Peer-Review Record:

A Model of Filamentous Cyanobacteria Leading to Reticulate Pattern Formation

Carlos Tamulonis and Jaap Kaandorp

Reviewer 1: Anonymous
Reviewer 2: Anonymous
Editor: Robert Haselkorn (The University of Chicago, USA) and John C. Meeks (University of California, USA), Guest Editors of Special Issue "Cyanobacteria: Ecology, Physiology and Genetics"

First Round of Evaluation

Round 1: Reviewer 1 Report

The authors present a model for the gliding motility of filamentous cyanobacteria, which involves elastic, gliding, and contact/cohesion forces. Simulations using the model address the observation of reticulate patterns in experiments with filamentous cyanobacteria. The authors find that for a certain parameter regime, they observe in their simulations patterns with characteristics similar to the characteristics of the experimental patterns. They also make quantitative measurements to describe the behaviour of the model trichomes.

The model is successful in producing local alignment of filaments and some sharp bends—both characteristics of the experimental system. They also explore behaviour in much of the available parameter space. However, their parameterization of the bending modulus is not acceptable because it leads to an unreasonably large (0.5 metre) persistence length for a bacterial trichome. As a result, I do not recommend that this paper should be published in its current form. While the authors might be able to find a similar phenomenological agreement with more reasonable parameters, it will change all of their results and amounts to a substantial revision.

Critical issue:

(1) The literature seems to indicate a low value of the bending modulus (alpha) that corresponds to a persistence length of 500 microns. The authors instead use alpha $= 2 \times 10^{-21}$ Nm^2, which is 1000× larger. With this value, the persistence length $=$ alpha/(k$_{bT}$) $= 0.5$ metres. This seems orders of magnitude too large, especially in the face of the results of Boal and Ng [44]. The authors need to be in a physical regime of parameter space in order to confront their results with observed patterns. Otherwise, they are just fitting a non-linear phenomenon with a (potentially inappropriate) non-linear model— in this case agreement does not provide firm insight into the physical system.

Other issues:

(2) In Section 3.3 the authors describe how an increase in system size indicated that one of the results presented earlier in the paper (at beta $= 0.5$) was an artifact of a smaller system size. This brings into question all results at the smaller system size, potentially including all the quantitative results of the paper. The authors should be able to argue that the other results will not depend on system size. The correlation length in Figure 3b is by eye more than the system size, indicating a potential qualitative change with increasing system size (which the authors find). The correlation length in other figures is less than the system size, indicating that they may be fine. The authors need to add some of this discussion to reassure the reader.

(3) The authors find that results at beta $= 0$ are most similar to the experimental reticulate patterns. In the force model, there are three forces: elastic, gliding, and contact/cohesion. The contact/cohesion force is presented in equation 12, and is proportional to epsilon. After Equation 23, the authors define beta $=$ epsilon/(zeta·v$_0$), implying that there will be no contact/cohesion forces when beta $= 0$. However, in the results of Figures 3, 4, and 5 the results for beta $= 0$ clearly show interactions between the filaments. The fourth paragraph of section 2.9 describes a hard-core interaction, which appears to be the source of the patterns when beta $= 0$. This hard-core interaction should be mentioned after Equation 12, since it is not simply an implementation issue but the dominant interaction with beta $= 0$.

(4) All the results depend on the cohesion forces, which are described by a Lennard–Jones function in Equation 12, in Section 2.5. However, this way of approximating cohesion is not well justified, and in Section 2.9 (several sections later) the authors mention that in reality the attractive force "does not really exist" and that the trichomes cohere after contact. The authors are using an LJ interaction to approximate contact adhesion. They should say why, and address how good of an approximation they expect this to be.

Other minor issues:

(5) In Section 2.4, it is stated that a stochastic process determines P, but no details are given. There should be some description, if brief, for this process. What distribution is used?

(6) The authors mention in the discussion that they may get stronger results for a deeper system, or for trichomes that may only glide freely near a surface or other trichome. In terms of the understanding gained from the modelling/simulations, how would these lead to qualitative changes in the observed patterns?

(7) The viscosity mu = 1 Pa s is used. This value is approximately 1000 times higher than the viscosity of water, which is the environment of filamentous cyanobacteria. A reference is given, but there should also be a brief justification in the text.

(8) The authors do not include a description of the reaction force for the top and bottom planes. A brief explanation would be appropriate.

(9) None of the references at the end of the paper contain journal volume or page numbers. They should.

(10) In general, and especially in the introduction, many statements are made without accompanying references.

(11) There are supplemental movies attached. These are not referred to in the text.

Round 1: Author Response to Reviewer 1

We would like to thank the reviewer for taking the time to do a thorough review of our paper. In response to the reviewers' comments:

(1) We initially attempted to use the bending modulus implied from Boal and Ng's study, however we found that during simulations this value seemed to be too low. The trichomes were completely flaccid and would collapse into a heap as soon as one collided with another trichome, unlike the smooth sinuous shape filamentous cyanobacteria often display.

In the paper we justify (at some length) the bending modulus we used, which we extrapolated from various sources, and explain why we believe the value in Boal and Ng's study is likely too low. The reviewer did not question the logic or the sources used to arrive at this value, but nevertheless seemed surprised at the result. The reviewer claims that the parameter falls outside of the physical regime, but doesn't offer a justification other than that the implied persistence length "seems too large". However, the large persistence length simply implies that the cyanobacteria are stiff enough that they are largely unaffected by thermal fluctuations. Despite this, the trichomes in our simulations are still quite flexible in practice, as can be seen in Figure 4. Even if the virtual trichomes are somewhat too stiff, we do not feel that this would have a major impact on the results.

(2) We added the following statement to the end of Section 3.3:

"The simulation results of the previous section for $\beta > 0.5$ are unlikely to be affected by the domain size, however, since the features in those simulations are on a much smaller scale than the domain size and are also more chaotic, as seen in the correlation length and the global alignment (Figure 5a,d)."

(3) We added the following clarification to the end of the non-dimensionalization section:

"In the subsequent sections, simulations for $\beta = 0$ use a modified interaction force such that there is no cohesion, but the hard core repulsion is maintained, *i.e.*, $F_0^c = 0$ for $h \geq 0$, otherwise $F_0^c = F^c$ with $\beta = 0.125$."

(4) We added two references to justify using the Lennard–Jones potential.

(5) We added the following clarification: "This is simulated by generating a pseudo-random number x following a uniform distribution $X \sim U(0,1)$, and reversing the gliding direction if $x < \omega \cdot \Delta t$.

(6) We amended the following sentences of the discussion:

"These differences may be due to the fact that we use a shallow domain (7.5 microns) to reduce the simulation run time, which may cause the pattern to be "squashed" as the domain ceiling prevents ridges from growing vertically"

"Restricting the virtual trichomes such that they may only glide when in contact with the substratum or another trichome may promote further aggregation of the trichomes from streams into ridges, as a trichome would be less likely to successfully break away from a stream because it would lose much of its propulsive force as it lost contact with neighboring trichomes and/or the substratum."

(7) We added the following sentence to Section 2.4:

"Gliding requires contact with some (semi-)solid substrate in order to provide a reaction force to the gliding mechanism. We assume that the trichomes are immersed in highly viscous medium that allows them to glide freely in all directions. This medium could consist of the EPS the trichomes produce copiusly when gliding [Hoiczyk2000]."

(8) The exact force is now given.

(9) The bibliography now has volume and page numbers.

(10) The reviewer did not provide any examples where he/she felt a reference was warranted. We cited 55 sources, which we feel offers reasonable background and justification for our assumptions.

(11) References to the movies were added to the results section.

Round 1: Reviewer 2 Report

The authors develop and analyze a model for the dynamics of filamentous bacteria gliding in two dimensions. Qualitative comparisons are made to the fossil record and modern experiments.

I have a few comments, suggestions, and questions. I do not feel that any of these points demand major changes to the manuscript, which is generally fine in the present form.

(1) Notation:

- L is used both for the length of the trichome Nl and in the non-dimensionalization as $\sqrt{\alpha/v_0\zeta}$.

- I have never seen $\langle\cdot,\cdot\rangle$ used to define an edge. It looks like an inner product. Is this standard?

- In Equations (5)–(8), is there any difference between subscripts and superscripts?
- It is confusing that κb is a vector that is not simply related to b. I initially thought that κ was a new parameter. Similarly, it is odd that e is a vector and [e] is a matrix. Why not use the Levi–Civita tensor?

- $\hat{h}$ looks like a unit vector parallel to h. Is there a reason that h only has a $\hat{\ }$ in Equation (12)?

(2) Walter (ref 7) describes the formation of "clumps" which are very similar to the structures describes by Shepard. Shepard and Sumner provide better images (Walter only shows sketches), but it might be worth mentioning that the phenomenon is more general. As I recall, Petroff *et al.* (ref 13) show similar patterns.

(3) In the force balance, the drag coeffcient is assumed to be the viscosity of water. Since the cells glide over a surface and next to other cells, it would seem that cell–cell friction and cell–surface friction might be at least as important as hydrodynamic drag. What about the viscosity of the material used in the "slime jet"?

(4) Following Equation (13), it is stated that "The interpolation ensures the net force and net torque of the interaction forces are null". This confuses me so I suspect that I have misunderstood something. In an overdamped system, such as this one, all forces and torques are balanced by drag on the cell. Is this all that is happening in this derivation? The text reads like certain forces and torques are assumed to vanish. Are these forces and torques introduced by the interpolation and the parameterization is chosen to cancel artifacts? Or, are there internal forces and and torques acting on the vertices that cancel as a result of Newton's second law? Please clarify this point.

(5) In the estimation of the Young's modulus it is assumed that the bending resistance is due to the cell wall. I suspect that this is a very poor approximation, accounting for the two order of magnitude difference between the estimated and benchmark values of Y. It would seem that the resistance is controlled or at least influenced by additional factors such as protein expression (notably MreB) and osmotic pressure. If my intuition is wrong (I am not an expert on cell morphology), I would appreciate knowing why.

(6) If I understand correctly, there are six important length scales in the problem, the size of the domain $\sqrt{DH}$, the spacing between trichomes $\rho^{-\frac{1}{3}}$, $L = \sqrt{\dfrac{\alpha}{v_0 \zeta}}, l, \Theta$, and the trichome length $\mathcal{L} = Nl$ (also denoted L in the manuscript), giving five dimensionless numbers. However, in the non-dimensionalization, the authors state that there are only two parameters: ρ and $\gamma = Nl/\theta$.

Unless I am mistaken, there are additional parameters like l/L, Nl/D, and N that the authors hold fixed but may influence the details of the numerics.

(7) It seems a little odd to posit an attractive force in the derivation (Equation (12)) and then ignore/dismiss it in the numerics ("… in reality the attractive force does not really exist, trichomes only cohere after they have come into contact" page 12). Clearly, this is done to (quite reasonably) avoid modeling steric interactions with hard bodies. It was a little distracting to think that sticky adhesion was decaying like $(\Theta/h)^6$. Perhaps note after Equation (12) that the attractive force decreases to zero at a finite distance in the numerics.

(8) The parameter space is rather large, and only a small subspace is explored in the numerics. This is fine, but it leaves a lot of interesting questions un-addressed. For example, is the transition from broad streams (Figure 4a) to loops (Figure 4d) continuous or are there bifurcations/phase transitions?

 Can you coarse grain this model to make it analytically tractable? The basic phenomena suggest something like an active shear-thinning uid? This would make it easier to understand the bifurcations in the dynamics and may require fewer parameters. Just something to think about.

(9) In this model, the density of cells is assumed to be constant. However, the timescale of the simulations is comparable to the doubling time of many bacteria. Because cell density is found to

be important in determining the morphology of communities, I suspect that reproduction is important in understanding the patterns. This point might be worth addressing in the text.

Notably, the introduction of reproduction does not introduce another parameter. As I recall, these cyanobacteria reproduce (with doubling time τ) by adding cells to the filament and that filaments periodically break (rate k_+). Thus, the length of a filament is not a model parameter. Rather, length is determined dynamically as $N \sim \tau k_+$

(10) This project would benefit immeasurably from a quantitative comparison to experiments. I understand that this is beyond the scope of this paper, but I strongly encourage future work in this direction.

(11) In the introduction, this project is put into the context of stromatolites. In the discussion/conclusion, I would appreciate to hear the authors thoughts on what this model teaches us about stromatolites. In the current form I am left to conclude only that communities of filamentous bacteria can develop reticulated patterns similar to forms observed in the fossil record. This conclusion does not offer much more than Shepard's experimental observation of this result. Can we use the details of this model to, for example, estimate the cohesion β of ancient cells? Do these results provide any insight into the identity of stromatolite building cells. As far as I can tell, nothing in this model suggests that photosynthesis (much less oxygenic photosynthesis) is required to form these patterns. It seems that this model shows that polygonal patters are a generic feature of elongated gliding cells and is independent of metabolism. A quick google search produced this video of mat of the sulfur-oxidizing bacteria Beggiatoa spp. http://vimeo.com/57205513 (note: I have no idea of the details of these observations so any similarity may be spurious).

Round 1: Author Response to Reviewer 2

We would like to thank the reviewer for his/her thorough review of our manuscript and thoughtful comments. In response to the reviewers' comments:

(1) The symbol used to denote trichome length was changed to an uppercase lambda. The $\langle \cdot, \cdot \rangle$ is often used to denote a tuple in computer science, in this case a pair. The superscripts should have been subscripts. The notation used in Equations (5)–(8) was adopted from Bergou et al's paper. h-hat is in fact a unit vector parallel to h, and we've clarified the text to this point.

(2) We added a few sentences to the introduction that expand a bit on similar structures in the Petroff and Walter references.

(3) Actually, as Reviewer 1 noted, we use a viscosity that is 1000 times greater than that of water (following Wolgemuth *et al.* 2005), for the very reasons the reviewer mentioned.

(4) The statement has been clarified and is now: "The sum of the interaction's opposing forces and torques is zero, thereby ensuring Newton's third law is respected."

(5) Our estimate is simplistic, however we were unable to find a reliable measurement of this parameter in the literature, and so we resorted to some simple extrapolations.

(6) There are indeed additional parameters. We have added a complete list to Section 2.8. Many are explored to some extent in the results (beta, reversal frequency, domain dimensions, trichome

density), whereas most of the physical characteristics of the trichomes are known from published sources (diameter, length, gliding speed, bending modulus), and finally N was simply chosen so that trichomes had a "smooth" appearance.

(7) To avoid confusion on this point we have replaced this statement with the following: "We find that these errors are infrequent and acceptable as a trade-off for increased computational performance." We have also added a comment to Section 2.5 regarding the Lennard–Jones interaction: "The attractive force has a short range, and is practically zero at h = 2θ."

(8) It would be interesting to explore the approach the reviewer suggested; we have not yet explored more coarse grained models.

(9) Shepard *et al.* claim that the formation of the reticulate pattern occurs on a faster scale than cell growth and division, and so we did not consider this in our model. Including this might make the results more robust, however, and we added a comment to the discussion.

(10) We had plans to include a quantitative comparison, however we were not able obtain a good dataset for doing so. It would be interesting to replicate Shepard's experiments taking care to collect enough good quality images/movies for a quantitative analysis on the patterns.

(11) Replicating Shepard and Sumner's results was our primary objective for this paper, as we state in the discussion. In future work we would like to increase the scale of the simulations by adding more trichomes and a deeper domain to (hopefully) get more robust reticulate formation. We would also like to do a quantitative analysis on experimental data to in order to fit the model to the data. Finally, we would like to see whether this same minimal system plus photomovement is sufficient to produce the cone-shaped structures documented by Walter, Petroff and others. If successful, we could then link macroscopic features of similar stromatolites back to the parameters of the trichomes, as well as understand the contribution of each factor in the model to building these structures. We have added this explanation to the conclusions section.

Second Round of Evaluation

Round 2: Reviewer 1 Report

With the exception of their response to one key point, the authors' responses are satisfactory. Their suggested bending modulus value implies a very high persistence length of 0.5 metres. In their response, the authors mention that with the lower value from Boal and Ng the trichomes collapse into a heap, and so a larger bending modulus was necessary. They also argue that a very large persistence length implies that the cyanobacteria are stiff enough to largely be unaffected by thermal fluctuations.

The paper can be published once the authors more clearly confront their disagreement with the results of Boal and Ng. In particular:

(1) At the end of the discussion of the bending modulus (in Section 2.8, on page 11), the implied persistence length and the irrelevance of thermal fluctuations should be explicitly mentioned.

(2) In Section 4, the discussion section of the paper, the topic of the bending modulus is returned to for a paragraph. This paragraph needs to explicitly address why the measurements of Boal and Ng were not used, *i.e.*, why Boal and Ng misinterpreted their data. In particular the authors should suggest how the measurements are significant measurements to not use. In particular this paragraph needs to

suggest how the measurement should be done to properly test their suggested values for the bending modulus, and so resolve the disagreement with Boal and Ng.

Round 2: Author Response to Reviewer 1

We have addressed the reviwer's requests:

(1) We added the following sentence to the end of Section 2.8:

"This value corresponds to a persistence length of 0.49 m, which implies that the trichomes would be practically unaffected by thermal fluctuations."

(2) We added the following paragraph to the discussion:

"Many of the model parameters, such as trichome length, diameter, gliding speed, reversal frequency, *etc.* are easily measured and are well known. However, the bending modulus is more difficult to measure directly. We attempted to use the bending modulus implied from the relation $\alpha = k_{BT}\,\varepsilon$ and Boal and Ng's (2010, p. 4625) measurements of the trichome persistence length, but the resulting value seemed too low compared to measurements of other bacteria and in practice the virtual trichomes appeared flaccid during simulations. It is possible that the flexure seen in Boal and Ng's trichomes was due more to the motility of the trichomes than random thermal fluctuations, in which case the above relation would no longer be valid, and a more complex model would be required to associate the observed geometry of the trichomes to their bending modulus. For example, Wolgemuth (2005) used an elastic model to estimate the bending modulus of *M. xanthus* by fitting the model to the flailing motions of a Myxobacterium stuck at one end."