2. REVIEW OF LITERATURE

The fact that myxobacters produce growth-inhibiting substances was described long ago, e.g. 1947 by Oxford in England, 1953 by Norén in Sweden, and 1955 by Katô in Japan. In an extensive study by Reichenbach and his colleagues in Germany it turned out subsequently that the myxobacters are indeed a rich source for antibiotics, and some of the compounds discovered by them are of practical importance. But before its discussion in detail, the organisms themselves should be characterized. They really are very unusual bacteria.

2.1.Characteristics of myxobacteria

2.1.1. Morphology

The vegetative cells of the myxobacters are unicelullar Gram-negative rods of <1.5 µm in width. They are either slender, flexible and tapered at the ends (cell type I) or cylindrical and more rigid with blunt, rounded ends (cell type II) (McCurdy 1989). The cells do not take stain readily by routine bacteriological techniques and are best observed by phase-contrast microscopy. They are never in chains or pairs. The cells tend to autolyze and form spheroplasts under a variety of conditions, e.g. anerobiosis, refrigeration temperatures, in the presence of certain cations, and in old cultures. One of their specialities is that the cells are capable of slow gliding movement when in contact with a solid surface or air-water interface at a rate of up to 10 µm/min but lacking flagella. Myxobacters typically produce an extracellular polysaccharide slime in which the cells are embeded or which is deposited as a trail behind gliding cells.

The myxobacters are distinguished from other Gram-negative bacteria by their content of 2- and 3-hydroxy fatty acids and the exclusive presence of menaquinones in their respiratory chains (Fautz *et al.* 1981). Although typically Gram-negative, the diaminopimelic acid-containing peptidoglycon in them is in relatively low amounts (0.6% dry weight) and organized in patches (White *et al.* 1968). The outer membrane lipopolysaccharide is similar to that of other Gram-negative bacteria, but differs in lacking heptose and in containing odd-numbered and isobranched fatty acids of 16 rather than 14 carbon length (Sutherland and Smith 1973; Rosenfelder *et al.* 1974). Some contain the unusual sugar 3-O-methyl-D-xylose (Weckesser *et al.* 1971). The cell membrane is marked by a predominance of branched, and the presence of diunsaturated fatty acids (Ware and Dworkin 1973). Myxobacters are more sensitive to actinomycin than other Gram-negative bacteria (Dworkin 1969).

All examined myxobacters, except *Nannocystis*, possess unipolar tufts of fimbriae (MacRae *et al.* 1977) which function as organelles of swarming (Dobson *et al.* 1979).

The vegetative colony of the myxobacters, known as swarm, has a characteristic appearance as a result of slime production and the tendency of cells to glide in more or less organized groups. The swarm is typically flat and thin with many concentric ridges or dense waves and with radiating lines or folds. It spreads extensively, sometimes rapidly, over the agar surface as a result of the outward movement of cells at the periphery. Frequently, the latter advance as groups forming tongue-like extensions or isolated clumps and streams. In the Polyangiaceae especially, the agar

beneath the swarm may be etched, eroded and penetrated. Some members of this family may possess prominent peripheral ridges, especially in contact with eubacterial colonies; others may separate into large clumps of migrating cells which furrow and penetrate deeply into the agar (McCurdy 1989).

Continued cultivation on rich media yields mucoid, non-spreading, non-fruiting or poorly fruiting colonies which resemble those of the non-gliding bacterial colonies. Such variants usually grow dispersely in liquid media. In some cases, these changes are associated with changes in the character of the slime (Grimm and Kühlwein 1973b). In other, perhaps most, instances, dispersed growth and loss of swarming is associated with the loss of fimbriae (Dobson *et al.* 1979).

The outstanding feature of myxobacters is their ability to produce fruiting bodies and myxospores. These morphogenetic processes make them by far the most sophisticated bacteria known, and the object of considerable scientific interest as model systems for the study of developmental mechanisms. Under optimal conditions, the myxobacters may go through the whole developmental cycle within 12-14 h (Reichenbach and Dworkin 1981). Fruiting body formation is initiated as a result of nutrient depletion. In *Myxococcus xanthus,* the omission of any one of the essential or strongly growth-limiting amino acids or starvation for carbon-energy or inorganic phosphate results in fruiting-body induction (Bretscher and Kaiser 1978). Upon induction, hundreds of thousands of cells aggregate at certain spots within the swarm. They aggregate into simple mounds in some species, while in others further morphogenesis may result in the

formation of complex structures including stalks and sporangia. In maturing fruiting bodies, the surviving cells differentiate into myxospores. In some species, autolysis of cells is a necessary evil (Wireman and Dworkin 1975). During fruiting body formation about 80% of the original vegetative population undergoes lysis; the surviving cells appear as myxospores. In two species at least (Reichenbach 1974; Qualls *et al.* 1978), light is required for fruiting. Fruiting bodies are the resistant or resting stage. In addition, they ensure a multicellular and, therefore, an adequately functional inoculum for the initiation of growth on a fresh substrate by organisms possessing a cooperative ("wolf pack") feeding habit (Dworkin 1972).

In those myxobacters where the myxospore is a microcyst, the hard slime coat surrounding the cell confers considerably more resistance to heat, dessication, mechanical disruption and ultraviolet light than is possessed by vegetative cells. The conversion of vegetative cells into microcysts without fruiting body formation can be induced by the addition of 0.5 M glycerol or other chemicals to liquid cultures (Dworkin and Gibson 1964; McCurdy and Khouw 1969; Reichenbach and Dworkin 1970; Gerth and Reichenbach 1978). However, such microcysts may differ from fruiting body microcysts in several physiological and structural properties (Kaiser *et al.* 1979).

2.1.2. Nutrition

Most myxobacters grow poorly or not at all on conventional bacteriological media, for example nutrient broth. They may be divided conventionally into two physiological groups; the

bacteriolytic, which includes all Myxococcaceae, Archangiaceae, Cystobacteraceae and most Polyangiaceae, and the cellulolytic, all of which are members of the genus *Polyangium*. The bacteriolytic myxobacters may be cultivated on agar media containing living or killed bacteria or yeasts which they lyse, and therefore have been called "micropredators" (Singh 1947). Their frequent observation on herbivorous dungs probably reflects the abundance therein of bacteria and other organic materials as sources of food. Most will also grow well on agar media containing enzymatically hydrolysed protein and salts including relatively high concentrations of Mg^{2+} or Ca^{2+}. However, cultivation on liquid media may present special problems, and a solid medium which supported good growth may fail to do so if agar is omitted. The cellulolytic myxobacters have very simple requirements. Nitrogen is supplied by NH_4^+ or NO_3^-, while carbon and energy are derived from cellulose, cellobiose or glucose. Most also yield good growth in casitone media and lyse dead bacterial cells (McCurdy 1989).

Myxobacter cultures emit characteristic musty odours. The bacteriolytic types often have a beet-like odour in fresh cultures, whereas the odour of many polyangia is streptomycetous which is due to the production of geosmin, as has been found in ***Nannocystis exedens*** (Trowitzsch ***et al.***1981).

The minimal nutritional requirements of most bacteriolytic myxobacters are still poorly known. Defined media originally reported for three species contained complex amino acid mixtures meeting both carbon-energy and nitrogen requirements (Dworkin 1962; Mayer 1967; Hemphill and Zahler 1968; McCurdy and Khouw

1969). *Myxococcus xanthus* could be cultivated on a medium containing pyruvate and asparatate as the predominant carbon-energy sources and amino acids leucine, isoleucine, valine and methione/vitamin B_{12} (Bretscher and Kaiser 1978). No definite obligate vitamin requirements have been demonstrated for most species, but some bacteriolytic Polyangiaceae appear to require, or are stimulated by vitamin B_{12} and perhaps other factors as well (McCurdy 1964, 1969b; Reichenbach 1970). None appear able to use sugars for carbon-energy, although some strains of *Stigmatella aurantiaca* produce detectable acid from glucose (Gerth and Reichenbach 1978). Furthermore, the addition of a polysaccharide such as starch, glycogen, agar or carboxymethylcellulose (Dworkin 1962; Schurmann 1967; McCurdy 1969b) as well as certain other soluble polymers often permits growth in liquid media that does not otherwise support growth.

2.1.3. Metabolism

The strictly aerobic, respiratory metabolism of the bacteriolytic myxobacters and their use of pyruvate and/or amino acids but not sugars as carbon-energy sources suggest that the tricarboxylic acid cycle is the main pathway of energy metabolism. Enzymes of the tricarboxylic acid cycle as well as typical aerobic bacterial electron transport systems have been detected in *Myxococcus xanthus* (Dworkin and Niederpruem 1964). *Stigmatella erecta* (McCurdy and Khouw 1969) and *Polyangium cellulosum* (Sarao *et al.* 1985). Some Embden-Meyerhof-Parans enzymes were detected in both organisms, although hexokinase was found only in *S. erecta* and *P. cellulosum*. The intermediary metabolism of the cellulolytic myxobacters has

not been studied at all but can be expected to differ from the bacteriolytic group (McCurdy 1989).

2.1.4. Genetics

The mol% G+C of the DNA of all myxobacters is 67-70 (Mandel and Leadbetter 1965; McCurdy and Wolf 1967) which differentiates them from the Cytophagales. Although the genomes of *M. xanthus* and *S. aurantiaca* were initially estimated to be 3 or 4 times larger than that of *Escherichia coli* (Zosman *et al.* 1978), it is probably in the range of 3.1-3.8 x 10^9 da or about 24-53% larger than the *E. coli* genome (Yee and Inouye 1981).

Genetic studies of the myxobacters have been limited largely to *M. xanthus* for which auxotrophic mutants, motility mutants and morphogenetic mutants have been isolated, resulting from spontaneous mutations or mutagenesis by ultraviolet light, nitrosoguanidine, ethylmethanesulfonate and ICR 191 (Burchard and Parish 1975; MacRae and McCurdy 1976; Hodgkin and Kaiser 1977; Grimm 1978).

The extreme instability of fruiting body formation in many species of myxobacters suggests that significant parts of the morphogenetic programme are subject to phase variation, as has been described for colonial morphology in *M. xanthus* (Burchard and Dworkin 1966; Wireman and Dworkin 1975; Burchard *et al.* 1977) and *Archangium violaceum* (Grimm and Kühlwein 1973a,b), or are plasmid-determined.

Endogenous cryptic plasmids have been found in strains of *Myxococcus fulvus* and *M. xanthus* (Brown and Parish 1976). Moreover, chloramphenicol resistance in MxFbc was correlated with the appearance of peak of extrachromosomal, covalently closed DNA molecules.

Resistance to chloramphenicol and other antibiotics as well has been transferred from *Pseudomonas fluorescens* and *E. coli* to *M. xanthus* (Brown and Parish 1976). Chloramphenicol resistance may also be transduced into *M. virescens* by the specialized transducing phage P1CM, with the accompanying appearance of plasmids not observed in the parental strain. DNA from the transduced myxococci could then be employed to clone certain genes in *E. coli* (Morris *et al.*1978).

It has been possible to transduce the transposon Tn5, carrying kanamycin resistance, from *E.coli* to *M. xanthus* by use of P1:Tn5. Only the Tn5 sequences were found in the transductants (Kuner and Kaiser 1981). Tn5 provides a good selectable marker for isolating and mapping mutants and for cloning *M. xanthus* genes in *E. coli*.

2.2. Habitats of myxobacteria

The distribution of myxobacters is worldwide. They are found mainly in four places: soil, dung, decaying plant material, and the bark of living and dead trees. They are primarily soil organisms that colonize other substrates only secondarily (Reichenbach and Dworkin 1981). The preferred substrate of a particular species may vary at different places. Thus, in North America, *chondromyces* spp. are common inhabitants of bark and

rotting wood, but rare in soil (Nellis and Garner 1964); in India, they are found regularly in soil in the rhizosphere of plants, on rotting wood, bark, and dung (Agnihothrudu *et al.* 1959; Singh and Singh 1971); in Europe, they are very rare organisms and have been found only in dung, rotting wood, and bark (Krzemieniewska and Krzemieniewski 1946; Dawid 1979).

Most known myxobacters occur in soil of neutral or slightly alkaline pH (Reichenbach and Dworkin 1981). *Cystobacter desciformis*, *Angiococcus disciformis* and *Polyangium sorediatum* prefer acid soils (Krzemieniewska and Krzemieniewski 1927). Although their numbers appear highest in temperate, cultivated soils, they have also been recovered from forest soils, marine and freshwater beaches and dunes, paddy field, grass land, mountain soils, underground limestone, tropical soils and arctic tundra. Acid soils and raw humus underground, e.g. the *Rhododendron* forest of West Virginia has been found to be totally devoid of myxobacters.

Dung of various animals, especially herbivores such as rabbit, hare, deer, mouse, sheep, goat etc. is an excellent source of myxobacters. Possibly, myxobacters pass unscathed through the digestive tract of animals (Kühlwein 1960) and deposit with dungs. However, the organisms as a rule appear to come from the surrounding soil after the dung has been dropped. This conclusion is based on the facts that aged dung is a better source of myxobacters than is fresh dung, that dung collected on soil rich in myxobacters results in a greater yield of strains than one from poor soil, and that the same organisms found on dung can be demonstrated in the surrounding soil (Reichenbach and Dworkin 1981). Several myxobacters, like *Myxococcus fulvus*,

M. virescens, *Cystobacter fuscus* and *Stigmatella erecta* are typical dung organisms. But, there is no myxobacter whose growth requires or is stimulated by dung components.

Myxobacters also occur on bark and rotting wood. They occur abundantly on bark of living elder (*Sambucus racemosa*), beech (*fagus sylvatica*) and black locust (*Robinia pseudoacacia*) (Dawid 1979). Bark is a reliable source for *Stigmatella aurantiaca* and *Chondromyces* spp. However, there are no myxobacters with an absolute dependence on bark and wood.

Only one species has been described as aquatic (Geitler 1924), but a number have been isolated from fresh water environments (Geitler 1925; Jeffers 1964; Brauss *et al.* 1967, 1968; Shilo 1970; Graff 1975; Hook 1977). It appears that there presence in these habitats are the result of runoff from adjacent soils. No marine myxobacters are known, although they have been isolated from shore material (Brockman 1976; Rückert 1975). So far, no strains of myxobacters have been isolated that tolerate full-strength sea water.

2.3. Enrichment, isolation and preservation of myxobacteria

2.3.1. Enrichment of bacteriolytic species

2.3.1.1. Incubation of natural materials

Bark of living or dead trees, rotting wood or dung pellets of herbivores are placed in Petri dishes lined with two or three layers of filter paper and soaked for several hours in distilled water containing Acti-Dione (30-50 µg/ml). Surplus water is poured off.

2.3.1.2. Soil baiting

A large Petri dish is half-filled with soil and moistened up to its water-holding capacity with Acti-Dione-containing distilled water (30-50 µg/ml). About a dozen autoclaved (121°C for 20-30 min) urine-free dung pellets from rabbits on an antibiotic-free diet are partly buried (half-way down) into the soil with a sterile forceps. (Krzemieniewska and Krzemieniewski 1926).

2.3.1.3. Singh plates

A thick paste of living or killed *Escherichia coli, Micrococcus luteus, Enterobacter aerogenes* or baker's yeast is spread as cross-streaks on 2% water agar (pH 7.0) containing 30 µg each of Acti-Dione and nystatin per ml. Small bits of soil or other inoculum are placed on the streaks (Singh 1947).

2.3.1.4. Water samples

Surface water from natural sources is drawn through 0.45 µm membrane filters coated with bacteria or yeast from approximately 10 ml of broth culture. The filter is then placed on 1.5% water agar containing Acti-Dione (30 µg/ml) and 100 mg $Cacl_2.2H_2O$ per litre and sometimes supplemented with 0.1% peptone (McCurdy 1989).

Alternatively, surface water is drawn through membrane filter. The filter is then put right-side up on the surface of rabbit dung agar (Gräf 1975).

All these preparations are incubated at about 27°C in freezer boxes to maintain relatively humid conditions. Myxobacters are detected by means of a dissecting microscope, with reflected

illumination for fruiting bodies and transmitted light for swarms. The cultures are checked daily, beginning with the second day. On agar enrichments, swarms are frequently detected even in the absence of fruiting body formation. The fresh organisms developed after 3-4 days are usually myxococci. They are followed by species of the Archangiaceae, Cystobacteraceae and finally, Polyangiaceae. Generally, no new development occurs after 3 weeks (McCurdy 1989).

2.3.2. Enrichment of cellulolytic species

Stanier's mineral salt agar (Stanier 1942) is overlaid with filter paper or a thin agar containing 0.5% cellulose powder and inoculated in the centre with finely crumbled soil or plant material. All sorts of organisms spread over the plate within the filter paper through the capillary water trapped between the fibres. Cellulose decomposers appear as bright yellow, orange, brown or even black areas in which cellulolytic activity becomes obvious after 2-3 weeks. The cellulose becomes slimy, acquires a glossy transparency, and later disappears completely.

2.3.3. Isolation of pure cultures

In the most simple cases, pure cultures of myxobacters can be obtained in one step. Frequent examination is necessary to choose the best time for transfer. Fruiting bodies usually contain myxobacterial cells exclusively, at least as long as they are young; and by carefully transferring material from fruiting bodies to a suitable agar medium, pure swarms can be obtained. An excellent tool for such delicate work is a glass rod drawn out into a fine tip (Reichenbach and Dworkin 1981). Another useful

tool for transfer is a stainless steel needle rubbed to a very fine, flattened tip (McCurdy 1989). Direct isolation from fruiting bodies is possible with *Myxocóccus* spp., because they produce soft-slimy fruiting bodies, and enough myxospores to initiate a swarm can be obtained by just touching the fruiting bodies at their tips. In case of the most complex fruiting bodies, the sporangia are borne on a stalk above the heavily contaminated surface and can be plucked off with the needle. The fruiting bodies of all other myxobacters are too intimately interwoven with the substrate to allow their transfer without picking up contaminated material (Reichenbach and Dworkin 1981).

The main contaminants to be dealt are other bacteria, especially, other gliding bacteria and small Gram-negative rods which remain trapped in the myxobacter slime. Nematodes and fungi, especially when fast growing, may also become a serious problem, and soil amoebae may multiply in great numbers on the food organisms.

If direct isolation is not possible, pure cultures are obtained by serial transfer of swarm edges which move ahead of contaminant bacterial growth. When serial transfers fail, pure cultures may be obtained by plating dispersed vegetative cells. Portions of swarm as free as possible from contaminating organisms are dispersed in DM (McCurdy 1963) in a blender with 200-µm glass beads. The suspension is serially diluted in DM and is surface- or pour-plated by use of *Escherichia coli* medium, SP agar or casitone-yeast extract agar (McCurdy 1989).

Myxobacters may be purified by heating. The fruiting bodies are suspended in 1 ml sterile water and incubated in water bath

for 10, 20 and 40 min. Samples are then transferred to a suitable medium. The useful temperature, too high for the contaminant but still withstood by the myxobacter, has to be found by trial and error. The working temperature for most of the myxobacters is 58°C (Reichenbach and Dworkin 1981).

Reichenbach (1983) described an excellent method for the purification of myxobacters in which antibiotics are used in high concentrations. As myxobacter fruiting bodies do not germinate in nutrient rich media, they can be treated in such media with high concentrations of otherwise toxic antibiotics. The contaminants would grow and are killed by the antibiotics. The fruiting bodies are then washed free of the antibiotics, transferred to a suitable growth medium on which they germinate and give rise to pure swarms.

The cellulolytic polyangia are difficult to isolate. They grow only slowly, and primary enrichments are rapidly overgrown with contaminants. Swarms should be transferred as soon as they are observed after dispersal and dilution. Suspensions are to be spread on the surfaces of both filter paper and cellulose agar overlay plates as well as Stanier's medium containing 1% cellobiose or glucose, and the transfer is repeated as soon as colonies appear. The swarms of polyangia often penetrate deep into the agar, and this may be exploited by inverting the agar and transferring the penetrating growth.

The final task is to check the purity of the isolated strain. The swarm material is transferred to trypticase broth, brain heart infusion and nutrient broth in which myxobacters grow only

slowly or not at all, in contrast to most contaminants. In addition, the culture may be inoculated into a suitable liquid medium in a shake flask; under such conditions, myxobacters grow only in clumps, flakes, or as a ring around the glass wall. Fast growth in uniform suspension is almost always an indication of contamination (Reichenbach and Dworkin 1981).

Microscopic examination often quickly reveals contaminants. Myxobacters are usually differentiated easily from other bacteria by their size, cell shape, and absence of flagellar motility.

2.3.4. Preservation

Vegetative cultures of myxobacters on suitable agar media, e.g. vy/2 agar kept at about 30°C usually remain viable for 2-3 weeks. They die out rapidly at temperatures of -18 to 4°C. In contrast, the mature fruiting bodies remain viable for a much longer time.

Almost all myxobacters capable of fruiting may be preserved as fruiting bodies dried on agar over filter paper in a vacuum evaporator. Swarm is taken on a small piece of sterile filter paper (3 x 1.5 cm) on water agar (Reichenbach and Dworkin 1981) and incubated at 28°C. Usually, large number of fruiting bodies develop within 2-3 days. These fruiting bodies are allowed to mature for about 8 days. The filter paper is then placed into a suitable container, such as a screw-capped tube, and dried in a vacuum dessicator over silica gel for a few dys. The containers are then tightly capped and stored at room temperature or at 6°C. Such dried fruiting bodies survive for 5-10 years. To start a growing culture, the filter paper is placed upside down on a suitable

medium. After 24 h. the filter strip is shifted to another place on the plate. When the paper is removed, many of the soaked and softened fruiting bodies stick to the agar surface.

Myxobacters may also be dried in skim milk (Reichenbach and Dworkin 1981). Fruiting bodies are suspended in skim milk, and a few drops of the suspension are then transferred to a freeze-dried milk plug in an ampule. The ampules are kept at 4°C for at least 10 min. Then they are dried at room temperature for 4 h in a vacuum dessicator. After drying, the ampules are filled with nitrogen gas and sealed. Such cultures retain their viability for at least 5-10 years when stored at room temperature. Experiments with true lyophilization and with protectives other than skim milk gave no better results.

However, the most convenient, but excellent method for the preservation of myxobacters is simply to grow them on slants of an appropriate medium in 5-10 ml screw-capped tubes and store them at -70°C. By use of this method, cultures have been maintained as long as 8 years (McCurdy 1989). Storage at ultra-low temperature is the safest means to preserve dispersed-growing strains, non-fruiting non-sporulating strains, and mutants.

A number of myxobacters can be converted to myxospores by glycerol induction technique (Dworkin and Gibson 1964). For those myxobacters that can not readily form fruiting body myxospores (e.g. developmental mutants) glycerol myxospores offer an advantage for storage.

To reactivate frozen cultures, the tubes are immersed into cold water to speed up thawing. The cells are transferred into

fresh medium immediately after liquefaction. Otherwise, the cells lyse within 15-30 min. The same happens if the temperature of the deep freeze rises above -30 to -25°C. For cells that are not easily reactivated or for fragile mutants, it may be useful to reactivate the cells by embedding them in a growth medium containing 0.6% agar. The soft agar gel may provide a matrix which protects cells that have become slightly damaged by freezing and thawing.

2.4. Classification of myxobacteria

Myxobacters were recognized as an independent group by Thaxter (1892). Even after hundred years, we know very little indeed about these interesting organisms.

The present classification of myxobacters is also based primarily upon the morphology of their fruiting bodies, myxospores, swarms and vegetative cells. This is in part for historical reasons, and in part because most of the physiological information is restricted to very few strains of myxobacters. In addition, the rather complex morphology of myxobacters suggests that a morphological classification is more feasible than is the case with most other bacteria.

Efforts are being made to add physiological characters to the morphological classification. But the results are scant so far. Comparison of a number of standard physiological tests did not identify useful key characters other than the Congo red reaction of slime (McCurdy 1969a). Potentially useful techniques of immunology (Grilione 1968) and DNA hybridization (Johnson and Ordal 1969) have not been exploited adequately here. A detailed

knowledge of a certain group may indeed reveal physiological characters of taxonomic relevance. Thus, members of the genus *Nannocystis* do not produce the carotenoid glycosides that are typical for almost all other myxobacters (Reichenbach and Dworkin 1981), and *Myxococcus stipitatus* is the only myxobacter that is colourless and lacks carotenoids ; it shows instead a strong fluorescence under ultraviolet light (Lampky and Brockman 1977).

The classification used in the latest edition of the Bergey's Manual (McCurdy 1989) is the same as that used in the eighth edition of the Manual (McCurdy 1974) and is based upon the formal revisions proposed by McCurdy (1969b, 1970, 1971a,b). Organisms of cell type I (the Myxococcaceae, Archangiaceae and Cystobacteraceae) were originally separated from those of cell type II (Polyangiaceae) on the basis of correlated differences in myxospore structures, adsorption of Congo red, and other cultural characteristics. Recent studies have confirmed the validity of the revised classification. Using 16S rRNA oligonucleotide cataloging, Ludwig *et al.* (1983) found the cell type I families to be closely related to one another but clearly separate from *Polyangium cellulosum* and *Nannocystis exedens* of the type II family. Furthermore, Fautz *et al.* (1981) reported significant differences between these two groups in hydroxy fatty acid content: the Polyangiaceae contained none, while members of the remaining families contained them abundantly. The family Polyangiaceae contains the largest numbers of recognizable species that form the most complex, intricate fruiting bodies.

Although no other classification has been formally published, Reichenbach (1974) and Reichenbach and Dworkin (1981) have

suggested a somewhat different classification (Summarized in Table 1). In it, the family Polyangiaceae is designated the Sorangiaceae, and the order Myxobacterales is divided into two suborders, the Cystobacterineae and Sorangineae, based mainly on cell shape, the way myxospores are formed, their fatty acid patterns, and adsorption of Congro red by the slime. The G+C ranges of the suborders seem to overlap almost completely with a slight tendency of the Sorangineae to the higher values. The suborders are divided into families, using the organization of fruiting bodies and the shape of the myxospores. The definition of genera and species is based on the same characters and on a few complementary physiological facts. This classification, however, poses nomenclatural problems. As noted by McCurdy (1970), *Polyangium vitellinum* is the oldest myxobacter in the literature and must, therefore, be the nomenclatural type not only of the genus but also of the family, and proposed suborder and, indeed, the entire order (McCurdy 1989).

Since cellulose degradation is a unique and stable character, Reichenbach and Dworkin (1981) proposed that the genus "*Sorangium*" be restricted to the cellulose decomposers. However, Jahn's (1924) *Sorangium schroeteri,* considered by Peterson (1974) to be the same as *Polyangium sorediatum* (Thaxter 1904), was not described as cellulolytic and, indeed, is too incompletely characterized for reidentification (i.e. no pure cultures, vegetative cells not described). If a type is designated for cellulolytic myxobacters, apparently it should be *Polyangium cellulosum* (Imshenetski and Solntzeva 1936; McCurdy 1970, 1989), although *Polyangium spumosum* if proved to be

Table 1. Taxonomy of the myxobacteria

Reichenbach and Dworkin (1981)	McCurdy (1989)
Order Myxobacterales	Order Myxococcales
Suborder Cystobacterineae	Family I. Myxococcaceae
Family Myxococcaceae	Genus I. *Myxococcus*
Genus *Myxococcus*	Family II. Archangiaceae
Genus *Corallococcus*	Genus I. *Archangium*
Genus *Angiococcus*	Family III. Cystobacteraceae
Family Archangiaceae	Genus I. *Cystobacter*
Genus *Archangium*	Genus II. *Melittangium*
Family Cystobacteraceae	Genus III. *Stigmatella*
Genus *Cystobacter*	Family IV. Polyangiaceae
Genus *Melittangium*	Genus I. *Polygangium*
Genus *Stigmatella*	Genus II. *Nannocystis*
Suborder Sorangineae	Genus III. *Chondromyces*
Family Sorangiaceae	
Genus *Sorangium*	
Genus *Polyangium*	
Genus *Haploangium*	
Genus *Chondromyces*	
Genus *Nannocystis*	

cellulolytic, could be an alternative candidate (McCurdy 1989).

Since very few genera of the Polyangiaceae have been studied in any detail, McCurdy (1989) was not in support of erecting additional genera such as *sorangium* or *Haploangium*. The latter was suggested for polyangia with solitary sporangia in spite of their occurrence in other species (e.g. *Polyangium vitellinum*). On the other hand, *Nannocystis* is so distinctive that McCurdy (1989) proposed its placement in a family separate from the Polyangiaceae.

The "Cystobacterineae" exhibit such a high degree of similarity in their 16S rRNA catalogues, McCurdy (1989) proposed to create a single family to include them all. In that case, the erection of a new suborder would then be superfluous.

The taxanomy of many of the Myxococcales is still largely based on the characteristics of the fruiting bodies, and still faces the same challenge as categorically put forward by Reichenbach and Dworkin (1981) that these structures are known to vary greatly in response to environemntal influences and either may not be formed or are arrested in their development as a result of mutation or unfavourable conditions. Imperfectly formed fruiting bodies of certain species simulate the mature fruiting bodies of other members of the group. Accordingly, in identifying these organisms, it is essential to study fruiting body formation, preferably with pure cultures, under a wide range of defined nutritional and environmental conditions and over a considerable period of time. This principle has not always been followed in the taxonomy of myxobacters, and the biochemical data conventionaly used in bacterial taxonomy are often lacking (McCurdy 1989).

The Myxococcales are unique among bacteria, in that in most cases the type materials are represented by herbarium specimens, not by type cultures. Such specimens, although often giving a good impression of fruiting body structure, offer little or no information about cells and spores. Since future work with these organisms will undoubtedly be based on the comparative study of pure cultures, it would be desirable, insofar as possible, to designate type or neotype strains of each species (McCurdy 1989).

2.5. Antibiotics from myxobacteria

Oxford (1947) had shown that a strain of *Myxococcus virescens* excreted into the medium substances that were inhibitory for *Staphylococcus aureus*. Immediately after that, reports on antibiotic activities turned out from a member of myxobacters (Finck 1950; Norén 1953; Katō 1955; Norén and Raper 1962), but no substances were isolated. It was assumed at that time that myxobacters could not be grown in liquid medium. This idea prevailed till about 1965. Peterson *et al.* (1966) demonstrated clearly for the first time antibiotic production by myxobacteria; they showed that some strains of *Polyangium (Sorangium)* produce an antibiotic (myxin) at the end of the logarithmic phase of growth. From *Myxococcus xanthus*, Norén and Odhan (1973) isolated some iso-branched fatty acids which inhibited the germination of *Fusarium* species. In 1977, the first complete structure of a myxobacter antibiotic, ambruticin, was elucidated (Connor *et al.*1977). This is a potent antifungal compound produced by *Polyangium (Sorangium) cellulosum*. Since then, search for new antibiotics from myxobacters is in progress.

2.5.1. Screening

In 1975, a team of workers in Gesellschaft für Biotechnologische Forschung mbH (GBF) in Germany had commenced a systematic search for new antibiotics from myxobacters. Roughly 800 strains of myxobacters were isolated and screened by them for antibiotic activity (Reichenbach *et al.*1988). Their screening of myxobacters employed simple growth inhibition tests, with a series of Gram-positive and Gram-negative bacteria, yeasts and moulds as indicator organisms (Reichenbach *et al.* 1984). Animal cell cultures, the brine shrimp *Artemia salina* and enzyme inhibition tests, e.g. for proteases, were also included. The isolates were shaken in suitable liquid media at 30°C, and tests for inhibitory substances were done time to time after 3 to 10 days separately with cell extracts and supernatants. About 60% of all myxobacters tested produced some kind of biological activity. With some genera of myxobacters (*Myxococcus*, *Stigmatella* and *Polyangium* the proportion was even higher and could indeed come close to 100%. The activities were directed against bacteria as well as eukaryotes. This was a remarkably high proportion of positive strains, and it was thus important to quickly find out what kind of compounds were responsible for the biological effects. Then they started to isolate the active principles and to elucidate their structures. This often turned out to be rather difficult because of very low initial yields of 0.1-10 mg/l. Their work was considerably facilitated after they themselves discovered that fermentation in presence of the adsorber resin Amberlite XAD-1180 usually leads to a substantial improvement in yields and a simplification of down-stream processing (Gerth *et al.* 1983). The

resin binds most of those secondary metabolites that are excreted into the medium, and thus reduces the danger of an end-product inhibition and at the same time stabilizes the active compounds. After an incubation period of 3-10 days, the resin was separated from the cell suspension. The compounds bound to the resin were eluted with methanol, and the harvested cell mass was extracted, e.g. with acetone. The remaining aqueous culture supernatant was freeze-dried to get hold also of definitely polar substances. The three fractions obtained in this way were then tested for biological effects. The use of adsorber resin also helps to avoid using large amounts of organic solvents which cause serious waste and effluent problems. They were successful to prepare the antibiotics sorangicin A and sorphen A on the 0.5-1 kg scale by this process.

2.5.2. Chemical structures

The results with myxobacters were very encouraging. Many antibiotics have been disovered (Table 2; Fig. 1). From the GBF, there turned up reports of discovering 38 structural types and more than 200 individual compounds within a relatively short time (1978-1990). Of these, 27(71%) of the basic structures proved to be new. In the group of 11 basic structures which were already known, more than half of the compounds isolated were new, so that in total 220 (94%) of all the compounds isolated were known before (Höfle and Reichenbach 1990). Compared with other producing organisms, such as actinomycetes, fungi and also plants, these relationships were exceptionally favourable. Even when specific screening methods are used, many more known compounds than new ones are still discovered from these sources.

Table 2. Antibiotics of myxobacterial origin

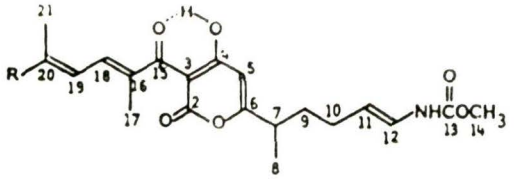
Trivial name	Structural type	Biological property	Producer	Reference
Myxin	Phenazine	Antibacterial and antifungal	*Polyangium (Sorangium)* sp.	Peterson *et al.* (1966)
Xanthancin (bacteriocin)	Protein	Antimyxobacterial	*Myxococcus xanthus*	McCurdy and MacRae (1974)
Bacteriocin	Protein	Antimyxobacterial	*Myxococcus virescens*	Brown *et al.* (1976)
Ambruticin	Polyether	Antifungal	*Polyangium cellulosum (Sorangium cellulosum)*	Connor *et al.* (1977) Ringel *et al.* (1977)
Fulvocin (bacteriocin)	Protein	Antimyxobacterial	*Myxococcus fulvus*	Hirsch (1977); Hirsch *et al.* (1978)
2,5-Epi-5,6-dihydroxy polyangioic acid		Antifungal	*Polyangium cellulosum* ssp. *fulvum*	Connor and Strandtmann (1978)

Table 2 (Contd)

Trivial name	Structural type	Biological property	Producer	Reference
Myxothiazol	Bisthiazole	Antibacterial, antifungal and insecticidal	*Myxococcus fulvus*; *Cystobacter disciformis* (*Angiococcus disciformis*)	Gerth *et al.* (1980); Kunze *et al.* (1989); Höfle and Reichenbach (1990
Myxovirescin	Macrolactam lactone	Antibacterial	*Myxococcus virescens*	Gerth *et al.* (1982)
Althiomycin	Bisthiazole	Antibacterial	*Cystobacter fuscus*	Kunze *et al.* (1982)
Pyrrolnitrin	Pyrrole	Antibacterial and antifungal	*Myxococcus fulvus*	Gerth *et al.* (1982)
Myxovalargin	Peptide	Antibacterial	*Myxococcus fulvus*	Irschik *et al.* (1983)
Myxalamid	Alaninolamide	Antibacterial and antifungal	*Myxocossus xanthus*; *Stigmatella aurantiaca*	Gerth *et al.* (1983)
Myxopyronin	Pyrone	Antibacterial	*Myxococcus fulvus*	Irschik *et al.* (1983)
Bacteriocin	Protein	Antimyxobacterial	*Myxococcus coralloides*	Munoz *et al.* (1984)
Stigmatellin	Chromone	Antifungal and antibacterial	*Stigmatella aurantiaca*	Kunze *et al.* (1984)

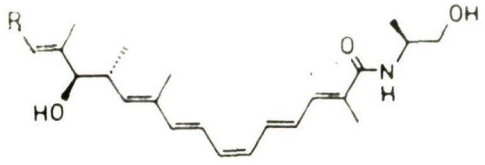
Table 2 (Contd)

Trivial name	Structural type	Biological property	Producer	Reference
Corallopyronin	Pyrone	Antibacterial	*Myxococcus coralloides* *(Corallococcus coralloides)*	Irschik *et al.* (1985)
Sorangicin	Polyether/macrolide	Antibacterial	*Polyangium cellulosum* *(Sorangium cellulosum)*	Jansen *et al.* (1985)
Angiolam	Macrolactam lactone	Antibacterial	*Cystobacter disciformis* *(Angiococcus disciformis)*	Kunze *et al.* (1985)
Aurachin	Quinolinealkaloid	Antibacterial and antifungal	*Stigmatella aurantiaca*	Kunze *et al.* (1987)
Glydobactin	Peptide	Antitumour	*Polyangium brachysporum*	Oka *et al.* (1988a and b)
Saframycin	Bisisoquinolinequinone	Antibacterial	*Myxococcus xanthus*	Irschik *et al.*(1988)
Myxochelin	Phenolic	Antibacterial	*Cystobacter disciformis* *(Angiococcus disciformis)*	Kunze *et al.* (1989)



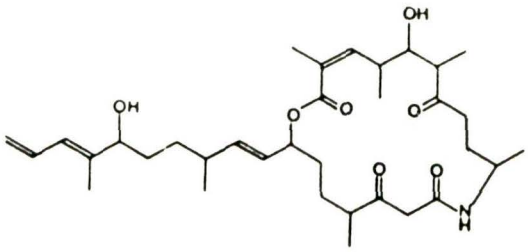
A: R = *n*-C_8H_7
B: R = *n*-C_4H_9

Myxopyronins

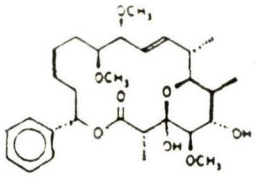


Myxalamid A: R = $CH_3CH_2(CH_3)CH$-
Myxalamid B: R = $(CH_3)_2CH$-
Myxalamid C: R = CH_3CH_2-
Myxalamid D: R = CH_3-

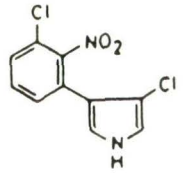
Myxalamids



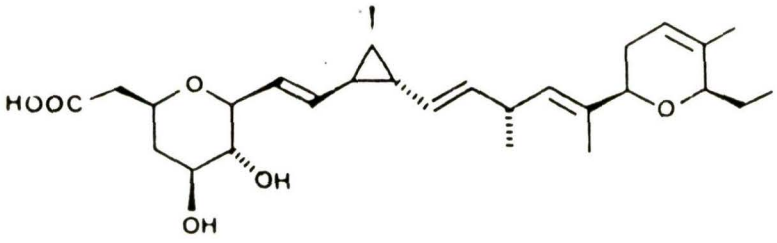
Angiolam A



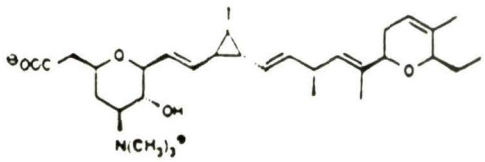
Soraphen



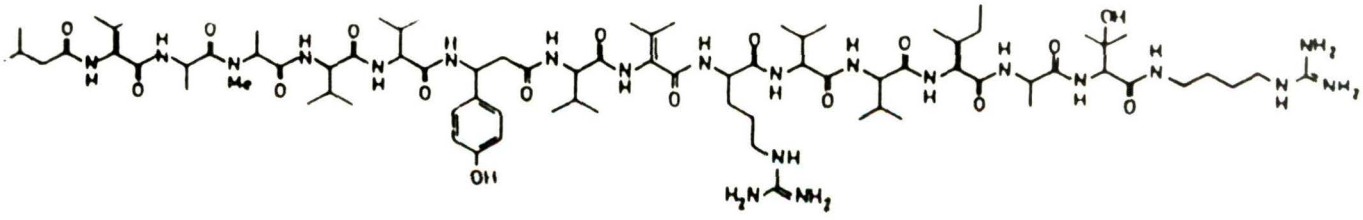
Pyrrolnitrin



Ambruticin S

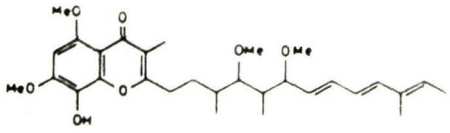


Ambruticin VS-1

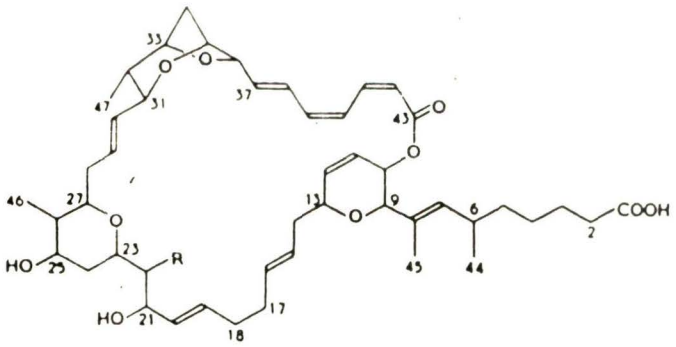


Myxovalargin

Fig. 1. Chemical structures of the antibiotics isolated from myxobacteria (continued in the next page)

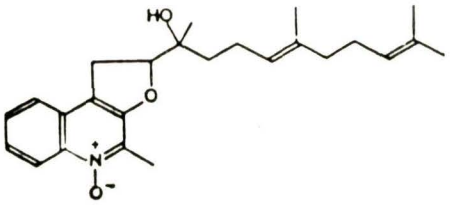


Stigmatellin

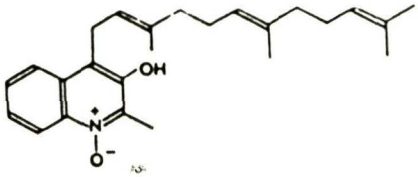


Sorangicin A: R = OH
Sorangicin B: R = H

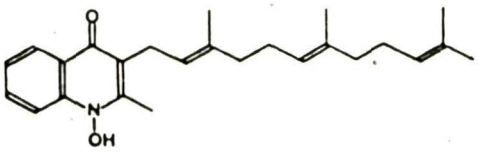
Sorangicins



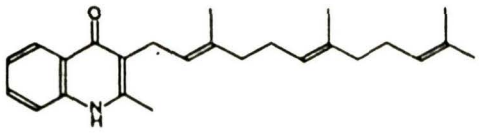
Aurachin A



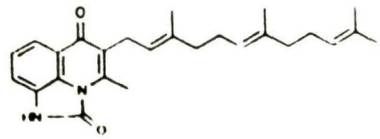
Aurachin B



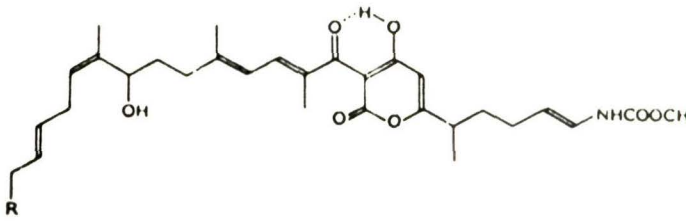
Aurachin C



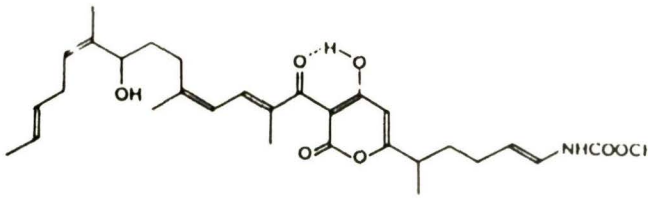
Aurachin D



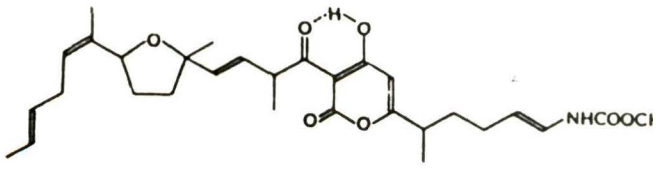
Aurachin E



Corallopyronin A: R = H
Corallopyronin B: R = CH_3



Corallopyronin A'



Corallopyronin C

Fig. 1. Chemical structures of the antibiotics isolated from myxobacteria

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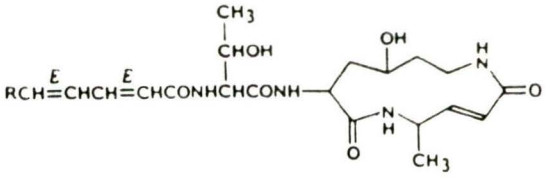
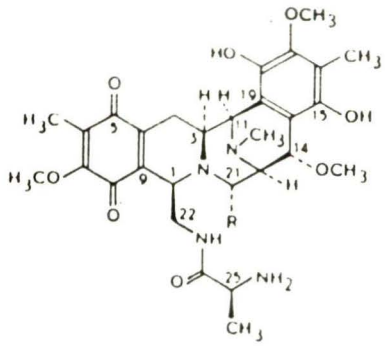
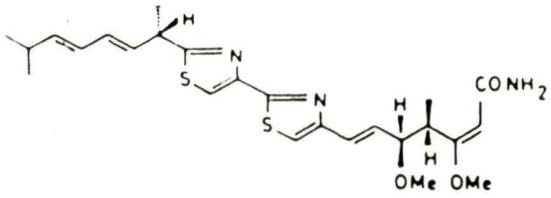


Glidobactin A: R = $CH_3(CH_2)_6$-
Glidobactin B: R = $CH_3(CH_2)_4CH{=}CH(CH_2)_2$-
Glidobactin C: R = $CH_3(CH_2)_4$-

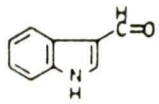
Glidobactins



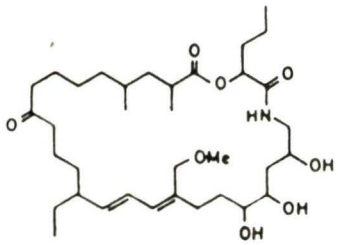
Saframycin Mx1 R = OH



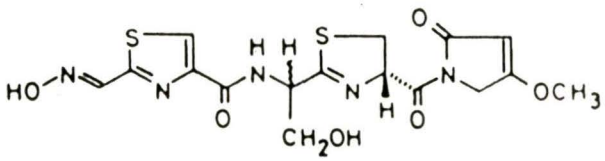
Myxothiazol



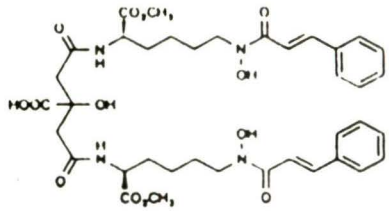
3-Formylinodole



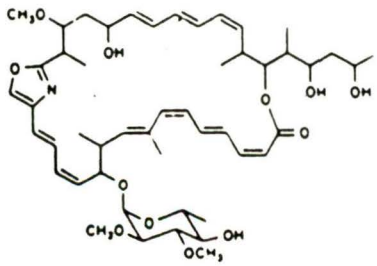
Myxovirescin



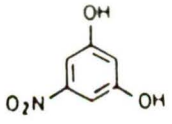
Althiomycin



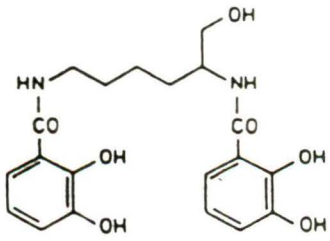
Nannochelin



Chivosazol



5-Nitroresorcinol



Myxochelin A

Fig. 1. Chemical structures of the antibiotics isolated from myxobacteria

Only two new basic structures from myxobacters have been described by other laboratories over the same period of time.

The antibiotics from myxobacters, indeed, belong to very different classes. While occasionally relatively simple compounds turned up, like indole-2-carboxaldehyde, indole-3-acetaldoxime, indole-3-acetonitrile and 5-nitroresorcinol, the biologically active substances usually were typical secondary metabolites with by no means trivial structures. With respect to their chemistry, the myxobacters are not specialized at all. There are aromatics, heterocycles, alkaloids, quinones, polyenes, polyethers, macrocyclic lactams, lactones and hemiacetals, glycosides and peptides, often in combinations, sometimes with halogen and the rare nitro group as substituents (Fig. 1). Aminoglycosides, β - lactams and modified bases of nucleic acids have not been found so far. The remarkable feature is that almost all inhibitors obtained from myxobacters were completely new structures. The exceptions are: pyrolnitrin (Gerth *et al.* 1982) that had been described earlier from *pseudomonads,* and althiomycin (Kunze *et al.* 1982) that was already known from streptomycetes. Further, Irschik *et al.*(1988) found a new natural saframycin, a structural type originally discovered in *Streptomyces lavendulae.* Also, the myxobacter iron-chelator, myxochelin A (Kunze *et al.* 1989) is chemically closely related to azotochelin from *Azotobacter,* which contains a lysine instead of the lysinol backbone. Bacteriocins, that only kill organisms closely related to the producer, are also made by myxobacters, e.g. fulvocin C which appears to be the smallest bacteriocin known (4673 da) and was the first one for which the complete amino acid sequence (45

amino acids) could be elucidated (Hirsch *et al.* 1978; Tsai and Hirsch 1981).

As is typical for secondary metabolism, myxobacters too tend to synthesize families of related compounds. Thus, more than 20 different myxovirescins could be isolated from a single strain, Mx v48, of *Myxococcus virescens* (Gerth *et al.* 1982; Trowitzsch *et al.*1982), about 35 different myxothiazols from *M.fulvus* strain Mx f85, and more than 10 different sorangicins including four glycosides from *Polyangium (Sorangium) cellulosum* strain So ce12 (Jansen *et al.* 1985; Irschik *et al.* 1987). While such chemical variability may become a nuisance during isolation, it still allows studies of the structure-activity relationship, often with variants that would be very difficult to synthesize (Reichenbach and Höfle 1989).

With respect to their secondary metabolism the myxobacters behave essentially like other producers of antibiotics. Thus, the synthesis of a certain antibiotic is strain, not species specific, and different strains of the same species may synthesize different antibiotics. There are, e.g. strains of *Myxococcus xanthus* that produce myxalamids, myxovirescins, saframycins, althiomycin, myxochelins or isoquinolins (Trowitzsch-Kienast *et al.* 1988). The important conclusion from this observation is that we can expect not only 32 (the number of myxobacter species) but an unlimited number of potential producers.

On the other hand, the same antibiotic may be found again in a different species, genera and families. Myxothiazol, for example, has been found in *Myxococcus fulvus, Cystobacter disciformis, Stigmatella aurantiaca* and *S. erecta*.

Often a strain synthesizes several, chemically totally different substances at the same time. Thus, S. *aurantiaca* strain Sg a15 synthesizes stigmatellin, myxalamids and aurachins.

2.5.3. Mechanism of action

The various myxobacter antibiotics act in different ways. Myxothiazol and stigmatellin both interfere with electron flow in the cytochrome b-c_1 segment of the respiratory chain (Thierbach and Reichenbach 1981; Thierbach *et al.* 1984). Aurachins C and D also block at this site. Interestingly, unlike the well-known antibiotic antimycin A, myxothiazol and stigmatellin bind near the outer, low potential (b_L) heme b-566 centre (the Q_o or ubiquinol oxidation site) (von Jagow and Engel 1981; Thierbach and Reichenbach 1981, 1983), and this very tightly, with apparent dissociation constants (Kd) of 10^{-10} M (in comparison, the Kd of strobilurin is 10^{-7} M, of oudemansin 5 x 10^{-7}M). Both inhibitors block the electron transport from QH_2 to the Rieske iron-sulphur protein and cytochrome c_1 as well as to the heme b_L domain, but obviously their specific binding sites differ slightly. This is suggested by a lack of cross-resistance to stigmatellin of myxothiazol-resistant mutants of *Saccharomyces cerevisiae* (Thierbach and Michaelis 1982; Thierbach *et al.* 1984), but can also be demonstrated by biochemical experiments. Both the antibiotics give rise to red shift in the spectrum of reduced cytochrome b_L which shows that they bind close to that domain. Unlike myxothiazol, stigmatellin appears to bind directly to the Rieske FeS cluster. This is indicated by a change in the EPR signals as

well as a dramatic shift in the mid point redox potential of the FeS protein from +290 to +540 mV (von Jagow *et al.* 1984; von Jagow and Ohnishi 1985). The effects of stigmatellin thus strongly suggest that the heme b_L and the FeS cluster are close neighbours and constitute the ubiquinol oxidation site.

Stigmatellin also turned out to be an extremely efficient inhibitor of electron flow in spinach chloroplasts (Oettmeier *et al.* 1985). It blocks at the reducing site of photosystem II (I_{50} = 52.5 nM) as well as at the cytochrome b_6/f complex (I_{50} = 59.0 nM) where it is the most potent inhibitor so far. Myxothiazol blocks the generation of the light-driven membrane potential in the phototrophic bacteria, *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*, probably by binding to the Q_2-FeS_R-cytochrome c_1 segment (Kotova *et al.* 1983).

Besides these, several inhibitors block the electron flow in complex I (NADH: ubiquinone oxidoreductase) of the respiratory chain: myxalamids, aurachins and pyrrolnitrin.

Among the vast number of known antibiotics there are only a few that specifically act on the eubacterial RNA polymerase (Reichenbach and Höfle 1989). It is remarkable that from myxobacters there have been found two completely new compounds of this type: myxopyronin/corallopyronin (Irschik *et al.* 1983, 1985) and sorangicin (Irschik *et al.* 1987). But the two antibiotics differ in the details of their mode of action. While pyronin antibiotics block chain elongation, like streptolydigin, sorangicin block chain initiation, like rifamycins. Ambruticin, which is only active against certain fungi and a few other eukaryotes, strongly inhibits the incorporation of uridine into macromolecular material and thus appears to interfere somehow

with RNA syntheseis in eukaryotes (Ringel 1978).

There are three myxobacterial antibiotics that block protein synthesis in eubacteria. Myxovalargin interferes with the binding of aminoacyl tRNA to the A site of the ribosome (Irschik and Reichenbach 1985). The angiolams equally inhibit bacterial protein synthesis, but the exact site of action is not known (Reichenbach and Höfle 1989). Althiomycin specifically blocks the puromycin reaction, i.e. peptide bond formation as was demonstrated with the substance isolated from streptomycetes (Fujimoto *et al.* 1970).

Only one compound from myxobacters is known to be active on the DNA level; saframycin. The saframycins attack DNA by at least 3 different mechanisms (Irschik *et al.* 1988). The most effective one is the formation of a covalent bond between the amino group of guanine and those saframycins that, like the myxobacter compounds, bear on OH- or CN-group on C-21. Again, the mechanism of action was elucidated with compounds obtained from streptomycetes.

2.5.4. Biosynthesis

Little is known about the biosynthesis of myxobacter antibiotics, i.e. the enzymes involved and their regulation. The antibiotics typically appear to contain amino acids, acetate, propionate and methyl groups from methionine (Trowitzsch-Kienast *et al.* 1986). This suggests that the secondary metabolites originate mainly from the amino acid and lipid metabolism, even though myxobacters efficiently synthesize sugars and the purine and pyrimidine bases. For many myxobacters, amino acids are the

preferred starting material for all biosynthetic activities. Some myxobacter antibiotics, e.g. sorangicins are pure polyketides and thus likely to be produced on a multienzyme complex. The same is the case with the peptide antibiotic myxovalargin (Reichenbach *et al.* 1988).

2.5.5. Fermentation

The biosynthesis of myxobacter antibiotics often takes place in the idiophase, i.e. late log to early stationary phase, e.g. that of myxovirescin (Gerth *et al.* 1982; Nigam *et al.* 1984). But, there also are cases in which productionis restricted to the log phase, e.g. that of pyrrolnitrin (Gerth *et al.* 1982), myxalamids (Gerth *et al.* 1983), myxopyronins (Irschik *et al.* 1983), stigmatellin (Kunze *et al.* 1984) and aurachin C (Kunze *et al.* 1987). The cultures of many myxobacters collapse soon after reaching the stationary phase. Sometimes, they become pitch black within 1 or 2 h due to autolysis and, probably, the liberation of phenol oxidases, thus those of *stigmatella aurantiaca*. The fermentations are performed at 30-32°C, typically with aeration rates between 0.01 and 0.3 v/v.min resulting in an oxygen saturation between 95 and 10%, sometimes even lower. The oxygen level may strongly influence productivity (Gerth *et al.* 1980) as well as the product spectrum (Kunze *et al.* 1987). Harvest usually is after 30-90 h of fermentation, but when antibiotic biosynthesis takes place during idiophase fermentations upto 10 days may be required. Besides oxygen, various nutrients may regulate the biosynthetic activities of myxobacters. An important factor is the type, and sometimes even the batch, of peptone used. Thus, for the

production of stigmatellin and aurachins, zein (maize gluten) was optimal (Kunze *et al.* 1984, 1987), for angiolam and myxalamids the single-cell protein, Probion (Hoechst) Gerth *et al.* 1983; Kunze *et al.*1985). The carbon:nitrogen ratio may be critical, as in the corallopyronin fermentation which gives a maximal yield with 0.6% peptone and 0.2% starch (Irschik *et al.* 1985). Also, examples of catabolite repressions by glucose, phosphate, amino acids and nitrate have been seen (Reichenbach and Höfle 1989).

Various strategies have successfully been applied to myxobacters for the improvement of yields. Sometimes a strain is inhibited by its own antibiotic, as in the case of myxovalargin, sorangicin and saframycin producers. In these cases, it is necessary to select resistant mutants. Often fed-batch fermentation gives superior results (Nigam *et al.* 1984). Most antibiotics are excreted into the medium, and fermentation in the presence of an adsorber resin may lead to substantially higher yields in addition to facilitating recovery (Gerth *et al.* 1983; Kunze *et al.* 1985). When growth and production are limited by ammonia production, on-line extraction of ammonia may have a dramatic effect (Reichenbach and Höfle 1989). Sometimes, feeding of a precusor strongly stimulates antibiotic production, e.g. anthranilic acid leads to a steep increase in the yield of aurachins in cultures of *Stigmatella aurantiaca*. Also, it has been found that alginate-immobilized myxobacters may synthesize antibiotics over a long time and with excellent efficiency (Reichenbach and Höfle 1989). Finally, chemostat cultures have been used with good results for the study of factors that influence antibiotic synthesis (Hecht *et al.* 1987).

2.6. Practical interest

Recently, myxobacters have become increasingly interesting in biotechnology as producers of lectins (Cumsky and Zusman 1979), of enzymes, e.g. proteases (Sudo and Dworkin 1972), restriction endonucleases (Mayer and Reichenbach 1978) and lytic enzymes (Hart and Zahler 1966; Hüttermann 1969), and of secondary metabolites like antibiotics (Rosenberg *et al.* 1973; Gerth *et al.* 1980, 1982,1983; Kunze *et al.*1982; Irschik *et al.* 1983).

Biotechnological use, however, implies large-scale fermentation and this is feasible only if inexpensive media can be used for production.

Investigations during the last 16 years have demonstrated that many new compounds can be obtained from myxobacters, and that they are amenable to the various strategies of yield improvement known from other microorganisms. Already yields of about 1 g/l have been achieved (Reichenbach and Höfle 1989). Large-scale fermentation of myxobacters is not a problem. It has been demonstrated that in many cases technical substrates like cornsteep powder, maize gluten, soya meal, yeast cells, or skim milk powder can be used with good results (Gerth *et al.*1984).

Sorangicin is a wide-spectrum, non-toxic antibacterial antibiotic with good activity in animals against intracellular bacteria. At present, saframycins command much interest as efficient antitumour compounds. The prospect of myxobacter variants is promising, because even slight structural modifications appear to influence substantially the *in vivo* activity of the antibiotic. Furthermore, there are several

antifungal compounds of low toxicity, e.g. ambruticin and several novel structures. Good activity of ambruticin against systemic mycoses has already been demonstrated (Lévine *et al*.1978).