Interactions Between Cells and Pre-Existing Slime via Elasticotaxis

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Abstract

Myxobacteria have the ability to move via elasticotaxis: cells follow lines of tension and compression in their agar support instead of swarming radially. The polysaccharide chains of agarose in agar become oriented by stretching or compressing a slab of agar. Myxobacterial cells push themselves along by secreting capsular polysaccharide from their back end, and the secreted polysaccharide is left behind as a slime trail. We propose that cells use elasticotaxis combined with reversal to align with and eventually glide along the slime trails produced by other cells. As the new slime being secreted by a cell crosses a slime trail left by another cell, interaction with the oriented polysaccharide chains of the trail causes the cell to rotate its long axis a bit in the direction of the trail. Having crossed the slime trail the cell will eventually reverse and re-cross the slime trail in the other direction, rotating a bit more. Re-crossing the slime trail will continue until the cell and its newly secreted polysaccharide are perfectly aligned maximizing the strength of the elasticotactic interaction. A model was created to test how long such alignment should take and possibly to calculate the elasticotactic force acting on the cell. Experimentally velocities of cells creating slime and gliding on already existing slime were gathered in order to calibrate the model.

Introduction

Myxobacteria

Myxobacteria is a naturally occurring, common bacteria that can be found in top soil around the world. They have been nicknamed the wolves of the bacteria world because they surround prey cells before devouring them and like wolves they travel in packs, swarms. This swarming is characterized by a coordinated motion of cells at high densities usually resulting in a radial movement outward from the point of inoculum when on a flat surface. Swarming bacteria is not defined by Myxobacteria; there are many types of bacteria that swarm: hyper-flagellated bacteria, Bacteriodetes, Flavobacteria, and Bacteriodes. Hyper-flagellated bacteria fall under swimming bacteria but the rest of the swarming bacteria are all gliding bacteria. Swimming bacteria are characterized by using flagella to swim in a liquid encapsulation; however understanding how bacteria glide is just beginning to finally be understood. Swarming, gliding bacteria have certain commonalities. First, for swarming to occur the cells have to be packed at high densities. The individual cells tend to be elongated with flexible bodies and due to their shape they usually move bi-directionally; periodically the cells reverse not by executing a U-turn, even with a flexible body this type of a movement from gliding cells is highly unusual, but by simply stopping and then, without any kind of turn, moving in the direction that opposes their previous movement. Also, the swarms are encased in a polysaccharide slime produced by the swarming bacteria. Finally, swarming, gliding bacteria are gram-negative and do not require chemotaxis, following chemical gradients, to move.[1]

Myxobacteria has certain traits that makes it very appealing to study when compared to other gliding bacterias. Since the cells are often found in top soil they can handle life on the surface whether that is soil or agar; they rapidly swarm meaning that studying their progression takes a shorter amount of time and their movements can be more obvious. Perhaps Myxobacterias most im-

portant aspect is that it behaves stably unlike or at least more so than other swarming bacteria. If we can understand how each aspect of Myxobacteria helps it to swarm we can hope to understand what is necessary for general swarming.[1]

Myxococcus xanthus

When choosing a specific strain of Myxobacteria to study there are certain things that make Myxococcus xanthus attractive. Much of the individual cells structure and how it moves is known making the isolation of unknown factors easier and many swarming mutants have been identified and tracked giving us a thorough knowledge of M. xanthus mutants as well as wild type. This study will in fact focus on a mutant. M. xanthus holds true to swarming bacteria with an elongated body, on average 5 microns long and .5 microns wide. It has been experimentally found that the average velocity of individual cells tends to be 4 microns/min, this includes not only the wild type cells but holds true as well for the mutants. They are able to move using two types of locomotion: adventurous- and social-motility.[1] See Fig.1.

On the head of the cell is the Social engine (S engine) made up of type IV pilus. These pilus reach out in front of the cell and grab on to the fibril networks between cells in front of them, thin polysaccharide chains that form on the surface of cells and create short bridges between cells when cells are close together. Once fully extended, pilus then retract back into the original cells body. This action not only pulls the cell forward but orients it in the direction of the cells in front of the original cell, helping to increase the order of the swarm. On the rear of the cell is the Adventurous engine (A engine), made up of a series of pores that emit a polysaccharide slime. This is considered adventurous locomotion because the slime blindly propels the cell forward. When the cell reverses, occurring on average every 8.8 +/- 2.2 minutes, it comes to a halt for approximately one minute and then the end that was previously emitting slime begins to lead the cell using the social motility.[1] The slime emitting pores are

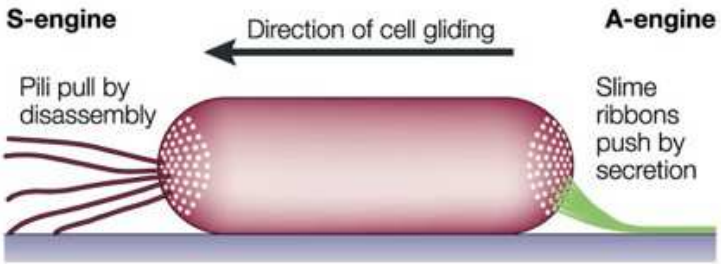

Figure 1: Diagram of an M. xanthus cell.

found elsewhere on the cell body, not just the poles, though not in as high of density; Wolgemuth et. al. found up to 250 pores at each end of the cell , while "in-between only a few scattered pores were found."[3] (Fig. 2)

Little is known about the slime that these cells emit and are encapsulated in. It is currently believed that the slime is not made up of a single polysaccharide and that the combination is possibly dependent on cell density and possibly

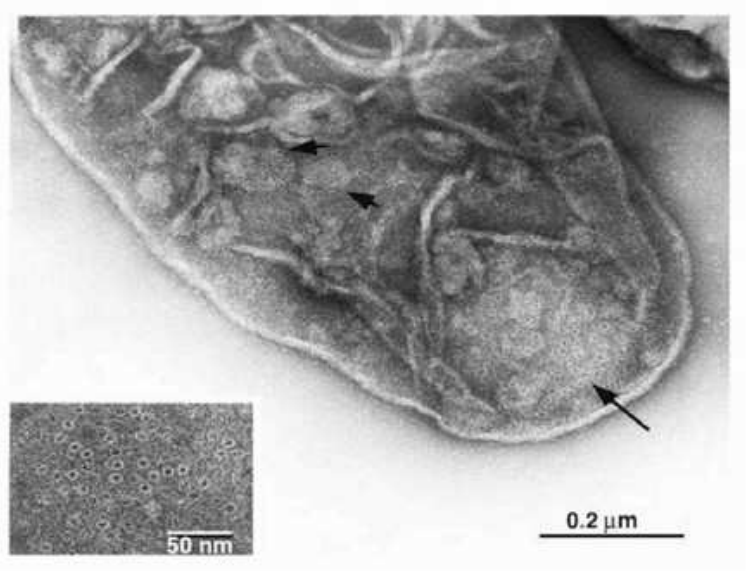

Figure 2: A magnified cell with visible slime nozzles. Compare the number on the end to over the rest of the body.

other factors. Polysaccharide in gels can take on many structures based on what the polysaccharide is for example a double helix.[4] The current investigation assumes that this is a rigid structure where the polysaccharide only fit together and can build onto each other in a certain way, like Lego blocks. The cells actually have a tendency to ride on top of the slime formed by a cell who had

been there before them and then follow this trail.

Elasticotaxis

Elasticotaxis was first realized in the early 1940s and was at that time defined as cells following lines of tension and compression. Cells normally spread radially outward from the point of inoculum. However, if there is a tension or compression acting on the agar they begin to swarm with a directionality as can be seen in fig. 3. The first experiments involved placing a rod underneath

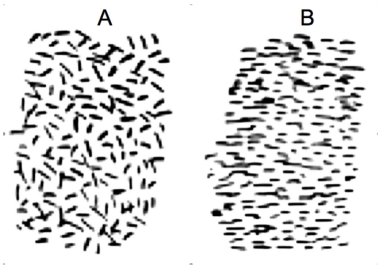

Figure 3: A) Cells normally swarming. B) Cells swarming along a line of tension.

a portion of the agar to create these tension lines (Fig. 4); they also ran an experiment with the dish sitting on its side to make sure these effects were not from gravity. When a small marble or the like is placed on the agar the cells can follow the tension lines to surround it; originally it was thought this surrounding mechanism, used to surround prey, was the only natural presentation of Elasticotaxis.[2] It is known to be exclusively an A motility phenomenon, S motility can be present but it in no way aides to elasticotaxis.[3] Now we know that these tension lines translate to long aligned ribbons of the polysaccharide making up the agar or surface.

Figure 4: The set up of the original experiment.

Our Hypothesis

We believe that elasticotaxis is also used for cells aligning themselves with pre-existing slime trail and then subsequently following these pre-existing slime trails, which are made up of these long aligned polysaccharide chains. Each time the cell crosses over the slime trail the elasticotaxis pulls it slightly more into alignment and this combined with the cell eventually reversing and running back into the same slime trail will eventually cause it to be completely aligned with the slime trail and it will travel back and forth on top of the slime.

Methods

To determine how different aspects of the cell and the slime contribute to the elasticotaxis interaction a model was created of a single cell crossing over a pre-existing slime trail. The model is made up of three key pieces. The first is the placement of the slime nozzles along the cell (Fig. 5). The slime nozzles are placed evenly over the center of the cells body with a blank space at the tail end of the cell; here it was assumed the slime nozzles would be creating slime behind the cell, creating a trail, not along the cell‘s body helping to realign with

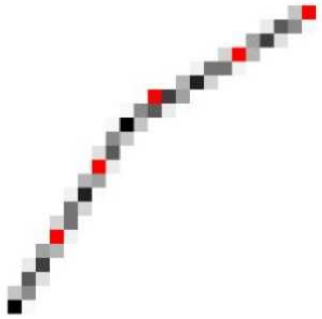

Figure 5: Location of the slime nozzles along the simulated cell.

a pre-existing slime trail.

The second is the cell movement. Using the x and y co-ordinates of the head position and the tail position a tail to head vector and angle is calculated and each point along the cell (the head point, mid point, and tail point) are moved one unit along this vector (Fig. 6). Each cell also has an internal reversal clock and with each time step taken the clock moves up one count; when the count reaches the reversal time the cell stops and rests for one minute and then the tail point and head point are switched.

The third is the readjustment of the cell when it comes in contact with the pre-existing slime trail. If one of the slime nozzles is lying on the slime trail then that nozzle is moved either upwards or downwards, to continue with the pre-existing momentum of the cell, into the next locked position. The locked position is the place where the slime on the cell and the trail can lock together, this locking interval is another aspect of the code that we vary. Since the locking positions are at set intervals when a cell nozzle is moved it is not always moved the same distance but to arrive at a certain locking interval. After the

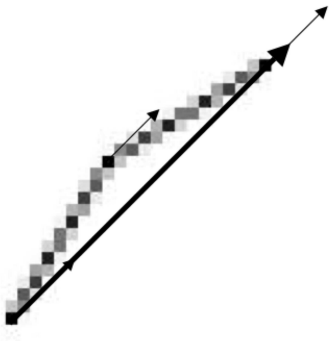

Figure 6: The vector each node of the cell is moved along when not interacting with the slime trail.

point along the cell has been moved, that half of the cell is readjusted to line up with it. If this causes the midangle to be greater than thirty degrees or less than negative thirty degrees, the limit set of how much the cell can bend, the midangle is set to the limit and the tail point is readjusted.

To put these all together first the cell is initialized, it has a length of 30 units and width of three units; its head node is placed at some random x and y. The front angle of the cell is a random angle between -89 and 89 degrees and a midangle of -30 to 30 degrees (Fig. 7). The x and y co-ordinates of the mid node and tail node are then determined from these angles. A random slime x value is determined somewhere within the area, this translates to a pre-existing slime trail infinitely in the y direction and from the random slime x to x + 3. The locations of the nozzles are then determined and if a nozzle lies in this x region it is then adjusted. Either once the nodes have been adjusted or it has been determined that no node is lying on the slime trail each point of the cell is moved one step.

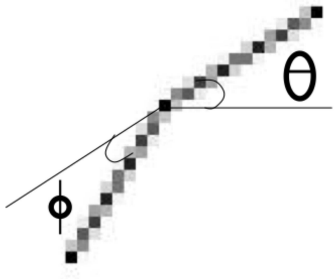

Figure 7: Angles used in initializing the cell.

To determine that the model is keeping with the experimental observation we can compare the model cell velocity with experimental velocity. Using MTrackJ a plug-in of ImageJ we were able to track several cells from an avi movie and determine their velocities when either on a slime trail or creating a slime trail. Challenges exist with this program though. The film goes in and out of focus making it difficult to determine exactly where the head of the cell is located (figure 5), also the quality of the image makes it difficult to determine whether the cell is on or creating a slime trail. We can compare if the difference in velocity between the two modes is the same between the experiment and the model.

Results

Using the tracking system relative velocities were calculated for cells creating new slime trails and gliding on pre-existing ones. Relative here is defined as pixels per frame; a true velocity was not needed since the difference is the only thing needed to calibrate the model. When cells are creating a new slime trail their relative velocity is 2.16 +/- .323 pixels/frame compared to 2.83 +/- .708

pixels/frame when gliding on a pre-existing slime trail. This velocity difference is expected either because the slime can be thought of as a lubricant for the cell to move along or perhaps by an elasticotactic force pulling the cell along the polysaccharide chains. The model itself is not yet complete. The first two pieces and the initialization parts of the model are completed while the interaction with slime still has bugs that are being worked out.

Future Work

Once the model is completed we plan to test how several attributes of the model affect the time necessary to completely align with the pre-existing slime trail; these attributes are original approach angle, number of slime nozzles along the cell body, and the repetitivety of the polysaccharide chain. We expect that the greater the original approach angle the less time it will take for full alignment since the cell is already greater aligned. Also, the more slime nozzles along the cells body we expect for it to align quicker since there are more times that the cell will be adjusted when crossing the slime trail. Finally, the more repetitive the polysaccharide chain is the slower we would expect the cell to completely align because the smaller the distances between lock in positions the cell will be adjusted a smaller amount overall.

Once this has been completed several things could be added to the model to make it more realistic and complex. The first of these would be to have the cell create a new slime trail as it moves. I see this as the most important complexity to be added. After this multiple cells could be added, this not only adds the obvious complexity but also allows for wild type cells since social motility is not an option for a cell without other cells to interact with.

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References

[1] Wu, Y. (2009). Behavioral Principles of Swarming Bacteria: A dissertaion.

[2] Stanier, R.Y. (1942). A Note On Elasticotaxis in Myxobacteria. *Journal of Bacteriology, 44(4),* 405-412.

[3] Wolgemuth, C., Hoiczyk, E., Kaiser, D., & Oster G. (2002). How Myxobacteria Glide. *Current Biology, 12,* 369-377.

[4] Rees, D. A. & Welsh, E. J. (1977). Secondary and Tertiary Structure of Polysaccharides in Solutions and Gels. *Angew. Chem. Int. Ed. Engl., 16,* 214-224.