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The Chemical Ecology of Predatory Soil Bacteria

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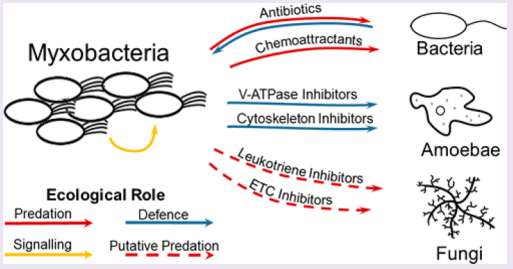
ABSTRACT: The study of natural products is entering a renaissance, driven by the discovery that the majority of bacterial secondary metabolites are not produced under standard laboratory conditions. Understanding the ecological role of natural products is key to efficiently directing our screening efforts, and to ensuring that each screen efficiently captures the full biosynthetic repertoire of the producing organisms. Myxobacteria represent one of the most common and diverse groups of bacteria, with roughly 2500 strains publically available. Fed largely through predation, the myxobacteria have developed a large repertoire of natural products that target other microorganisms, including bacteria and fungi. Many of these interactions can be observed in predation assays, providing direct evidence for environmental interactions. With a focus on *Myxococcus xanthus*, this review will highlight how recent advances in myxobacteria are revealing the chemical ecology of bacterial natural products.



Bacterial natural products frequently serve as both drug leads and molecular probes, harnessing compounds developed over millions of years of evolution for human use.[1] While these compounds have led to many useful therapeutics and greatly improved our understanding of the cell, in many cases it is not clear what role they serve in the natural environment. For example, roughly 70% of the 12 000 bioactive secondary metabolites isolated from actinobacteria exhibit antibacterial activity *in vitro*.[2] This activity has led to the development of many effective antibiotics,[1] but at low concentrations antibiotics also modulate bacterial gene expression, triggering community behaviors like biofilm formation and virulence gene expression.[3] It has been hypothesized that these subinhibitory effects demonstrate that antibiotics are used in nature not as weapons but as interspecies signals.[4]

The advent of genomic sequencing has made it apparent that the vast majority of natural products are not produced in appreciable quantities during normal laboratory growth, and that initial screens have captured only a small subset of the available diversity.[5,6] Understanding the ecological role of natural products is key to isolating new compounds with high efficiency, ensuring that each screen efficiently captures the biosynthetic repertoire of the producing organisms. To date, most natural products appear to be produced in direct response to environmental stresses,[7,8] especially from other microorganisms.[9,10]

While our picture of this complex web of microbial interactions is at best incomplete, recent research has begun to provide new pieces to the puzzle. The clearest results have so far been obtained from a fascinating family of predatory soil bacteria, the myxobacteria.

THE SOIL ENVIRONMENT IS RICH IN PREDATORY BACTERIA

Life in the soil is dependent on access to a number of key nutrients, chief among them the carbon-containing compounds that drive ATP synthesis. These compounds are obtained from one of three sources. The first source is plants, which release sugar through their roots to symbiotic bacteria, which in turn fixate nitrogen or produce secondary metabolites that ward off plant pathogens. The second source is complex organic matter from dead organisms, but it must be digested by dedicated saprophytes before consumption.[11] Soil organisms themselves serve as the final source of energy. Predation by amoebae can account for 60% of all bacterial death,[12] while bacteria like *Bdellovibrio bacteriovorus* survive solely on nutrients from prey species.[13] Dormant bacteria have a number of physical defenses against predation,[14] which largely limits predation to growing cells. In semiarid environments, both bacterial growth and predation can be further limited to a narrow window of activity following rainfall.[12]

Many bacterial species are not obligate predators but use predation as a supplementary source of nutrients.[13,15] This is especially true for saprophytes, and a recent study has shown that many *Streptomyces* spp. can use dormant Gram-positive or Gram-negative bacteria as their sole nutrient source.[15] Environmental isolates fed on bacteria without coaxing, while lab strains like *Streptomyces coelicolor* A3(2) were able to survive by predation only after serial passage through nutrient-poor, prey-rich media. This effect was only observed on agar plates, regardless of prey cell density in liquid culture. The authors focused on the production of small-molecule antibiotics as a means of predation,[15] but this lack of growth may stem more from the nature of *Streptomyces* digestive enzymes. With some

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bacterial predators, it has been observed that survival by digestion of dissolved proteins requires a minimum concentration of predatory cells, below which the excreted enzymes do not reach an energy efficient concentration.[16]

In vivo identification of predatory soil bacteria with ^{13}C-labeled *Escherichia coli* has highlighted the role of bacteria from the *Myxobacteria*, *Xanthomonadaceae* (lysobacter-related), and *Bacteroidetes* families.[17] Best known of these are the myxobacteria, which form predatory "wolf-pack" aggregates on agar, swarming and dissolving prey species (Figure 1). The

Figure 1. Ecological interactions of *M. xanthus*. (A) Approximately 100 000 *M. xanthus* cells aggregate to form a single fruiting body, with ~10 000 ultimately differentiating into myxospores.[96] (B) *M. xanthus* cells (left) feeding on *E. coli* (right). Cells move radially from the point of inoculation and do not alter their course to more effectively encounter prey.[97] (C) *B. subtilis* cells form spore-filled megastructures in response to *M. xanthus*. The predatory myxobacteria are dyed with congo red.[71] (D) *S. coelicolor* (right) produces the blue antibiotic actinorhodin and begins the transition to white aerial hyphae in response to *M. xanthus* (left).

myxobacteria are nearly ubiquitous in soil and have been found on every continent on Earth, including Antarctica.[18] Analysis of a collection of 16S rRNA from 182 soil samples found that myxobacteria ranged from 0.4% to 4.5% of the total fraction of bacterial cells.[19] Further analysis of a single Chinese site showed that at 4.1% of the total rRNA signal, myxobacteria comprised the fifth most prevalent family of bacteria, with a species diversity second only to the actinobacteria.[19]

As a major component of the soil, myxobacteria have a significant impact on protists, fungi, and other bacteria in the soil. Their role as predators implies that many of these interactions will be antagonistic, while the presence of larger predators (amoebae, nematodes) places myxobacteria in the role of prey as well. This makes the myxobacteria an excellent model system for determining the diverse chemical biology of bacterial natural products.

MYXOCOCCUS XANTHUS IS A POTENT NATURAL PRODUCT PRODUCER

The model species *Myxococcus xanthus* forms the basis of our understanding of myxobacteria.[20] Originally thought incapable of producing bioactive natural products, sequencing of the *M. xanthus* DK 1622 genome in 2006 revealed a minimum of 18 biosynthetic gene clusters.[21] Analysis using the ANTISMASH server (antismash.secondarymetabolites.org) increases the total to 24 putative clusters, totalling 14.5% of the 9.1 Mb genome.[22] This is comparable to the model streptomycete *Streptomyces coelicolor* A3(2) (27 clusters, 10.6% of 8.7 Mb),[5] indicating that *M. xanthus* is a major potential source of natural products.

To date, 10 compounds with distinct molecular scaffolds have been isolated from *M. xanthus* spp., though not all have known biological activity (Figure 2). The pan-species biosynthetic repertoire of *M. xanthus* has been determined from LC-MS analysis of 98 strains, 20 of which came from a single German site.[23] This analysis found 48 compounds with molecular masses between 300 and 1200 Da, with an abundance of nitrogen-containing compounds.

Of these compounds, DKxanthene, myxalamide, myxochelin, myxochromide, and four additional compounds identified by only mass and retention time were found in more than 90% of the tested strains, suggesting that these compounds provide a significant fitness benefit to *M. xanthus*. Conversely, 11 compounds were found in less than 3% of strains, suggesting that they provide either a minor fitness benefit or are readily replaced by other compounds with similar functions. Between these two extremes are 29 compounds that are present in many—but not all—strains. A large subset of these are almost exclusively coproduced, suggesting that they are either close analogues or exert a synergistic mode of action (Figure 3). Conversely, some compounds are rarely or never coproduced, suggesting that they are antagonistic or fulfill highly similar roles (Figure 4). Unfortunately, there is little other information on the bulk of these compounds. Only the antibiotic myxovirescin has been extensively studied (*vide infra*), though subsequent work determined the structure of c506, now called myxoprincomide.[24]

In the broader context, over 600 chemically distinct natural products have been isolated from myxobacteria,[2] the majority of which are produced during exponential growth.[25−29] Production during exponential growth suggests that these compounds provide a fitness benefit during normal bacterial feeding and cell division. This is in sharp contrast to the natural products of streptomycetes, which are produced primarily during nutrient limitation.[8] Some compounds, such as the antibiotics saframycin and althiomycin, have been found in both streptomycetes and myxobacteria.[28,30−32] Both of these compounds are produced during exponential growth in myxobacteria but during the stationary phase in streptomycetes, suggesting that the timing of their production is linked to their ecological role, not their *in vitro* antimicrobial activity.

Both saframycin and althiomycin are very rare metabolites of myxobacteria (<1% of *M. xanthus* strains),[23] suggesting that the biosynthetic genes required for these compounds were acquired in the recent past by horizontal gene transfer from either streptomycetes or a third, uncultivated bacterial family. This theory is especially convincing for althiomycin, which has also been isolated from the Gram-negative gammaproteobacteria *Serratia marcescens* Db10.[33] This strain of *S. marcescens* produces the antibiotic during stationary phase and appears to have acquired the biosynthetic genes in the distant past. The G+C content of these genes matches the broader *Serratia* genome (59% vs ~70% for *Streptomyces* and *Myxococcus*), and the genes are not flanked by obvious transposable genetic elements.

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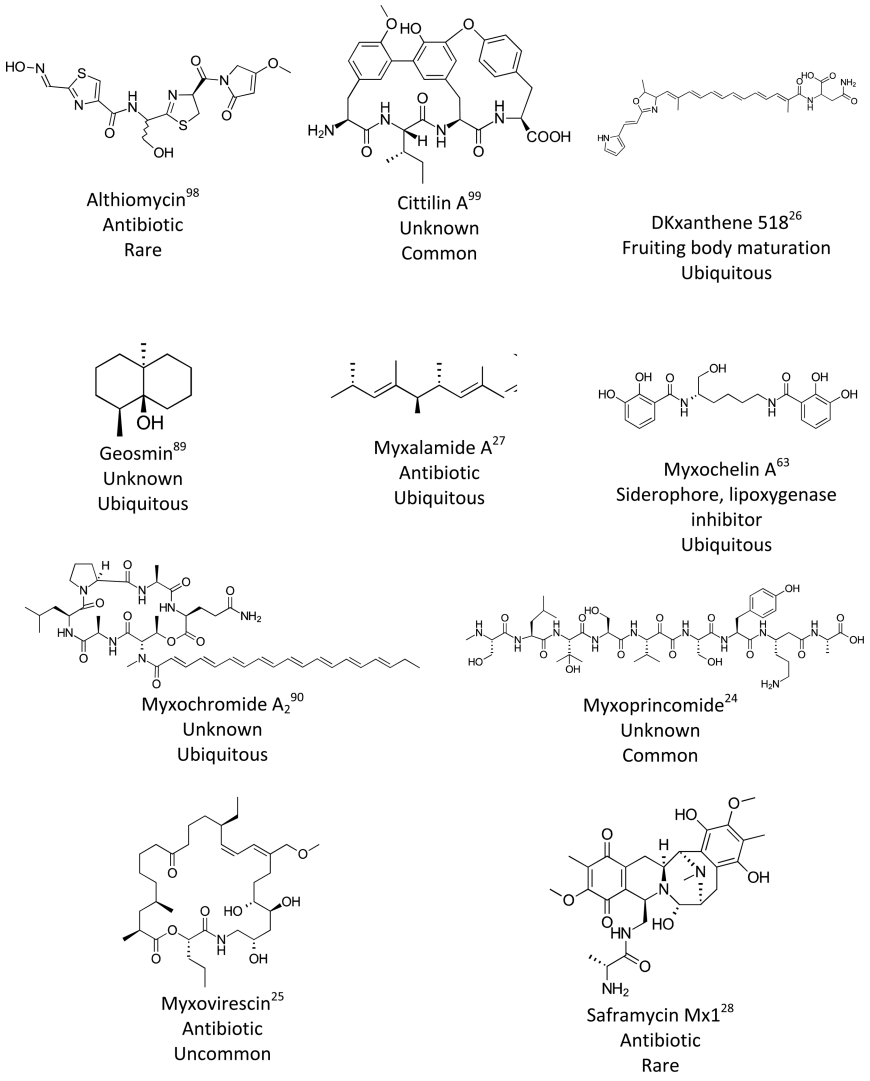

Figure 2. Representative examples of natural product scaffolds identified in *Myxococcus xanthus* species. Ubiquitous = found in at least 90% of surveyed strains, common = found in the majority of strains, uncommon = found in 5−50% of strains, rare = found in less than 5% of strains.[23]

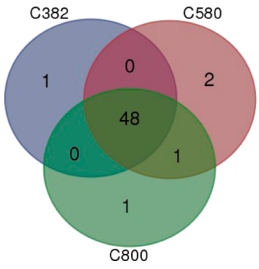

Figure 3. Compounds C382, C580, and C800, commonly coproduced in *M. xanthus* spp. Their structures and biological activity are currently unknown. Numbers within the circles indicate occurrence across distinct *M. xanthus* isolates, while the names of the compounds correlate to their observed *m*/*z*.[23]

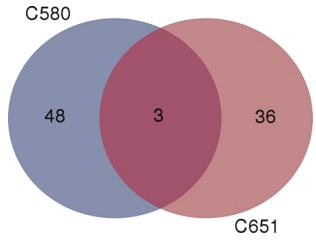

Figure 4. C651 and C580, two compounds that are rarely coproduced in *M. xanthus*. Their structures and biological activity are currently unknown. Numbers within the circles indicate occurrence across distinct *M. xanthus* isolates, while the names of the compounds correlate to their observed *m*/*z*.[23]

MYXOBACTERIA ARE OBLIGATE SOCIAL BACTERIA

The life cycle of *M. xanthus* depends on broad coordination between myxobacterial clones, starting from when cells first seek nutrients as "wolf-packs" (Figure 1).[20,34] Lacking both flagella and cilia, individual cells are proposed to move through the helical motion of focal adhesion proteins that run down the length of the cell.[35−37] In wolf-packs, cells also extend and retract type IV pili at their leading end, adhering to nearby cells.[35]

Periodically during predation, cells will reverse direction *en masse*, leading to a characteristic rippling effect on agar. These reversals are required for efficient digestion of colonies of prey bacteria[37] and are triggered by the Frz chemosensory complex.[35] Synchronization of these cellular reversals is not

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fully understood but is thought to occur through direct cell−cell contact and not *via* diffusion of a chemical signal.[36]

As *M. xanthus* cells move, they also secrete from the trailing pole of the cell a complex mix of polysaccharides.[38] This slime trail, visible by light microscopy, aids in the movement of subsequent cells. The exact composition of the slime has not been determined, but *M. xanthus* is able to use the trails of other bacterial species, who also exhibit increased movement on *M. xanthus* slime.[38]

Secretion of both slime and digestive enzymes diverts significant resources from growth and cell division, imposing a fitness penalty on *M. xanthus*. High cell densities dramatically reduce the individual costs of these activities, favoring the emergence of pack behavior. This can be observed through deletion of the gene coding for type IV pili, *pilA*, as deficient cells rapidly redevelop pack behavior after serial passage through nutrient-poor agar environments.[39]

MYXOBACTERIA EXHIBIT PLANNED DORMANCY

Under nutrient-poor conditions, almost all myxobacteria form large fruiting bodies, complex superstructures that originally led to these bacteria being classified as eukaryotes (Figure 1).[34,40] Within the fruiting body, some cells develop into dehydration-resistant myxospores, while all cells become less susceptible to grazing by protists and *C. elegans*.[41] Full maturation of the fruiting body is an energy-intensive process, which requires that approximately 90% of the myxobacterial cells autolyse.[41]

Formation of fruiting bodies is coordinated in part by A-signal, a quorum sensor of *M. xanthus*.[42] A mix of free hydrophobic amino acids, A-signal appears to be produced *via* degradation of *M. xanthus* surface proteins by exogenous proteases and is reduced in strains lacking *asgA* and/or *asgB*. Release of these (uncharacterized) proteases begins 2 h after starvation, initiating fruiting body development if the total concentration of A-signal exceeds 10 μM.[42] After 6 h of starvation, *M. xanthus* cells produce a second fruiting body developmental signal by releasing the exogenous protease PopC. This protease cleaves the outer membrane protein p25 to give the C-signal, p17.[43,44] While cells lacking *asgA* or *asgB* are deficient in both A-signal and PopC, the addition of A-signal to these strains does not lead to PopC production. However, heterologous expression of PopC is sufficient to trigger fruiting body formation absent A-signal, suggesting that A-signal and C-signal act independently and that the C-signal triggers a later step in the fruiting body formation pathway.[45] Fruiting body formation can be reversed by the addition of nutrients within 24−30 h of starvation, through rapid proteolysis of the transcription factor MrpC.[46,47]

Formation of fruiting bodies, secretion of degradative enzymes, and slime trail formation are all community behaviors that require significant resource allocation by each cell, potentially allowing a small subpopulation to reap the benefits of community behavior without contributing resources. As a result, these species appear to have developed a number of measures to exclude "cheating" cells that do not contribute to swarm fitness. Simply growing laboratory strains of *M. xanthus* in different environments for several weeks is enough to produce lineages which do not recognize the ancestral strain as self, leading to antagonistic behaviors on agar and exclusion from fruiting bodies.[48] This behavior was not linked to mutations in traA, a kin-recognition protein that allows *M. xanthus* to swap outer membrane components with neighboring cells.[49] Membrane swapping allows *M. xanthus* cells with dysfunctional outer membrane proteins to gain functional copies from healthy cells[50] but is also used to transfer toxic proteins between cells from closely related lineages.[51]

SMALL-MOLECULE SIGNALS FOR PREDATION AND KIN INTERACTIONS

The *M. xanthus* DK 1622 genome contains a multitude of regulatory proteins and membrane receptors, implying that the bacteria can recognize many different environments.[21] Strangely, this functionality is not reflected in wolf-pack behavior.[52] When inoculated adjacent to prey colonies, *M. xanthus* cells move in all directions from the point of inoculation and do not appear to alter their movement in response to prey metabolites or signal molecules (Figure 1).[53] The same is not true for prey species.[52] While nearly immobile on the hard agar plates used in many predator−prey interaction studies, on low-weight (0.3%) agar plates *E. coli* can move up to 60× faster than *M. xanthus*. Rather than fleeing *M. xanthus*, these prey swim straight toward the wolf-packs and are rapidly digested. Movement toward *M. xanthus* cells was significantly reduced in *tsr* or *tar* knockouts, genes necessary for chemotaxis toward free serine and aspartate, respectively. Chemotaxis was directed exclusively toward starving *M. xanthus* cells, over a time frame that matches the production of A-signal (*vide supra*). A-signal thus seems to act as both an *M. xanthus* quorum sensor and as a lure for prey like *E. coli*. Digestion of the first *E. coli* cells will lead to an increase in local amino acid concentrations, strengthening the lure and raising the concentration of nutrients high enough to halt fruiting body formation.

To date, only the DKxanthenes have been implicated in intraspecies communication in *M. xanthus*.[26] These pigments give *M. xanthus* cells their characteristic yellow color and have long been used to distinguish between two types of *M. xanthus* cells. "Yellow" cells exhibit normal swarming, DKxanthene production, and development behavior, while "tan" cells are impaired in these activities but survive longer under starvation conditions.[41,54] Cells can switch between tan and yellow, and wolf-packs and fruiting bodies contain a mixture of both types.[54]

Unlike many natural products from *M. xanthus*, the DKxanthenes are produced during stationary phase and are not secreted from the cell.[26] Eliminating DKxanthene production in an obligate yellow lineage of *M. xanthus* extended the time required for fruiting body formation from 12 to 24 h and similarly extended the time needed for myxospore formation from 3 days to a minimum of 1 week (only 18% of spores had formed by this point). The addition of purified DKxanthene restored approximately 25% of spore formation after 3 days of starvation. Mixing DKxanthene-producing and DKxanthene-deficient cells in a 1:1 ratio also restored some spore formation, but only to 1.3% of wild-type production.[26] The mechanism of DKxanthene-induced spore formation has not been reported.

ANTIBIOTIC PRODUCTION AIDS IN *M. XANTHUS* FEEDING

Many strains of *M. xanthus* produce a macrocycle called myxovirescin, also known as antibiotic TA (Figure 2).[25,55] This compound was isolated from *M. xanthus* by Rosenberg and colleagues[55] then independently isolated and structurally characterized by Trowitzsch and colleagues from *Myxococcus*

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virescens Mx v48.[25] Myxovirescin was initially thought to interfere with peptidoglycan cross-linking, as it blocked incorporation of radio-labeled diaminopimelic acid and UDP-GlcNac into *E. coli* peptidoglycan.[55] However, recent studies have shown that myxovirescin has no direct effect on peptidoglycan synthesis. Rather, myxovirescin binds to the signal peptidase LspA, preventing the proper processing of many different pro-lipoproteins, including those key for peptidoglycan synthesis.[56] Resistance to myxovirescin can therefore be conferred by either overexpression of LspA or expression of a mutated LspA. *M. xanthus* has adopted the latter approach. There are four different *lspA*'s in the genome of *M. xanthus* DK1622, including two in the myxovirescin biosynthetic cluster.[57]

The ecological role of myxovirescin is well understood, thanks in large part to the work of Wall and colleagues.[58] As with many antibiotic producers, on hard agar *M. xanthus* DK1622 is able to create a zone of inhibition in a soft agar overlay of *E. coli* cells. This zone is lost in a myxovirescin-knockout mutant, demonstrating that myxovirescin is the primary diffusible antibiotic in this system (the hydrolytic enzymes produced by *M. xanthus* are unlikely to readily diffuse through soft agar).

The ability of *M. xanthus* to prey on *E. coli* was significantly impeded in a myxovirescin-knockout strain, with *E. coli* survival extending from approximately 40 h after inoculation to 120.[58] However, rates of killing similar to wild-type could be restored by adding a subinhibitory concentration of the protein synthesis inhibitor spectinomycin to the agar (1/4 MIC). Effective *M. xanthus* predation also correlated to increased movement speed across a nutrient-poor, prey-rich agar plate. Both the absence of myxovirescin and presence of myxovirescin-resistant *E. coli* led to reduced movement rates, implying that *M. xanthus* was less effective at degrading *E. coli* in these environments.

As the bacteriostatic antibiotic spectinomycin was able to restore effective predation to *M. xanthus* at significantly below its MIC,[58] antibiotic production appears necessary to impair the *E. coli* response to predation but not to kill the prey species. The interchangeability of spectinomycin and myxovirescin also suggests the latter can be readily replaced by other natural products. Myxovirescin is only secreted by ∼30% of *M. xanthus* strains,[23] but to date a broadly produced antibiotic with a similar spectrum of activity has not been reported in *M. xanthus*.

MYXOCHELIN IS A SIDEROPHORE WITH MULTIPLE FUNCTIONS

Free iron is a scare resource in soil, and the vast majority of microorganisms either secrete iron-binding siderophores or use the siderophores of nearby strains.[59,60] *M. xanthus* is no different, and production of the iron-binding myxochelins was ubiquitous in the 98 strain survey.[23] However, these compounds appear to have functions beyond iron uptake.

Originally isolated from the myxobacterium *Angiococcus disciformis* An d30 under iron-limiting conditions,[61] the myxochelins were recently reisolated from the actinomycete *Nonomuraea pusilla* TP-A0861.[62] These authors found that the noncytotoxic myxochelin A inhibited tumor invasion of murine colon 26-L5 cells *in vitro* at a low micromolar concentration.[62] A separate study has shown that the myxochelins inhibit proliferation of K-562 cells *via* inhibition of 5-lipoxygenase, an iron-containing enzyme essential for the development of leukotrienes.[63] While the effect of myxochelin on fungal orthologues of 5-lipoxygenase has not been directly investigated, *Myxococcus* spp. broadly inhibit the growth of fungi and can feed directly on fungal cells.[18,64]

M. XANTHUS TARGETS THE ELECTRON TRANSPORT CHAIN

Analogues of myxalamide are ubiquitous in environmental *M. xanthus* strains[23] and were first characterized in 1983.[27] Produced during exponential growth in *M. xanthus* Mx x12, the various myxalamide analogues reach a combined concentration of 120 mg/L by the onset of a stationary phase, indicating a key role in the *M. xanthus* lifecycle. While sensitive to both light and oxygen, the myxalamides are primarily excreted after production and are unlikely to act as cellular antioxidants. Indeed, the myxalamides are cytotoxic to both fungi and Gram-positive bacteria and when injected are highly toxic to mice. All of these toxicities are linked to inhibition of the electron transport chain (ETC), specifically the reduction of cytochrome *b* by NADH.[27]

Research into the myxalamides has focused largely on their biosynthesis,[65] though the high titer and pan-species prevalence suggests an important role in *M. xanthus* fitness. Indeed, myxobacteria are the predominant natural source of electron transport chain inhibitors.[66,67] It is unclear if this prevalence is due to chance, or if ETC inhibition provides a fitness benefit over other more common forms of growth inhibition (e.g., inhibition of glycan biosynthesis, protein synthesis, or nucleic acid metabolism).

PREY SPECIES EMPLOY PHYSICAL AND CHEMICAL DEFENSES

Predation by *M. xanthus* represents a significant challenge to the growth of other soil microorganisms, imposing selective pressure on prey species.[14,17,18,68,69] Biofilms appear to act as a general physical barrier to predation, rendering *E. coli* less susceptible to killing by both *M. xanthus* and *Caenorhabditis elegans*.[14] Strains that are not prodigious biofilm producers may evade predation in multispecies biofilms,[70] while others display more elaborate adaptations. Exposing cultures of the Gram-positive soil bacterium *Bacillus subtilis* to *M. xanthus* leads to "mega-structures," large spore-filled cell aggregates that rise up to 200 μm above the agar plate.[71] Fruiting bodies are found in close proximity to these mega-structures, indicating that they are largely resistant to *M. xanthus* predation.

Prey strains have also developed chemical defenses. The addition of live *M. xanthus* to actively growing *B. subtilis* colonies was shown to result in complete digestion of standard laboratory strains (168), while the majority of environmental cells survived (NCIB3610, 68%).[69] Gene knockout and compound addition studies confirmed that the increased survival was due to production of bacillaene, a bacteriostatic antibiotic known to inhibit protein synthesis in both Gram-positive and Gram-negative bacteria.[72] Pure bacillaene also conveyed *M. xanthus* resistance to susceptible *E. coli* cells.[69]

The defensive role of bacillaene is consistent with earlier studies employing streptomycetes, which demonstrated that antibiotic-producing strains were able to prevent invasion by competing strains, but were no better at claiming territory already occupied by susceptible strains.[73] Exposing *S. coelicolor* to *M. xanthus* also results in a 20-fold increase in production of the antibiotic actinorhodin while accelerating the formation of aerial hyphae (Figure 1).[74] More generally, this "firewall"

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approach also matches the physicochemical properties of many antibiotics, which adhere strongly to soil and thus may accumulate close to producing strains.[75,76]

NATURAL PRODUCTS OF NOTE FROM OTHER MYXOBACTERIA

Myxobacteria represent one of the most common and diverse bacterial orders,[19] leading to a wealth of natural products.[2] Of these, compounds from *Sorangium cellulosum* are of particular interest, especially the clinical antitumor compound epothilone.[77] *S. cellulosum* has the largest reported bacterial genome (14.8 Mb, larger than that of *S. cerevisiae*).[78] Unlike *M. xanthus*, *S. cellulosum* does not form predatory wolfpacks, instead deriving the majority of its nutrients from the degradation of organic matter. As the name suggests, *S. cellulosum* is able to degrade cellulose and can grow with paper as the only carbon source.[18] The natural product repertoire of *S. cellulosum* contains several eukaryotic cytoskeleton inhibitors,[79] including the microtubule inhibitor epothilone and tubulin polymerase inhibitor disorazol.[80,81] While the soil sorption capability of these compounds is unknown, as cytoskeleton inhibitors they likely work to prevent the engulfment of *S. sorgangium* by amoebae and other eukaryotic predators of bacteria.[82]

S. cellulosum produces another fascinating natural product, carolacton.[83] At nanomolar concentrations, carolacton is bactericidal to growing *Streptococcus mutans* biofilms. Planktonic cells are unaffected, and while large membrane pores appear on carolacton addition, the antibiotic does not directly disrupt the membrane.[84] Biofilms can be rendered resistant by knocking out either the transcription factor CysR or the serine/threonine protein kinase PknB,[84,85] though neither protein appears to regulate the other. At this point, only activity against *S. mutans* biofilms has been observed, and it is ultimately unclear whether carolacton can target soil biofilms. Self-regulation or inhibition of other myxobacteria is another potential role, as *S. cellulosum* has genes coding for over 300 serine/threonine protein kinases.[78]

Not all myxobacterial natural products inhibit the growth of microorganisms. The archrazolids and apicularens are two families of nanomolar eukaryotic vacuolar ATPase inhibitors, found in *Archangium* and *Chondromyces* spp., respectively.[86] Inhibition of v-ATPases prevents proper acidification of eukaryotic lysosomes and may impair digestion of myxobacteria by amoebae. Early work with myxobacteria noted that "amoebae grow luxuriantly and produce cysts in crude cultures that also contain actively growing myxococci."[87] As these compounds are also effective against mammalian enzymes, the archazolids are currently under investigation as potential cancer chemotherapeutics.[88] To date, no v-ATPase inhibitors have been isolated from *M. xanthus*, though this organism produces at least 12 natural products of unknown function (*vide infra*).

MANY *M. XANTHUS* METABOLITES HAVE NO KNOWN FUNCTION

The compounds presented here represent only a small subset of the biosynthetic capabilities of the myxobacteria. Thousands of myxobacteria have been isolated to date, and roughly 2500 unique strains are publically available from the Leibniz Institute DSMZ. Many strains are known for production of only a single bioactive metabolite, while the biosynthetic potential of others is completely unknown.

M. xanthus is the most extensively studied myxobacterial species. It is known to produce at least 10 natural products in the laboratory environment, with genetic clusters coding for perhaps 14 more (Figure 2).[21,22] Of those with characterized structures, four have unknown activity. Three of these compounds, geosmin, myxochromide, and myxoprincomide, are ubiquitous in myxobacteria, implying that they convey a large fitness advantage.[23,89] The concentration of these compounds is generally low in laboratory culture (<1 mg/L),[24,90] implying that they are either effective at low concentrations or that production is upregulated by unknown stress conditions. This is equally true for many compounds predicted by genomic analysis, which may be expressed below detectable levels or have bioactivities that are not readily detectable in standard assays.[6,91,92]

The last compound of known structure and unknown activity is the macrocyclic peptide cittilin.[93] Produced by *Streptomyces* A 9783 and a majority of *M. xanthus* species,[94] the role of cittilin in soil ecology is currently unknown. Early reports indicate that cittilin is a modest inhibitor of the neurotensin receptor of guinea pigs (IC_{50} = 30 μg/mL),[94,95] though this seems unrelated to growth in the soil environment.

CONCLUSION

Myxobacteria are uniquely situated for research into the chemical ecology of soil natural products. As obligate predators and occasional prey, the effects of their secondary metabolism can be readily evaluated through a combination of genetic knockouts and simple predation assays. At the same time, their social lifestyle and altruistic behavior drives rapid speciation, leading to a plethora of strains and natural product classes.

While the ecological role of many antibiotics is largely unknown,[3,4] *M. xanthus* research suggests that many act as chemical defenses against predation. Production of bacillaene by *B. subtilis* significantly reduces grazing by *M. xanthus*,[69] though the antibiotic is bacteriostatic and has no direct killing effect.[72] Production of actinorhodin by *S. coelicolor* has a similar effect,[74] while the antieukaryotic natural products of *Sorangium* spp. suggest this firewall approach is common to myxobacteria as well.[77,80] Long-term resistance to predation appears to require physical barriers, as both *Bacillus* and *Streptomyces* construct spore-filled structures when challenged with *M. xanthus*.[71,74] Biofilms appear to form a similar role, providing resistance to grazing by both *M. xanthus* and *C. elegans*.[14]

M. xanthus does use at least one antibiotic as a molecular weapon, as myxovirescin expression improves predation against susceptible *E. coli* strains.[58] While pure myxovirescin can inhibit the growth of these strains *in vitro*, its activity can also be replaced by subinhibitory concentrations of spectinomycin. This implies that full growth inhibition is not required for effective *M. xanthus* predation.

The role of natural products is tied to the local environment, and several myxobacterial compounds appear to fill multiple roles. The simple amino acids that form A-signal both act as a quorum sensor during fruiting body formation and serve as a lure to draw in prey species like *E. coli*.[45,52] The principle siderophore of *M. xanthus*, myxochelin,[61] also appears to serve a dual purpose as a low micromolar inhibitor of eukaryotic 5-lipoxygenase and may contribute to the antagonistic interactions between myxobacteria and fungi. Myxochelin may also find a role in cancer therapy.[64]

From prey to protists to other myxobacteria, *M. xanthus* exhibits broad antagonistic activity in laboratory and soil

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environments. Key to many of these interactions is the production of natural products, which allows researchers to directly visualize complex interspecies interactions with genetic knockouts and simple *in vitro* competition assays. At present, these experiments suggest that many microorganisms use natural products as molecular firewalls that guard against competition or predation, while myxobacteria may find similar compounds useful for incapacitating prey. However, to date less than a third of the biosynthetic repertoire of *M. xanthus* has been well characterized. How the remainder will alter our understanding of bacterial chemical ecology remains to be seen.

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Notes

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KEYWORDS

Antibiotics: Small molecules produced by microorganisms that inhibit the growth of other microorganisms in a laboratory setting

Chemical ecology: The study of chemicals involved in interactions between living organisms

Chemotherapy: The use of small molecules to inhibit the growth or proliferation of pathogenic bacteria, fungi, viruses, or cancer cells

Myxobacteria: A broad family of deltaproteobacteria, characterized by their formation of fruiting bodies and excretion of a polysaccharide slime

Myxococcus xanthus: The best studied of the myxobacteria; predatory bacterium that subsist primarily by digesting other living microorganisms

Natural products: Compounds produced by natural organisms, which often display biological activity

Natural product biosynthesis: The production of natural products by living organisms

Secondary metabolites: Small molecules produced by organisms that do not directly contribute to cell division or growth

Sorangium cellulosum: A myxobacterium that survives largely *via* digestion of cellulose and other dead organic matter; producer of a number of interesting natural products and record-holder for the largest bacterial genome (14.8 Mb)

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