Hot or Cold: The effect of temperature on the growth rate of *Tetrahymena thermophila*

Ashkan Afshari, Khashayar Noroozzadeh, Janek Nowicki, Kimberly Truong

Abstract

Tetrahymena thermophila is a ciliated eukaryotic protozoan that lives in temperate freshwater ecosystems. In this experiment, we examined the effect of temperature on the growth rate of *T. thermophila*. We predicted that decreasing temperature leads to a decrease in growth rate. In our experiment, *T. thermophila* was incubated at three different temperatures 25^{o}C, 30^{o}C, and 35^{o}C; with a set of four replicates for each temperature. Cell counts were taken at 0, 2, 4, 6, and 8 hours after incubation, and cell concentrations were calculated from those counts. Based on a one-way ANOVA, the p-value was 0.013. We rejected our null hypothesis and provided support for our alternate hypothesis which stated that temperature has an effect on the growth rate of *T. thermophila.*

Introduction

Tetrahymena thermophila is a freshwater, unicellular eukaryote found commonly in habitats such as ponds and lakes, (Cassidy-Hanley 2012). It is larger than most mammalian cells, measuring 30 x 50 μm, and feeds via phagocytosis (Collins & Gorovsky 2005). This organism possesses two nuclei that have separate germline and somatic functions the micronucleus houses sexual reproduction genes and the macronucleus is the centre of gene expression (Eisen et al. 2006). Asexual reproduction and replication of *T. thermophila* occurs through conjugation, which occurs between nutritionally starved, sexually mature cells (Collins & Gorovsky 2005).

According to Cassidy-Hanley (2012), *T. thermophila* has a rapid replicative growth rate, with a doubling time of less than 2 hours. This growth rate contributes to its importance as a model organism for biological research; and because it is of such importance in research, preparing an optimal environment for reproduction is essential. We sought to specifically investigate temperature as an environmental factor, as it has been found to play an important role in the growth rate of *T. thermophila* (Cassidy-Hanley 2012).

The optimal doubling time for *T. thermophila* occurs at 35^{o}C, with a generation time of 2 hours (Cassidy-Hanley 2012). The primary goal of our study was to investigate the effect of decreasing temperatures below this optimum. To do this, we measured the effect of temperature on the growth rate of *T. thermophila* when incubated at three different temperatures, 25^{o}C, 30^{o}C, and 35^{o}C, over a period of 8 hours. Our hypotheses were as follows:

H_0: Temperature does not have an effect on the growth rate of *T. thermophila*.

H_A: Temperature does have an effect on the growth rate of *T. thermophila*.

We predicted that decreasing temperature would result in a decreased growth rate. Nägel and Wunderlich (1977) found that nucleocytoplasmic transport in a *Tetrahymena* species was severely inhibited when temperature was decreased, which may have negative implications on *T. thermophila*'s growth rate. Indirect support may also come from evidence that the reverse may be true, which means, increasing temperature will increase growth rate. For example, an increase in temperature has been shown to enhance the rate of phagocytosis, which in turn increases food uptake and thus rate of reproduction (Frankel & Nelson 2001).

Our study is important for two reasons: Firstly, temperature is an important factor for the growth rate of *T. thermophila*, thus it is crucial to know how altering temperature affects their growth. Secondly, although there are many studies done on the effect of increasing temperature beyond the optimum, there are comparatively few on the effect of decreasing temperature.

Methods

All cells used in this study were wild-type *T. thermophila*. The initial stock cell culture was diluted with SSP medium (containing 2% proteose peptone, 0.1% yeast extract, 0.2% glucose, and 33 μM $FeCl_3$). Glutaraldehyde was used a fixative.

Replicate preparation

Using sterile technique, we transferred 3mL of culture into 12 10-mL test tubes (Figure 1). For each of our three treatments (incubation in water baths kept at temperatures of 25°C, 30°C, and 35°C), we incubated four replicates. Since we predicted that decreasing temperature results in a decreased growth rate, we designated the set of replicates that were to be incubated at 35°C as our treatment control, as 35°C is well-supported in literature as being *T. thermophila*'s optimum growth temperature (Cassidy-Hanley, 2012).

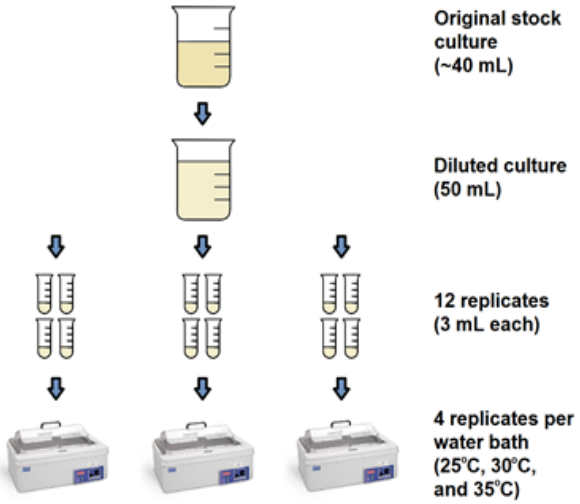

Figure 1. Steps taken to prepare 12 replicates for three treatments (25°C, 30°C, 35°C)

Data collection

We incubated each set of four replicates in water baths kept at temperatures of 25°C, 30°C, and 35°C. Cell counts of all replicates were conducted at two- hour intervals, with final counts being made 8hr after incubation. Cells were fixed with 20% glutaraldehyde and counted using a haemocytomer using an Axiostar microscope under 100X magnification.

Operational definition of growth rate

We defined the growth rate for each of our replicates as the slope of the linear best fit line. This was obtained by graphing calculated cell concentrations for each replicate (cells/mL) vs. time (hours). As the equation for a linear graph is given by the form, the

derivative with respect to this equation, equivalent to the slope, represents the rate of change in cell concentration (y) over time (x), a rate we conflated with the growth rate of *T. thermophila* for the purposes of this experiment.

Statistical analysis

Using these data, we calculated the average slope value/growth rate and 95% confidence intervals (CI). As we defined growth rate of *T. thermophila* to be analogous to the slope values, we used these values in a one-way ANOVA with α of 0.05 to test the statistical significance of our results.

Results

As observed in Figure 2, growth rate was lower at 25°C than at 30°C and 35°C. The growth rate at 30°C is the highest observed instead of at 35°C, however there is almost complete overlap of 95% confidence intervals with the growth rate data for 35°C. The p-value was calculated to be 0.013; the calculated F-value, 5.43, is higher than the critical F value, 4.26. The 95% confidence intervals for mean growth rate across all temperatures were all fairly large, indicating wide variance/uncertainty in growth rate among the replicates.

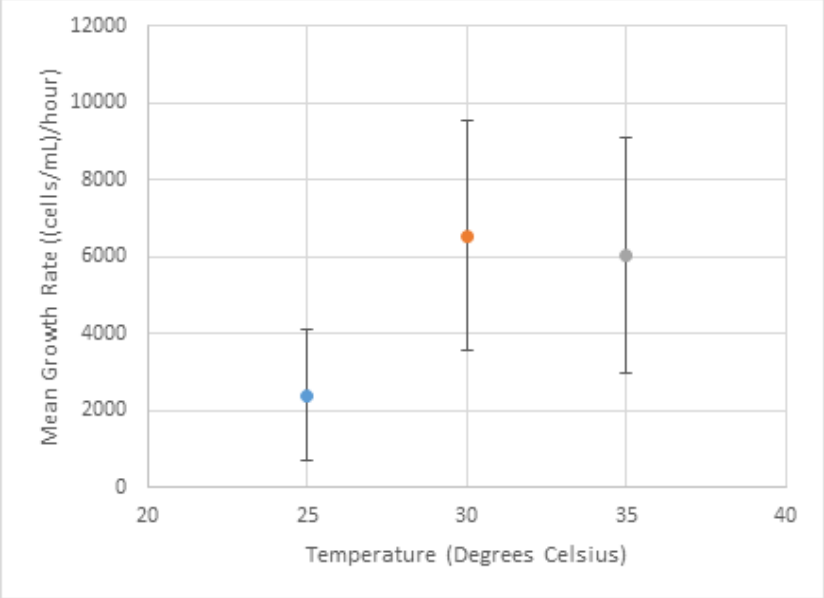

Figure 2. Mean growth rate of *T. thermophila* calculated for each of the 3 treatments (incubation at temperatures 25°C, 30°C, and 35°C) calculated from t = 0h to t = 8h. Error bars represent 95% confidence intervals, n = 4. p=0.013

Discussion

Based on our one-way ANOVA results ($p < 0.05$), we reject our null hypothesis and provide support for our alternative hypothesis, which states that temperature does have an effect on the growth rate of *T. thermophila.* Our prediction that decreasing temperature leads to decreased growth rate is somewhat supported by the data, as the mean growth rate was lowest for the treatment temperature of 25^{o}C. However this difference was not statistically demonstrated as we did not run a test that specifically compared data from the 25^{o}C treatment to another data point. This can also be applied to our finding that the highest growth rate was not at the predicted optimum temperature of 35^{o}C, but instead at 30^{o}C (Figure 2). We do note that the difference between the calculated rates at 30^{o}C and 35^{o}C is fairly small (especially when considering 95% CI) compared to the rate at 25^{o}C. This small difference in growth rate between the two temperatures has been previously observed in other studies (Frankel & Nelsen, 2001).

A possible biological explanation for this result may lie in the formation process of *T. thermophila*'s food vacuoles. Food vacuole formation is associated with a faster rate of phagocytosis, and faster phagocytosis indicates faster and more efficient energy and nutrient uptake (Jacobs et al. 2006). This in turn contributes to greater capacity to synthesize biomolecules and increase reproduction rates. From this we can infer that faster food vacuole formation would be associated with higher growth rates. Jacobs et al. (2006) found that the optimal temperature range for food vacuole formation in *T. thermophila* was between 28^{o}C to 30^{o}C, a finding corroborated in a study by Luan et al. (2012), which found that the highest average number of food vacuoles occurred at a temperature of 30^{o}C.

There is also some support in existing literature with regard to why growth rate was lowest at 25^{o}C. Nägel and Wunderlich (1977) found that the nucleocytoplasmic transport of RNA and assembled ribosomes is severely impaired at lower temperatures in a similar

Tetrahymena species. As this process is vital to many aspects of protein synthesis (among other biological functions), we can infer that inhibition of this process has negative implications for the viability of *T. thermophila* growing at lower temperatures. Likewise, Thormar (1962) observed that the exposure of a similar *Tetrahymena* species to extreme cold caused inhibitory effects on cell reproduction, positing that this is due to the activation and inactivation of temperature-sensitive macromolecular structures within the organism.

Another interesting observation we noted in this study was the relative plateau or decrease of cell concentration levels observed at t = 2h (Figure 2), even though t = 2h was ostensibly *T. thermophila*'s doubling time (Cassidy-Hanley, 2012). This may be a result of temperature sensitive periods that *T. thermophila* exhibit following sudden temperature shifts, which usually result in delays in cell division (Frankel, Mohler & Frankel, 1980). This phenomenon, called the excess-delay phenomenon, can cause observable "lags" in cell concentration increases. It may explain why cell concentration measurements did not increase at that time.

Due to the size and amount of overlap between the 95% confidence intervals for mean growth rates of all treatments, there is a risk that we may have committed a type I error (i.e., the false rejection of a true null hypothesis). We must look at possible reasons for this wide uncertainty and variance in our data. Our main source of uncertainty is the small sample size. In fact, 95% confidence intervals, a measure of uncertainty/variation, become larger with decreasing sample size. Other sources of uncertainty or variation include human error during the experiment, such as contamination or pipetting errors, which can all affect cell concentration. Subjectivity of cell counts also contributed to variation, as all four members of our group conducted counts. We attempted to calibrate our haemocytometer counts by all counting one sample and observing the same number of cells. However, due to the number of

counts we each conducted, it is likely that this part of our procedure was still vulnerable to subjectivity.

Conclusion

We rejected our null hypothesis and provide evidence to support our alternate hypothesis which states that temperature does have an effect on the growth rate of *T. thermophila*. As temperature decreases the rate of growth decreases for *T. thermophila*. We hope for our study to become a useful source of information for further studies on *T. thermophila* and its interplay with temperature.

Acknowledgments

First of all, we would like to thank to our outstanding professor Dr. Carol Pollock and the University of British Columbia for providing the opportunity to take such an engaging course. We also would like to thank Dr. Carol Pollock for the constant instruction and guidance throughout the process of this experiment. In addition, we also appreciate our teaching assistant, Jason Wong and technician, Mindy Chow for providing us with equipment, materials, and advice throughout this experiment.

Literature Cited

Cassidy-Hanley, DM 2012, *Tetrahymena* in the Laboratory: Strain Resources, Methods for Culture, Maintenance, and Storage [online], *Methods in Cell Biology*, vol. 109, pp. 237-276, doi: 10.1016/B978-0-12-385967-9.00008-6.

Collins, K & Gorovsky, MA 2005, *Tetrahymena thermophila* [online], *Current Biology*, vol. 15, no. 9, pp. R317-R318. doi:10.1016/j.cub.2005.04.039

Eisen, JA, Coyne, RS, Wu, M, Wu, D, Thiagarajan, M, Wortman, JR, Badger, JH, Ren, Q, Amedeo, P, Jones, KM & Tallon, LJ 2006, Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote [online]. *PLoS Biol*, vol. 4, no. 9, p. e286. doi:10.1371/journal.pbio.0040286

Frankel, J, Mohler, J & Frankel, AK 1980, The relationship between the excess-delay phenomenon and temperature-sensitive periods in *Tetrahymena thermophila*, *Journal of Cell Science*, vol. 43, no. 1, pp. 75-91.

Frankel, J & Nelsen, EM 2001, The effects of supraoptimal temperatures on population growth and cortical patterning in *Tetrahymena pyriformis* and *Tetrahymena thermophila*: A Comparison [online], *The Journal of Eukaryotic Microbiology*, vol. 48, no. 2, pp. 135-146, doi:10.1111/j.1550-7408.2001.tb00296.x

Jacobs, ME, DeSouza, LV, Samaranayake, H, Pearlman, RE, Siu, KW, & Klobutcher, LA 2006, The *Tetrahymena thermophila* phagosome proteome [online]. *Eukaryotic Ce*ll, vol. 4, no.12, pp. 1990-2000. doi:10.1128/EC.00195-06

Luan, E, Miller, G, Ngui, C, and Siddiqui, F 2012. The effect of temperature on food vacuole formation by *Tetrahymena thermophila* [online]. *The Expedition*, vol. 2.

Nagel, W & Wunderlich, F 1977, Effect of temperature on nuclear-membranes and nucleocytoplasmic RNA-transport in *Tetrahymena* grown at different temperatures, *Journal of Membrane Biology*, vol. 32, no. 2, pp. 151-164.

Thormar, H 1962, Effect of temperature on the reproduction rate of *Tetrahymena pyriformis*, *Experimental Cell Research*, vol. 28, no. 2, pp. 269-279. doi:10.1016/0014-4827(62)90283-5