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Myxobacteria, producers of novel bioactive substances

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Myxobacteria are soil bacteria that move by gliding and have an astonishing life cycle culminating in fruiting body formation. In a research program at the Gesellshaft für Biotechnologische Forschung over the past 25 years the organisms have been shown to be a rich source of potentially useful secondary metabolites. So far about 80 different basic compounds and 450 structural variants have been characterized. Many of those compounds were new. It is particularly remarkable that myxobacteria specialize in mechanisms of action that are very rare with other producers. Thus 20 new electron transport inhibitors, 10 substances that act on the cytoskeleton, four inhibitors of nucleic acid polymerases, and one inhibitor of fungal acetyl-CoA carboxylase, a novel mechanism of action, have been found. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 149–156.

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Introduction

Secondary metabolites are synthesized by animals, plants and microorganisms, and have been selected for optimal efficacy by nature for millions of years. They often are available soon after their discovery in quantities that allow in-depth testing, and sometimes can be directly produced for application. Alternatively, they may provide essential structural ideas, without which synthesis programs are difficult to design. Microorganisms are particularly valuable as producers because they can be cultivated in bioreactors (up to 400 000 l), so that production of a compound becomes independent of time, locality and climate. Further, microorganisms can be used for biotransformations in order to produce chemical derivatives that are difficult and expensive to make by purely chemical methods.

Relatively few microorganisms are good producers of secondary metabolites. Among bacteria, this is the case mainly with the Actinomycetales (around 8000 compounds), the genus *Bacillus* (1400 compounds), and the pseudomonads (400 compounds). Therefore efforts have been made for many years to discover new groups of producers. This has indeed been achieved, although in very few cases. In fact, only two new bacterial groups have been added to the producers already known 50 years ago: the cyanobacteria and the myxobacteria. In both cases, about 10 years of basic microbiological research had first to be invested in the development of methods for isolation, cultivation and preservation, before the organisms became amenable to studies of their secondary metabolism. Unfortunately, such basic research is no longer in favor with students, scientists, and financing agencies. As a reward, in both cases many completely new compounds have been found, some with very interesting properties and potential for application, and still more compounds can be expected from them. This can be taken as a demonstration that bio-diversity is indeed an asset. The following discussion will be restricted to myxobacteria.

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What kind of organisms are myxobacteria?

Myxobacteria live preferentially in places that are rich in microbial life and organic matter, *viz.*, soil, rotting plant material, dung of various animals and on the bark of living and dead trees. They are found everywhere, in all climate zones and vegetation belts, but are particularly numerous in semiarid, warm areas, e.g., in Egypt, northern India, or the southwestern USA, probably because they produce desiccation- and, when dry, heat-resistant myxospores. On the other hand, they have also been isolated from freshwater and from marine sediments, although only recently truly marine, halotolerant myxobacteria have been obtained from the Pacific around Japan [9]. While myxobacteria have been reported that poke holes in cells of green algae and fungi and empty them out, myxobacteria pathogenic for humans, animals or higher plants have never been observed. More information and references about the ecology of myxobacteria can be found in recent reviews [22,24].

How does one isolate myxobacteria? In two ways. The majority of species live by degrading proteins and even whole cells of other microorganisms, mainly bacteria and yeasts, and require peptides and amino acids as nutrients. Those species are best obtained by inoculating streaks of living *Escherichia coli* on plain water agar with small bits of soil or rotting plant material. The cultures are incubated at 30°C, and after a couple of days the first myxobacteria may appear. One group of myxobacteria, members of the genus *Sorangium*, are cellulose degraders. They have to be enriched on filter paper pads placed on a mineral salts agar with KNO_3 as the only nitrogen source. The cellulose degraders need more time for their development. Patches of decomposed cellulose may be expected after 12–14 days at 30°C. Many details about isolation procedures have been described in the literature over the past 100 years (for a summary and evaluation, see Ref. [24]).

How do we recognize myxobacteria? Myxobacteria have two characteristics that allow us to distinguish them readily from other

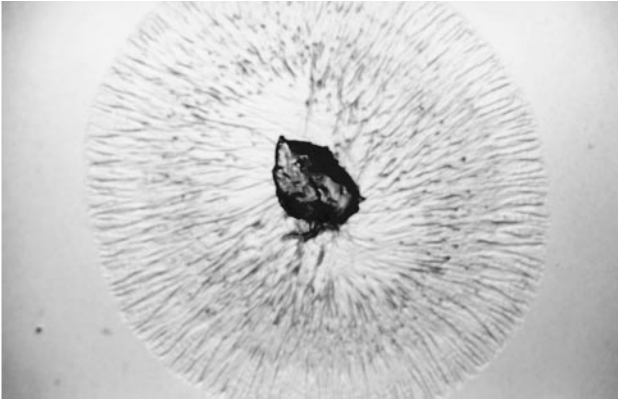

Figure 1 Young swarm colony of *Cystobacter violaceus*, still without fruiting bodies. Diameter of swarm 15 mm.

bacteria. Their cells move by gliding, or creeping, over the surface of or within the substrate, so that colonies spread over the culture plate, sometimes covering it completely within 6–8 days (Figure 1). Such swarm colonies are thin, translucent and film-like, so that they are difficult to see, and are recognized best under a dissecting microscope with oblique illumination, and even then only by an experienced eye. Gliding motility is a property of many bacteria in most branches of the bacterial phylogenetic tree. Yet the structure of myxobacterial swarms is typical enough to allow us to distinguish them from other gliders. A second characteristic of myxobacteria is unique among bacteria: under starvation conditions the cells start to aggregate within the swarm, pile up and produce fruiting bodies. Those fruiting bodies may be very simple in shape and structure, e.g., a knob consisting of slime and myxospores as in the genus *Myxococcus* (Figure 2). However, there are also remarkably sophisticated designs, like those of the genus *Chondromyces*: little treelets with white, sometimes branched slime stalks bearing clusters of bright orange sporangioles at the tips of the stalks and branches (Figure 3). This cooperative morphogenesis has so far defied all mechanistic explanations, but is presently being investigated by several laboratories. Myxobacterial fruiting bodies measure between 20 and 1000 μm in size, usually are bright yellow, orange, red, brown, or black, and normally appear in large numbers, particularly with the cellulose degraders (Figure 4), so that they often can be seen with the naked eye and are easy to recognize under a dissecting microscope. The number of cells involved in the construction of a single fruiting body is in the order of 10^4 to 10^6.

Within the maturing fruiting body, a cellular morphogenesis takes place during which vegetative cells shorten and fatten and convert into the myxospores mentioned above. While vegetative cells are rod-shaped and often rather large, typically 4–12 μm long and 0.7–1.2 μm wide (Figure 5), myxospores are usually much shorter, in some species even spherical, and when mature always optically refractile under the phase contrast microscope (Figure 6). The myxospore is the survival strategy of the myxobacteria. The fruiting body, on the other hand, probably guarantees that a new life cycle is started by a cell community rather than an individual cell. More about the life cycle of the myxobacteria can be found in a number of reviews [2,3,24].

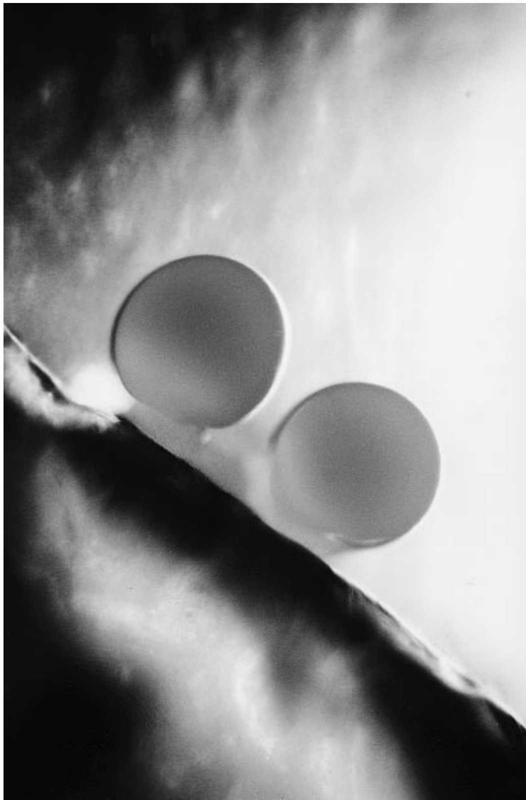

Figure 2 Fruiting bodies of *Myxococcus fulvus*, soft slime balls on a short pedicel. Diameter around 250 μm.

Figure 3 Fruiting body of *Chondromyces robustus*, a new species, consisting of a white slime stalk and a cluster of bright orange sporangioles. About 320 μm high.

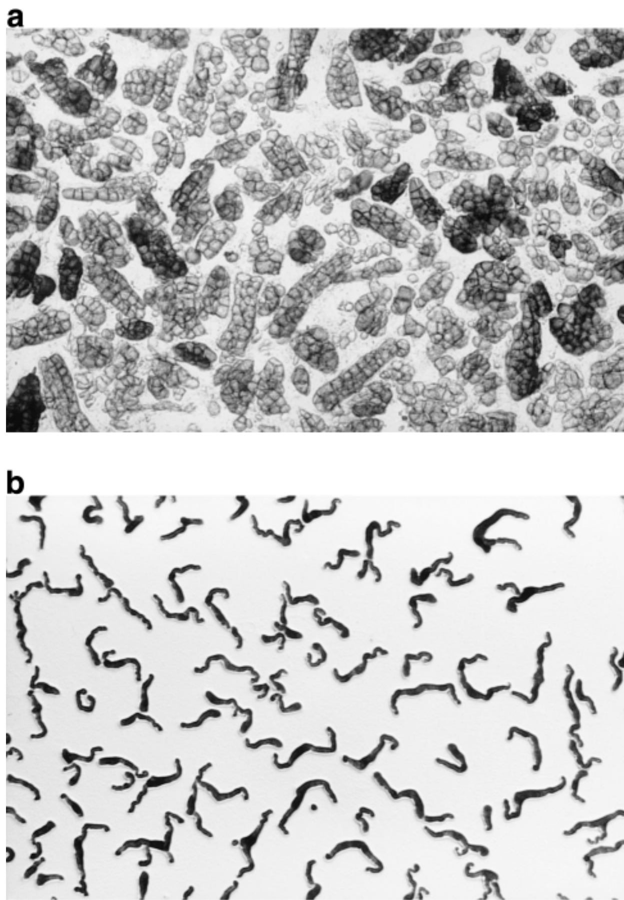

Figure 4 Fruiting body populations. (a) *Sorangium cellulosum* fruiting bodies, small sporangioles (diameter 10–20 μm) tightly packed in parcels of variable size, are produced in enormous numbers within macerated filter paper. (b) Fruiting bodies of *Corallococcus coralloides* on the surface of an agar plate, long, slender ridges, 35–75 μm wide, of cartilaginous consistency.

Myxobacteria are Gram-negative bacteria. Their cells appear in two basic shapes: either slender with tapering ends, or cylindrical with blunt, rounded ends. Those two cell types characterize two suborders. The mechanism of gliding motility is still elusive. A recent hypothesis, based on electron microscopic observations showing a complex apparatus in the periplasmic space, assumes that the cells are propagated by a contraction wave in the surface created by a conformational change in that apparatus [4,14]. There is an excellent recent review about gliding motility in bacteria [32].

What else is known about myxobacteria? As mentioned, all myxobacteria specialize in degradation of biomacromolecules. This may be the explanation for fruiting body formation. A cell community is more efficient in producing high levels of extracellular enzymes and is less sensitive to diffusion losses than an individual cell. In fact, myxobacteria are well known for their intercellular communication systems that help to keep the cells together. The intercellular signals may be of a mechanical (e.g., pili) or chemical (e.g., pheromones [5,8,16,19]) nature. In the swarm, the cell community lives in a common slime sheet and thus is virtually stationary. This may explain why myxobacteria produce so many antibiotics. Like the equally stationary actinomycetes, the myxobacteria can not simply move to some other place but are forced to defend their niche against competitors.

Until the 1960s it was generally believed that myxobacteria can not be grown in liquid media, but it turned out that this is not correct. With many strains one soon gets suspension cultures, and quite a few myxobacteria have been grown since in bioreactors, some even at an industrial scale of 50–70 m^3. For media, technical substrates, like corn steep powder, soya flour and meal, yeast cells, technical starch, skim milk, and various peptones are satisfactory. The useful temperature range for fermentations is between 28°C and 34°C, the pH range is 6.8–7.8. The organisms are strictly aerobic, but oxygen demand is relatively low because of rather slow growth rates, normally with doubling times between 4 and 14 h. The maximum cell densities may be as high as 8×10^9 cells ml^{-1}.

Myxobacteria have the largest genomes of all bacteria: 9.5–10 Mbp. Their DNA has a high G+C content, between 66 and 72 mol%. In almost all myxobacteria thus far investigated there is a peculiar hybrid nucleic acid, msDNA, in a high copy number per cell, but without a known function [1]. msDNA consists of a piece of single-stranded DNA (in *Myxococcus xanthus* 162 bases long) which is covalently linked to a single-stranded RNA (77 bases long). The free end of the DNA hybridizes with one end of

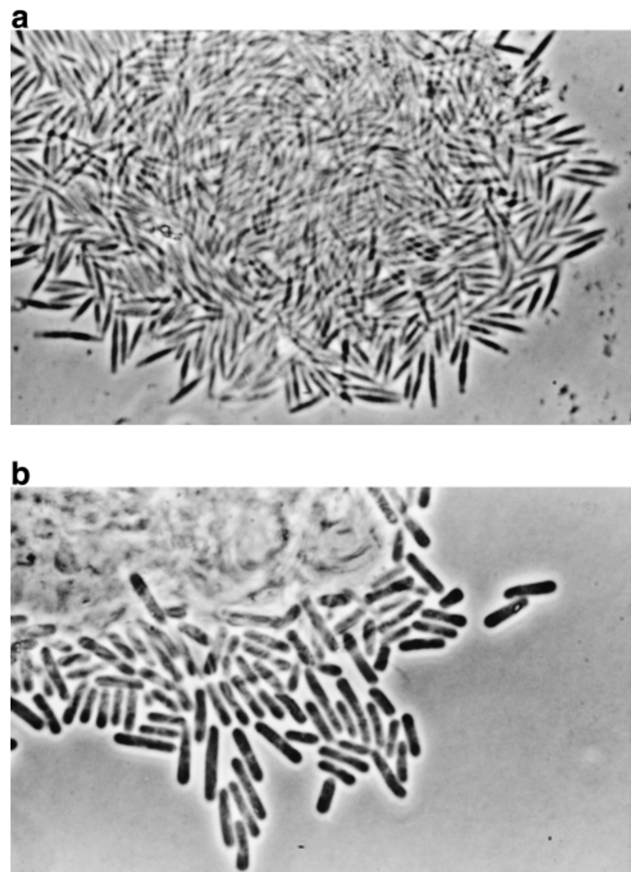

Figure 5 Vegetative cells of two suborders of myxobacteria. (a) Cells of a representative of the suborder Cystobacterineae: slender cells with tapering ends, mostly 4.8–5.8 μm long. (b) Cells of members of the suborder Sorangineae are cylindrical with rounded ends, in this case in the same length range as above but clearly more robust. Both pictures in phase contrast and at the same magnification.

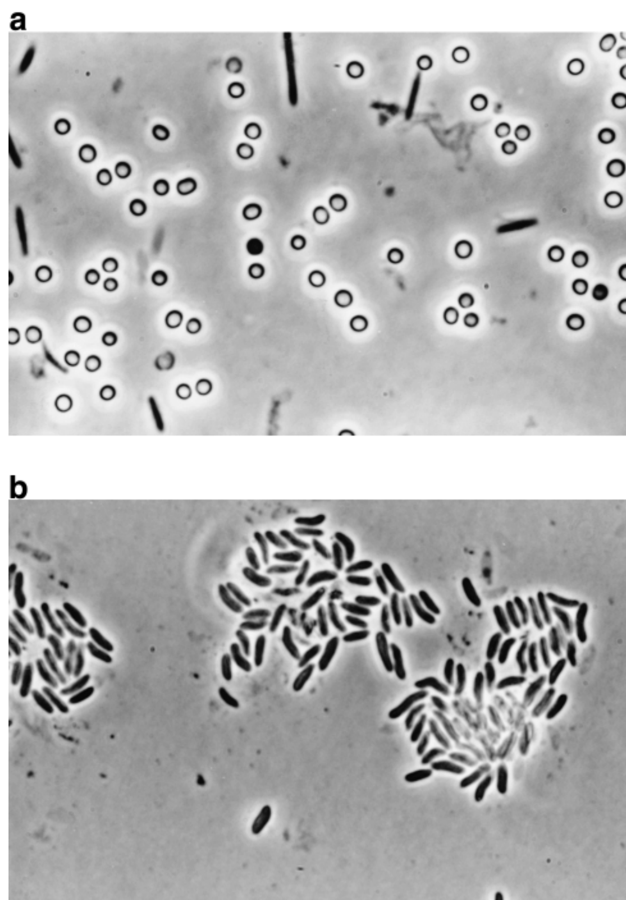

Figure 6 Myxospores. (a) Spherical myxospores of *Myxococcus fulvus*, 1.3–1.6 μm in diameter. Between mature myxospores, a dark, immature myxospore and vegetative cells are seen. (b) Rod-shaped myxospores of *Cystobacter ferrugineus* 3.3–3.9 long. Optical refractility is not apparent at this high magnification. Both pictures show myxospores from fruiting bodies in phase contrast.

the RNA. msDNA appears to be produced by reverse transcriptases [12].

In the past 25 years several gene-transfer systems (coli phage P1, conjugation with *E. coli*, general transducing myxophages, electroporation) have been developed for myxobacteria and were applied to problems of gliding motility, carotenoid synthesis, cell signalling, and morphogenesis. More recently, myxobacterial polyketide synthase and peptide synthetase genes have also been identified and analyzed (for reviews, see Refs.[3,27]).

The taxonomy of myxobacteria rests mainly on morphological characteristics, *viz.*, the shape and structure of vegetative cells, myxospores, swarm colonies, and fruiting bodies. Also, 16S rRNA sequences have been determined for many strains. Myxobacteria are a phylogenetically coherent group but can be subdivided into two or three suborders [13]. Subsequently they were allocated to the delta branch of the Proteobacteria [31]. About 40 species are recognized that are classified in one order, Myxococcales, with two suborders, four families and 12 genera. Recently more species and genera have been found, and a revision of the taxonomy is under way. Information about the classification of myxobacteria, mostly together with extended descriptions of species and the group as a whole, can be found in several modern treatises [3,15,21,24,33].

Myxobacteria as producers of secondary metabolites

What types of secondary metabolites are found in myxobacteria? So far, we have discovered, isolated and characterized around 80 different basic structures and 450 structural variants from myxobacteria. The structure elucidation and derivatization work is done by Professor Dr Gerhard Höfle and his group in the Department of Chemistry of Natural Products at the GBF. In addition hundreds of derivatives of compounds of practical interest have been prepared. Also, total syntheses of quite a few myxobacterial compounds have been published by laboratories worldwide. The results of those investigations have been summarized in several reviews [7,25–27]. In those reviews all original publications are cited, so that only very recent articles are mentioned here.

Most myxobacterial substances are moderately lipophilic, linear or cyclic polyketides and peptides. We found only one modified sugar and one modified nucleotide (unpublished data). Many compounds combine a polyketide sequence with an amino acid, often in the form of a heterocycle. In addition to typical secondary metabolites several other remarkable substances have been isolated from myxobacteria: three different iron transport metabolites; cholestenols and lanosterol, steroids unequivocally synthesized by myxobacteria (which is extremely rare among prokaryotes); ceramides and cerebrosides; and carotenoid glycosides esterified with fatty acids. Certain myxobacteria synthesize geosmin, so that their cultures have an intense earthy smell.

The myxobacterial polyketides belong to very different chemical classes: macrocyclic lactones and lactams, sometimes even classical macrolides with a sugar attached; polyethers; polyenic compounds; alkaloids; aromatic compounds. The peptides often are depsipeptides containing hydroxy acids in addition to—sometimes very unusual—amino acids: e.g., β-amino acids, 4-methylazetidinecarboxylic acid, homoproline, and even more exotic specimens. About 40% of the compounds were completely novel structures, others were still new but contained structural elements that were known before from other organisms, e.g., streptomycetes and cyanobacteria. Some were strikingly similar to compounds isolated from marine sponges, tunicates, and molluscs. Only rarely were structures encountered that were identical to known compounds. Thus, myxobacteria produce althiomycin originally discovered in streptomycetes, pyrrolnitrin known from pseudomonads, and a saframycin almost identical with compounds isolated from streptomycetes and related to compounds found in pseudomonads and marine sponges. Quite a few myxobacterial compounds are chlorinated, several contain the rare nitro group, and tartrolon is one of only four natural products known to complex boron. Clearly, myxobacteria are a rich source of new structural ideas.

Are there unusual microbiological problems in production of secondary metabolites with myxobacteria?

Most of the problems are the same as with other producers, and the same or similar solutions also can be applied to myxobacteria. With myxobacteria the ability to synthesize a certain compound is normally a strain and not a species characteristic. However, certain compounds have been found in only one species. Thus, *S.*

cellulosum yielded so far more than 30 new compounds, only a few of which have been discovered in another myxobacterium, not even as chemical variants. Interestingly there is a sharp division line between the two suborders: only one substance, pyrrolnitrin, has been found in both. This is remarkable because it is often said that the genes for secondary metabolites are freely exchanged between unrelated bacteria, and even between eukaryotic and prokaryotic organisms. Why then should myxobacteria belonging to different suborders always select genes for different substances? After all, the habitats are more or less identical. Another peculiarity, also shown by other producers, is that a strain may synthesize at the same time — less often under different culture conditions — two or more chemically unrelated compounds. Thus *Chondromyces crocatus* strains produce in parallel six or seven different compounds. A strain of *Stigmatella aurantiaca* synthesizes at the same time stigmatellin, myxalamid and at least four different aurachins. All six compounds are electron transport inhibitors which block with high efficiency the mitochondrial respiratory chain at different sites in complexes I and III. Individual strains may produce different compounds in varying combinations, e.g., one *S. cellulosum* strain synthesizes sorangicin, disorazol, chivosazol and sulfangolid, another one disorazol, icumazol and soraphen. Sometimes large families of structural variants are found, and this again is a strain characteristic. Thus we obtained from one *M. fulvus* strain 30 different myxothiazols, from another one only two. One *Myxococcus virescens* strain synthesizes 20 different myxovirescins, a *S. cellulosum* strain nearly 50 different soraphens. Of course, many variants are produced in trace amounts, so that they can only be isolated when large-scale fermentations (several hundred liters) are performed. Interestingly, the main component is normally also the most active compound, which suggests a selection for efficacy. This is corroborated by derivatization programs: rarely producing a derivative with the same activity as the parent compound, normally activity is much less or zero; a compound that is better is a very rare event.

It is generally assumed that microbial secondary metabolites are preferentially synthesized during the late logarithmic and stationary growth phases, the so-called idiophase, when metabolism is no longer fully occupied with growth. However, with myxobacteria this often is not the case. Many substances are synthesized from the beginning of growth, or shortly after. In fact, the stationary phase may be very short, because the culture breaks down soon after growth ceases. This is particularly dramatic with *St. aurantiaca* strains growing in a peptone medium: within 2 h after stationary phase is reached, the cells lyse and the medium becomes pitch black. On the other hand, there also are examples of myxobacterial production processes with a clear idiophase. Thus, many *Sorangium* compounds are produced for a long time into stationary phase.

Myxobacteria sometimes produce secondary metabolites only if a limitation is introduced into the fermentation process. Myxovirescin is synthesized in the long transition period to stationary phase. The production rate depends on the peptone-uptake rate with a very sharp optimum. Oxygen supply is often critical for yield: sometimes a good supply of oxygen is essential, in other cases a limitation. In the beginning, yields are usually low, between 0.5 and 20 mg l^{-1}. This is, however, not always the case. One strain of *M. xanthus* produced 120 mg l^{-1} myxalamid from the beginning, and wild strains of *Sorangium* yielding 50–70 mg l^{-1} of soraphen have been found. Therefore it is advisable first to check the strain collection for a better producer before starting work on a new compound. In some cases, fermentation yields could be increased to 1–2 g l^{-1} by process and strain improvement applying the usual methods. During strain improvement work three myxobacterial peculiarities may become cumbersome. Firstly, most strains grow as lumps and flakes when first inoculated into liquid media, and homogeneous cell suspensions often are obtained only after weeks and months of repeated transfers in liquid medium. Secondly, plating normally does not yield single cell colonies in high numbers, and efficient plating media have to be developed for every strain. And finally, colonies develop only slowly, within 8–14 days. Most myxobacterial compounds are excreted into the medium. Adsorption to an Amberlite type resin, e.g., XAD-16 (Rohm & Haas, Darmstadt, Germany) has been found very useful, from screening to industrial-scale fermentations. The resin removes the compound from the equilibrium, preventing end product inhibition, modification and degradation of the compound. It also facilitates harvest substantially.

So, could myxobacterial compounds be produced by fermentation? There is no recognizable reason why not. Of course, every group of producers has its own problems, but those problems have been solved in other groups and will be solved also with myxobacteria. Myxobacteria can be handled experimentally like other bacteria. So far only a few scientists have tried to improve myxobacterial strains and production processes, so that experience on how to proceed best is very limited. New strategems may have to be developed differing from those that work with, say, the streptomycetes. But it has been demonstrated that yields can be improved 1000-fold (e.g., for soraphen from 0.5 to 2000 mg l^{-1}), that myxobacteria grow readily on technical substrates, and that 50–100 m^3 fermentations are possible without particular problems. Some of the more specific questions of myxobacterial fermentations are discussed in a recent review [23].

Are there many more myxobacterial compounds to be expected?

Probably yes, based on five observations. (1) We still discover, with very limited capacity, between four and eight new basic structures per year. (2) We see in HPLC diode array scans many peaks with new retention times and UV spectra. (3) While some compounds are seen relatively often, others have been found only once or twice, and still more such rare compounds must be present. (4) Over a period of 20 years, we isolated and screened just 6000 strains of myxobacteria. This is a very small number if compared, e.g., with the number of various actinomycetes screened by many laboratories over decades. (5) There are some groups of myxobacteria that yielded very few compounds, while from others we obtained many different substances. It is difficult to accept that those rare producers really should have an underdeveloped secondary metabolism. This question can be answered with the methods of modern molecular genetics, by which the presence of relevant genes can be demonstrated in the respective genomes.

How do myxobacterial compounds act?

The many different compounds from myxobacteria also show quite different mechanisms of action. There are inhibitors of prokaryotic

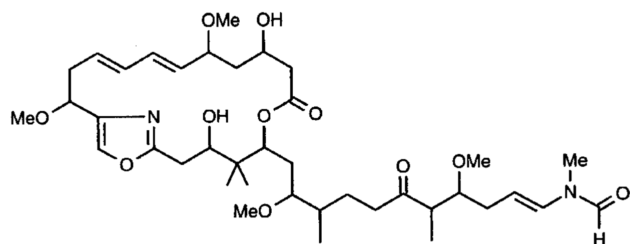

Figure 7 Rhizopodin, a substance that leads to a reorganization of the actin skeleton.

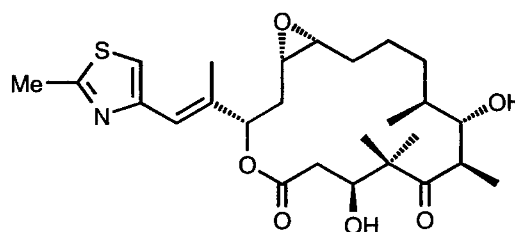

Figure 9 Epothilon A, a paclitaxel mimic, stabilizing microtubuli. Epothilon B with a methyl next to the epoxide is 10 times more active.

(myxovalargin) and eukaryotic (gephyronic acid) protein synthesis, compounds that stimulate potassium export from Gram-positive bacteria (tartrolon), and compounds that bind to DNA (saframycin). In some cases, the mechanism of action has not yet been elucidated, e.g., for the highly cytotoxic vioprolids or for the antifungal, leupyrrin. Also, there are myxobacterial compounds which do not show any biological or biochemical effects in our test battery. On the other hand, myxobacteria appear to specialize in mechanisms of action that are extremely rare with other producers. This emphasizes that it is worthwhile to exploit biodiversity and look for new groups of producers.

Thus far we isolated nearly 20 different, new electron transport inhibitors [10,28] (see also Refs. [18,35]). They act at different sites in the mitochondrial respiratory chain, many with extremely high efficacy. Some block in complex I (NADH: ubiquinone oxidoreductase), others in complex III (bc_1-complex), one inhibits bacterial cytochrome oxidase. Myxothiazol and stigmatellin played a substantial role in elucidating the biochemistry of complex III. Stigmatellin was also useful in the characterization of the cytochrome b_6/f complex in photosynthesis, for which it is the most efficient inhibitor known (shown with spinach chloroplasts). The binding sites of both substances have been studied by cocrystallization with their respective complexes [36].

Another group of myxobacterial substances block specifically eubacterial RNA polymerases. Sorangicin stops chain initiation, like the rifamycins. In fact, it shows considerable cross-resistance with rifampicin. Myxopyronin and corallopyronin are inhibitors of chain elongation, like streptolydigin. Ripostatin appears to inhibit chain initiation but shows no cross-resistance with sorangicin or rifampicin. It is moderately active against Gram-positive bacteria, inactive on Gram-negatives, but inhibits *E. coli* RNA polymerase *in vitro*. A structural variant of ripostatin also inhibits fungi, but only at very high concentrations (20–80 μg ml^{-1}). The efficacy of the substance is probably limited by insufficient penetration. For animal cells, ripostatin is barely toxic. A more recent discovery is etnangien (unpublished), which also inhibits certain viral reverse transcriptases. Its exact mechanism of action remains to be elucidated.

An exciting discovery was that about 10% of our myxobacterial compounds interact specifically with the cytoskeleton of eukaryotic cells. Not only could such compounds become valuable tools in basic research for a better understanding of those very complex systems, they also might become useful drugs for the control of cancer. Thus far we isolated 10 different, novel basic structures of this type, four of them working specifically on actin, four on tubulin, and two showing less systematic effects but still clearly affecting the cytoskeleton and inducing apoptosis. All of those substances are extremely efficient inhibitors of cell cultures, the most active one showing IC_{50} values around 3 pg ml^{-1}.

The first such compound that we found was rhizopodin (Figure 7). It was discovered because the culture broth of a specific *Myxococcus stipitatus* strain induced L929 mouse fibroblasts to develop long, thin, branched extensions, or runners, resembling the rhizopodia of certain protozoa. The minimal dose producing that effect turned out to be 5 nM. A study on the mechanism of the phenomenon revealed that, at a concentration of 100 nM, the stress fibers in the cells disappeared completely within 3 h. Later F-actin was reconstituted in the form of a cloud of short fibers around the cell nucleus. Long F-actin fibers could be seen only in the runners. As the F-actin ring required for cell separation can not form, cell division becomes impossible. The cells enlarge substantially, become multinucleate, but stay alive for weeks. The effects of rhizopodin resemble those of latrunculin B, but are elicited at 10-fold lower concentrations. While they take a little longer to appear, they are irreversible, in contrast to those of latrunculin B [6].

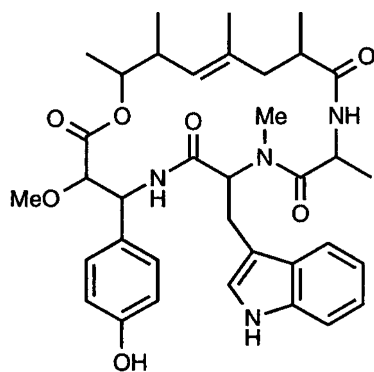

Figure 8 Chondramid A, closely related to jasplakinolide from a marine sponge, stabilizes actin like phalloidin.

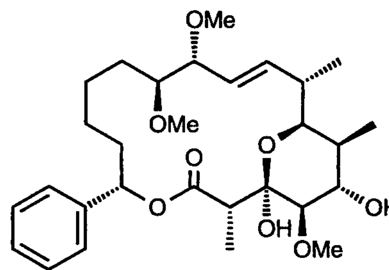

Figure 10 Soraphen $A_{1\alpha}$, an inhibitor of fungal acetyl-CoA carboxylase.

A second myxobacterial compound affecting actin is chondramide A and its structural variants (Figure 8). The chemical structure of this compound is closely related to that of jasplakinolide, or jaspamide, obtained from a marine sponge. Chondramide stabilizes actin, as does phalloidin, a toxin of green and white deathcaps. In fact, it appears to have the same binding site on actin, as it competes efficiently with phalloidin for binding. In contrast to phalloidin, chondramide penetrates all kinds of mammalian cells. IC_{50} values for different tumor cell lines are between 3 and 85 nM. *In vitro*, the chondramides induce and stimulate actin polymerization, also under non-polymerizing conditions, which is not the case with phalloidin [29].

In the past 3 years, the myxobacterial compound, epothilon (Figure 9), attracted much interest because it was demonstrated to be a paclitaxel mimic, i.e., it stabilizes microtubuli within the cell, so that functional mitotic spindles can no longer be built. With mitosis made impossible, the cell enters apoptosis. As epothilon also kills multidrug-resistant cells, including paclitaxel-resistant ones, is well soluble in water, and can be produced by fermentation, it is a promising candidate for an antitumor drug. Several syntheses of epothilon have been published, but so far are unlikely to be economically competitive with the fermentation product [17,30,34].

Another new compound, tubulysin, has the opposite effect on tubulin: it leads to a complete breakdown of the microtubuli within hours (Sasse, Khalil *et al.*, GBF, unpublished data). This was also demonstrated *in vitro*. Apicularen A also effects the tubulin cytoskeleton, but probably indirectly [11]. This compound shows a structural relationship to the salicylihalamides and the lobatamides, compounds from marine organisms.

One myxobacterial substance, soraphen (Figure 10), even has a novel mechanism of action. This substance blocks specifically and with high efficacy fungal acetyl-CoA carboxylase. It also acts on the rat liver enzyme, but not on that of higher plants [20]. It would have been a wonderful agricultural fungicide, but had to be given up because of unacceptable side effects.

Presently no myxobacterial product, or a drug derived from a myxobacterial compound, is on the market. But several compounds and their mechanisms of action were of sufficient interest to initiate industrial projects. It remains to be seen whether in the end one of those compounds is able to pass the many thresholds which are on the road to application.

Do myxobacterial strains that produce a certain metabolite have a uniform geographic distribution?

This seems not to be the case. Soraphen and epothilon are two important metabolites with potential application. Therefore we tried to obtain as many wild strains as possible producing them. We found the compounds only in strains of *S. cellulosum*, and screened all strains of that organism in our collection, about 1600, for synthesis of those compounds (Dr Klaus Gerth, GBF, personal communication). The result with strains isolated from European samples is shown in Table 1. While North, Central and Western Europe supplied a substantial number of strains but only few producers, the results were much better with strains from Mediterranean countries. A weak point of the statistics is that the quality of the habitat could not be considered, because detailed data about the sampling sites were usually lacking.

Table 1 Geographic distribution of *Sorangium cellulosum* strains synthesizing important metabolites[a]

Total number of strains from		Strains producing		
		Soraphen (%)	Epothilon (%)	Either of the two (%)
Europe	*616*	*9 (1.5)*	*7 (1.1)*	*16 (2.6)*
North, West and Central	209	2 (0.9)	0	2 (0.9)
Mediterranean and Southeastern	353	6 (1.7)	7 (2.0)	13 (3.7)
Atlantic Islands	54	1 (1.9)	0	1 (1.9)

[a]The number of strains screened was 1600.

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