Computational Analysis of *Myxococcus xanthus* Gliding Motility with Varying Cellular Growth Rates Laura Batista & Akeisha Belgrave Ph.D, Biophysics Laboratory

Introduction

In this experiment, the model organism Myxococcus xanthus was used to observe and analyze the effects of varying growth rates in adventurous motility. Understanding different aspects of motility and the peptidoglycan layer can be an important factor in developing new ideas and strategies to combat motile pathogenic bacteria cells and prevent infections. In this experiment, *M xanthus* was grown at different growth rates in hopes of creating a quantifiable difference in velocity based on PG cross-link density.

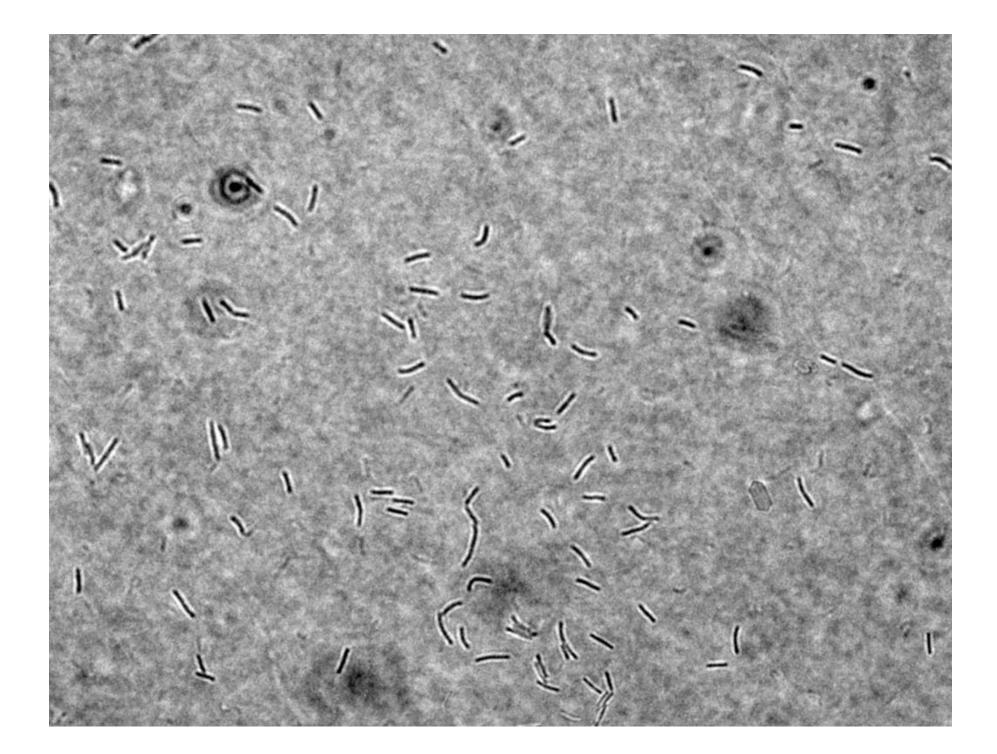


Figure 1. DZ2- \triangle PilA-AlgZ-fyp grown in 100% CYE (L. Batista, 2021)

Hypothesis

Decreasing cross-link density in the peptidoglycan layer will disrupt FAs positioning and reduce cell velocity.

Significance

Motility is a crucial factor in virulent bacteria cells, especially those which affect mucus membranes, therefore, further understanding of bacterial cell motility can potentially develop new strategies to limit motility driven infections (Kao, 2014).



Myxococcus xanthus is a gram negative, predatory soil bacterium. It is composed of two specific motility mechanism which allow it to either glide or twitch depending on the solid surface at which these are found on.

Social or S-motility allows *M. xanthus* cells to move along soft, moist surfaces and is mediated by type IV pili. It allows these cells to move in swarms across surface without the aid of flagella. Type IV pili are polymers of the major pilin protein that are displayed on the surfaces of many Gram-negative bacteria (Melville, S., Craig, L. 2013). Adventurous or A**motility** allows *M. xanthus* wild-type cells to move along dry/hard surfaces.

The ability to switch between two different motility mechanism makes *M. xanthus* a great model organism to study motility and its interdependence to motile pathogenic bacteria cells.

Background

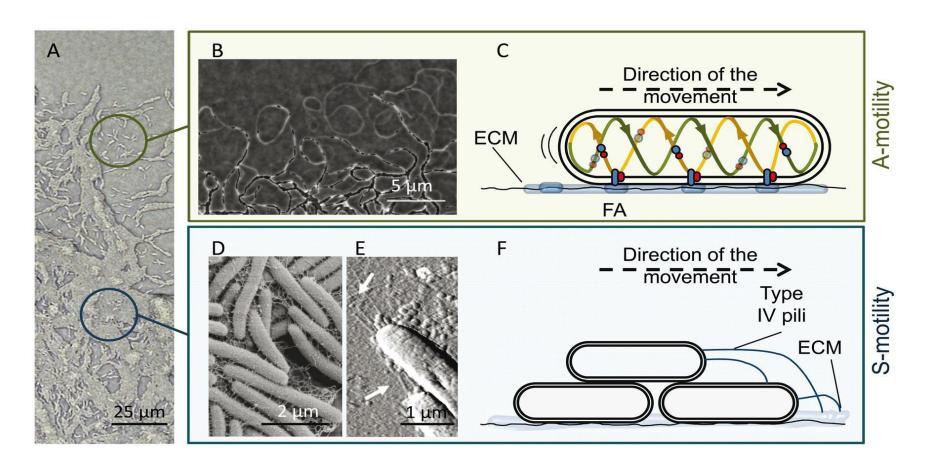


Figure 2. Photo showing the two *M. xanthus* motility systems . Photo by Muñoz-Dorado, 2016.

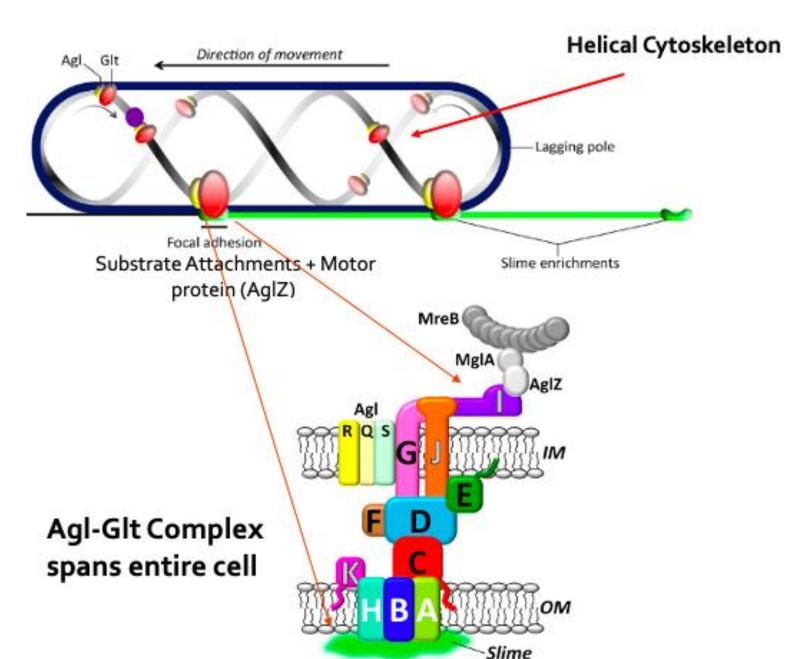


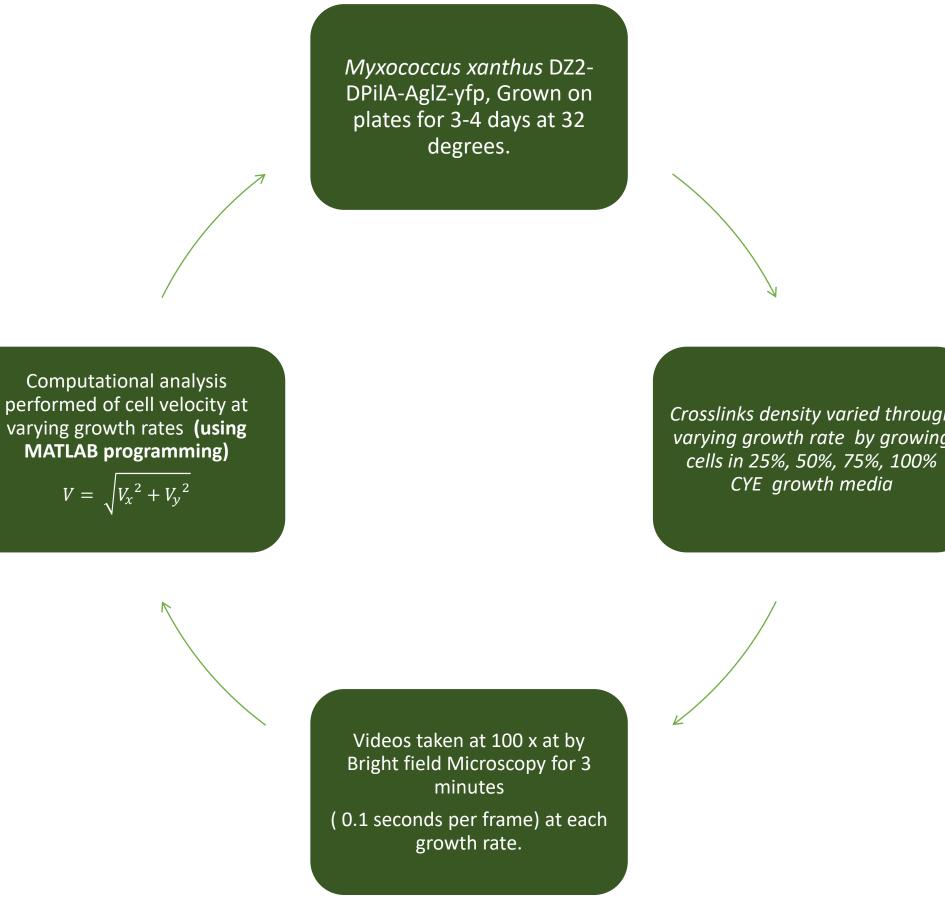
Figure 3. The Agl-Glt complex helps power gliding motility in M. xanthus cells. Photo Credit: Islam, S and Mignot, T., 2015

Data points were analyzed using a self-written program via Matlab. Magnitude of the velocity was computed using the X and Y values of the position of the cell over time. A total of 40 cells, 10 per each concentration was measured using this computational analysis in order to identify the trends in velocity between cells grown in different concentrations.

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Methods

Experiments were performed on DZ2- \triangle PilA-AlgZyfp, in which Type IV pili is deleted.



Unfiltered cell X-Directed motion of (M.xanthus in 100%)

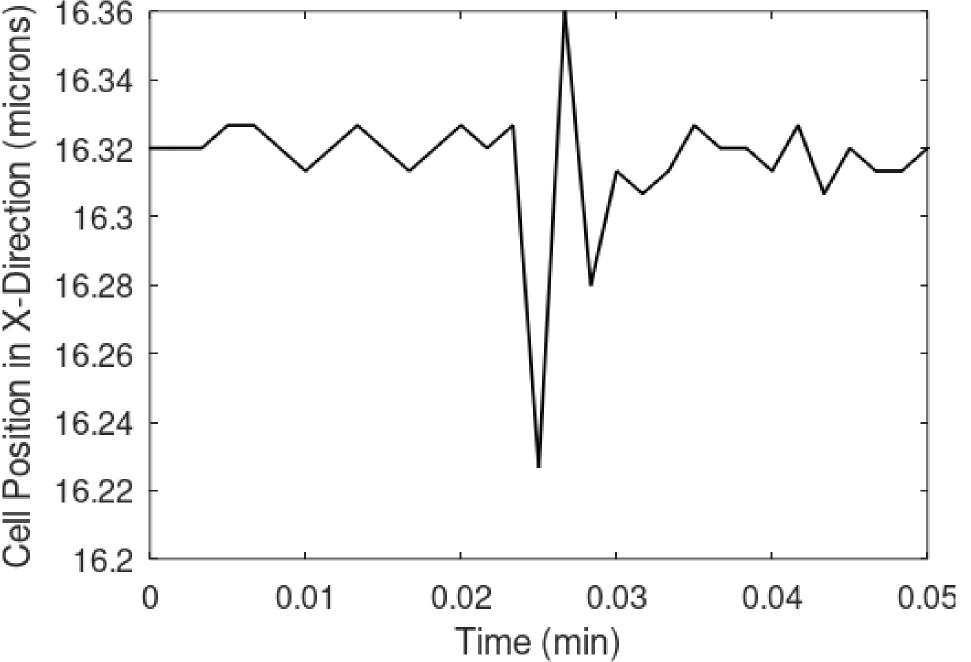
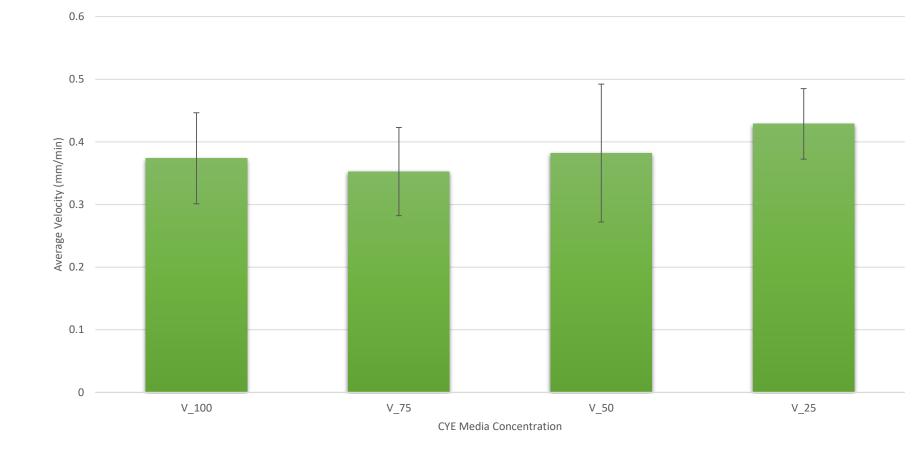


Figure 4. X and Y position of cell grown at 100% CYE (control) measured using Octave program (Similar to Matlab).

Despite the insignificance in the p-value, a slight increase in velocity can be observed in fig.5. This can be interpreted as the bacteria cells growing at a lower concentration (25%) were in in fact able to efficiently turnover newly synthesized PG material, therefore providing more support to the AGL-GLT complex as it moves throughout the cell length and propels the cell forward

Average Velocity of M. xanthus in varying growth rates





For future directions, a larger sample size of 25-100 cells will be used in order to eliminate any inaccuracy. Osmic shock assay will also be carried out to confirm cross-link density between cells grown at different concentrations, along with changing growth rates using different temperatures instead of diluted nutrients.



https://doi.org/10.1083/jcb.201412047 https://doi.org/10.1016/j.semcdb.2015.10.033.

Harrisburg

Results

Figure 5. Average velocity of M. xanthus in different concentrations of CYE media. (P>0.05).

Conclusion / Future Directions

References

Balagam, R., Litwin, D. B., Czerwinski, F., Sun, M., Kaplan, H. B., Shaevitz, J. W., & Igoshin, O. A. (2014). Myxococcus xanthus gliding motors are elastically coupled to the substrate as predicted by the focal adhesion model of gliding motility. *PLoS* Computational Biology, 10(5), e1003619. https://doi.org/10.1371/journal.pcbi.1003619

Bui, N. K., Gray, J., Schwarz, H., Schumann, P., Blanot, D., & Vollmer, W. (2009). The peptidoglycan sacculus of Myxococcus xanthus has unusual structural features and is degraded during glycerol-induced myxospore development. *Journal of bacteriology*, *191*(2), 494–505. https://doi.org/10.1128/JB.00608-08

Jakobczak B, Keilberg D, Wuichet K, Søgaard-Andersen L (2015) Contact- and Protein Transfer-Dependent Stimulation of Assembly of the Gliding Motility Machinery in Myxococcus xanthus. PLoS Genet 11(7): e1005341. https://doi.org/10.1371/journal.pgen.1005341

Nan, B. Mauriello, E. Sun, I. Wong, A. Zusman, D. (2010). A multi-protein complex from Myxococcus xanthus required for bacterial gliding motility. *Molecular Biology.* 76(6) p. 1539-1554. https://doi.org/10.1111/j.1365-2958.2010.07184.x Treuner-Lange, A., Macia, E., Guzzo, M., Hot, E., Faure, L. M., Jakobczak, B., Espinosa, L., Alcor, D., Ducret, A., Keilberg, D., Castaing, J. P., Lacas Gervais, S., Franco, M., Søgaard-Andersen, L., & Mignot, T. (2015). The small G-protein MgIA connects to the MreB actin cytoskeleton at bacterial focal adhesions. *The Journal of cell biology*, *210*(2), 243–256.

Mignot, T. (2007). The elusive engine in *Myxococcus xanthus* gliding motility. *Cell. Mol. Life Sci.* 64, 2733–2745 https://doi.org/10.1007/s00018-007-7176-x

Salim T. Islam, Tâm Mignot. (2015). The mysterious nature of bacterial surface (gliding) motility: A focal adhesion-based mechanism in Myxococcus xanthus. Cell and Developmental Biology. Vol 46 143-154.

Mauriello, E. M., Mouhamar, F., Nan, B., Ducret, A., Dai, D., Zusman, D. R., & Mignot, T. (2010). Bacterial motility complexes require the actin-like protein, MreB and the Ras homologue, MgIA. The EMBO journal, 29(2), 315–326. https://doi.org/10.1038/emboj.2009.356

Sun, H. Yang, Z. Shi, W. (1999). Effect of cellular filamentation on adventurous and social gliding motility of Myxococcus xanthus. Molecular Biology Institute. 96 (26) 15178-15183; https://doi.org/10.1073/pnas.96.26.15178 Zhang, H. Venkatesan, S. Nan, B. Myxococcus xanthus as a Model Organism for Peptidoglycan Assembly and Bacterial Morphogenesis. *Microorganisms* 2021, 9,916. https://doi.org/10.3390/ microorganisms9050916