The Effect of Temperature on Flagellar Growth Rate of *Chlamydomonas reinhardtii*

Amy Chan, Robert Chung, Andrew Hefford

Abstract

Chlamydomonas reinhardtii are simple motile unicellular green algae that contain two anterior flagella. Due to the importance of flagella in many organisms for cellular processes we decided to determine the effect temperature change had on flagellar assembly. We used a change in pH to remove the flagella and then incubated the organism at temperatures of 10, 20 and 30 degrees Celsius. Incubated samples were taken every 5 minutes, fixed with IKI and then the flagella were measured via microscopy. Our results showed that the 20˚C and 30˚C incubations had the most rapid flagellar assembly with growth rates of 0.13 µm/min and 0.15 µm/min respectively and the 10˚C incubation had a slower flagellar assembly rate of 0.038 µm/min. Using ANOVA and Tukey's post hoc statistical analysis our results showed that there was a significant difference in flagellar assembly between the 20˚C and 10˚C ($p = .003$) incubation as well as the 30˚C and 10˚C incubation ($p = .001$). However, the flagellar assembly at our 30˚C and 20˚C incubation was not significantly different ($p = .322$).

Introduction

Chlamydomonas reinhardtii is a simple motile unicellular green alga found in many different environments around the world. *C. reinhardtii* has many interesting features, but one of its most prominent features, is its two anterior flagella, which range from 10 to 12 µm in length (Harris 2001). Flagella can be extremely important for cellular survival and can be used for motility, adherence, or signalling between mating gametes (Mitchell 2000). Research has shown that certain mutations in the flagella of *C. reinhardtii* will negatively affect how the flagellum assembles at temperatures above or below 20 degrees Celsius, the optimal growth temperature of *C. reinhardtii* (Huang *et al.* 1977, Lien and Knutsen 1979). There are many reasons the flagellum assembly can be reduced at non-optimal temperatures, but some of the main reasons include the denaturing of flagellar structural proteins, a decrease in the kinetics of intraflagellar transport proteins or the denaturing of the basal body (Huang *et al.* 1977, Piperno *et al.* 1998).

Since flagella can be important for many organisms' cellular survival and reproduction we thought it would be worthwhile to examine how temperature affects flagellar assembly in a model organism such as *C. reinhardtii*. Our goal was therefore to test whether different temperatures would have an effect on the flagellar assembly in wild-type *C. reinhardtii*. Past research projects have shown that the cell density of *C. reinhardtii* peaks at about 22 degrees Celsius (Fagdaghi *et al.* 2011) and research by Gerber (1973) showed that the polymerization of tubulin proteins in bacterial flagella was hindered above 37 degrees Celsius. This research led us to develop two separate hypotheses shown below and visually demonstrated in Figure 1:

H_{a1}: Lower than optimal temperature (22˚C) will decrease flagellar assembly rate for the wild-type strain of *C. reinhardtii.*

H_{o1}: Lower than optimal temperature (22˚C) will not affect or increase flagellar assembly rate for the wild-type strain of *C. reinhardtii.*

H_{a2}: Higher than optimal temperature (22˚C) will decrease flagellar assembly rate for the wild-type strain of *C. reinhardtii.*

H_{o2}: Higher than optimal temperature (22˚C) will not affect or increase flagellar assembly rate for the wild-type strain of *C. reinhardtii.*

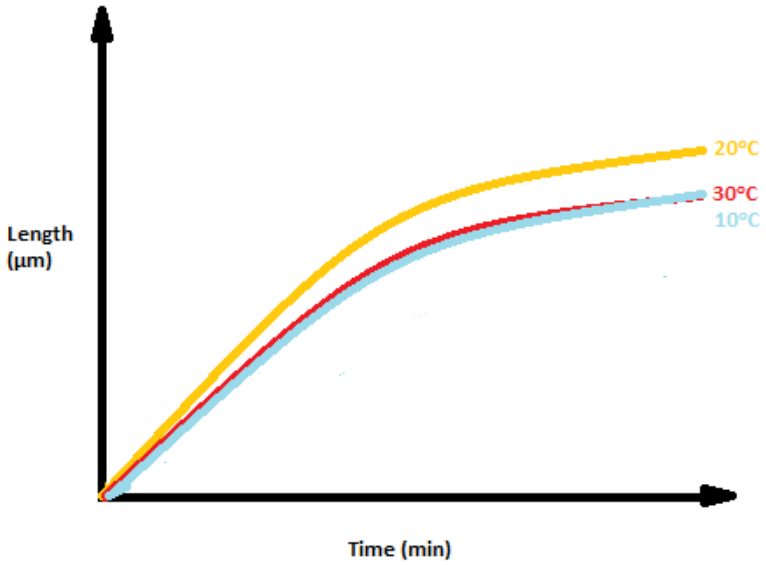

Figure 1. A prediction model of length of flagella growth per minute for *C. rheinhardtii* was made based on our predictions.

Methods

We performed three replicates at 10^{o}C, 20^{o}C, and 30^{o}C. In respect with each replicate, we measured the flagella length in micrometers of five cells to minimize biological variation. We calibrated the AXIO microscope with a micrometer at the beginning of each replicate to ensure accurate measurements. For the initial step we transferred 10 mL of *C. reinhardtii* culture into a 20 mL beaker. We then deflagellated the organism by rapidly reducing the pH to dissolve the tubulin complexes that comprise the flagella. As illustrated by Figure 2, we kept the culture constantly stirred using a magnetic stirrer, and measured with a pH meter. We then obtained a pH of 4.5 within 30 seconds by adding 0.5 N acetic acid drop-wise. After one minute, we restored the pH to 6.8 by adding 0.5 N KOH drop-wise. As soon as possible, we removed three drops of the deflagellated culture into a separate tube as a time zero control. In addition for each replicate, we set aside three drops of the non-deflagellated culture as a control to reference against.

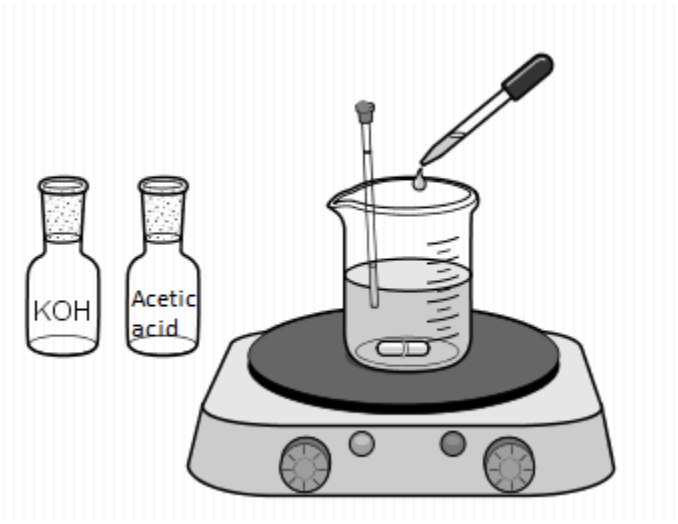

Figure 2. Set-up of equipment used for deflagellation of *Chlamydomonas*.

We checked the deflagellation with a microscope using a 16 µL sample taken from the deflagellated culture. Next, we poured 10 mL of this culture into a 15 mL centrifuge tube. We then centrifuged this tube for 5 minutes on setting 2 (low speed) as a higher setting will cause cells to lyse. After centrifugation, we discarded 7 mL of supernatant and added 3 mL of culture medium to the pellet. We then placed this tube in a water bath at the specified temperatures. At five-minute intervals from zero to 25 minutes, we removed 180 µL and added it to a microcentrifuge tube with 20 µL IKI solution to fix the cells. At each time step, we measured the length of flagella growth of five cells with a microscope using the ocular micrometer. Hence we averaged the growth rate of the five cells (pseudo-replicates) to calculate a mean growth rate for each replicate. We repeated the aforementioned steps for each replicate to achieve the setup in Figure 3.

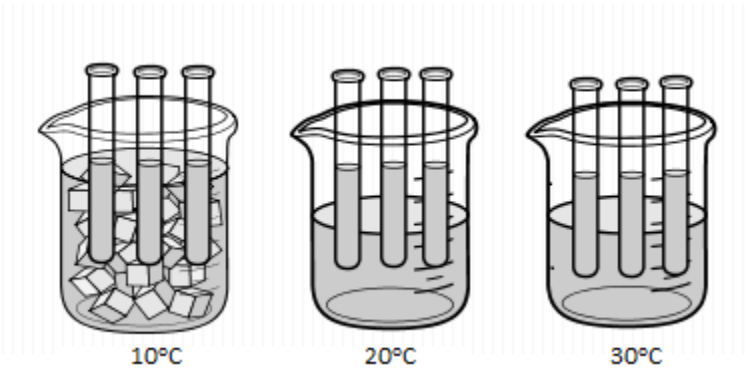

Figure 3. The set-up for the three replicates at three different temperatures.

Results

The average growth rates for each treatment at 10ºC, 20ºC, and 30ºC were calculated to be 0.036 µm/min, 0.13 µm/min, and 0.15 µm/min with confidence intervals of ±0.014, ±0.021, and ±0.021 respectively.

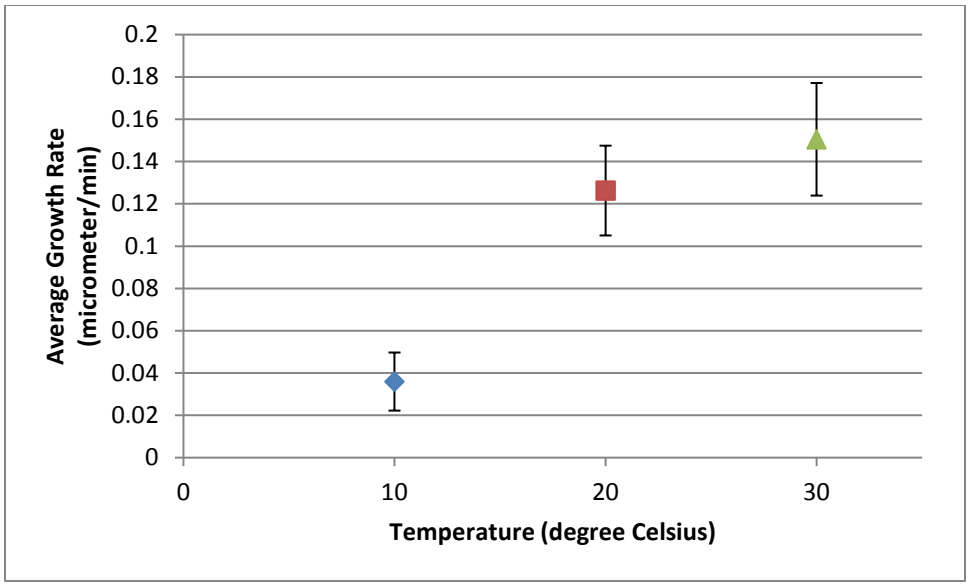

Figure 4. The average growth rates of wild-type *C. reinhardtii* at temperatures of 10°C, 20°C, and 30°C. Bars represent 95% confidence intervals, n = 3 for each temperature.

By inspection of Figure 4, we can see there is a difference between the average growth rate at 10°C and 20°C because the confidence intervals do not overlap. Furthermore, another significant difference is noted between 10°C and 30°C likewise. However, there is not a significant difference between the growth rates at 20°C and 30°C because the confidence intervals overlap. From the figure, we can see a trend that warmer temperatures have less of an impact on the growth rate of flagella of *C. reinhardtii* and conversely, colder temperatures have a greater impact on the growth rate. Average growth rates of flagellar length increased at temperatures of 10°C and 20°C.

The temperature treatment of 10°C was found to be significantly different from 20°C and 30°C whereas the treatment groups of 20°C and 30°C were not significantly different.

In order to confirm the significant differences in the ANOVA test, we used a post hoc Tukey analysis that simultaneously compares all means in a pairwise manner. The family-wise error rate was taken into account by adjusting each treatment's confidence level interval to

97.8% to obtain a joint confidence interval of 95%. Thus, the chance of type I errors were reduced when making these multiple comparisons. As illustrated in Figure 5, treatments 20°C and 30°C were not significantly different from the inclusion of the zero and the calculated p-value of 0.322, which is indicative of no significant difference. In addition, treatments 10°C and 20°C are significantly different as this comparison does not contain zero and has a p-value of 0.003. Similarly, treatment 10°C and 30°C are significantly different with a p-value of 0.001.

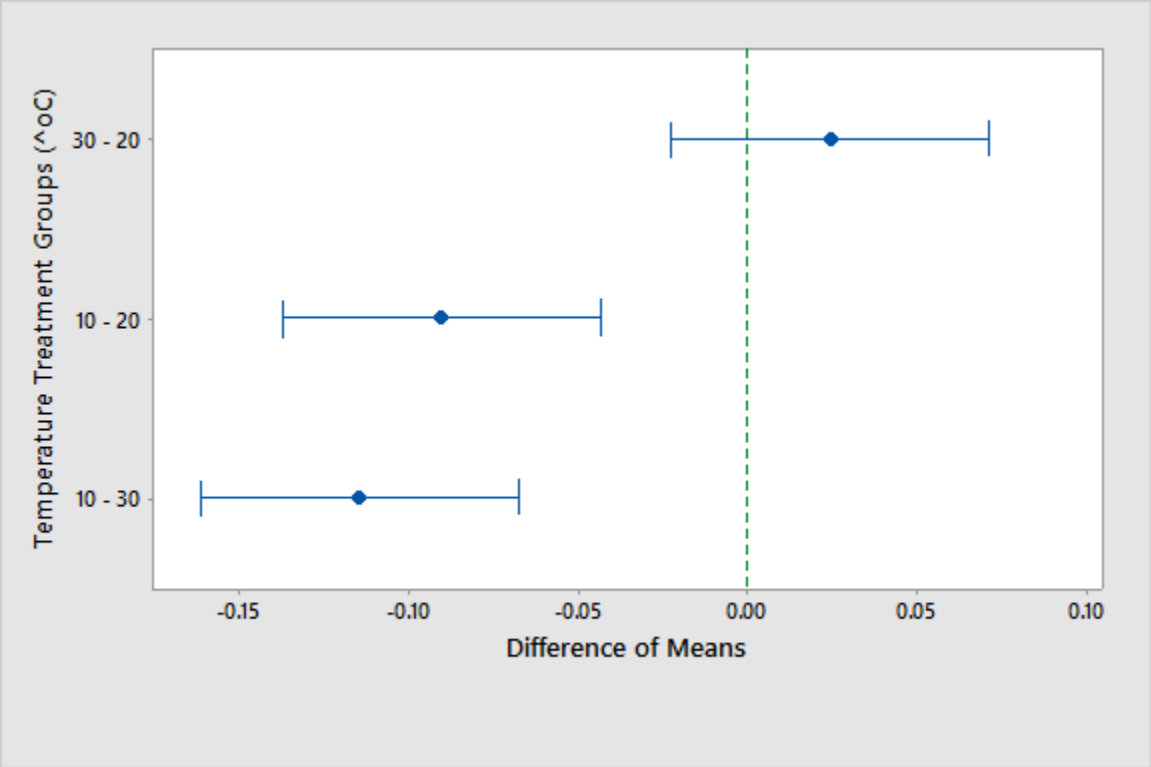

Figure 5. Post hoc analysis of Tukey's pairwise method.

Discussion

Analysis of flagella assembly at 10, 20 and 30° Celsius, allows us to support one of our hypotheses and reject the other. From the results and the analysis using the one-way ANOVA test, we were able to provide support for our H_{a1} and reject H_{o1}. The flagella regeneration rate at

10^{o}C was significantly lower when compared to the optimal temperature of 20^{o}C. When exposed to the cold treatment, a greater number of *C. rheinhardtii* were unable to reassemble their flagella. The flagella reassembly rate for the cold temperature is lower because *C. rheinhardtii* likely experienced decreased growth outside the optimal temperature ranges, 20^{o}C - 25^{o}C (Harris 2001). The flagella protein, flagellin, decreases in structure stability resulting in decreased flagellar polymerization rates at subcritical temperatures (Gerber *et al*. 1973). As well, the decline in growth rate at suboptimal temperatures is due to limited photosynthesis and respiration (Robarts and Zohary 1987). The cells exposed to the colder treatment also appeared to be lysed and were much smaller in size than the organisms grown at the optimal temperature and appeared to have numerous smaller chloroplasts. However, the smaller size may be a result of mating, as *C. rheinhardtii* shed their cell walls in order to allow their pair of gametes to fuse (Goodenough *et al*. 1975). Also, during different time intervals of flagella reassembly, *C. rheinhardtii* appeared to have a circular vacuole protruding from their body as opposed to the round appearance they originally had. The visible circular membranes were likely a result of the resorption of the flagella in response to the pH shock (Sanders and Salisbury 1994). We observed a large amount of variation in the flagella lengths at various temperatures. This is likely due to the small number of *C. rheinhardtii* that could be looked at for their flagella length because many of the organisms were not able to reassemble.

From the data and statistical values obtained from the one-way ANOVA test, we were not able to provide support for our H_{a2} and therefore failed to reject our H_{o2}. The flagella regeneration rate at the 30^{o}C treatment level was not significantly different from the optimal temperature of 20^{o}C. This finding suggests that the flagellar growth rate of *C. rheinhardtii* was not affected by the increase in temperature. Previous studies show that 32^{o}C appears to be a

restrictive threshold for *C. rheinhardtii* flagella growth (Adams *et al*. 1982, Huang *et al*. 1977). Huang *et al.* (1977) found that there was increased impairment of flagella functionality and flagella reassembly as the temperature increased from the optimal temperature to the restrictive temperature. Our findings are inconsistent with previous research where it was shown that flagella reassembly is inhibited at 32^{o}C (Huang *et al*. 1977). The organisms do not exhibit a decrease in flagella regeneration rate at higher temperatures, and even showed a higher rate of regeneration than seen at the accepted optimal temperature. Although our results are inconsistent with the literature, it is possible that the organisms were acclimated to the warm temperature. Tanaka *et al.* (2000) found that *C. rheinhardtii* were able to maintain functionality of their photosynthetic machinery even when exposed to 35^{o} C, which is higher than the previously stated restrictive temperature. Since the *C. rheinhardtii* did not exhibit a decrease in flagella regeneration rate, it may be possible that many of them were already acclimated to the higher temperature, 30^{o}C. Because the results of the higher and optimal temperatures do not appear to be statistically significant, the threshold for thermal stability of the flagella may be higher than previous literature has stated. To understand how high temperatures affect the rate of flagella regeneration of *C. rheinhardtii*, it would be useful to observe the regeneration rate at a higher temperature to observe the effects of extreme temperature on the organism.

Throughout the experiment our group was careful to minimize any error however there were still a few sources of error that may have occurred. One of the main steps in our experiment, which may have caused error, was due to time constraints we had to use pseudo-replicates. This simply means that our treatment was applied (i.e. deflagellation) to all three replicates at the same time and not individually. By using pseudo-replicates, it rendered our samples to be more prone to human error when deflagellating the organisms. In order to

minimize the biological variation in our measurements we measured five cells at each of our time points and took an average of these measurements.

Conclusion

Our studies demonstrate a significant difference in the growth rates of flagella of *C. reinhardtii* at different temperatures. We observed that at temperatures of 10ºC, the organism had lower rates of flagellar regeneration than at higher temperatures of 20ºC and 30ºC. This allowed us to support our hypothesis H_{a1} which speculated that the assembly rate of flagella would be decreased at less than optimal temperatures, whereas we were unable to support our hypothesis H_{a2} which speculated that the assembly rate would be decreased at greater than optimal temperatures. This project provides information on the flagellar properties of *C. reinhardtii*, which can be applied to other flagellated species, improving our understanding how the temperature affects microbial species.

Acknowledgments

We would like to thank the University of British Columbia for providing us with the resources to perform this experiment. We would also like the thank Dr. Carol Pollock and Dr. Maryam Moussavi for their patience, advice and assistance. In addition, we thank Shannan May-McNally and Mindy Chow for their advice on our experiment and for setting up our materials.

Literature Cited

Adams, G. M. W., Huang, B. and Luck, D. J. 1982. Temperature-sensitive, assembly-defective flagella mutants of *Chlamydomonas reinhardtii*. Genetics, **100**(4): 579-586.

Fadaghi, F., Jayasundera, K., Krishnan, D., and Liu, X. 2011. The Effect of Temperature on cell density of wild Type and mutant *Chlamydomonas reinhardtii*. The Expedition, **1**(1)

Gerber, B. R., S. Asakura, and F. Oosawa. 1973. Effect of temperature on the *in vitro* assembly of bacterial flagella. Journal of Molecular Biology **74**(4): 467-487.

Goodenough, U. W. and Weiss, R. L. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. III. Cell wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. The Journal of Cell Biology, **67**(3): 623-637.

Harris, E. H. 2001. *Chlamydomonas* as a model organism. Annual Review of Plant Physiology and Molecular Biology, **52**(1): 363-406.

Huang, B., Rifkin, M. R. and Luck, D. J. 1977. Temperature-sensitive mutations affecting flagellar assembly and function in *Chlamydomonas reinhardtii*. The Journal of Cell Biology, **72**(1): 67-85.

Lewin, R. A., T. H. Lee, and L. S. Fang 1981. Effects of various agents on flagellar activity, flagellar autotomy and cell viability in four species of *Chlamydomonas* (chlorophyta: volvocales). Symposia of the Society for Experimental Biology, **35**: 421-37

Lien, T., and Knutsen, G. 1979. Synchronous growth of *Chlamydomonas reinhardtii* (Chlorophycae): A Review of Optimal Conditions. Journal of Phycology, **15**(2): 191-200.

Mitchell, D.R. 2000. *Chlamydomonas* flagella. Journal of Phycology. **36**(2): 261-273.

Piperno, G., E. Siuda, S. Henderson, M. Segil, H. Vaananen, and M. Sassaroli. 1998. Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects. The Journal of Cell Biology, **143**(6): 1591-1601.

Robarts, R. D. and Zohary, T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. New Zealand Journal of Marine and Freshwater Research, **21**(3): 391-399.

Sanders, M. A., and Salisbury, J. L. 1994. Centrin plays an essential role in microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*. The Journal of Cell Biology, **124**(5): 795-805.

Song, L. and Dentler, W. L. 2001. Flagellar protein dynamics in *Chlamydomonas*. Journal of Biological Chemistry, **276**(32): 29754-29763.

Tanaka, Y., Nishiyama, Y. and Murata, N. 2000. Acclimation of the photosynthetic machinery to high temperature in *Chlamydomonas reinhardtii* requires synthesis de novo of proteins encoded by the nuclear and chloroplast genomes. Plant Physiology, **124**(1): 441-450.