low speed centrifugation (Behmlander and Dworkin, 1991). Fibrils can restore cohesion, chemotaxis, and fruiting body development to mutants that cannot produce fibrils by simply adding them back to cells (Chang and Dworkin, 1994; Yang et al., 2000b; Kearns et al., 2002). The functions of the many proteins in the matrix are as yet unknown. One fibril protein, a zinc metalloproteinase known as FibA, is essential for PE chemotaxis to dilauroyl PE and PE-16:1ω5c/16:1ω5c but not dioleoyl PE (Kearns et al., 2002).

The excreted polysaccharide appears to contain amino sugars, among others (Sutherland and Thomson, 1975; Sutherland, 1979). Slime from fruiting bodies resembles in its overall composition that produced in vegetative cultures. In *Mx. xanthus*, fibril polysaccharide is composed of galactose, glucosamine, glucose, rhamnose and xylose (Behmlander and Dworkin, 1994). A large extracellular protein-polysaccharide-lipid complex with proteolytic activity has been isolated from culture supernatants of *Mx. virescens* (Gnosspelius, 1978b). A glycopeptide with a heat-stable blood anticoagulant activity, named "myxaline," has been obtained from the culture broth of *Mx. xanthus* (El Akoum et al., 1987; Masson and Guespin-Michel, 1988). The glycan part contains *N*-acetyl amino sugars, and the peptide is rich in glutamic acid and serine. The excreted slime must be chemically different in the three suborders of myxobacteria, since Congo red (0.01% [w/v] aqueous solution) stains the slime of the Cystobacterineae but not that of the Sorangineae (McCurdy, 1969b) or the Nannocystineae.

Myxobacterial Colonies

On media with a relatively low nutrient content (like VY/2 and CY agar), colonies of myxobacteria spread over the agar surface. Such colonies are called "swarms." With a few exceptions the colony does not move as a whole but simply increases in diameter because of gliding motility and cell division.

The appearance of a swarm depends on the species and the medium. On rich media, such as those with a high peptone content (around 1%), myxobacterial colonies may remain small and more or less compact; sometimes they are even circular, convex, and with an entire edge like ordinary bacterial colonies. The typical myxobacterial colony is, however, a swarm sheet consisting of cells and excreted slime; much of the latter

is probably composed of interwoven slime trails. The swarms often contain conspicuous veins, rings, and ridges or even depressions in the agar surface. Such patterns can be characteristic for specific taxonomic groups (see below). The swarm sheet can sometimes be loosened from the agar surface with water (e.g., with *Cystobacter* and *Stigmatella*); sometimes, however, it sticks tenaciously to the agar surface, occasionally to such an extent that the agar is torn into pieces when the aging swarm sheet contracts (especially with *Sorangium*). In some cases, the swarm sheet becomes so tough that it is very difficult to cut with the inoculating loop (with *Archangium*, *Cystobacter* and *Melittangium*); in other cases, it is soft and slimy and can easily be scraped off the plate (with *Myxococcus*). Often the myxobacteria penetrate the agar (even 1.5% agar), and the swarm spreads below the agar surface, sometimes penetrating to the very bottom of the dish (often with *Polyangium*). In such cases, the swarm may completely disappear below the surface, and its former position can be recognized only by a shallow depression. All members of the Sorangineae and Nannocystineae tend to grow within the agar. However, some members of the Cystobacterineae also do so, although here it is often a consequence of swarm degeneration. Some myxobacteria corrode the agar surface to the extent that they produce long tunnels or large, cavern-like holes in the plate. This is typical of *Polyangium* and especially of *Nannocystis*.

The swarm edge usually shows characteristic flame-like protrusions, or flares. Sometimes wide, tongue-like depressions are produced (by *Chondromyces* and other members of the Sorangineae), or isolated narrow trenches radiate from the swarm center far into the surrounding agar surface (*Polyangium* and *Nannocystis*). Occasionally, massive ridges are also seen at the swarm edge (*Chondromyces*, *Polyangium* and *Nannocystis*). At the swarm edge, the swarm may consist of only one cell layer, but as a rule the swarm sheet is composed of many layers that quickly pile up behind the margin. When growing on very lean media, most of the swarm may be only one cell thick, although even then the bacteria tend to concentrate themselves in certain areas, such as along radial tracks. In large swarms, most of the cell population may be found in the outer regions, and if no fruiting bodies or myxospores have been produced, the interior may be completely deserted. The speed of the advancing edge is in the range of 1 mm in 9 h (*Cb. violaceus* at 22°C; Reichenbach, 1965b) to about 1 mm in 1.6 h (*Cystobacter* and *Stigmatella* strains at 30°C).

Various taxa may be recognized by their swarm morphology. When plate cultures are flooded with an aqueous 0.01% (w/v) Congo red solution for 5–10 min, the swarm sheets of the Cystobacterineae stain a deep violet-red, while those of the Sorangineae and Nannocystineae remain unstained (McCurdy, 1969b). The swarms of *Myxococcus* and *Corallococcus* typically develop meandering radial veins, which may be particularly conspicuous on CY agar; the swarm sheet usually remains soft and slimy. In contrast, long, more or less straight, branching veins are seen in the swarms of *Cystobacter*, *Archangium*, *Melittangium* and *Stigmatella*. On VY/2 agar, the swarms of most Sorangineae are sunk into the agar and appear as wide shallow depressions at the surface. *Chondromyces* and *Sorangium* often develop long, cable-like veins on the surface that sometimes curl into a ring at one end. Also, isolated rings are often seen in those swarms. With *Chondromyces* swarms, the swarm edge typically consists of a series of tongue- and shell-like shallow depressions. *Polyangium* swarms on VY/2 agar often penetrate the agar to the bottom and characteristically are surrounded by a more or less perpendicular, curtain-like zone composed of tiny, well-separated cell clumps. *Polyangium* and *Nannocystis* growing on streaks of autoclaved *E. coli* often produce long, radiating, narrow trenches in the agar surface, with small cell clusters (and, later on, fruiting bodies) at the end. The two can often be distinguished because the tracks and cell clumps of *Nannocystis* are more delicate. On mineral salts-glucose media, like CA2 and CK6 agar, *Sorangium* often produces swarms with a beautiful, dense network of branching cable-like veins.

Note that the swarm structures described above are usually only seen with strains that have recently been isolated. The swarms of strains that have been transferred many times, or that have been adapted to growth in liquid media, are often very different. Such swarms may be soft and slimy, lacking all morphological differentiation, and, in the case of the Cystobacterineae, sometimes no longer grow on the surface but are sunk into the agar.

A colony dimorphism has been described for *Cb. violaceus* and *Mx. xanthus* and is probably quite common among myxobacteria (Reichenbach, 1965c; Grimm and Kühlwein, 1973a; Grimm and Kühlwein, 1973b; Grimm and Kühlwein, 1973c; A. C. Burchard et al., 1977). This dimorphism may regulate cell motility and consequently the spreading behavior of the colonies. The dimorphism in *Cb. violaceus* affects the slime, the capacity to fruit, and the ability to grow as a homogeneous cell suspension. Also, pigmentation can be affected (deep and pale violet in *Cb. violaceus*, tan or yellow in *Mx. xanthus*). Usually one colony type is more stable, but segregation in both directions is possible, sug-

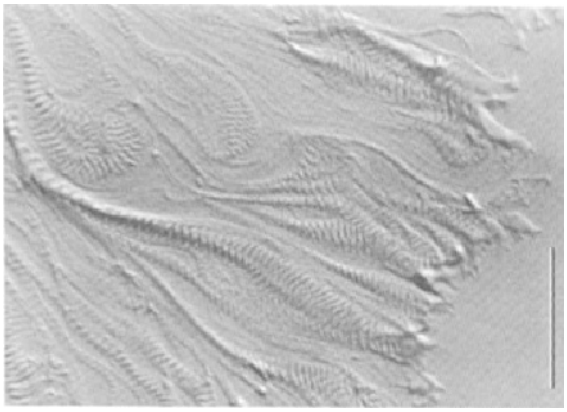


Fig. 6. Oscillating ridges or ripples. *Stigmatella erecta* on Casitone agar; dissecting microscope, oblique illumination. Bar = 200 μm.

gesting a phase variation. Both cell types may serve essential functions in nature. In *Mx. xanthus*, the tan cells preferentially make spores in spite of the fact that tan locked mutants are incapable of completing sporulation unless yellow cells are provided (Laue and Gill, 1995). The results suggest that the yellow cells provide a factor enabling spore maturation.

A striking feature of myxobacterial swarms is the ability of the cells to organize their movement in waves that have been referred to as "ripples," "oscillatory waves," or "traveling waves." Dense fields of regularly arranged ridges are found in long, parallel rays. They are positioned parallel to one another and perpendicular to the direction of the ray. The distance between the ridges is uniform and is approximately equal to the width of the ridges (Fig. 6). In *Mx. xanthus*, a distance of 45 μm from crest to crest has been measured (Shimkets and Kaiser, 1982). The phenomenon was discovered when movies of developing *Corallococcus* and *Myxococcus* swarms were first made. They showed that the ridges actually move, so that the whole field appears to oscillate; hence, the ridges were called "oscillating waves" (Reichenbach, 1965a; Reichenbach et al., 1965f; Reichenbach et al., 1965g). In fact, the movements of the ridges may even speed up and slow down, or pulsate, over a period of several hours. The movement of the ridges is fast enough to be recognized under the dissecting microscope; a speed of about 2 μm/min has been measured in *Mx. xanthus* (Shimkets and Kaiser, 1982). Traveling waves were later found in many other myxobacteria. They appear to occur with all Cystobacterineae and occasionally are also observed with members of the Sorangineae, e.g., *Chondromyces*. With *Sg. aurantiaca*, even waves travelling in opposite directions along the same path have been seen (Reichenbach et al., 1980).

The mechanism of cell movement in the traveling waves has been examined extensively in *Mx. xanthus* (Shimkets and Kaiser, 1982; Sager and Kaiser, 1994; Sager, 1996; Igoshin et al., 2001; Welch and Kaiser, 2001; Borner et al., 2002). Rippling can be induced by purified peptidoglycan and peptidoglycan components, such as *N*-acetyl-glucosamine, *N*-acetylmuramic acid, diaminopimelic acid, and D-alanine (Shimkets and Kaiser, 1982). The CsgA cell signaling protein is essential (Shimkets and Kaiser, 1982). Cell movement occurs along the same axis as the rippling movement and is periodic (Sager and Kaiser, 1994; Sager, 1996; Welch and Kaiser, 2001). The periodicity of individual cells matches the period of macroscopic rippling. When two cells moving in opposite directions meet end to end, they reverse their gliding direction. This periodicity is thought to be linked to the C signal, a cell contact-mediated signaling molecule. Mathematical models quantitatively reproduce the experimental observations and illustrate how intracellular dynamics, contact-mediated intercellular communication, and cell motility can coordinate to produce this behavior (Igoshin et al., 2001; Borner et al., 2002). These models of traveling waves illustrate how cell-contact interactions rather than reaction diffusion can regulate biological pattern formation.

Finally, the shape of myxobacterial swarms may become distorted by stress lines in the agar plate produced, for example, by mechanical pressure and stretching, a phenomenon known as "elasticotaxis" (Stanier, 1942). Elasticotaxis is due to A motility (Fontes and Kaiser, 1999).

Fruiting Bodies

The outstanding trait of the myxobacteria is their ability to form multicellular fruiting bodies (Figs. 7–10), which serve as the housing for the resistant resting cells, the myxospores. The processes that lead to their formation make the myxobacteria the object of considerable interest as model systems for the study of development. There are some excellent reviews focusing on different aspects of fruiting body and myxospore development: Kaiser (1989), Dworkin (1991), Dworkin (1996), White (1993), Shimkets and Dworkin (1997c), Shimkets (1999), and Ward and Zusman (2000).

DESCRIPTION OF FRUITING BODIES. The terminology used to describe the structural components of myxobacterial fruiting bodies is illustrated in Fig. 13. Although complete consistency with usage of terms in other biological systems is not possible, terms that have already acquired a well-defined meaning in other systems should be avoided if they are inappropriate. The term "fruiting body" has a long tradition of

use and may be acceptable in connection with myxobacteria, although in biology, fruiting usually implies sexual processes, and no such events seem to be involved in the case of the myxobacteria. The term "sporangium" has also been applied to these structures, but this term is even more suggestive of sexuality and is best avoided. Most types of fruiting bodies are composed of myxospores, and the walled portions containing these myxospores are in fact communal cysts; they have traditionally been referred to as "cysts." However, since the term "cyst" in microbiology is applied to quite a different structure (i.e., dormant, encapsulated single cells), we propose to use the term "sporangiole" instead and to accept its obvious shortcomings. The advantage of the term sporangiole over "sporangium" is that it is a relatively unusual term and has been used only in connection with a restricted group of fungi (Mucorales), where it is not even used with a consistent meaning. Its use here is thus less likely to cause confusion. Incidentally, the term sporangiole was applied to these myxobacterial structures by Link (1809) in the very first description of a myxobacterium, *Pl. vitellinum* (Fig. 14). The simplest myxobacterial fruiting bodies are mounds of either soft or hardened slime, in the latter case often having bizarre shapes (*Corallococcus* and *Archangium*) or in the former typically having a knob or head with a constriction or a stalk at the base (*Myxococcus*).

The size of myxobacterial fruiting bodies varies between 10 and 1000 µm or greater depending on the species. Many can be seen with the naked eye, especially as they are normally produced in large numbers and often are brightly colored in hues of yellow, orange, red, brown or black. The distribution of fruiting bodies over the swarm usually is random, but sometimes fruiting bodies arise in concentric rings or along radial veins or in various patterns controlled by elasticotaxis (Stanier, 1942a). Fruiting proceeds in the swarm from the center to the periphery. The shape, structure and color of fruiting bodies is species specific—in fact myxobacterial species are mainly defined by their fruiting bodies—but similar shapes were developed by completely unrelated species, e.g., *Stigmatella* and *Chondromyces*, or *Cystobacter*, *Polyangium*, *Byssophagia*, *Jahnia* and *Sorangium*. Also, the shape and color of fruiting bodies may vary substantially within the species.

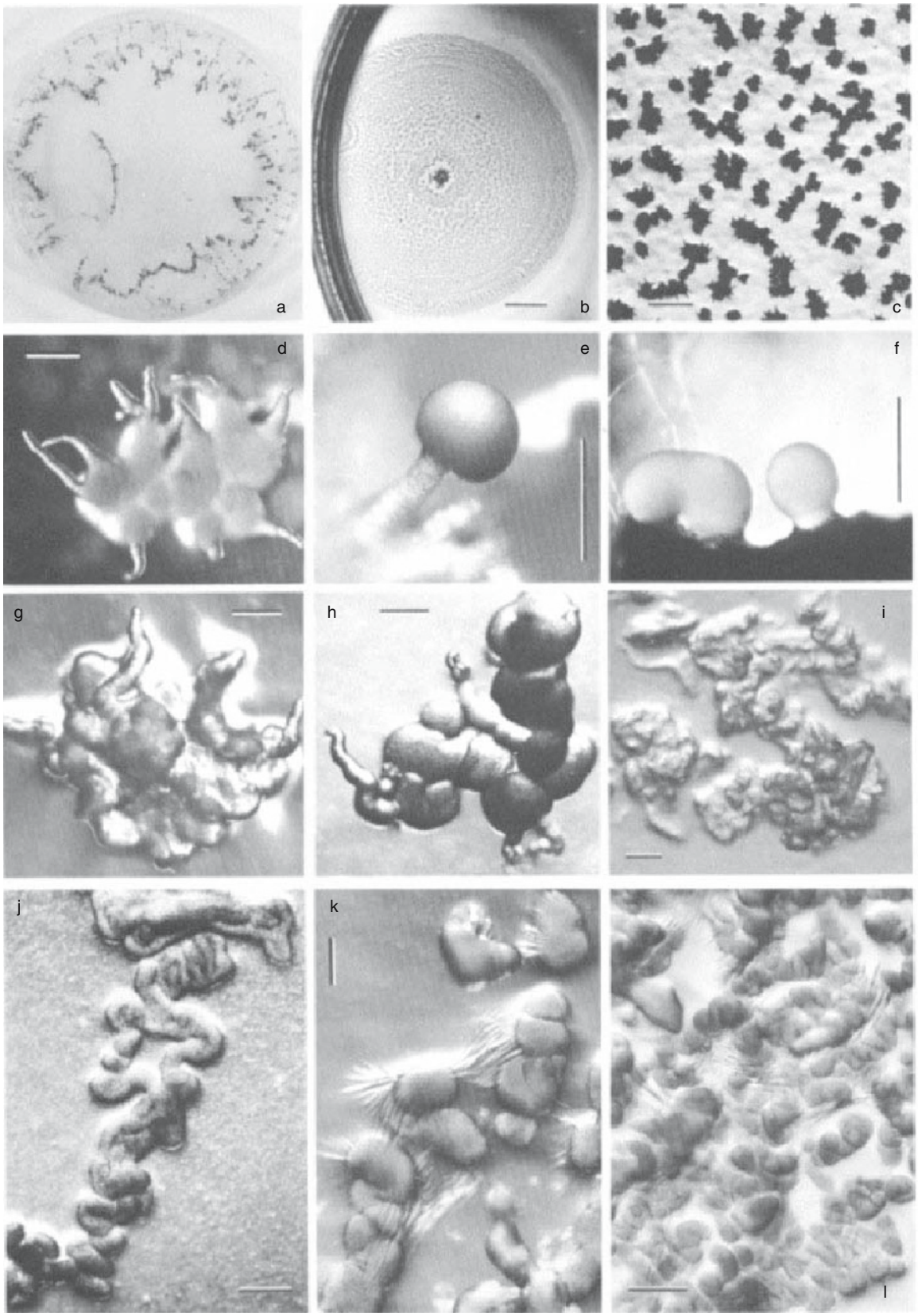
Most myxobacteria enclose their myxospores in sporangioles, i.e., containers with well-defined walls; the interior of the sporangioles is always hard and tough. The sporangioles may occur singly or in groups; they may rest either directly on or in the substratum or on simple or branched slime stalks.

The following key gives a survey of the different fruiting body types and their relation to the various myxobacterial taxa. Photographs of fruiting bodies of most species are provided for reference (Figs. 7–10). Keep in mind, however, that fruiting body structure and morphology often degenerate quickly after a few transfers upon isolation. Also, strains may be isolated that produce degenerate fruiting bodies from the very beginning. In such cases, only completely atypical knobs and ridges of slime and myxospores are formed. In addition, the fruiting bodies of several myxobacteria have also been studied with the scanning electron microscope, and some very impressive pictures have been obtained (e.g., Brockman and Todd, 1974; Stephens and White, 1980b; Galván et al., 1981; Stephens et al., 1982).

Key to the Fruiting Body Types of Myxobacteria

1. Naked masses of slime and myxospores 2
1′. Myxospores encased in sporangioles 3
2. Fruiting body soft-slimy . . . . . *Myxococcus* (*Kofleria*)
2′. Fruiting body hard, cartilaginous. . . . . *Corallococcus*, *Archangium*
3. Sporangioles single 4
3′. Sporangioles in groups 5
4. Single sporangiole located on or in the substrate . . . . . *Nannocystis*, *Haploangium*
4′. Single sporangiole on top of a stalk . . . . . *Melittangium*, *Stigmatella erecta*
5. Group of sporangioles on or in the substrate . . . . . *Cystobacter*, *Pyxicoccus*, *Polyangium*, *Sorangium*, *Nannocystis*, *Haliangium*, *Byssophaga*
5′. Group of small spherical sporangioles that are often empty and then look glassy transparent, arranged in extended, dense sheets, or often in chains . . . . . *Hyalangium*
5″. Coils of sporangioles sitting on a slime cushion or soft slime stalk . . . . . *Jahnia*
5‴. Cluster of sporangioles on top of an unbranched stalk . . . . . *Stigmatella aurantiaca*, most *Chondromyces* species
5⁗. Clusters of sporangioles on a branched stalk . . . . . *Chondromyces crocatus*

More or less the same morphological types of fruiting bodies are found in the suborders Cystobacterineae and Sorangineae (with the qualification that the Sorangineae produce only fruiting bodies with sporangioles). Still, the course of morphological differentiation may be different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with seemingly identical fruiting body types. Thus, *Chondromyces* and *Sg. aurantiaca* both form little treelets, but the stalks and sporangioles are produced in a different way in the two cases. While *Chondromyces* excretes a slime stalk that lifts the undifferentiated cell mass upward, and differentiation of the sporangioles occurs as the last step, *Sg. aurantiaca* piles up a mass of cells with approximately the shape and size of the final fruiting body, then the cells either withdraw from the stalk area or degenerate and lyse in that part, and the sporangioles mature (Fig. 11).

As has already been mentioned, stalks, sporangiole walls, and pigments are produced during fruiting but nothing is known about the chemical composition of these structures and substances. Under the electron microscope, the stalk of *Cm. crocatus* is seen to consist of numerous parallel tubes, which are approximately the diameter of a cell and run in the direction of the long axis of the stalk (McCurdy, 1969a; Abadie, 1971b). The stalk of *Sg. aurantiaca* was found to be composed of tubules wedged against each other and containing cell debris but only rarely a cell or myxospore; the stalk was surrounded by a thin, dense surface layer resembling the wall that surrounds the sporangioles (Voelz and Reichenbach, 1969). The stalk of *Sg. erecta* also was found to be cell free and to consist of slime, although in this case tubules could not be distinguished (Galván et al., 1987). In contrast, scanning electron micrographs suggested that the stalk of *Sg. aurantiaca* is cellular (Stephens and White, 1980b). The contradiction is not yet resolved; perhaps the fruiting bodies examined with the scanning electron microscope had not yet fully matured. In any case, under the light microscope the stalk of *Sg. aurantiaca* looks translucent, unpigmented, and white and thus completely different from the opaque, dark brown sporangioles.

The base of fruiting body stalks is often clearly disk-shaped. The disks apparently arise early during fruiting body development when the aggregated cells move on a circular path at the site of fruiting.

Little is known about the arrangement of the cells within the developing fruiting body, but it appears that this arrangement, and perhaps rearrangements by migrations of certain cell populations, may play a major role during fruiting body morphogenesis (O'Connor and Zusman, 1989; Sager and Kaiser, 1993). Many species surround the cell mass with a tough wall, often after a subdivision of the original mass into portions of approximately equal size, thus creating sporangioles, either single or in clusters. In *Me. boletus*, a very regular pattern of three or more layers of strictly parallel cells has been observed (Jahn, 1924). The cells are perpendicularly arranged on the outer surface of the fruiting body, and it has been suggested that they are responsible for the secretion of the sporangiole wall. In developing *Cb. fuscus* sporangioles, the cells in the outer layers lie parallel to the surface.

The wall of the sporangioles is very thin, about 30 nm in *Cm. crocatus* (Abadie, 1971b) and 200–300 nm in *Cystobacter* (Vahle, 1910; Jahn, 1924). It dissolves in potassium hypochlorite solution (Vahle, 1910) and is intensely colored by pigments that are not identical with those of the cells and cannot be extracted by any of the normal solvents (Vahle, 1910). The chemical composition and the origin of the wall are not known. It has been suggested that cells lying at the surface excrete the wall (Jahn, 1924). Or perhaps it is formed by fusion of the cells forming the top layer. In *Me. boletus*, the cells in the developing sporangiole are arranged in a regular pattern in rows of parallel rods standing perpendicular to the surface. Thus, in this case it seems that the wall is excreted from one cell pole only (Jahn, 1924). How the size of the sporangiole is determined within the undifferentiated cell mass is one of the many unsolved questions in myxobacterial morphogenesis. During germination the sporangiole wall is ruptured mechanically, and an empty husk remains after the cells leave.

Fruiting Body Morphogenesis

The fruiting bodies are produced by the coordinated movement of 10^5 to 10^7 swarm cells that

Fig. 7. Myxobacterial fruiting bodies. a) *Cystobacter ferrugineus* on VY/2-agar; culture is 4 weeks old; the organism has spread all over the plate and produced numerous dark brown fruiting bodies; the diameter of the plate is 9 cm. (b–d) *Corallococcus coralloides*: b) swarm on VY/2 agar, with numerous fruiting bodies; the delicate swarm edge and the clear lysis zone in the yeast agar are well recognizable; c) field of fruiting bodies at higher magnification; d) a single fruiting body, in situ on the agar surface. e) *Myxococcus stipitatus*, fruiting body at the edge of a piece of filter paper, in situ. f) *Myxococcus fulvus*, fruiting bodies on a soil crumb, in situ. (g–j) *Archangium*-like fruiting bodies: g) *Archangium gephyra*, large fruiting body in situ on agar surface; h) *Cystobacter ferrugineus*, fruiting body with only rudimentary differentiation into sporangioles, in situ on agar surface; i) *Cystobacter*, probably *Cb. ferrugineus*, with totally degenerated fruiting bodies, in situ on agar surface; j) *Cystobacter* strain with typical "*Archangium*" fruiting bodies, in situ on agar surface; k) *Cystobacter velatus*, fruiting bodies in situ on agar surface, covered with a delicately plicated slime sheet; and l) the same organism at lower magnification; one can see some of the enormous number of fruiting bodies that may be produced under suitable conditions. Bar = 5 mm in b), 500 µm in c), 200 µm in l), and 100 µm in the remaining micrographs.

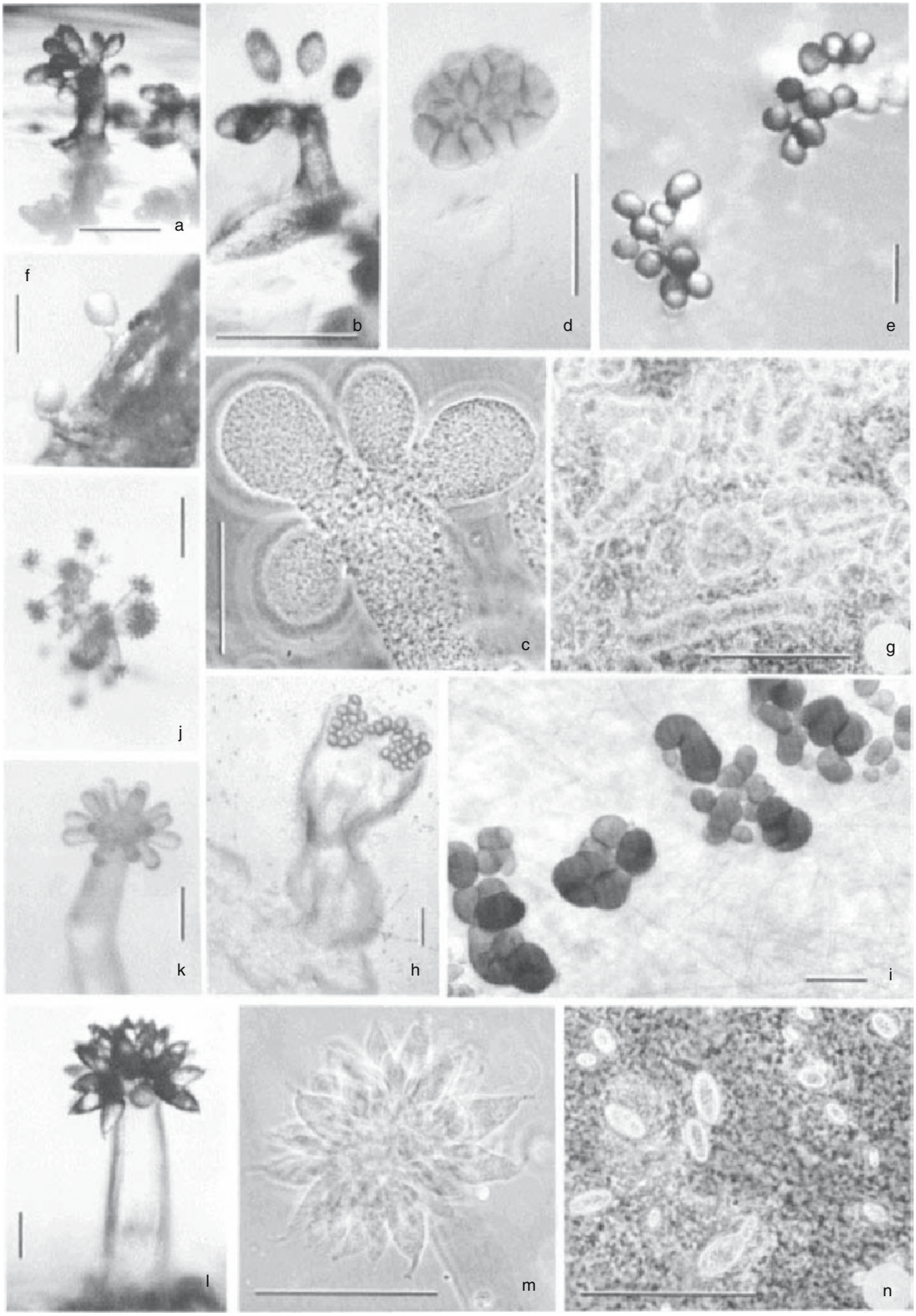
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Fig. 8. Myxobacterial fruiting bodies. (a–c) *Stigmatella aurantiaca*: a) fruiting body in situ on an agar surface; b) fruiting body with its sporangioles on exceptionally long peduncles, in situ; c) early developmental stage of a fruiting body, mounted in "Miracle Mount" (Cunningham, 1972) to make it transparent; the stalk still consists mainly of cells that later migrate into developing sporangioles or degenerate; the young sporangiole in the upper left corner shows the developing sporangiole wall that ends in a collar at the base of the sporangiole; phase contrast. d) Although this fruiting body strikingly resembles those of certain *Polyangium* (see h) or *Sorangium* species (see g), it still is that of a *Cystobacter*, *Cb. minus*, as is clearly shown by the shape of the vegetative cells; interference contrast. e) *Stigmatella erecta*, fruiting bodies in situ on agar surface. f) *Melittangium lichenicola*, fruiting bodies in situ on a piece of wood. g) *Sorangium cellulosum* on filter paper, cautiously squeezed slide mount; the shape of the fruiting bodies is often controlled by the orientation of the remnants of the wood fibers and tracheids, along and within which they develop; phase contrast. h) *Polyangium* sp., fruiting body in crude culture in situ on agar surface; note the deep path that has been etched into the agar surface by the migrating bacterial mass (compare with Fig. 2c) before it encased itself in sporangioles; the tiny dots all over the agar surface are encysted amoebae. i) *Jahnia thaxteri*, fruiting bodies on filter paper, in situ. (j–m) *Chondromyces apiculatus*: j) cluster of fruiting bodies on surface of filter paper, in situ; dissecting microscope; k) early (club) stage of sporangiole differentiation; dissecting microscope; l) mature fruiting body; m) small, mature, fruiting body in "Miracle Mount" (Cunningham, 1972) to make it transparent; the myxospores within the turnip-shaped sporangioles can just be recognized; the slime stalk, in contrast, is cell free; phase contrast. And n) *Nannocystis exedens*; the fruiting bodies are single sporangioles of very variable size and are embedded in the agar substrate; slide mount, phase contrast. Bar = 50 μm in (c), 200 μm in (i), 400 μm in (j), and 100 μm in the remaining pictures.

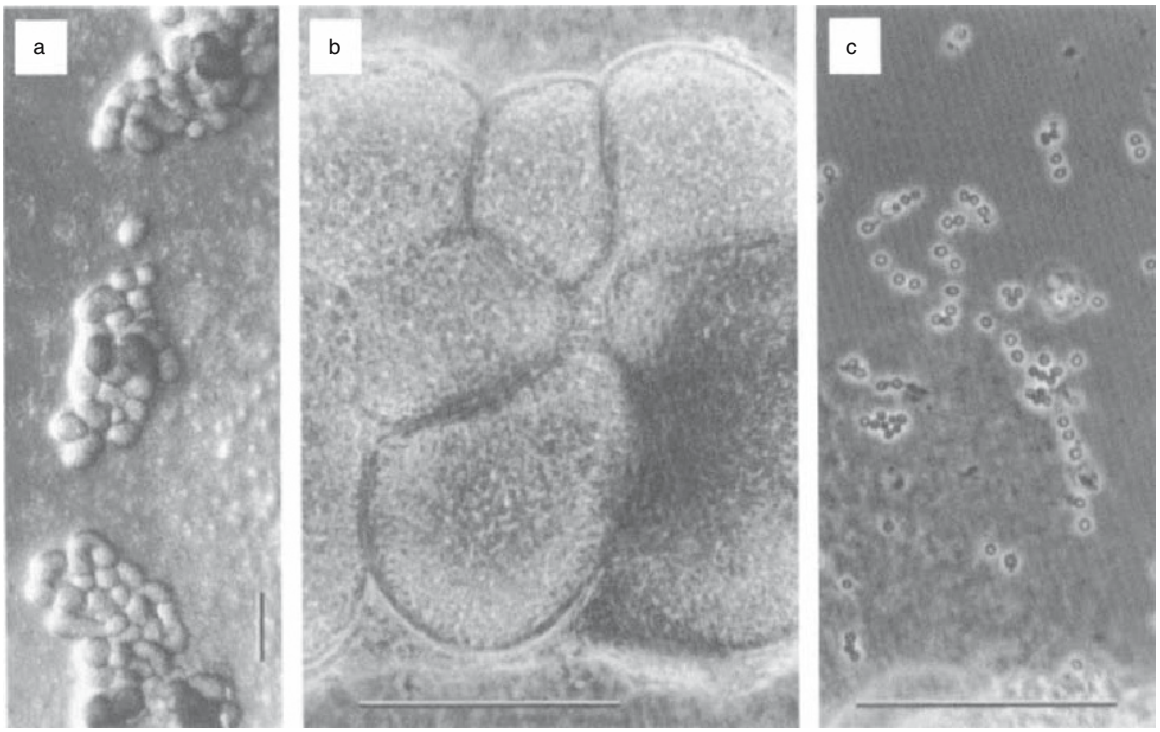

Fig. 9. Myxobacterial fruiting bodies: *Pyxicoccus fallax*. a) In crude cultures and on natural substrates the sporangioles tend to be disk-shaped and loosely stacked but, as shown here, in pure culture, they are more or less spherical and tightly attached to each other. b) At high magnification, the walls of the sporangioles, although only about 1 mm thick, become clearly recognizable. c) When the sporangioles are crushed, they release optically refractile, spherical myxospores, shown here at the same magnification as the sporangioles in (b), which look exactly like *Myxococcus* myxospores. (b) and (c) are in phase contrast. Bar = 100 μm in (a), 50 μm in (b) and (c).

retain their physical individuality throughout this process of cooperative morphogenesis (Fig. 12). Formation of fruiting bodies can schematically be divided into a number of developmental stages: 1) environmental signal(s) to direct the vegetatively growing cells to a developmental path (Kaiser, 2000b); 2) cell aggregation or accumulation; 3) emergence of molecules on the cell surface that mediate cell adherence; 4) rearrangement or clustering of the cells within the original undifferentiated mass; 5) creation of the specific shape of the fruiting body, perhaps the most fascinating event in the whole sequence; 6) production of special structural

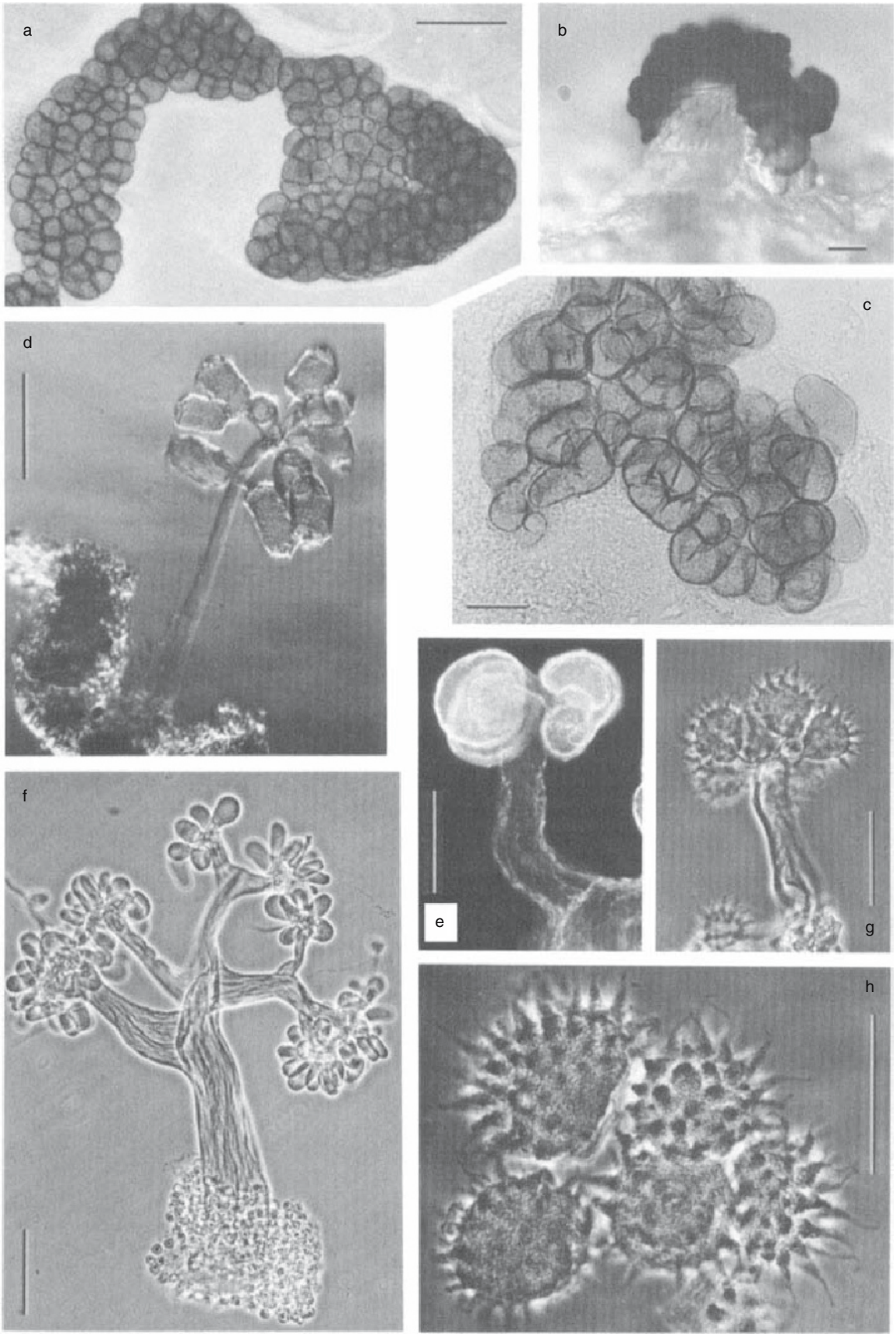
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Fig. 10. Myxobacterial fruiting bodies. a) *Sorangium cellulosum*, on VY/2 agar in situ (compare with Fig. 9). (b and c) *Jahnia thaxteri*; b) on agar surface in situ; a mass of tightly packed sporangioles is situated on a stalk-like cushion of slime: the whole fruiting body looks like a false morel; c) when mounted in "Miracle Mount" (Cunningham, 1972), the individual sporangioles can easily be distinguished. d) *Chondromyces pediculatus*, fruiting body on a soil crumb, embedded in Miracle Mount. (e and f) *Chondromyces crocatus*, both in Miracle Mount: e) a young, not yet fully differentiated stage; in dark field illumination, the translucent, cell-free stalk and the densely packed cell masses on its top are clearly distinguished; f) mature fruiting body; at the base of the stalk some encysted soil amoebae are seen. (g and h) *Chondromyces lanuginosus*, both in Miracle Mount: g) survey of the whole fruiting body; and h) view from above showing the end face of the sporangioles covered with little tails. (f to h) in phase contrast. All bars = 100 mm.

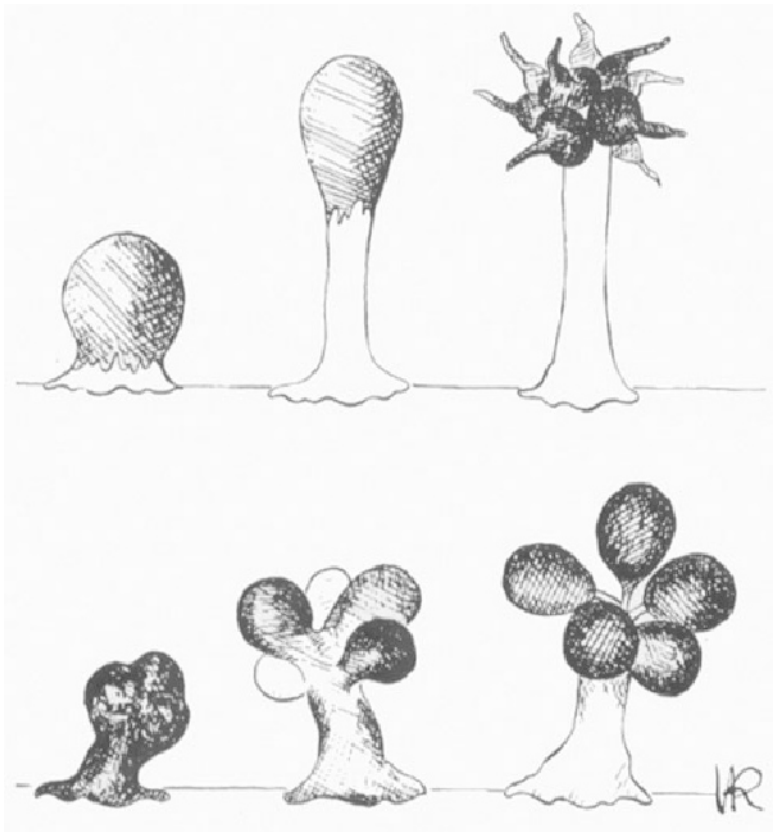


Fig. 11. Similar fruiting body shapes may be reached in different ways, as shown here for *Chondromyces apiculatus* (above) and *Stigmatella aurantiaca* (below), two organisms formerly thought to be closely related. The hatched areas indicate where the cells are located at the various stages.

elements (stalks and sporangiole walls; sporangial walls are produced only after the shape of the fruiting body has been obtained); and finally, 7) during the maturation phase, conversion of the vegetative cells into myxospores (White, 1993). In addition, cell-cell signals are exchanged throughout the entire process.

Under optimal conditions, the bacteria may go through the whole developmental cycle within 12–24 h. Fruiting body induction as well as the individual steps of differentiation are subject to control by environmental factors such as nutrients, pH, divalent cations, and temperature. In at least two cases (*Cm. apiculatus* and *Sg. aurantiaca*), normal development takes place only in illuminated cultures (Reichenbach, 1974a; Reichenbach, 1974b; Qualls et al., 1978; Stephens and White, 1980a). The course of fruiting body formation has been documented for different genera and species in several scientific films (Reichenbach et al., 1965d; Reichenbach et al., 1965e; Reichenbach et al., 1965f; Reichenbach et al., 1965g), which show the sequence of events during fruiting.

Fruiting body formation by *Mx. xanthus* has been the subject of the most intense scrutiny, and a great deal is known about the various signals that are exchanged during conversion of the vegetative cells to the simple *Myxococcus* fruiting body (Fig. 8). Five such signals, A through E, have been identified and are described in detail in Shimkets (1999). On the other hand, the signal(s) that directs movement of the vegetative cells into the aggregation center has remained mysterious. While it is intuitively plausible that the process is driven by chemotaxis, and indeed,

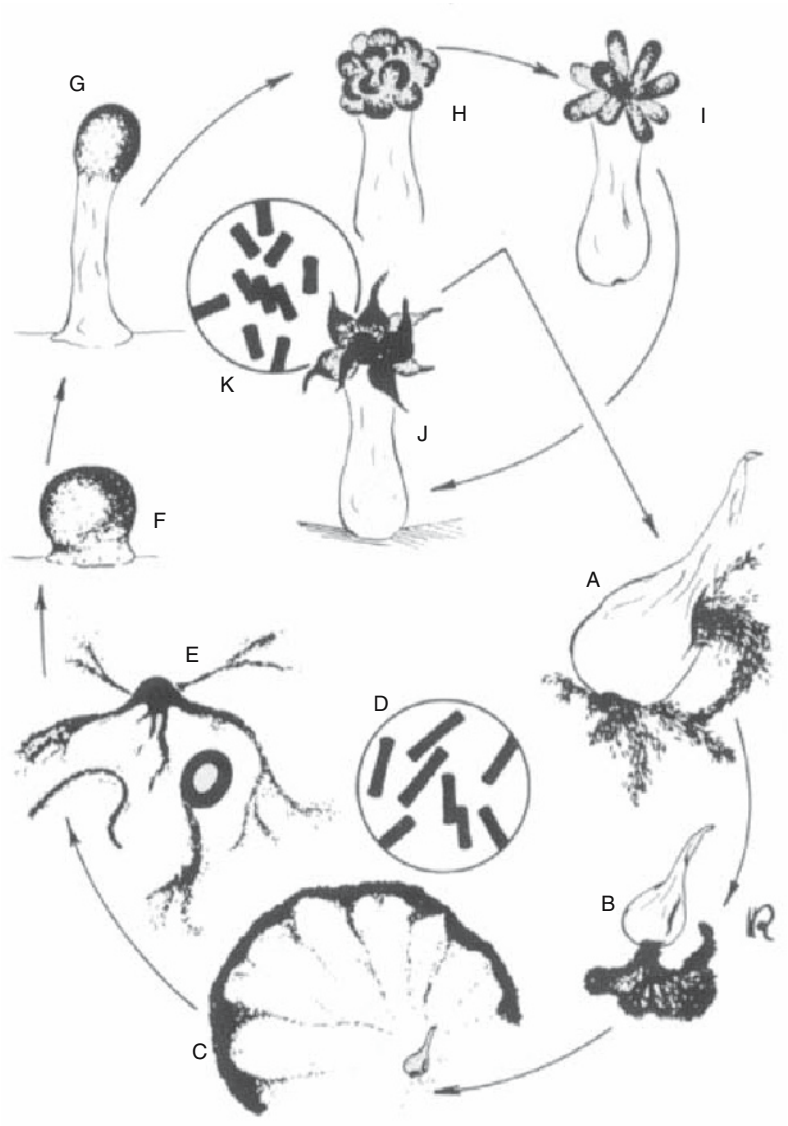


Fig. 12. Cooperative morphogenesis in myxobacteria. The organism shown in the diagram is *Chondromyces apiculatus*. Symbols: A, germinating sporangiole; B, C, development of a swarm colony; D, vegetative cells; E, aggregation of vegetative cells at various places within the swarm. F to J, fruiting body development; F, knob stage; G, excretion of the slime stalk, cells all concentrated in the terminal knob; H, the terminal mass begins to differentiate, forming wart-like protrusions which later elongate into (I) club-like structures and finally mature into (J) turnip-shaped sporangioles; K, myxospores.

chemotactic behavior has been demonstrated (see the section Gliding Motility in this Chapter), whether chemotaxis per se directs the cells into the centers has not been demonstrated.

Myxospores

Within the maturing fruiting body, a cellular morphogenesis takes place, i.e., the production of myxospores. In the suborder Cystobacterineae, this always means a rather dramatic shortening and fattening of the vegetative rods into spheres or at least into short, fat rods. The myxospores surround themselves with a capsule that may, however, be rather thin and recognizable only under the electron microscope. Also, they become optically refractile. In the suborders Sorangineae and Nannocystineae, the shape change is much less extensive. The vegetative cells may become somewhat shorter and slightly constricted in the middle. Often they also become optically refractile and may (e.g., in *Cm. crocatus*; Abadie, 1971b) or may not develop a thin capsule. The fine structure of myxospores, formerly also called "microcysts," has been studied with six organisms: *Mx. xanthus* (Voelz and Dworkin, 1962; Voelz, 1966a; Bacon and Eiserling, 1968; Kottel et al., 1975), *Sg. aurantiaca* (Reichenbach et al., 1969b; Voelz and Reichenbach, 1969), *Sg. erecta* (Galván et al., 1987), *Archangium gephyra* (Galván et al., 1992), *So. cellulosum* (Lampky, 1976), and *Cm. crocatus* (McCurdy, 1969a; Abadie, 1971b). *Myxococcus xanthus* has a very thick, layered capsule, which can be seen under the light microscope, especially after staining with India ink. The capsule appears to be reduced in thickness during germination and is left back by the emerging vegeta-

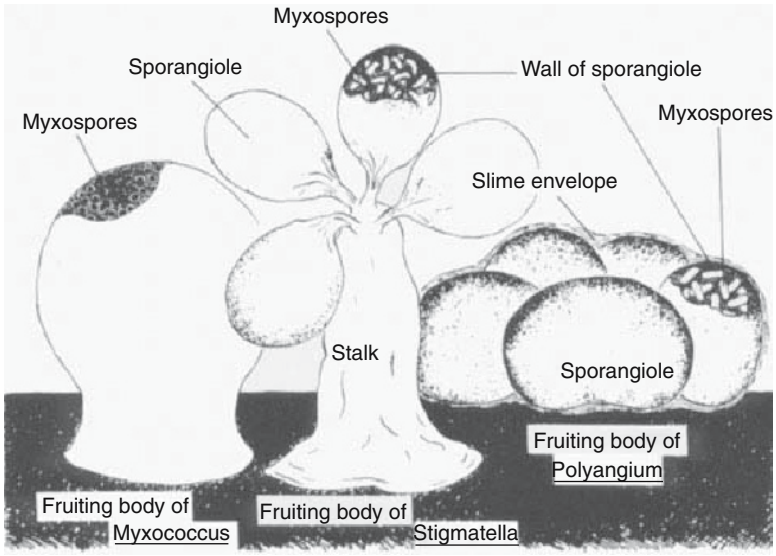


Fig. 13. The terminology for myxobacterial fruiting bodies and their structural components.

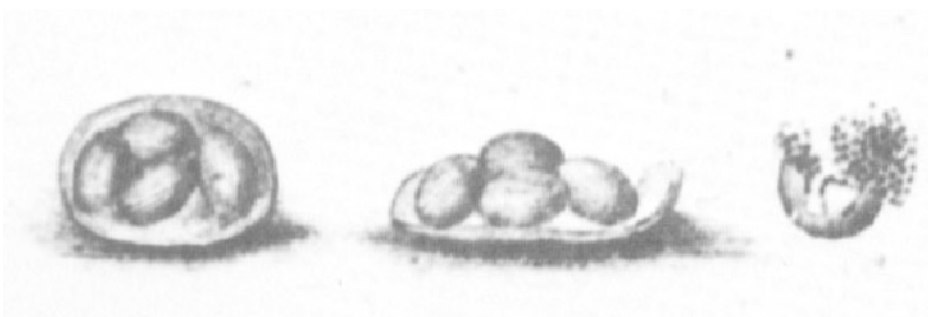


Fig. 14. *Polyangium vitellinum*, probably the first drawing of a myxobacterium to be published (Link, 1809). The original description of this still-valid genus and species reads as follows: "Among the Gasteromycetes in front of *Endogene* is to be inserted: *Polyangium*. Sporangium spherical or distortedly ovoid. Sporangioles very large in relation to the sporangium, ovoid, crammed full with a granular material. The sporangium consists of a thin, translucent, gelatinous, vesicular membrane enclosing four to six ovoid sporangioles which are filled with a colored granular mass. Sporidia could not be distinguished. *P. vitellinum*, with small yolk-colored spots, is to be counted among the smallest (species) of the order. It is found on old tree trunks, either singly or in groups of two or three, and can be recognized by its intense yolk color. Discovered by my friend Ditmar. Is to be inserted among the Gasteromycetes after *Tremella*." (Translated from the Latin.)

tive cell as an empty shell. The cell wall is retained intact during sporulation. In the cytoplasm, polyphosphate and polysaccharide granules can be seen. In glycerol-induced spores (see below), about 75% of the capsule is a polysaccharide consisting entirely of galactosamine and glucose (Kottel et al., 1975). Artificially induced myxospores fold their outer membrane into vesicular and tubular structures. An interesting open question is what happens with the gliding apparatus during shape change.

With S*g. aurantiaca* fruiting body myxospores, the outer membrane also becomes ruffled or folded; in artificially induced myxospores, these folds form densely packed membrane bodies between the cytoplasmic membrane and the capsule. Presumably, the outer membrane cannot be melted in during cell conversion and therefore is conserved in this way. As artificially induced cells change their shape much faster than cells in developing fruiting bodies, the phenomenon is more pronounced in the former. In both types of myxospores, polyphosphate and polysaccharide granules can be seen; the surface of the latter is densely covered by small, dark, ribosome-like particles. Essentially the same type of myxospores is found in fruiting bodies of *Sg. erecta* and *Ar. gephyra*; the latter may contain large, fusiform bodies with a crystalline internal structure, probably protein crystals. Fruiting body

myxospores of *So. cellulosum* seem to be devoid of a capsule. They are full of large, electron translucent granules, probably polysaccharide, which is also found near the poles of vegetative cells. In fact, phase (bright–pole) regions are observed under the phase contrast microscope in older vegetative cells. Mesosome-like membrane stacks are seen deep in the interior of the myxospores. Fruiting body myxospores of *Cm. crocatus* were found with and without a capsule. Also in this case mesosomes were present. Further, polysaccharide granules, slime vesicles, a ruffled outer membrane, and areas of vesicular or tubular outer membrane structures do occur. The arrangement of myxospores within the sporangioles is normally at random, but for *Me. boletus* an orientation strictly perpendicular to the outer wall has been described (Jahn, 1924). In *Cystobacter fuscus* sporangioles, a pattern of surface-parallel myxospores can be seen impressed on the inner face of the wall (Jahn, 1924). In cultures, myxospores are often produced free, outside of fruiting bodies.

With the discovery that the addition of glycerol to suspension cultures of *Mx. xanthus* induces the vegetative cells to convert synchronously within 90 min into myxospores (Dworkin and Gibson, 1964a), a well-defined experimental system for the study of morphogenesis in myxobacteria became available. This led to a host of studies on the events during induction and conversion. Nevertheless, as yet, there are no compelling insights into the mechanism of glycerol induction or sequence of the events leading to the formation of the myxospore. Details can be found in the reviews mentioned in the beginning of this section. Many other myxobacteria can also be induced to sporulate by adding chemicals to suspension cultures. *Stigmatella aurantiaca* can be induced by 40 different chemicals acting on at least three different receptors, as is suggested by reciprocal induction-resistant mutants (Gerth et al., 1993; Gerth and Reichenbach, 1994). Induction in *Sg. aurantiaca* is also possible by high temperatures just below the temperature maximum, and inductions by heat and by chemicals complement each other. This could mean that stress receptors and heat shock proteins play a role in myxospore induction, possibly within fruiting bodies, too (Heidelbach et al., 1993).

Myxospores are clearly designed for survival under unfavorable environmental conditions. Myxospores of *Mx. xanthus* are much more resistant to elevated temperatures (60°C), ultraviolet light, and sonic vibration than vegetative cells are (Sudo and Dworkin, 1969). In contrast to vegetative cells, myxospores are completely desiccation resistant and can be lyophilized. Dried myxospores may survive for at least 20 years at room temperature. In the dried state, temperature resistance of myxospores becomes especially impressive. Strains of *Myxococcus*, *Corallococcus*, *Archangium*, *Cystobacter*, *Polyangium* and *Sorangium* survived for 30 min at 140°C; *Corallococcus*, *Archangium*, *Polyangium* and *Sorangium* survived for 45 min; and *Archangium* and *Polyangium* even survived for 60 min. But 140°C for 240 min or 145°C for a short time killed all myxobacteria (H. Reichenbach, unpublished results).

Heat resistance of myxospores is often useful for the isolation of pure strains. To eliminate contaminants, fruiting body suspensions can be heated at 58°C for 15 or 40 min with good success. In *Mx. xanthus*, myxospore resistance to heat and desiccation appears to be accompanied by the accumulation of trehalose up to 1.1 μg/mg protein (McBride and Zusman, 1989a). To discuss here other details of the numerous studies on morphogenesis in myxobacteria is impractical; they will be found in several of the reviews mentioned at the beginning of this section (however, see also Sudo and Dworkin [1973], Shimkets [1987], Shimkets [2000], Ward and Zusman [2000], and White and Schairer [2000]).

Functions of Myxospores and Fruiting Bodies

The function of the myxospore is intuitively obvious. The metabolic quiescence of myxospores of *Mx. xanthus* (Dworkin and Niederpruem, 1964b) and their resistance to desiccation, temperature extremes, and UV light (Sudo and Dworkin, 1969) allow the organism to withstand environmental extremes and to persist for long periods during conditions that may not be optimal for growth. However, the function of the fruiting body is more subtle; "The most important question involving life history strategies is Why does one have fruiting bodies at all . . . ?" (Bonner, 1982).

While Bonner's question pertained to *Dictyostelium discoideum*, it is equally applicable to the myxobacteria. The function of the fruiting body, whether structurally simple as in *Myxococcus* or complex as in *Chondromyces*, relates ultimately to the "wolf pack" mode of feeding that is characteristic of all myxobacteria (Dworkin, 1973a). All the myxobacteria that have been so examined feed by means of excreting a battery of hydrolytic enzymes that enable them to degrade macromolecular debris or to lyse and feed on other cells (see the section Lysis of Other Bacteria in this Chapter). As a result, they are at the mercy of diffusion—of their enzymes away from the cell and of solublized nutrients toward the cell. Thus, feeding at a high cell density and thereby concentrating the hydrolytic enzymes and the resultant products is an advantage.

Rosenberg et al. (1977) has experimentally verified this hypothesis in *Mx. xanthus*. One may therefore view the fruiting body as a device for concentrating the cells, prior to their entrance into the resting state, so that upon germination they may immediately be present at a sufficiently high cell density to generate a swarm and thus to feed efficiently. The particular organization of the sporangioles that is characteristic for each species may simply reflect the optimal packaging of the myxospores, so that upon germination, multiple emerging swarms are poised to begin feeding at their optimal cell densities.

Lysis of Other Bacteria

The ability of the myxobacteria to lyse and feed on other cells and to hydrolyze a variety of insoluble macromolecules is well documented (Rosenberg and Varon, 1984), is characteristic of the myxobacteria, and accounts for the life style and multicellular proclivity of this group. This ability was observed early in the history of the myxobacteria, and the literature is full of such reports (e.g., Beebe, 1941; Singh, 1947; Nolte, 1957; Margalith, 1962; Bender, 1963; Raverdy, 1973). For this activity, a battery of cell-wall lytic enzymes, lipases, nucleases, polysaccharidases and nucleases as well as antibiotics and bacteriocins are produced (Rosenberg and Varon, 1984).

Studies on bacteriolytic activities led to the first studies on myxobacterial exoenzymes (e.g., Norén, 1960a; Bender, 1962; Hart and Zahler, 1966, Haskå, 1969; Hüttermann, 1969; Sudo and Dworkin, 1972). Some of the responsible enzymes have since been purified to varying degrees and have been biochemically characterized. While a complete bacteriolysis obviously requires many different enzymes, all of the studies on bacteriolytic enzymes of myxobacteria have concentrated on peptidoglycan-degrading enzymes. A glucosaminidase, an amidase, and an endopeptidase cleaving D-Ala/diaminopimelic acid and D-Ala/Lys bonds and proteases have been obtained from culture supernatants of *Mx. xanthus* (Sudo and Dworkin, 1972). Three bacteriolytic enzymes have been isolated and highly purified from *Mx. virescens* (Haskå, 1972a; Haskå, 1972b; Haskå, 1974); two of them proved to be endo-β-*N*-acetylglucosaminidases and the third an endopeptidase which could be purified by adsorption to montmorillonite followed by selective desorption (Haskå, 1974; Haskå, 1975). The fact that the bacteriolytic enzymes retain some of their activity when they are adsorbed to clay minerals may be of ecological relevance (Haskå, 1981). The addition of C11 through C15 fatty acids (about 2 mM) to suspensions of the target bacteria increased the efficiency of the bacteriolytic enzymes substantially (Haskå et al., 1972c). In contrast to the two myxococci, *Cc. coralloides* excreted an enzyme with a muramidase (i.e., lysozyme) activity (Harcke et al., 1972). (Additionally, the "*Sorangium*" isolate whose enzymes are often discussed in this connection was in reality a species of *Lysobacter*.)

Little is known about the enzymes with which microorganisms other than bacteria are attacked. Living cells of four yeast species could not be degraded by *Mx. xanthus* enzymes, but they were digested after a prior treatment with cysteine or organic solvents or after heating; in contrast, living cells of the yeast-like fungus *Pullularia* (*Dematium*) *pullulans* were partially decomposed (Bender, 1963). Isolated cell walls of *Saccharomyces cerevisiae* also could not be degraded by enzymes from *Mx. fulvus* Mx strain f80 without a brief heat treatment (Borchers, 1982). Apparently, the mannoprotein layer on the outside of the yeast cell wall protects the susceptible material. Two inducible enzymes have been demonstrated in the culture supernatant of *Mx. fulvus* Mx f80: 1) a laminarinase with a high specificity for β-1,3-glucosidic bonds cleaving laminaran endolytically with laminaritriose as the main product; and 2) a β-1,3-glucanase that can remove a high-molecular-weight polysaccharide other than β-1,3-glucan from the cell wall. The yeast cell wall is not completely broken down by *Mx. fulvus* Mx f80: The inner, fibrillar, glucan layer and the bud scars remain undigested, as can be seen under the electron microscope.

Several *Mx. xanthus* and *Mx. fulvus* strains were shown to control populations of the cyanobacterium, "*Phormidium luridum*," with predator-prey cycles of 9 days under experimental conditions. As few as 50 *Myxococcus* cells per 100 ml containing 10^7 cells of the prey were sufficient to start a lytic cycle (Burnham et al., 1984). The myxobacteria produced a lysozyme type enzyme (Daft et al., 1985). However, evidently because of insufficient supply of inorganic nutrients, myxobacteria cannot control natural blooms of cyanobacteria (Fraleigh and Burnham, 1988). In two cases myxobacteria have been observed to eat holes into the cell wall of the fungi *Cochleobolus* and *Rhizoctonia* (Homma, 1984) and of the green alga *Cladophora* (Geitler, 1924), respectively. The myxobacteria enter the cells and produce fruiting bodies inside.

Most myxobacteria excrete strong, diffusible proteases, as can be readily seen through the clearing action that occurs around colonies growing on skim milk agar. A few of these enzymes have been further characterized: From the culture supernatant of *Mx. virescens*, at least three proteases have been obtained (Gnosspelius,

1978b). One of them has been purified and was found to be an alkaline serine protease (26 kDa) that preferentially cleaved peptide bonds between hydrophobic amino acids. Three different proteases have also been demonstrated in the culture supernatant of *Mx. xanthus* FB (Coletta and Miller, 1986). They have tentatively been classified as alkaline serine proteases. There were qualitative differences among the proteases of different *Mx. xanthus* strains. A coagulase, myxocoagulase (57 kDa), has been isolated from the culture broth of *Mx. fulvus* strain NK35 (Bojary and Dhala, 1989). The enzyme was clearly different from *Staphylococcus* coagulase; it did not contain sugar and was active only on rabbit plasma.

While myxobacteria can hydrolyze many other macromolecules by means of diffusible exoenzymes, those enzymes have not been characterized so far. Thus, many myxobacteria decompose starch; quite a few are potent chitin degraders, e.g., all strains of *Cb. fuscus*, *Cb. ferrugineus*, *Sg. aurantiaca* and *Sg. erecta* and many strains of *Me. lichenicola* and *So. cellulosum*. Also, many myxobacteria appear to attack xylan. Two enzyme activities could be demonstrated in the culture supernatant of the cellulose degrader *So. compositum*, growing in a liquid medium with suspended cellulose powder. The enzymes produced only glucose and cellobiose from cellulose. One was a cellobiase and was destroyed by heating at 55°C for 10 min. After that, an enzyme releasing cellobiose from cellulose was still intact (Couke and Voets, 1968). These two enzymes probably did not constitute the entire cellulase complex of the organism; rather another endocellulolytic enzyme may exist that was not released from the cells. In fact, in plate cultures of *So. cellulosum* on filter paper or on cellulose powder agar, the lysis zone always exactly coincides with the size of the swarm colony. Thus, there seems to be no diffusible cellulase efficient enough to break down the crystalline cellulose completely.

During fruiting body formation of *Mx. xanthus* autolysis of a major part of the cell population takes place before sporulation begins. Approximately 85% of the vegetative cells in the population undergo lysis, the surviving cells completing myxospore formation and formation of the fruiting bodies (Wireman and Dworkin, 1977). Varon et al. (1986) have characterized the activities necessary for this developmental autolysis and have isolated five different autocides. Among these, AM I is a mixture of fatty acids and AM V is a phosphoethanolamine with unsaturated fatty acids (Gelvan et al., 1987). In the latter case, the cells release the fatty acids apparently through the action of a phospholipase; also in AM I, unsaturated fatty acids are the most efficient autocides. While AM I and AM V act specifically on *Myxococcus* species, the other autocides have wider specificity, acting on a variety of myxobacteria. Mutants that are resistant to autocides are no longer able to produce fruiting bodies.

Taxonomy and Identification

(Note: Much of the material in this section is taken verbatim from the recent revision of the taxonomy of the myxobacteria by Hans Reichenbach in the second edition of *Bergey's Manual of Systematic Bacteriology* [Reichenbach, 2005]).

All known myxobacteria are united in the single order Myxococcales. The name was changed from Myxobacterales (McCurdy, 1989) in accordance with Rule 9 of the International Code of Nomenclature of Bacteria (which requires that the name of an order be derived from the name of the type genus).

The phylogenetic position of the myxobacteria has been put on a solid base by 16S rRNA/DNA studies (Ludwig et al., 1983; Shimkets and Woese, 1992; Sproer et al., 1999). These studies have shown that the myxobacteria are a phylogenetically coherent group within the Deltaproteobacteria (Stackebrandt et al., 1988). Also apparent is that the order Myxococcales is divided into three suborders: a major division that separates the suborders Cystobacterineae and Sorangineae (Reichenbach, 1974b) and a second one separating the Nannocystineae (Reichenbach, 2005).

As discussed earlier in this chapter, the members of the suborders differ in cell shape, myxospore development, colony structure, fatty acid and carotenoid pattern, and the chemical composition of their slime (Congo red reaction). The suborders are divided into families based on the organization of the fruiting bodies and the shape of the myxospores. The definition of genera and species is based on the same characteristics and on a few complementary physiological facts. There are different possibilities for splitting and rearrangement, and in the absence of more compelling arguments, personal preference must still determine which route to follow. All in all, we can distinguish three suborders, five families, 17 genera and about 50 different species of myxobacteria, a number that while far from complete, is not likely to increase dramatically in the future. Note, however, that myxobacterial taxonomy cannot yet be regarded as settled and we are still confronted with major problems in distinguishing species, e.g., in the genera *Corallococcus*, *Archangium*, *Cystobacter*, *Nannocystis*, *Sorangium* and *Polyangium*.

Table 3. Taxonomic survey of the myxobacteria.[a]

Order: Myxococcales
Suborder: Cystobacterineae
Families and genera:
Myxococcaceae
Myxococcus
Corallococcus (formerly *Chondrococcus*)[2]
Pyxicoccus nov. gen.[b]
Cystobacteraceae
Archangium
Cystobacter
Melittangium
Stigmatella
Hyalangium nov. gen.[b]
Suborder: Sorangineae
Families and genera:
Polyangiaceae
Sorangium
Polyangium
Haploangium
Chondromyces
Byssophaga nov. gen.[b]
Jahnia nov. gen.[b]
Suborder: Nannocystineae
Families and genera:
Nannocystaceae
Nannocystis
Kofleriaceae
Kofleria nov. gen.[b]
Haliangium nov. gen.[b]

[a]Three recently described and unusual genera, *Anaeromyxobacter* (Sanford et al., 2002), *Enhygromyxa* (Iizuka et al., 2003b), and *Plestiocystis* (Iizuka et al., 2003a), are missing from Table 3 and the illustrated key (Fig. 15) because they have not yet been characterized thoroughly enough to accurately place them in this taxonomic scheme.

[b]These novel genera are validly described in Reichenbach (2004).

A survey of the taxonomy of myxobacteria derived from the taxonomy presented in *Bergey's Manual* (Reichenbach, 2005) is presented in Table 3. An annotated and illustrated key to the genera follows in Fig. 15. Note that three recently described genera, *Anaeromyxobacter* (Sanford et al., 2002), *Enhygromyxa* (Iizuka et al., 2003b) and *Plesiocystis* (Iizuka et al., 2003a), do not appear because they have not been characterized thoroughly enough for accurate placement.

Illustrated Key to the Genera of Myxobacteria

1. Vegetative cells are slender rods with more or less tapering ends, cigar- , boat- or needle-shaped, about 3.5–12 μm long and 0.6–0.8 μm wide. Swarms (e.g., on VY/2 agar) tend to remain thin, film-like, and often show a striking surface structure in form of radial veins and fields of tiny ridges or waves. Swarm edge usually has delicate fringes and flame-like protrusions. Myxospores arise through a striking cellular morphogenesis: the vegetative cells always shorten and fatten substantially. Myxospores always seem to have a capsule, which, however, is often seen only under the electron microscope. Slime and swarm sheets are stained with Congo red. The fatty acid pattern is dominated by branched-chain fatty acids; 2- and 3-hydroxy fatty acids are present in substantial amounts. All species are of the bacteriolytic type.

Suborder: Cystobacterineae 2

1′. Vegetative cells are stout, cylindrical rods, sometimes almost cube-shaped, with broadly rounded ends, about 2.5–8 μm long and 0.6–1.0 μm wide. Swarms (e.g., on VY/2 agar) tend to sink into and to penetrate the agar, sometimes to the bottom of the plate; they usually form shallow pits and bowl-like depressions; their surface structure is often less pronounced, although radial veins, ring-shaped ridges, and fanlike structures may be produced. The agar surface in the swarm area may be more or less corroded. Often, the cells concentrate at the swarm edge as a band or a massive ridge, or they form spherical or kidney-shaped clusters that migrate away from the center and leave deeply etched paths in the agar surface. Myxospores differ only slightly from vegetative cells in shape and seem to possess no capsule or, at the most, a very thin one; they are, however, optically refractile. Slime and colonies do not stain with Congo red. Branched-chain fatty acids are reduced in the fatty acid pattern, and hydroxy fatty acids are completely absent. Several species are cellulose decomposers.

Suborder: Sorangineae 5

1″. There are two types corresponding to two families. A. Swarm colonies without a slime sheet, on certain media tend to corrode the agar plate very deeply, transforming it into a spongy mass. Vegetative cells, short rods. Fruiting bodies tiny to moderately sized sporangioles, solitary or aggregated. Or, B. Vegetative cells are slender rods with rounded ends, like those of the Sorangineae but more delicate, 4–6 μm long and 0.6–0.7 μm wide. Swarm colonies with a well-developed slime sheet and massive, branching veins, resembling the colonies of the genus *Cystobacter*. Fruiting bodies not known.

Suborder Nannocystineae 10

2. Myxospores are regular spheres or ovoids with a smooth surface and a heavy capsule, diameter 1.2–2.5 μm. Vegetative cells are 3–6 μm long, boat- or cigar-shaped. Swarms often consist of a relatively soft slime sheet, although occasionally tough and tenacious sheets are also produced.

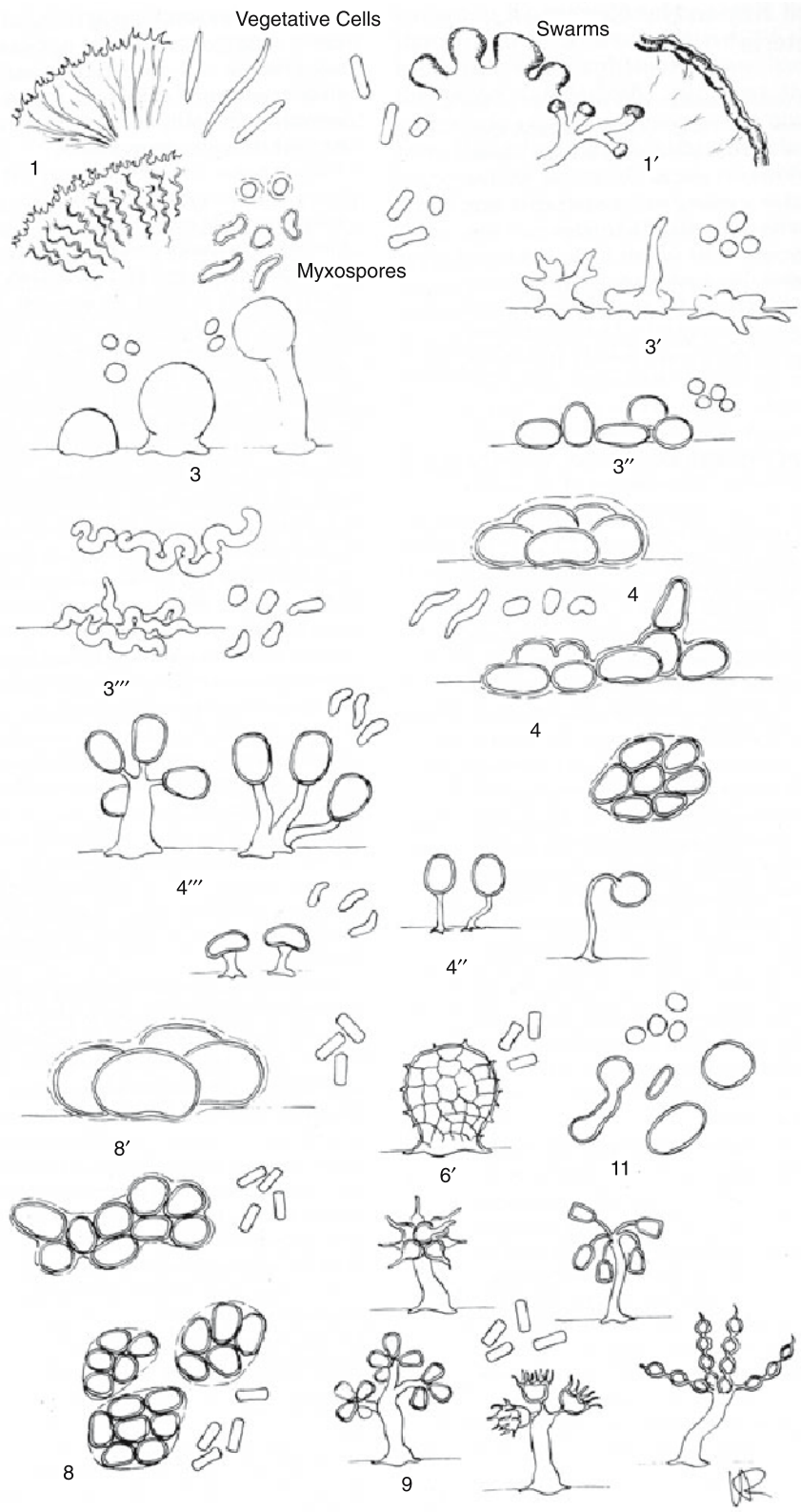


Fig. 15. A diagrammatic illustration of the key to the myxobacterial genera. The numbers in the figure refer to those in the key. The cells shown next to the fruiting bodies are myxospores.

The surface structure of the swarm is often rudimentary, but characteristic meandering radial veins may develop. Fruiting bodies are simple, spherical, soft-slimy mounds and heads or are cartilaginous columns and ridges that are sometimes branched, both without a distinct outer wall, or are sporangioles with a tough wall.

Family: Myxococcaceae 3

Comments: The differentiating characteristic of the family is the smooth, spherical myxospore. Myxospores that are nearly spherical are found with three other myxobacteria (*Archangium*, *Cystobacter* and *Nannocystis*), which can, however, easily be distinguished by the characters mentioned below.

3. Fruiting bodies are soft-slimy spherical heads or drops, often constricted at the base, in one species with a long slime stalk . . . . . *Myxococcus*

Comments: In agar cultures the shape of the fruiting body often deviates from the type, and only flat, cushion-like mounds or ridges are produced. There are four species: *Mx. fulvus* has white, pink, brick red, or even violet fruiting bodies with small myxospores (1.2–1.8 μm). *Mx. xanthus* and *Mx. virescens* produce orange and greenish yellow fruiting bodies, respectively, and have large myxospores (1.8–2.5 μm); they may be only one species, although, apparently, typical *Mx. virescens* strains produce very large myxospores and often gray, fruiting bodies on VY/2 agar. *Myxococcus stipitatus* has white-to-brownish fruiting bodies with a long slime stalk; these bodies are often found in groups on a common cushion-like base. Fruiting-body myxospores are small and they are often, but not always, clearly oval in shape. The swarms show a strong yellow fluorescence with UV light illumination at 366 nm; however, whether this quality is exclusive for the species is not yet established.

3′. Fruiting bodies are tough cartilaginous columns or ridges, sometimes staghorn- or coral-like, branched, very variable in shape, often more or less deeply rooted within the agar . . . . . *Corallococcus*

Comments: This genus has formerly been called *Chondrococcus*. The name is improper because it has been previously used for an alga. In *Bergey's Manual* (McCurdy, 1989), the genus is united with *Myxococcus*, but we prefer to retain it and suggest a new generic name. Because of its enormous variability, many species have been described in this genus, only two or three of which may be realistic. But a final decision will require more information. *Cc. coralloides* has small myxospores (1.2–1.8 μm) and produces relatively large pink or red fruiting bodies that are often branched or bear finger- or horn-like projections. Strains that also have small myxospores and produce large numbers of tiny, brownish fruiting bodies in dense fields have been described as *Cc. exiguus*. They represent either a species of their own or a variety of the former. *Chondrococcus macrosporus* has large myxospores (1.8–2.4 μm) and sturdy, yellow fruiting bodies. In contrast to the other two species, it is relatively rare.

3″. Fruiting bodies consist of sporangioles with a wall. Sporangioles are spherical, arranged in packages and sheets . . . . . *Pyxicoccus*

Comments: The third genus of the family, which is now called "*Pyxicoccus*," is somewhat confusing. The origin of the genus was a bacterium described by Thaxter (1904) as *Myxococcus disciformis*. As the spherical myxospores of the species are enclosed in sporangioles with a wall, Jahn (1924) created a new genus, *Angiococcus*, for it, apparently without ever having seen the organism. In spite of the fact that the same organism had been reported again from Poland (Krzemieniewska and Krzemieniewski, 1926), it was later concluded that *Angiococcus* does not exist (Peterson, 1966). But then the bacterium was rediscovered in the fossa of an alkaline bog (Hook et al., 1980). So the old species and genus names were reanimated, and a neotype strain of *Angiococcus disciformis* was deposited (ATCC 33172). The morphology of the organism suggests that it is really Thaxter's organism. But it does not belong to the Myxococcaceae. In the last edition of *Bergey's Manual* it was consequently classified as "*Cystobacter disciformis*." It has vegetative cells and a swarm morphology as is found in that genus. Its present classification is supported by 16S rRNA/DNA sequence data, which place the neotype strain close to *Cystobacter minus* (Sproer et al., 1999). There are, however, also organisms with spherical myxospores, a swarm morphology (as is characteristic for the Myxococcaceae), and a 16S rRNA/DNA sequence that position them close to *Myxococcus* (Sproer et al., 1999). For organisms of this type, exemplified by strain An d1, a new genus name, *Pyxicoccus*, will be used here. This avoids confusion, especially as it is not yet established whether the species *disciformis* really belongs to the genus *Cystobacter*. If not, the genus name *Angiococcus* may have to be used for it again. Note that *Pyxicoccus* fruiting bodies often degenerate into *Corallococcus*-like structures. The 16S rRNA/DNA study also proved that *Pyxicoccus* may easily be confused with other myxobacteria, as two of the three strains

tentatively identified as *Angiococcus disciformis* clustered in the Archangium group (Sproer et al., 1999).

3‴. Fruiting bodies consist of irregular masses of hardened slime (in which the myxospores are embedded) and lack an outer wall. They are either cushions with a bulging, brain-like surface or ridges that consist of meandering rolls often with rising, fingerlike projection . . . . . *Archangium*

Comments: The variability of *Archangium* fruiting bodies, and the degeneration of the fruiting bodies of other myxobacteria to *Archangium*-like structures, makes it impossible at the moment to distinguish different species. In fact, it is not even possible to recognize *Archangium* reliably, and it would be more honest to name such strains "*Archangium*-like myxobacteria." *Chondromyces serpens* is the first species for which *Archangium*-type fruiting bodies have been described, but because there are doubts about its identity, the later name, *Ar. gephyra* Jahn 1924, is used for relevant strains. This genus was formerly classified in a genus of its own, Archangiaeceae, but is now attached to the Cystobacteraceae. *Archangium violaceum* must be regarded as a degenerated *Cystobacter*, *Cb. violaceus*, under which name the organism was originally described.

2′. Fruiting bodies consist of sporangioles with a distinct outer wall, often with stalks. Myxospores are short, fat rods. Vegetative cells are slender rods with tapering ends, either boat- or needle-shaped.
Family: Cystobacteraceae 4

Comments: Aberrant fruiting bodies are common in cultures.

4. Fruiting bodies are clusters of sporangioles that sit directly on the substrate, often embedded in a clearly recognizable, common, transparent slime envelope or covered by a slime sheet . . . . . *Cystobacter*

Comments: There are myxobacteria with very similar fruiting bodies in the suborder Sorangineae, and some of these were originally united with the present *Cystobacter* species in a common genus, *Polyangium*, which, incidentally, is the oldest generic name in myxobacterial taxonomy (Link, 1809). The original family name was Polyangiaceae. Subsequently, it was realized that the genus *Polyangium* was heterogeneous. The genus was therefore split, and the Sorangineae-type species were united with species of the genus *Sorangium*. For reasons of priority, the name of the newly defined genus was *Polyangium*, and for the remaining species, the old generic name *Cystobacter* Schroeter 1886 was reactivated. The genus *Sorangium* disappeared completely, and as a consequence the family name Sorangiaceae was also eliminated and replaced by Polyangiaceae, which now, of course, had a completely different meaning than before. Here we redefine the genus *Sorangium* and replace it in the Polyangiaceae.

The species of the genus *Cystobacter* are not well studied, and it is not clear at the moment how many there are. The type species, *Cb. fuscus*, is readily recognizable from its large, glistening, chestnut brown sporangioles. *Cystobacter ferrugineus* has large, elongated, dull, dark brown sporangioles; they are often arranged in chains that tend to curve upwards in a fingerlike fashion. Both species have relatively long and slender, often slightly curved myxospores and are strongly chitinolytic. There are strains that produce light to reddish brown and often kidney-shaped sporangioles covered by a thin, translucent, and delicately plicated slime sheet; the myxospores are short, fat rods; these strains clearly represent a separate species, *Cb. velatus*. Other strains form somewhat smaller but still large, dull, dark brown, spherical sporangioles that contain myxospores in the shape of short, fat rods; they often produce a deep violet pigment and appear to constitute the species *Cb. violaceus*. Still other strains form tiny, light to golden brown, spherical sporangioles that are tightly packed together in small clusters and are often embedded in the agar substrate, resembling *Sorangium* or *Polyangium* fruiting bodies in all these respects; but the vegetative cells are long, slender needles, the myxospores are short, fat rods, and the swarms are of the *Cystobacter* type. These organisms belong to *Cb. minus*. The problem of *An. disciformis* has already been discussed.

4′. Vegetative cells are delicate, slender rods with tapering ends. Fruiting bodies consist of small spherical sporangioles that are often empty and then look glassy transparent, arranged in extended, dense sheets, or often, in chains. Myxospores are short rods to irregularly spherical, optically refractile. Swarm colonies have a thin but tough slime sheet with fine veins and adsorb Congo red, producing a purple red color. They are of the proteolytic–bacteriolytic nutritional type . . . . . *Hyalangium*

Comments: This organism differs substantially from the *Cystobacter* species discussed above and is therefore classified in a different, new genus. The distinguishing characteristics are as follows. The vegetative cells are shorter and more delicate than those of the *Cystobacter* spe-

cies. The sporangioles are small and often empty and glassy. They are arranged in monolayer sheets or in short chains and do not pile up or do so only slightly. The swarm colonies have a delicate slime sheet with very fine veins. The only species known so far resembles somewhat "*Cystobacter disciformis*," which, however, has much different fruiting bodies. Also, some traits are like those of *Polyangium minus* in the original description of Krzemieniewska and Krzemieniewski (1926). The identity with that organism cannot be ruled out with certainty, but even if the two organisms should be the same, a reclassification would be required.

4″. Fruiting body consists of a tiny sporangiole sitting on a delicate, white stalk . . . . . *Melittangium*

Comments: Most species were formerly attached to the genus *Podangium*. The type species of *Podangium*, *Pd. erectum*, has since been transferred to *Stigmatella*; the remaining species have been united in the genus *Melittangium*, so that the genus *Podangium* is now dissolved. There are at least three *Melittangium* species: *Me. boletus* has sporangioles that resemble mushroom caps and are located on very short stalks. *Mellitangium lichenicola* forms spherical, light brown sporangioles on short and relatively sturdy stalks. *Mellitangium alboraceum* is described as having a spherical sporangiole on a long and bent stalk. There are also strains that produce very tiny and delicate fruiting bodies; they may represent still another species, *Me. gracilipes*. The myxospores are always short, fat rods, and the vegetative cells are long, slender needles.

4‴. Fruiting bodies consist of dull, orange-brown to dark brown, ovoid sporangioles, which are arranged as either several on a common stalk or each on a stalk of its own . . . . . *Stigmatella*

Comments: *Stigmatella* is easily distinguished from *Melittangium*: its fruiting bodies are much coarser, and the vegetative cells are boat-shaped and of moderate length. The myxospores are short, fat rods and often S- or C-shaped. There are two species: *Sg. aurantiaca* with several sporangioles attached to a common stalk, often via tiny peduncles, and *Sg. erecta* with one sporangiole on each stalk. Clear as this may appear, the two species still are sometimes difficult to differentiate, because *Sg. erecta* often produces several fruiting bodies side by side on a common base and sometimes with partially fused stalks, so that the resulting structure may be confused with a *Sg. aurantiaca* fruiting body. These two species occupy different ecological niches: While *Sg. aurantiaca* almost always appears on rotting wood, *Sg. erecta* is found preferentially on dung and sometimes in soil. *Stigmatella erecta* was formerly classified as *Podangium erectum* (see "Comments" on *Melittangium*), *Sg. aurantiaca* as *Chondromyces aurantiacus*.

5. The suborder Sorangineae contains only one family. Vegetative cells are slender, cylindrical rods with blunt, rounded ends. Often containing optically bright granules of reserve material. The fruiting bodies always consist of sporangioles, that may be single or, more often, clustered in sedentary sori or borne on a slime stalk. In cultures, strains often refuse to produce fruiting bodies, yet degenerate fruiting bodies, as are so typical for the Cystobacteraceae, are only rarely formed. The myxospores in the fruiting bodies are morphologically not very different from vegetative cells, perhaps somewhat shorter and slightly constricted around the middle. But they are true, optically refractile resting cells, which are as desiccation and heat resistant as the myxospores of the two previous families. The swarm colonies tend to etch the agar, producing radial tracks, holes, and pits in the surface of the plate, and often penetrate the substrate deeply. In the genera *Polyangium* and *Byssophaga*, discontinuous swarm colonies with scattered cell clusters do also occur besides the more typical, coherent swarms with slime sheets and veins. Congo red is not adsorbed to the slime of the swarms. Most species are of the proteolytic–bacteriolytic nutritional type, but some are cellulose degraders and, in contrast to all other myxobacteria, may be cultivated on very simple media with an inorganic nitrogen source and a sugar as the only carbon source.

Family Polyangiaceae 6

6. Fruiting bodies consisting of several sporangioles clustered together 7

6′. Fruiting bodies consisting of large, solitary sporangioles, golden yellow, with a wrinkled or netlike surface structure when mature, found on bark and decaying wood . . . . . *Haploangium*

Comments: This genus is easily recognized by its peculiar fruiting bodies that so far have only been encountered on the bark of living or dead trees in North America and in Europe. It also has a special position in that these are the only myxobacteria that have resisted all efforts to cultivate them. (The "*Haploangium*" species, described and cultivated by Singh and Singh, 1971, were obviously misnamed because their vegetative cells were long, slender rods with tapering ends.) There may be two species that differ in size: *Ha. rugiseptum* with large sporangioles and *Ha. minus* with small ones.

7. Sporangioles located directly on or within the substrate 8

7′. Sporangioles borne on long, white stalks. 9

7″. Sporangioles orange to red brown, in convoluted, brain-like chains, often on a prominent slime cushion . . . . . *Jahnia*

8. Grows in the form of independently migrating cell companies, or pseudoplasmodia, that finally contract into intensely cinnabar- to carmine-red knob-like masses, resembling *Myxococcus* fruiting bodies. Fruiting bodies are clusters of large, red sporangioles, but are only exceptionally produced. Cellulose is decomposed . . . . . *Byssophaga*

8′. Fruiting bodies consist of tiny sporangioles that are tightly packed together and consequently are often polyhedral rather than spherical. A large number of the small parcels thus formed may lie together in more or less densely packed masses, but also the parcels themselves may become rather large. The fruiting bodies are often produced in enormous quantities, especially on digested filter paper, so that they determine the color of the swarm: bright yellow, orange, all shades of brown, and even pitch black. The vegetative swarm is usually more or less bright orange. Decomposes crystalline cellulose (filter paper) . . . . . *Sorangium*

Comments: We propose to restrict this genus to the cellulose decomposers. Cellulose degradation is such an unusual and stable character and in addition parallels a series of other morphological, physiological and biochemical characteristics in the respective strains that a separation of these cellulolytic species seems justified. The organisms have not been sufficiently studied to allow satisfactory classification of species. In addition, the original descriptions do not always indicate whether an organism was a cellulose decomposer, because not all strains have been cultivated. Also, the organisms are very variable. There appear to be at least three species: *Sorangium compositum* produces yellow-orange fruiting bodies, *So. cellulosum* brown or gray ones, and *So. nigrum* black ones, but this characteristic appears insufficient to differentiate the species reliably. There are many other morphological, chemosystematic, and physiological differences among strains, but the taxonomic relevance of these observations is presently still under study.

8″. Fruiting bodies consist of more or less spherical sporangioles that may be yellow, orange, brown or gray. Several of them may be embedded in a common, translucent slime envelope . . . . . *Polyangium*

Comments: For some time, this genus also contained the species of *Cystobacter* (see there for further comments). *Polyangium vitellinum* produces large, golden yellow sporangioles in groups of 1–20 in a common envelope. Apparently, in culture the color often changes to greenish yellow. *Polyangium luteum* has smaller, golden yellow sporangioles embedded in a yellow slime envelope. *Polyangium aureum* may be another species of this group of polyangia with orange sporangioles. There are further strains with small sporangioles that are densely clustered together in the same way as with certain *Cystobacter* and *Sorangium* strains. The fruiting bodies are light brown or gray in color and usually are embedded in the agar substrate. Probably, all strains of this type can be classified as *Pl. fumosum* and *Pl. spumosum. Polyangium parasiticum* seems to parasitize fresh-water algae; it produces red-brown sporangioles within the emptied alga cells. This species has not been reported since its first description in 1925, and its parasitic growth and even its appearance in freshwater may have been an exceptional situation. The polyangia are somewhat difficult to handle and are not well-studied organisms. There are some doubts whether the present genus is really homogeneous.

9. Fruiting bodies consist of a conspicuous, unbranched or branched white slime stalk, bearing a cluster of bright orange sporangioles. . . . . *Chondromyces*

Comments: The myxobacteria with the most beautiful and complex fruiting bodies are found in this genus. There are six species. One species, *Cm. aurantiacus*, had to be transferred to the genus *Stigmatella. Chondromyces crocatus* has a branched stalk with clusters of small, ovoid sporangioles at the ends of the branches. All other species have unbranched stalks. *Chondromyces apiculatus* produces conspicuous, turnip-shaped sporangioles, and *Cm. pediculatus* produces bell-shaped sporangioles, often drooping on long, delicate peduncles; both species are relatively common, the former especially on rotting wood, the latter often in soil and decaying plant material. *Chondromyces catenulatus* produces chains of sporangioles, and several chains originate from one stalk. *Chondromyces lanuginosus* (formerly *Synangium*) has a cluster of a few large sporangioles, each of which ends in a ring of long tips; the sporangiole thus resembles the young fruit of a hazelnut. *Chondromyces robustus* has large, fat, more or less spherical sporangioles with one to three tips.

10. Vegetative cells are short, stout, cylindrical to somewhat bulging rods with rounded to squarish ends. Fruiting bodies consist of spherical to ellipsoidal, sometimes irregular sporangioles. Most of the sporangioles are solitary, scattered on top and within the agar plate; many strains also produce dense clusters, sheets, and packets of sporangioles. The size of the sporangioles usually varies very widely. Myxospores are very short rods, ellipsoidal or spherical. Swarm colonies etch and corrode the agar more or less, depending on the medium, from shallow depressions and pits (on yeast agar) to deep holes and channels, often transforming the agar plate into a spongy mass down to the bottom of the dish.
Family Nannocystaceae 11

Comments: This family belongs to the third suborder of the Myxococcales, the Nannocystineae, together with the family Kofleriaceae. The suborder can presently be defined only by 16S rRNA/DNA sequence data, which clearly show a separate position of the organisms classified here (Sproer et al., 1999). The family, however, with only one genus, *Nannocystis*, is clearly defined by its phenotypic characteristics. Some of the more unusual myxobacteria appear to belong to this taxonomic group, in particular psychrophilic myxobacteria (Dawid et al., 1988).

10′. Vegetative cells are delicate, slender cylindrical rods with rounded ends. Swarm colonies consist of a slime sheet with more or less prominent radial veins, do not etch the agar, resemble the swarms of the Cystobacteraceae but do not adsorb Congo red. Produce usually many small to large globular knobs all over the swarm surface and dense, spherical cell aggregates within the agar resembling sporangioles, but without a wall, and sporangioles and myxospores have yet to be found. Besides terrestrial organisms also some marine, halophilic myxobacteria appear to belong to this group.
Family Kofleriaceae 12

11. Fruiting bodies are tiny, ovoid or spherical, solitary sporangioles that are produced in large numbers and are mostly embedded in the substrate. Myxospores are spherical or ovoid. Vegetative cells are often very short, stout, almost cube-shaped. Swarm is etched more or less deeply into the agar. The agar plate may become completely corroded . . . . . *Nannocystis*

Comments: Presently only two species, *Na. exedens* and *Na. pusilla*, are known, but probably other species do exist. *Nannocystis exedens* is easily recognized by its typical swarm pattern, which is especially conspicuous on water agar with streaks of living or autoclaved *E. coli*, and by its unique fruiting bodies. The latter may become considerably larger and very irregular in shape when they are produced on the agar surface. Some strains produce composite fruiting bodies that consist of clusters of small sporangioles that are more or less tightly squeezed together. Such strains may represent a different species ("*Na. aggregans*"). *Nannocystis* is perhaps the most common myxobacterium, although it is often overlooked; it is present in virtually every soil sample that contains any myxobacteria. While *Nannocystis* fits, in general, rather well with the other members of the order Myxococcales, it shows certain peculiarities that clearly set it apart, such as the nature of its carotenoid pigments and its synthesis of cholestenols.

12. Vegetative cells are long, slender, cylindrical rods with rounded ends. Swarm colonies have radial veins in a tough slime sheet, and there are numerous small to very large globular masses, or knobs, all over the swarm. Congo red is not adsorbed, and the agar is not etched. Mature fruiting bodies have not been observed but may consist of sporangioles. Cells within the knobs are long, cylindrical rods, optically refractile. They are of the proteolytic-bacteriolytic nutritional type, chitin degraders, and found in soil and similar substrates. Only one species, *Ko. flava*, is described. . . . . *Kofleria*

12′. Marine myxobacterium. Vegetative cells have rounded, blunt ends. Yellow colonies are spread and usually slightly sunk into the agar surface. Fruiting bodies are yellow to brown and consist of one or more sessile sporangioles in dense packs. They are moderately halophilic, requiring 1–3% NaCl for optimal growth (Fudou et al., 2002) . . . . . *Haliangium*

Applications

The most promising opportunity for biotechnological applications with myxobacteria is in the production of secondary metabolites that inhibit the growth of other organisms. The ability of the myxobacteria to produce antibiotics has been known for some time (Oxford, 1947; Norén, 1953; Kato, 1955; Norén and Raper, 1962). Apart from trivial fatty acids that inhibit the germination of fungal spores (Norén and Odham, 1973), until relatively recently, no chemical structure was proposed for any of these inhibitors. The first myxobacterial antibiotic for which the chemical structure has been elucidated was ambruticin, a potent antifungal compound from *So. cellulosum* (Ringel et al., 1977). In the past two decades, about 100 basic structures and 600 structural

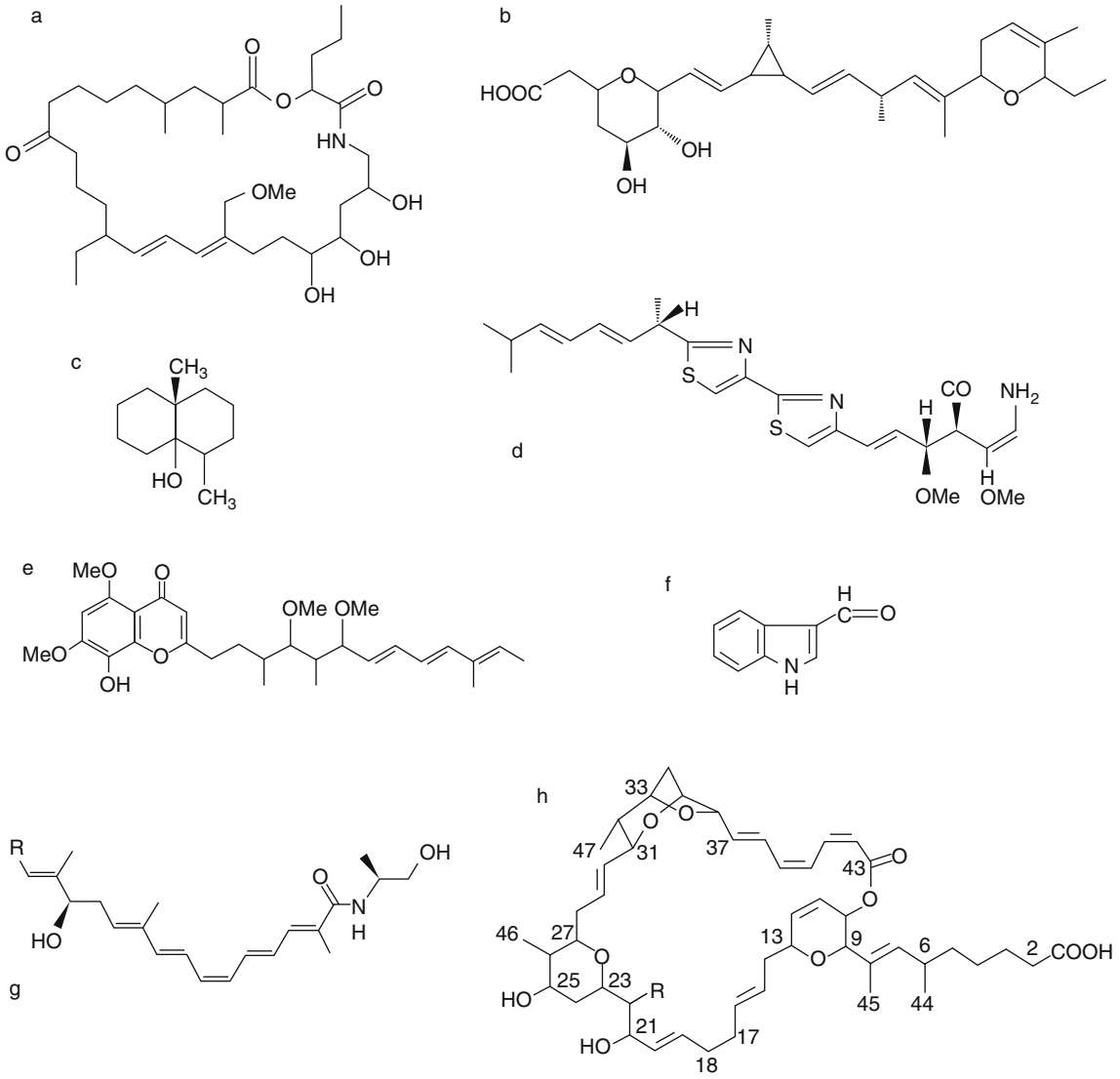


Fig. 16. A few examples of secondary metabolites produced by myxobacteria. a) Myxovirescin A; the compound, or variants of it, has been described as antibiotic TA and as megovalicins; b) ambruticin; c) geosmin; d) myxothiazol; e) stigmatellin; f) 3-formylindole; g) myxalamid; R is 2-butyl, 2-propyl, ethyl, and methyl for myxalamids A, B, C, and D, respectively; and h) sorangicin; R is OH and H for sorangicin A and B, respectively.

variants have been discovered in myxobacteria and have been fully characterized chemically (Reichenbach and Höfle, 1999; Reichenbach, 2001). The more significant insights can be summarized as follows: 1) A high proportion of myxobacterial strains (about 50% of the Cystobacterineae and almost 100% of the *Sorangium* strains) produce some kind of compound with biological activity. 2) The substances produced are chemically very different, including aromatic, heterocyclic, and polyenic compounds, alkaloids, macrocycles, polyethers, and peptides. A few examples are shown in Fig. 16. 3) Most of these compounds have not been isolated from other organisms and thus appear to be unique to the myxobacteria. The only exceptions are pyrrolnitrin and althiomycin; saframycin has been found with a new variant. 4) The ability to produce a specific compound is typical for particular but not all strains of that species. In fact, different strains of the same species may produce totally different antibiotics, but the same antibiotic may also be found in different species and even genera and families. Interestingly, the border between the suborders is also a division line for secondary metabolism, and so far only a few compounds have been found that are produced by representatives of the different suborders (e.g., pyrrolnitrin). 5) A strain may produce, at the same time, two or more compounds that are

totally different chemically. 6) Often a family of chemical variants of the same basic structure is synthesized by a strain; up to 40 different variants have been isolated from one single strain. 7) The initial yields are usually low (0.5–20 mg/liter), but with conventional methods, yield increases to more than 1 g/liter have already been achieved. 8) Interesting mechanisms of action have been discovered; compounds have been discovered that are specific inhibitors of eubacterial RNA and protein synthesis or are extremely efficient inhibitors of electron transport in respiration and photosynthesis. Two of the latter are used as specific biochemical probes and are commercially available (myxothiazol and stigmatellin). Among the compounds discovered also are iron transport metabolites of the myxobacteria (myxochelin and nannochelin). 9) An astonishing number of myxobacterial compounds interfere with the eukaryotic cytoskeleton, either actin (rhizopodin and chondramide) or tubulin (disorazol and epothilone).

At the moment, several of the myxobacterial inhibitors are being studied for potential application and large-scale production. The most promising of these is epothilone, which is currently being evaluated in phase three human clinical trials as an antitumor drug by three different pharmaceutical companies (Gerth et al., 1996). Epothilone isomers stabilize polymerized microtubules, leading to mitotic arrest at the G_2-M transition and cytotoxicity in proliferating cells (Bollag et al., 1995).

Antibiotic TA was discovered in *Mx. xanthus* strain TA (Rosenberg et al., 1973). Published chemical data, specifically NMR data (Rosenberg et al., 1982), suggest that the compound may be identical to one of the 20 variants of myxovirescin (Gerth et al., 1982; Trowitzsch et al., 1982; Trowitzsch-Kienast et al., 1989). This identification is further supported by the observation that myxovirescins are produced by many *Mx. xanthus* and *Mx. virescens* strains (H. Reichenbach, unpublished observations). In fact, the same antibiotic has been described a third time from another *Mx. xanthus* strain (Onishi et al., 1984). Furthermore, the megovalicins (Miyashiro et al., 1988; Takayama et al., 1988) are identical with previously published variants of myxovirescin; a new species, *Mx. flavescens*, has been created to describe the producing organism (Yamanaka et al., 1987), which cannot, however, be distinguished from existing species.

Another good opportunity for application may lie in the field of myxobacterial proteins and enzymes, a potential that has not yet been thoroughly investigated. Thus, restriction endonucleases (Morris and Parish, 1976; Mayer and Reichenbach, 1978) and special proteases could be of interest in the future. A lectin is produced by *Mx. xanthus* during development (Cumsky and Zusman, 1981; Nelson et al., 1981). *Myxococcus xanthus* also secretes a blood anticoagulant, myxaline, which is a heat-stable glycopeptide (El Akoum et al., 1987; Masson and Guespin-Michel, 1988). The same or a similar compound is also produced by other myxobacteria.

The efficient secretion of proteins observed with myxobacteria is unusual for Gram-negative bacteria and points to another conceivable application, as expression hosts for foreign proteins (Breton et al., 1984b; Breton et al., 1986; Nicaud et al., 1984). *Myxococcus xanthus* has been shown to secrete more than 50 different proteins into the medium, amounting to about 4% of the intracellular protein. This is impressive even if the actual number may be somewhat lower because some of the bands may have been due to proteolytic activity (Nicaud et al., 1984). Apparently, the outer membrane of the myxobacterial cell is less of a barrier than in most other cells, perhaps due to its more dynamic behavior. Two *E. coli* periplasmic enzymes, acid phosphatase and TEM2 β-lactamase, have been shown to leave the periplasm quickly when they are expressed in *Mx. xanthus* and appear in the extracellular medium (Breton and Guespin-Michel, 1987). The extracellular pectate lyases of *Erwinia* are also expressed in *Mx. xanthus* and secreted into the medium, although in this case the myxobacterial proteases inactivate the lyase (Breton et al., 1986).

There are no known myxobacterial pathogens for humans, animals or plants. The fish pathogen *Chondrococcus columnaris* is a *Cytophaga*-like bacterium and has been wrongly classified as a myxobacterium. Because of their many aggressive enzymes, myxobacteria may occasionally contribute to the deterioration of materials such as rawhide or cellulose fabrics (e.g., Heyn, 1957), but their importance in that respect is doubtful. However, although not yet demonstrated experimentally, myxobacteria reasonably play a substantial role in solubilizing large macromolecules, cell carcasses, and other biological detritus. As myxobacteria may reach rather high population densities in soil, it has been argued that they could become antagonistic to useful soil organisms like *Azotobacter* (Callao et al., 1966). However, although a suppression of *Azotobacter* by *Mx. xanthus* can indeed be demonstrated in the laboratory, whether this occurs in nature is an open question. Myxobacteria are obviously able to destroy cyanobacteria in mixed cultures in aqueous environments, and their possible use to control cyanobacterial water blooms has been suggested (Burnham et al., 1981; Burnham et al., 1984; Daft et al., 1985). But that potential has not yet been demonstrated under practical condi-

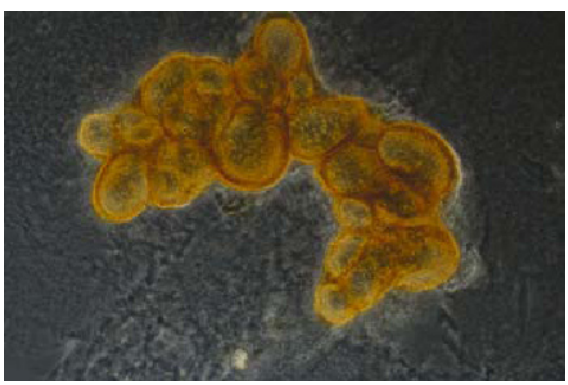
tions. Still another application of myxobacteria in environmental problems may be their use as pollution indicators (Gräf, 1975; Trzilová et al., 1980; Trzilová et al., 1981). This scheme is presently being perfected and may eventually become a useful addition to conventional biological water analysis.

Certain chemical compounds produced by myxobacteria may occasionally become troublesome. Many myxobacteria produce a strong earthy smell, which, in the case of *Na. exedens*, has been demonstrated to be due to geosmin (Trowitzsch et al., 1981). While that smell may be pleasant in connection with soil, it is disagreeable in drinking water, from which it has to be removed.

In conclusion, the economic potential of the myxobacteria is only beginning to be exploited and many potential products have yet to be adequately assessed.

Color Plates

1. *Pyxicoccus fallax* nov.gen., nov.spec., strain Ang D2, fruiting bodies on agar surface consisting of numerous small sporangioles.



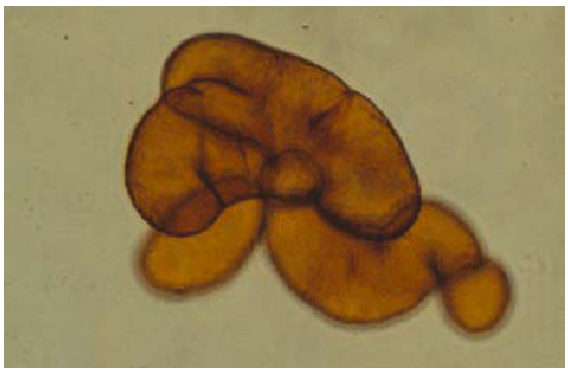
2. *Pyxicoccus fallax* nov.gen., nov.spec., fruiting body in slede mount at high magnification showing sporangioles with clear walls.

3. *Cystobacter fuscus*, mature fruiting body on agar surface, sporangioles with typically shining surface.

4. *Cystobacter fuscus*, developing fruiting bodies on agar surface.

5. *Cystobacter ferrugineus* strain Cb2056, fruiting body on agar surface with dull sporangioles.

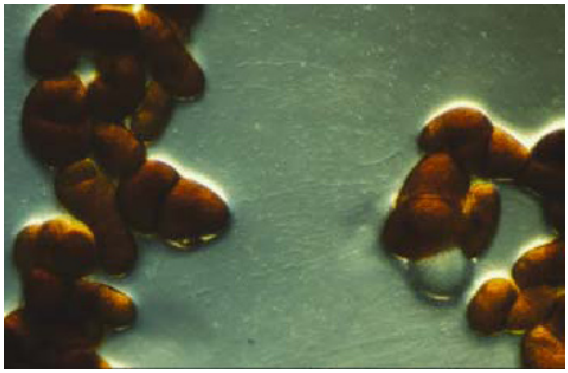
8. *Cystobacter velatus* strain Cb v37, fruiting bodies on agar surface with upward looping chains of sporangioles.



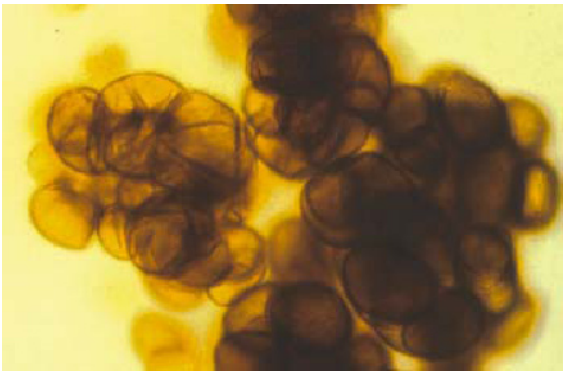
6. *Cystobacter ferrugineus* strain Cb2056, fruiting body in slide mount, several sporangioles show the elongated shape typical for that species.

7. *Cystobacter velatus*, fruiting bodies on agar surface, spoangioles covered with a delicate, plicated slime sheet.

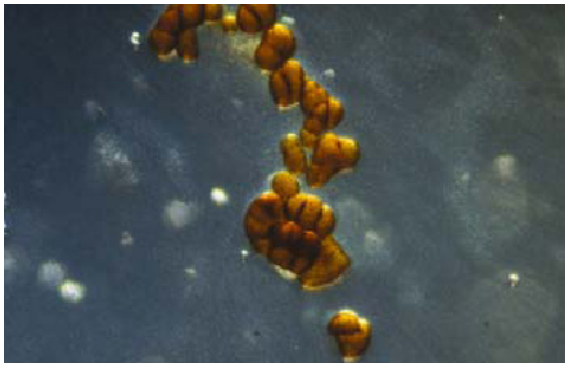
9. *Cystobacter violaceus*, original (now lost) type strain, violet swarm colonies on agar plate.



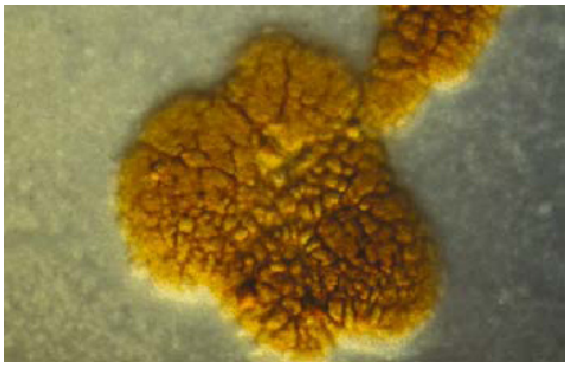
10. *Cystobacter violaceus* strain Cb vi67, fruiting body on agar surface.



13. *Cystobacter gracilis* nov.spec., strain Cb g53, fruiting body on agar surface under cover glass.

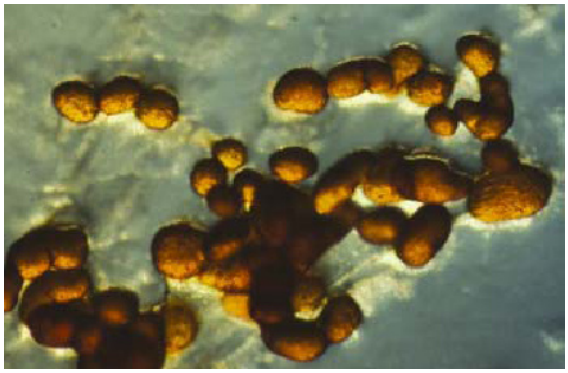


11. *Cystobacter* minor strain Cb m29, fruiting body on agar surface.



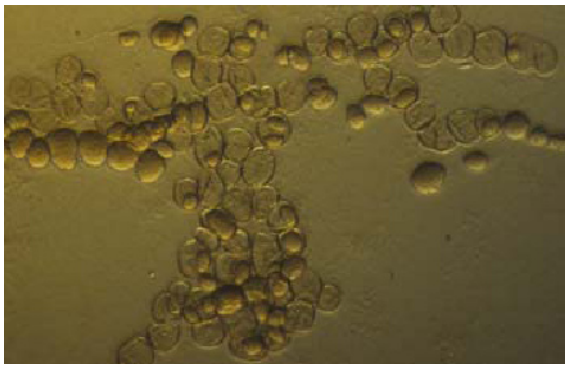
12. *Cystobacter* minor strain Cb m29, fruiting body on agar surface.

14. *Cystobacter miniatus* no.spec., strain Cb mi1, fruiting body consisting of densely packed sporangioles in a column-like, erect mass typical for that species.



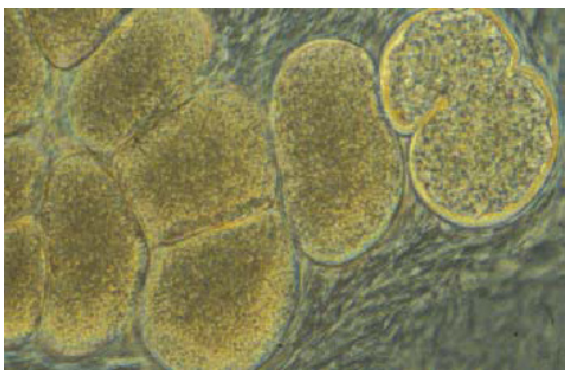
15. *Cystobacter badius* nov.spec., fruiting bodies on agar surface; besides chains of sporangioles, there also are found individual sporangioles, often on a short pedicel.

18. *Jahnia thaxteri* nov.gen., nov.spec., strain Pl t3, fruiting body on agar surface under cover glass; a wound chain of large sporangioles is sitting on a more or less obvious slime cushion.



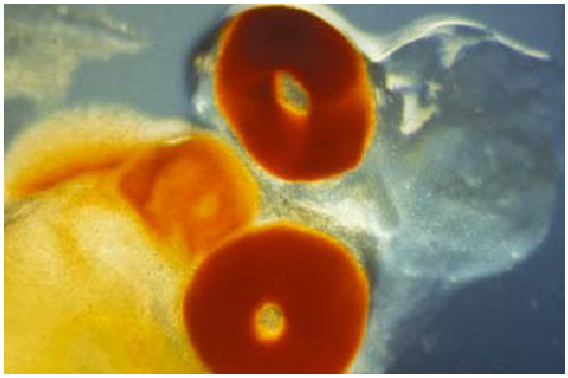
16. *Hyalangium minutum* nov.gen., nov.spec., strain Hy m3, fruiting bodies on agar surface under cover glass, with tiny sporangioles in a dense, mostly monolayer sheet; many sporangioles are empty looking, as if made of glass, as is typical for this organism.

19. *Byssophaga cruenta* nov.gen., nov.spec., strain By c1, swarm pseudoplasmodia on agar surface.

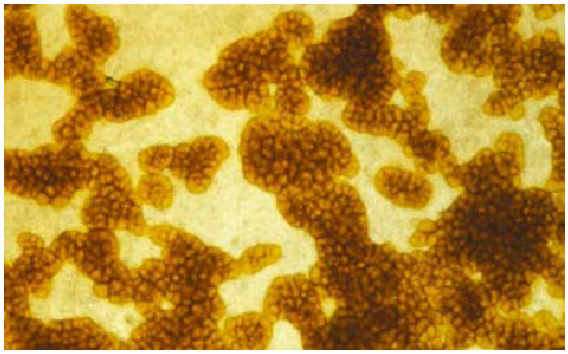


17. *Hyalangium minutum* nov.gen., nov.spec., strain NOCB-2, sporangioles in slide mount at high magnification, some of them showing invaginations of the sporangiole wall.

20. *Byssophaga cruenta* nov.gen., nov.spec., strain By c2, type strain), piled up cell mass on agar surface resembling a Myxococcus fruiting body.

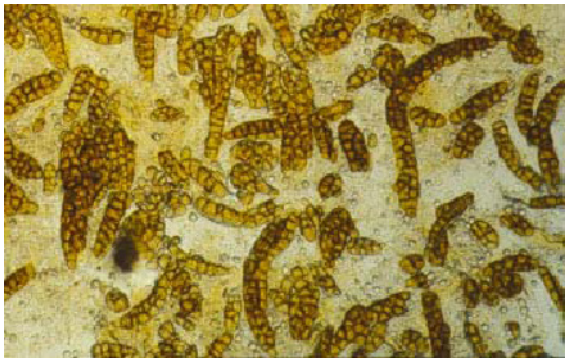


21. *Byssophaga cruenta* nov.gen., nov.spec., strain By c2 (type strain), cells assembled in massive rings on the agar surface.



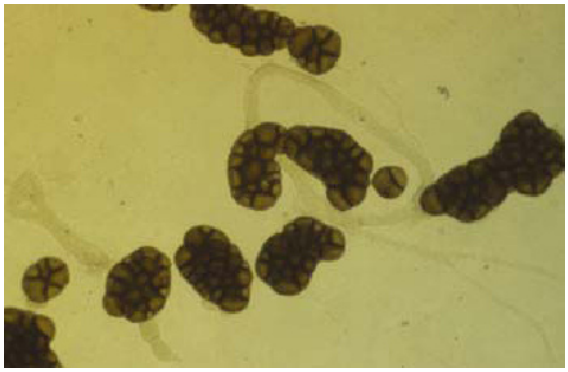
24. *Sorangium cellulosum* strain So ce1569, fruiting bodies on agar surface under cover glass.

22. *Byssophaga cruents* nov.gen., nov.spec., strain By c1, fruiting body on agar surface under cover glass consisting of large, densely packed sporangioles.

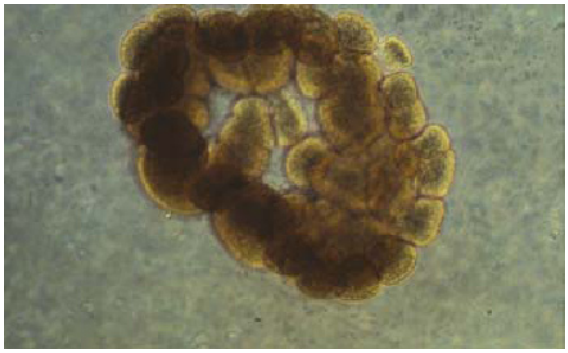


25. *Sorangium nigrum* nov.spec., strain So ce1600, fruiting bodies from macerated filter paper in slide mount.

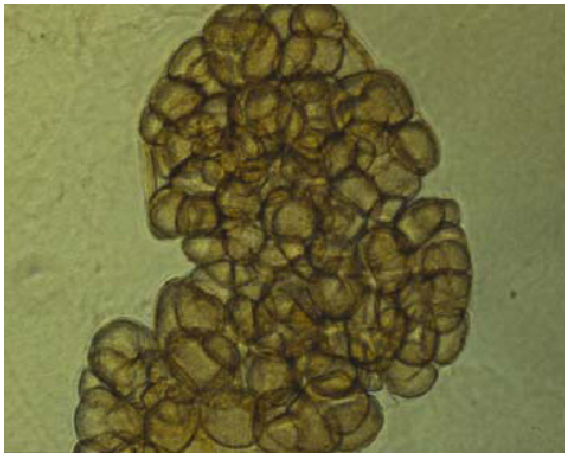
23. *Sorangium cellulosum* strain So ce1569, fruiting bodies from macerated filter paper in slide mount; the tiny sporangioles are often arranged in chains because they were produced along cellulose fibers.



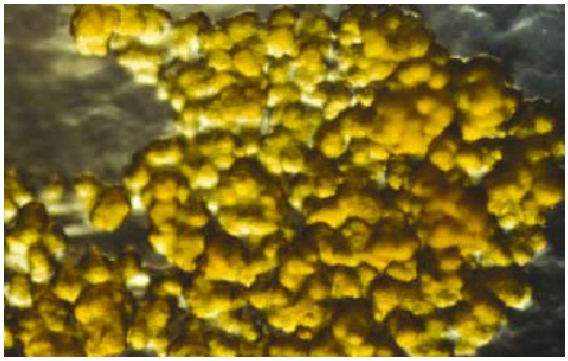
26. *Sorangium nigrum* nov.spec., strain So ce 1602, fruiting bodies on agar surface under cover glass.



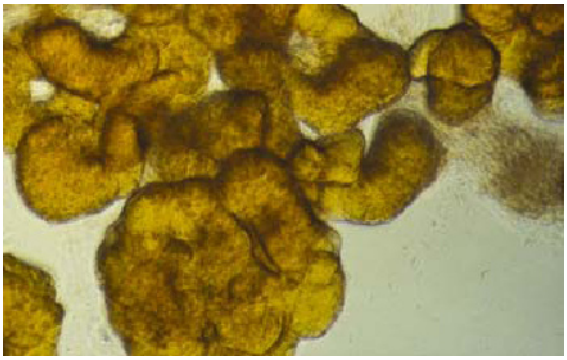
27. *Polyangium fumosum* strain Pl fu12, fruiting body on agar surface under cover glass.



28. *Polyangium spumosum* strain Pl sm4, fruiting body in slide mount.



29. *Kofleria flava* nov.gen., nov.spec., strain Pl vt10, growth on agar surface with numerous spherical knobs which are, however, not fruiting bodies.



30. *Kofleria flava* nov.gen., nov.spec., strain Pl vt10, nodules in slide mount.

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