The predatory life cycle of *Myxococcus xanthus*

Ryan Keane¹,² and James Berleman¹,²

¹Department of Biology, Saint Mary's College, Moraga, CA 94556, USA
²Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

*Myxococcus xanthus* is a predatory bacterium and a model system for social behaviour in bacteria. *M. xanthus* forms thin biofilms, where cells work together to colonize new territory, invade prey colonies and lyse prey cells. Prey-cell lysis occurs at close proximity, and utilizes antibiotics such as myxovirescin, hydrolytic enzymes such as the protease MepA and extracellular outer-membrane vesicles that may facilitate delivery. Many questions about the mechanism of prey lysis remain, as well as a complete understanding of the vast hydrolytic and secondary metabolite potential present in the *M. xanthus* genome. However, it is clear that predation presents unique challenges for this bacterium, which are solved, in part, through the social behaviours at the disposal of *M. xanthus*. Here, we discuss the life cycle of *M. xanthus*, and the hypothesis that multicellular behaviour in this organism is critical to, and derives from, the challenges of growth as a bacterial predator.

**Introduction**

*Myxococcus xanthus* is a predatory bacterium that can lyse other bacteria and grow on the nutrients released (e.g. Berleman & Kirby, 2007; Xiao *et al.*, 2011). *M. xanthus* also forms elaborate biofilms with architecture that includes macroscopic fruiting bodies where vegetative cells differentiate into spores (e.g. Kaiser, 2003; Huntley *et al.*, 2011). The predatory behaviour of *M. xanthus* has been compared to a multicellular ‘wolfpack’, but the mechanism of predation and the connection to multicellularity are still being investigated (Berleman & Kirby, 2009). This review covers recent work that has begun to unravel the novel mechanism by which *M. xanthus* utilizes a combination of antibiotics, hydrolytic enzymes and subcellular structures to lyse target prey (Fig. 1). A common theme of investigations into predatory behaviour is that motility and multicellular behaviour are both integral to predation (Fig. 2). *M. xanthus* cells utilize motility for a myriad of cooperative, multicellular behaviours, including scouting, branching, rippling and fruiting-body aggregation (detailed explanations are given in the Cellular development and multicellular behaviour section). As we continue to refine our understanding of the mechanism of predation, we also hope to better characterize how acquiring nutrients through predation results in the wolfpack behaviours needed for a predatory life cycle (Fig. 3).

*M. xanthus* is found in soil environments all across the globe (Dawid, 2000; Fiegna & Velicer, 2005). It is a member of the *Myxobacteria*, a family of organisms characterized by a diverse range of multicellular structures, as well as their cellulolytic and proteolytic activities (Hyun *et al.*, 2008). Cellulolytic myxobacteria are important to the global carbon cycle as degraders of plant biomass, while proteolytic myxobacteria are micropredators that impact carbon flow and composition of microbial communities (Lueders *et al.*, 2006). The myxobacteria have large genomes for bacteria and produce a wide range of secondary metabolites with many undiscovered activities (Goldman *et al.*, 2006). The ability of myxobacteria such as *M. xanthus* to lyse both Gram-negative and Gram-positive species has interesting implications for both microbial ecology and antibiotics research. The full impact of myxobacteria on microbial communities is difficult to assess, but they may be a resource for production of novel chemical structures. Secondary metabolite production for predation by myxobacteria in general is likely to be important for the evolution of attack and defence mechanisms that can specifically act as virulence factors in pathogens (Erken *et al.*, 2013; Pukatzki & Provenzano, 2013).

The observation that the myxobacteria are predators goes back to at least 1962 (Noren & Raper, 1962), with the distinction that while many bacteria may release toxic compounds that may inhibit the growth or cause lysis of neighbours, only a predator grows on nutrients released by lysing neighbour cells. Several early studies characterized the range of susceptible prey, explored the connection of hydrolytic enzymes to predation, and examined the antibiotic production of myxobacteria species (Singh, 1947; Hart & Zahler, 1966; Noren & Raper, 1962; Rosenberg *et al.*, 1973). In addition to this general characterization of myxobacteria, there have also been tremendous genetic analyses of *M. xanthus* focused on the motility, cell-signalling and morphogenesis of *M. xanthus* into fruiting bodies (Nan & Zusman, 2011; Sun *et al.*, 2000; Abellón-Ruiz *et al.*, 2014; Huntley *et al.*, 2011).

Compared to other micropredators, *M. xanthus* is more versatile than obligate predators such as *Bdellovibrio*...
species. Whereas individual *Bdellovibrio* cells invade Gram-negative prey cells in a non-cooperative and species-specific manner (Lerner et al., 2012; Hobley et al., 2006), *Myx. xanthus* is able to lyse a wide range of Gram-negative and Gram-positive bacteria in a non-species-specific manner (Mendes-Soares & Velicer, 2013). Other soil microbes show cooperative traits, such as *Bacillus subtilis*, which forms specialized cell types and secretes secondary metabolites that exhibit antibiotic potential at high concentrations (Vlamakis et al., 2008; Liu et al., 2010). However, it is often unclear whether the studied organism actually produces sufficient concentrations of these metabolites for lysis *in situ*, nor is it obvious whether the organism then derives growth substrates directly from its lysed neighbours. Interestingly, *Myx. xanthus* and *Bac. subtilis* demonstrate autolytic and cannibalistic activity, respectively, under certain lab conditions (Lee et al., 2012; Berleman et al., 2006; Shank et al., 2011), although there is evidence that both species prefer predation to lysis (Berleman et al., 2006; Nandy et al., 2007). Indeed, it seems likely that within microbial communities of mixed species, cannibalism may only occur under extreme...
circumstances. Thus, in order to understand lysis of prey cells, we must consider both molecular mechanisms and the impact on the community.

**Mechanisms of prey lysis**

*Myx. xanthus* was first isolated and cultured from cow dung, which suggested a preference for growth on macromolecules (Beebe, 1941). The discovery of lytic enzymes in culture extracts of *Myx. xanthus* indicated predatory potential (Hart & Zahler, 1966). In addition, for *Myx. xanthus*, access to peptide substrates may be critical to life-cycle regulation. *Myx. xanthus* lacks the genes required for the synthesis of three branched-chain amino acids – leucine, valine and isoleucine – all of which are essential for growth (Goldman *et al.*, 2006). In addition,
the lack of phenylalanine or tryptophan in growth medium is sufficient to trigger fruiting-body formation (Dworkin, 1963). It is likely that one of the primary ways *M. xanthus* acquires these essential molecules is through predation.

The mechanism of *M. xanthus* predation is distinct from other microbial predators such as *Bdellovibrio* species, which rely on cell invasion or prolific antibiotic producers such as *Penicillium notatum* (Berleman & Kirby, 2009). With *M. xanthus*, prey-cell death occurs at very close range, and may require cell–cell contact, but without prey-cell invasion or engulfment (Fig. 1a, c). The wolfpack comparison is based on the idea that at higher cell densities, there should also be a higher concentration of extracellular antibiotics and hydrolytic enzymes. However, individual *M. xanthus* cells are also competent predators, as one cell can lyse a micro-colony of ~20 *Escherichia coli* cells and will typically lyse all cells in the micro-colony before moving on (McBride & Zusman, 1996).

This indicates that the close-proximity requirements may be due to limited diffusion and/or the delivery mechanism used to lyse prey.

*M. xanthus* secretes several secondary metabolites with antibiotic properties, including myxovirescin, myxalamid and cierrezin compounds, along with several classes of hydrolytic enzymes used to digest prey macromolecules (Fig. 1b) (Berleman et al., 2014). *M. xanthus* utilizes myxovirescin (also called antibiotic TA) to kill *E. coli*, perhaps by targeting its type II signal peptidase (Xiao et al., 2012). Myxovirescin-deficient (Δtau1) mutants showed no deficiency in killing Gram-positive *Micrococcus luteus*, but a high deficiency in killing Gram-negative *E. coli*, indicating that myxovirescin is essential for lysing some, but not all, prey (Xiao et al., 2011).

Subcellular structures may also play a critical role in predation. *M. xanthus* produces prolific outer-membrane vesicles (OMVs), some of which facilitate connections...
between cells (Remis et al., 2014; Palsdottir et al., 2009). OMVs contain a complex mixture of secondary metabolites, hydrolytic enzymes, chaperones and proteins of unknown function; OMV fractions purified from cells retain some lytic activity (Evans et al., 2012, Berleman et al., 2014). Included in this is the protease MepA, which is responsible for most extracellular protease activity in *Myx. xanthus* (Fig. 1b). MepA is a 1708 aa protein that has three conserved domains which help predict the function of this protease (http://www.ncbi.nlm.nih.gov). An N-terminal fungalisin/thermolysin propeptide (FTP) motif is predicted to prevent premature activation, restricting MepA activity, perhaps until it reaches its target localization in OMVs or until contact with suitable substrates. The central zinc-dependent M36 peptidase domain contains the conserved active site motifs HEXXE and EXXXD, as well as an integrated protease-associated (PA) motif thought to form a lid structure that may also play a role in preventing premature or untargeted activity. Finally, a C-terminal calcium-dependent cadherin domain is predicted to mediate cell–cell interactions. Together, these predictions support the hypothesis that MepA is utilized during cell–cell contact for macromolecule degradation, and since there are many more peptidases in the *Myx. xanthus* genome it remains to be determined what other enzymes are involved. Considering these results, and the large size of the *Myx. xanthus* genome and its expansions in secondary metabolism and hydrolytic pathways, we suspect that there is still much more to learn about the predatory mechanism (Goldman et al., 2006). For instance, *Myx. xanthus* predation is also affected by its environment. In an aqueous environment, predator–prey contact mediated by matrix polymers such as exopolysaccharide (EPS) was needed to lyse *E. coli*, but was not necessary for lysis of other prey species (Pan et al., 2013). In other studies, ecological variables, such as agar surface type, prey-cell density and prey-cell envelope, impacted the first 24 h of *Myx. xanthus* predation (Hillesland et al., 2007); however, almost all sensitive prey were lysed by 14 days. This evidence indicates that *Myx. xanthus* is able to eventually overcome transient resistance to predation (Hillesland et al., 2007) and provides further support for *Myx. xanthus* as a highly versatile bacterial predator.

Under stringent conditions, where prey are the sole nutrient source, the ratio of *Myx. xanthus* cells produced for every one *E. coli* cell killed is \( \sim 0.2 : 1 \) (Berleman & Kirby, 2007). By comparison, smaller predators like *Bdellovibrio* species produce new cells at a ratio of \( \sim 5 : 1 \) for every *E. coli* cell lysed (Sockett, 2009). *Myx. xanthus* predatory success can be measured through a variety of predation parameters, and depends upon the prey species that is encountered. Some species, such as *E. coli*, are easily lysed by *Myx. xanthus* (Pan et al., 2013; Mendes-Soares & Velicer, 2013), while others, such as *Curtobacterium citreum*, are highly resistant to predation (Mendes-Soares & Velicer, 2013). Although *Myx. xanthus* migrates rapidly when *Bacillus bataviensis* is the prey substrate, very little *Bac. bataviensis* was killed in comparison to other bacterial prey sources used, such as *E. coli* and *Mic. luteus* (Mendes-Soares & Velicer, 2013). Compared to other micropredators, *Myx. xanthus* appears to have a wide range of potential prey, but more work is required to determine what mechanisms distinguish a species that will be lysed from one that is resistant to lysis.

### Cellular development and multicellular behaviour

The size and shape of individual cells have long been used as a means of distinguishing developmental stages within a species (Justice et al., 2014). *Myx. xanthus* vegetative cells are flexible rods that are motile on most solid surfaces (Fig. 2a); spores, in comparison, are non-motile and spherical in shape (Fig. 2b) (Beebe, 1941). Cell morphology can also help distinguish areas of differing multicellular development. For example, spores are typically found within fruiting-body structures, while rod-shaped cells roam the spaces between these fruiting bodies as peripheral rods with a distinct protein profile (O’Connor & Zusman, 1991). At the cellular level, the term development in *Myx. xanthus* refers to the transition between vegetative cells and spores. However, at the multicellular level, development is more complex and encompasses a wide range of transitions, with four characteristic morphological structures observed: scouting, branching, rippling and aggregating structures (Fig. 2c–f). This review subsequently refers to all multicellular processes as behaviours to avoid confusion with cellular development.

We use the term ‘scouting’ to refer to the colony morphology or behaviour where groups, individuals and trails are observed in patterns similar to Fig. 2(c). We use the term ‘branching’ to refer to colony morphology or behaviour where branches are observed similar to Fig. 2(d). We use these morphological terms to avoid confusion with the genetic controls of motility. *Myx. xanthus* has two genetically distinct motility systems: social (S) and adventurous (A). S-motility relies on type IV pili and EPS, while A-motility relies on some integration of rotational periplasmic motors and focal adhesion complexes (Kaiser, 1979; Li et al., 2003; Nan et al., 2011; Wartel et al., 2013). These motility systems have been thoroughly reviewed in detail recently and are not the main subject of this review (Kaimer et al., 2012; Pathak et al., 2012; Nan & Zusman, 2011). These two gliding motility systems are synergistic and both may contribute to the morphological patterns observed (Mauriello et al., 2010). 'Rippling' refers to the \( \sim 50–100 \) \( \mu \)m wide wave-like morphology as shown in Fig. 2(e). 'Aggregating' refers to the larger, 0.1–1 mm wide domed structures that form which contain vegetative cells (Fig. 2f), which upon cellular differentiation become mature fruiting bodies.

The diverse structures of *Myx. xanthus* form through the behaviours of vegetative cells, but only one of the four
multicellular behaviours – aggregating into fruiting bodies – is associated, to date, with a definitive change in cell type, as fruiting aggregation typically precedes sporulation. The other three well-characterized multicellular behaviours instead are utilized for nutrient acquisition through either colonization of new territory or degradation of prey. Spores are metabolically dormant, but transfer of spores to fresh medium results in germination and return to the vegetative cell type. Germination occurs at surprisingly low nutrient levels, as even fruiting medium used to induce sporulation (Hagen et al., 1978) can suffice for germination. Furthermore, all four of the above-mentioned multicellular behaviours are often observed simultaneously when *Myx. xanthus* contacts suitable prey (Fig. 2g).

It is crucial, therefore, to examine why all of these behaviours can occur simultaneously in close proximity. Although fruiting-body formation is often referred to as a developmental programme, models of population-wide, uniform decision making (Kroos, 1990; Giglio et al., 2011) seem insufficient at explaining the role of multicellular structures observed during predatory behaviour. Indeed, many bacterial species will convert a fraction of the population to dormant cells or spores under low nutrient stress (Berleman & Bauer, 2004; Bacun-Druzina et al., 2007; Lindsay et al., 2006), yet *Myx. xanthus* fruiting bodies have an unmatched level of cell-type segregation. The formation of aggregates prior to sporulation is an intriguing aspect of *Myx. xanthus* behaviour, in particular since, during predation, aggregation does not require starvation, and can also occur as a response to subtle decreases (e.g. a twofold decrease) in prey availability (Berleman & Kirby, 2007). This prompted us to consider all multicellular behaviours in terms of how they may contribute to nutrient access to promote a predatory life cycle.

### Scouting behaviour

All forms of multicellular behaviour in *Myx. xanthus* require motile cells that can direct cell movement (Kaimer & Zusman, 2013). *Myx. xanthus* has two genetically distinct motility systems: S-motility and A-motility (Kaiser, 1979; Li et al., 2003; Nan et al., 2011; Wartel et al., 2013). Both A- and S-motility systems utilize multicellular behaviour and shared resources: trails, EPS and transferable outer-membrane proteins such as CgLB, Tgl and TraAB (Ducret et al., 2013). CgLB and Tgl can be shared among cells, possibly via OMV transport (Rodriguez & Spormann, 1999; Nudleman et al., 2005; Remis et al., 2014).

During surface colonization, a scouting pattern of trails that consist of uncharacterized polysaccharide and OMVs (Wartel et al., 2013; Remis et al., 2014) is formed by cells migrating at low cell density that provides a preferred path of travel for other cells to follow (Fig. 2c) (Wolgemuth et al., 2002). This low-cell-density behaviour of *Myx. xanthus* is a characteristic trait that results in a fuzzy appearance to colony edges on harder surfaces (Shi & Zusman, 1993). Scouting behaviour depends primarily on A-motility, which drives independent cell movement, but cell groups are also observed and, since cells leave a trail for neighbouring cells to utilize, even isolated cell movement benefits neighbours (Fig. 2c) (Kaiser, 2003). The TraAB system mediates the exchange of lipoproteins such as the Cgl proteins, and cglB strains are rescued by close proximity to CgLB-producing cells (Pathak et al., 2012; Pathak & Wall, 2012). In addition, *Myx. xanthus* strains deficient in proteins controlling A-motility also showed predatory deficiencies (Pham et al., 2005), suggesting that these cell-to-cell interactions may also impact predation. Other predators, such as *Bdellovibrio*, have also been observed to utilize A-motility homologues for predation (Lambert et al., 2011).

### Branching behaviour

At higher cell densities, cells coalesce into a branching morphology that facilitates the faster migration of larger cell groups (Fig. 2d) (Kaiser & Crosby, 1983; Berleman et al., 2011). Branching morphology occurs primarily as a result of S-motility, which has requirements of high cell density, high nutrient levels and cooperation (Berleman et al., 2011). At high surface wetness, only branching morphology is observed (Hillesland et al., 2007). Both EPS and the Tgl protein are shared between cells to promote branch morphology (Hu et al., 2012; Rodriguez-Soto & Kaiser, 1997). Comparatively, movement through S-motility is more rapid and more energy consuming than movement via A-motility (Hillesland & Velicer, 2005). Although it is convenient to separate these behaviours for genetic analyses, they can occur simultaneously and may function synergistically during predation, suggesting that *Myx. xanthus* uses these two morphologies to adapt to varying levels of prey and other nutrients (Hillesland & Velicer, 2005). In support of this, a recent study showed that both motility systems are crucial for regulating *Myx. xanthus* predation of the legume symbiont *Sinorhizobium meliloti*, indicating how important both motility behaviours are for successful predation (Pérez et al., 2014).

### Rippling behaviour

The presence of prey can dramatically alter the multicellular behaviour of *Myx. xanthus*. At low cell density, cells make prolonged contact with prey and stimulate reversals when needed to return to any remaining prey cells (McBride & Zusman, 1996). At high density, wave structures are formed during predation of other micro-organisms, as *Myx. xanthus* cells sweep back and forth across the available prey; this behaviour is referred to as rippling (Fig. 2e) (Berleman et al., 2006). On a cellular level, rippling is the frequent reversal of *Myx. xanthus* cells based upon predator–prey signalling (McBride & Zusman, 1996; Berleman et al., 2008) and predator–predator signals, such as the contact-dependent C signal (Welch & Kaiser,
2001). Cellular reversals serve to maintain cell–cell contact with prey, which is necessary for *Myx. xanthus* to lyse *E. coli* cells (McBride & Zusman, 1996). Predatory rippling is dependent on the product of *csgA*, which encodes a short-chain alcohol dehydrogenase that may act on lysophosphatidylethanolamine lipids for either cell recognition or movement (Avadhani *et al.*, 2006). On a molecular level, cell reversals are triggered by a pole-to-pole change in motility proteins, such as FrzZ, MglB and MglA, which then causes the *Myx. xanthus* cell to reverse direction (Eckhert *et al.*, 2014; Kaimer & Zusman, 2013; Keilberg & Søgaard-Andersen, 2014).

Rippling is not only a characteristic form of multicellular behaviour that occurs during predation, but also a strategy to increase predatory efficiency. During rippling, waves of cells form perpendicular to the direction of migration and a rapid, directed migration through prey colonies occurs called predataxis. As a group, cells can direct movement through prey with little random drift away from the prey colony (Berleman *et al.*, 2008; Zhang *et al.*, 2012). Rippling behaviour serves to minimize predator–predator contact and maximize predator–prey contact, a process that is expected to promote contact-dependent prey-cells lysis. The presence of prey cells, or prey macromolecules such as *E. coli* peptidoglycan, nucleic acids and proteins, induces rippling in *Myx. xanthus* colonies, suggesting that rippling acts as both a predatory and scavenging action for *Myx. xanthus* (Berleman *et al.*, 2006). It has further been shown that the harsh treatment of *Myx. xanthus* cells in starvation assays or mono-culture lab conditions that cause cell death will also induce rippling (Berleman *et al.*, 2006). These observations prompt caution when examining proteinaceous cell–cell signals that could also stimulate predatory rippling or interpreting experiments on spontaneous rippling when cell death and subsequent feeding have not been monitored.

In large populations and in the absence of prey, *Myx. xanthus* cells reverse with low frequency, moving across large areas and gradually changing directions in large arcs through cell bending and surface perturbations (Shi *et al.*, 1996; Berleman *et al.*, 2008). Conversely, in the presence of prey, *Myx. xanthus* cell behaviour is observed with frequent reversals (Berleman *et al.*, 2008). The frequency of reversals is directly proportional to prey-cell density, indicating that when large amounts of prey are available cells reverse often and become ‘trapped’ within the area that contains prey. This is similar to the ability of *E. coli* to remain in areas with chemoattractant by tumbling whenever a decrease in attractant is detected (Berg & Brown, 1972). In addition, strains defective in regulating reversals, such as *frz* mutants, show defects in predation both as individuals and as groups (Berleman *et al.*, 2008; McBride & Zusman, 1996). The predatory defects of motility mutants is likely due in part to an inability to access prey with direct cell–cell contact: individual *frz* mutants lack the ability to reverse in order to maintain contact with prey. In larger populations, the lack of cell reversals causes defective rippling wave structures and eliminates rapid expansion through prey colonies (Berleman *et al.*, 2008). Thus, the regulation of cell reversals is essential for multicellular rippling behaviour, and provides a mechanism for *Myx. xanthus* to access and then remain in the vicinity of an insoluble nutrient source such as prey.

### Fruiting behaviour

Fruiting is the result of the congregation of >10⁶ vegetative cells into aggregates that become stable, dormant fruiting bodies after cell differentiation into spores (Fig. 2f). Under strict lab conditions of high cell density and starvation, *Myx. xanthus* will form spore-filled fruiting bodies through a predictable programme of morphological changes and cell development (Kroos, 1990; Giglio *et al.*, 2011). During predatory interactions with *E. coli*, *Myx. xanthus* will also form fruiting bodies in a predictable manner that includes response to environmental cues, rather than following a strict programme (Fig. 2g) (Berleman & Kirby, 2007). Aggregates form predictably at the borders of an inoculum of *E. coli*, regardless of the ambient nutrient levels present. While fruiting aggregation occurs across a wide range of nutrient concentrations, cells within aggregates only transition to spores at very low nutrient levels. Fruiting-body maturation in *Myx. xanthus* is one of several examples of population bifurcation in bacteria (Kearns & Losick, 2005). Fruiting-body formation allows part of the population of *Myx. xanthus* to go dormant, while peripheral rods remain active in a persister-like state (O’Connor & Zusman, 1991). Since fruiting bodies also form during growth of *Myx. xanthus* on suitable prey, rippling and aggregation have been proposed to work together as a mechanism for population management that is critical to the predatory life cycle of *Myx. xanthus* (Berleman & Kirby, 2009). In support of this, *relA* and *asgD* mutants, which are blocked at an early step of fruiting behaviour under mono-culture conditions, form aggregates similar to wild-type in the presence of *E. coli* prey, but remain defective in sporulation (Berleman & Kirby, 2007). Conversely, when *Myx. xanthus* encounters an increase in prey levels, fruiting-body aggregation is inhibited.

The transition from a *Myx. xanthus* rod shape with dimensions of 5 µm length and 0.8 µm diameter to a 1–2 µm³ coccus shape (spore) can occur extremely fast – in 4 h or less in a glycerol-induced model of sporulation (Müller *et al.*, 2012). To date, no time-based model of sporulation within a fruiting body has been developed. This change requires reorganization of the shape-maintaining cytoskeletal protein, MreB, as well as activity of *nfs* and *exo* genes (Müller *et al.*, 2012). Specifically, the Agl machinery is responsible for rotating the Nfs protein complex around the cell envelope during sporulation (Wartel *et al.*, 2013). Shape change is followed by production of a thick outer wall consisting of the spore coat protein S, in addition to glycocomaminoglycan, N-acetylgalactosamine, glucose and glycine (Inouye *et al.*, 1981; Kottel *et al.*, 2006).
1975; Holkenbrink et al., 2014). Together, these changes allow spores to survive harsh environmental conditions in a dormant state.

**Predatory life cycle**

It is unusual to think of bacteria as predators, but a full understanding of *Myx. xanthus* behaviour and development requires us to do so. Fig. 3 shows the life cycle of *Myx. xanthus*, indicating how all of the morphological structures discussed above relate to promoting growth as a predator. Vegetative cells (Fig. 3a) use scouting (Fig. 3b) and branching behaviour (Fig. 3c) to access new territory. Contact with prey cells stimulates reversals that cause individual *Myx. xanthus* cells to become trapped in micro-colonies of prey until pre-cell lysis is complete (Fig. 3d). At higher cell densities, contact with prey yields rippling behaviour that allows rapid, directed migration through prey colonies (Fig. 3e). During predation, sudden decreases in prey availability stimulate fruiting aggregation (Fig. 3f), likely through the same mechanism that causes individual *Myx. xanthus* cells to remain trapped in prey micro-colonies. Prolonged starvation causes cells within aggregates to trigger cell development to spores and become mature fruiting bodies (Fig. 3g), while cells outside of aggregates maintain rippling, branching or scouting behaviour. There is often a stark contrast between rippling behaviour on one side of aggregates and colonizing behaviour on the other (Fig. 2). Fruiting bodies can remain dormant, allowing for spores (Fig. 3h) to wait out long periods of nutrient deprivation, before beginning the cycle anew by germinating into vegetative rods (Fig. 3a).

Many bacterial species undergo cell development to a dormant cell or spore under low nutrient stress (Berleman & Bauer, 2004; Bacun-Druzina et al., 2007; Lindsay et al., 2006), but most bacteria lack the morphological complexity of *Myx. xanthus* or the myxobacteria in general. A reasonable hypothesis then is that multicellular behaviours in *Myx. xanthus* occur to support predation and growth on large, insoluble macromolecules. In support of this, there are a number of genetic correlations between multicellular behaviour and predation. Two bacterial enhancer-binding proteins (eEBPs), MXAN4899 and HsfA, participate in control of both processes (Jelsbak et al., 2005; Volz et al., 2012). Alone, MXAN4899 acts as both an inhibitor and an activator of secondary metabolites such as myxovirescin and Dkxanthen; MXAN4899 regulates A-motility and was additionally found to be critical for fruiting-body formation (Jelsbak et al., 2005; Volz et al., 2012). MXAN4899 also regulates type IV pilus formation by regulation of *pilA* (Volz et al., 2012). Similarly, the eEBP HsfA regulates fruiting-body formation and co-regulates S-motility with MXAN4899. HsfA and MXAN4899 form a hetero-oligomeric complex that controls S-motility by regulating DNA expression of motility proteins such as DkxB and DkxCC. As mentioned earlier, S-motility has recently been hypothesized to be crucial for predation (Pérez et al., 2014).

Another genetic correlation between fruiting and predation involves the *groEL1* and *groEL2* genes, which encode chaperone proteins. If both *groEL1* and *groEL2* are deleted, lethality occurs; however, a deletion of only one of the *groEL* duplicates did not reduce *Myx. xanthus* viability (Li et al., 2010). The GroEL1 protein has been shown to be a vital heat-shock protein that is also critical for growth and development: *groEL1* deletion causes a delay and reduction in fruiting-body formation, but no deficiency in predation. Meanwhile, deletion of *groEL2* caused predatory deficiency (Li et al., 2010). Wang et al. (2014) further found that *groEL2* is needed for synthesis of myxovirescin, and that exogenous addition of myxovirescin enabled *groEL2* mutants to kill *E. coli*. Interestingly, both *GroEL1* and *GroEL2* were found in high abundance in OMVs, suggesting that both copies of GroEL play a role in maintaining the structure of extracellular proteins that could be involved in coordinating multicellular processes such as predation and fruiting aggregation (Berleman et al., 2014).

Additionally, *csgA*, *sdeK* and three *asg* genes (essential for various aspects of fruiting-body formation under starvation conditions) are needed for predation (Pham et al., 2005; Pathak et al., 2012). The involvement of *csgA* in predation is peculiar as it is not expressed when cells are grown in high-nutrient liquid broth (Kim & Kaiser, 1990). Further experiments are needed to determine whether *csgA* is induced by prey or macromolecules on surfaces. Pham et al. (2005) observed that both wild-type *Myx. xanthus*, and *csgA*, *asgA* and *sdeK* mutants, lysed *Serratia marcescens*, regardless of whether the agar was rich or minimal medium. This implies that, in some cases, pathways important to predation are activated if prey is present regardless of the amount of ambient nutrients available in the environment (Pham et al., 2005). Furthermore, this suggests that the preferred source of nutrition for *Myx. xanthus* may be from prey macromolecules.

**Conclusion**

The fruiting bodies formed by myxobacteria are some of the most complex and fascinating morphological structures observed in bacteria. Mature fruiting bodies may provide several benefits, such as pronounced height and colouration for distribution, or serve as a source of an ‘instant swarm’ of high cell density (Rosenberg et al., 1977), such that *Myx. xanthus* cells can germinate together and maintain a cohesive pack. But for us to arrive at a more thorough understanding of this fascinating multicellular behaviour, we must continue to examine the unique aspects of how *Myx. xanthus* grows, in addition to how it starves. In addition to the long-term benefits, fruiting behaviour also serves an immediate benefit after a decrease in prey availability: reduction of the local population of active *Myx. xanthus* cells (Berleman et al., 2007). Scouting, branching and rippling behaviours are also prominent forms of multicellular behaviour for this species, which
each serve to increase access to nutrients for growth and predation. Fruiting aggregation therefore plays an important role in the predatory life cycle by preventing overpopulation, and maintaining an efficient balance of predator and prey. It will require further study to determine whether the tremendous diversity of multicellular structures in the myxobacteria family has evolved as a general coupling to the peculiar lifestyle of scavenging nutrients through lytic, proteolytic and cellulolytic processes. To put this in a broader perspective, Myx. xanthus aggregates, while impressive in their formation, are often smaller (<0.1 mm height) than typical bacterial colonies (~0.5 mm height). From this perspective, the ability of Myx. xanthus to form flat, spreading colonies with thin layers of cells in scouting, branching and rippling morphologies is as distinctive as its ability to form aggregates.

Another distinctive attribute is the novel mechanism of predation employed by Myx. xanthus, which involves a combination of antibiotic secondary metabolites, hydrolytic enzymes and subcellular extracellular vesicles that work together to promote contact-dependent prey-cell lysis. The expansive genomes of the myxobacteria likely serve both as a reservoir of biochemical potential and as a practical scaffold for synthetic biology of complex metabolites. In addition to the biochemical potential, the survival of E. coli and other potential pathogens in the environment is a public-health concern (van Elsas et al., 2011), and Myx. xanthus thus plays an intriguing role as a natural biological-control agent. Furthermore, the ability of Myx. xanthus to perform gliding motility may allow it to access otherwise inaccessible prey sources (Lueders et al., 2006). Yet, the usefulness of myxobacteria to prevent the control of pests remains largely unexplored. As we gain a better understanding of the complex community structures formed by microbes, it behooves us to look deeper into population dynamics and the impact of microbial predators for just this reason.

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