The Effect of Changes in Temperature on the Doubling Time of Wild-Type *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila is a single celled protozoan commonly found in freshwater habitats (Cassidy-Hanley 2012). In this study, we investigated the relationship between changes in temperature and doubling time in *Tetrahymena thermophila*. In our experiment, *T. thermophila* was incubated at three different temperatures: 30°C, 35°C, and 40°C. Our data were collected at three hour intervals from 0h to 9h, with a final sample taken at 10h. The population size was determined by placing 20 µL of fixed *T. thermophila* cells onto a haemoctyometer and counting the cells using the Axio Star microscope. The doubling time was lowest at 35°C (4.22 h) compared to the doubling time at 30°C (7.37 h) and 40°C (5.63 h). Based on calculations of the one-way ANOVA, we failed to reject our null hypothesis ($p = 0.49$); therefore the doubling time of *Tetrahymena thermophila* was longer or the same at 35°C. Although we failed to reject our null hypothesis, trends were still consistent with previously conducted experiments, stating that growth rates were highest at optimal temperature (Frankel and Nelsen 2001).

Introduction

Tetrahymena thermophila is a ciliated single-celled protozoan commonly found in freshwater habitats such as streams, lakes and ponds (Cassidy-Hanley 2012). The organism displays nuclear dimorphism, in that it has two nuclei which serve different purposes. It has a diploid germline micronucleus which stores genetic information required for sexual reproduction, and a somatic macronucleus which is expressed during vegetative growth and replication (Gorovsky 1973). Due to its large cell size (40-50 µm) and phagocytotic behaviour, *T. thermophila* cells have been commonly called "animalcules" (Orias *et al.* 1999).

T. thermophila is a useful model organism for molecular research as it has a rapid doubling rate of two hours and can grow in a variety of media (W.M. Keck Science Department). Amongst the possible conditions used to grow *T. thermophila*, temperature was the focus of this study because temperature is correlated with key metabolic functions. Laun *et al.* (2012) found that an increase in temperature resulted in a significant increase in the number of food vacuoles observed in *T. thermophila.* An increase

in temperature increases the efficiency of phagocytosis, which in turn increases food uptake and results in faster reproduction. Nevertheless, *T. thermophila* can only tolerate temperatures up to a certain point before cell growth starts to decline (Frankel and Nelson 2001).

According to results of previous studies, *T. thermophila* grows at an optimal temperature of 36°C with a generation time of two hours; however growth slows down at approximately 39°C and 39.5°C (Frankel *et al*. 2001). Therefore, the goal of our study was to further examine the effect of temperatures above and below optimal temperature on the doubling time of *T. thermophila*. Although the literature considers the optimal temperature of *T. thermophila* to be 36°C, we considered optimal temperature to be 35°C. The hypotheses for this study are as follows:

H_A: The doubling time of *Tetrahymena thermophila* is the shortest at the optimal temperature (35°C).

H_o: The doubling time of *Tetrahymena thermophila* is longer or the same at the optimal temperature (35°C).

Although many experiments have been done on the effect of temperature on *T. thermophila* growth, our study further details how temperature ranges outside of optimal affect *T. thermophila.*

Methods

Stocks and Media

All cells used in this study were of wild type *T. thermophila* strain B2086. The cells were diluted using SPP media composed of: 2% proteose peptone, 0.1% yeast extract, 0.2% glucose, and 22 µM $FeCl_3$. The total concentration of *T. thermophila* stock solution was calculated by conducting an initial cell

count using 1 mL of the 30 mL stock solution fixed with 100 µL of gluteraldehyde. We counted an average of 50 cells using the Fuchs-Rosenthal (FR) haemocytometer and calculated the initial cell count to be 275 000 cells/mL. To get our desired concentration of 30 000 cells/mL, we diluted 3.27 mL of stock *T. thermophila* with 26.73 mL of standard growth medium.

Sample Preparation and Light Microscopy Analysis

We pipetted 2 mL of the prepared solution from a 125 mL Erlenmeyer flask into 12 labelled 10 mL test tubes using proper sterile technique (Figure 1). For each of our three treatments temperatures, we incubated 4 replicates (Figure 2) in water baths at 30°C, 35°C and 40°C; 30 °C was the lower limit, 40°C was the upper limit, and 35°C as the control. These designations were determined based on previous studies by Frankel and Nelsen (2001), in which they found optimal growth of *T. thermophila* between 35-39°C. We assumed the initial cell count at time 0 hour was equivalent to the initial concentration. Subsequent cell counts were conducted at 3 hour intervals using the same procedure. An additional sample was taken at the 10th hour. For every count, a 100 µL sample was pipetted from each replicate into a 500 µL labelled micro-centrifuge tube. The samples were fixed using 10 µL of gluteraldehyde. A 20 µL sample from each of the 12 tubes was transferred onto a FR haemocytometer and examined with the Axio Star microscope using phase microscopy at 100X total magnification (Figure 3). A maximum of 100 cells was counted in the 1mm x 1mm grids; we excluded those on the borders or outside the grid range.

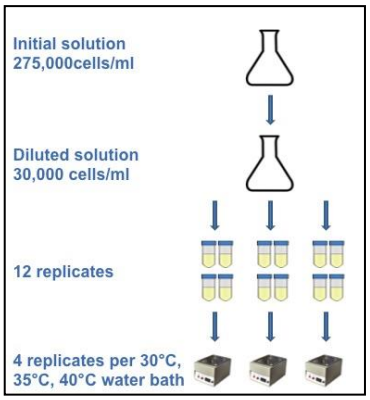

Figure 1: Flow chart of the steps taken to prepare the 12 replicate test tubes (10 mL) for the 3 water bath treatments (30°C, 35°C, and 40°C).

Figure 3: Four replicates for 40°C treatment, each containing 2 mL of stock solution with a total concentration of 60,000 cells.

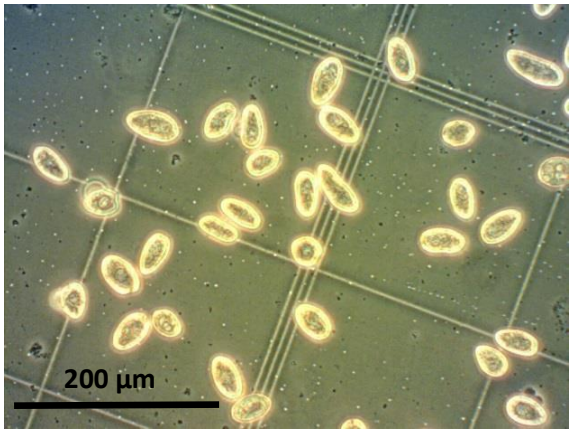

Figure 3. *T. thermophila* initial cell count on a FR haemocytometer under the Axio Star microscope using phase microscopy at 100X total magnification.

Statistical Analysis and Qualitative Analysis

After the data collection process was complete, we graphed population against time for each replicate at each of the three temperatures. After analyzing these graphs, we defined the exponential growth period to be between 6 hours and 10 hours. We then calculated doubling time during the exponential growth period and graphed the means of the replicates against temperature. The doubling time was calculated using the formula $T_d = (t_2-t_1)* \log(2) / (\log (Q_2/Q_1))$ (where $t_2 = 10h$, $t_1=6h$, and Q represents the population density at each corresponding time). This calculation assumes a constant growth rate between t_1 and t_2. A one-way ANOVA and 95% confidence intervals were used to statistically evaluate the results obtained.

Qualitative analysis on the samples was conducted by observing the samples for cloudiness which accounts for bacterial contamination or high population densities of *T. thermophila* (Cassidy-Hanley 2012).

Results

We observed our samples at each time interval (0 h, 3 h, 6 h, 9 h, and 10 h) prior to placing them on the haemocytometer and noted their appearance. There was no cloudiness in any of our samples.

Before we started counting the number of cells, we observed each sample under the microscope and did not find any visible contamination.

The population of each replicate was graphed against time for each of the three temperatures and the approximate exponential growth period was determined to be from 6 hours to 10 hours based on these graphs. The mean doubling time between 6 hours and 10 hours of *T. thermophila* for 30°C, 35°C, and 40°C was graphed with 95% confidence intervals (Figure 4). These results show that the shortest doubling time (t = 4.22 h) is in fact at the optimal temperature (35°C). Doubling time is greater above (t = 5.63 h at 40°C) and below (t = 7.37 h at 30°C) the optimal temperature, and is largest below the optimal temperature. The trend of the data shows that doubling time increases as you deviate away from optimal temperature. However, temperatures below optimal lead to a much larger doubling time than temperatures above optimal temperature.

The 95% confidence intervals at 30°C, 35°C, and 40°C are 5.60 h, 1.95 h, and 1.50 h respectively. By observing the 95% confidence intervals of the three means (Figure 4) we see that they all overlap and therefore the differences in the means are not significant. The confidence interval is largest for the mean doubling time at 30^{o}C since there was a lot of variation in the replicates at this temperature. A one-way ANOVA statistical analysis was performed on the doubling times for each replicate at all three temperatures. Based on the ANOVA results, we calculated F value = 0.77, F critical = 4.26, and p-value = 0.49. Since the F value is less than the F critical value and the p-value calculated is greater than 0.05, we fail to reject H_o which states that the doubling time of *T. thermophila* is longer or the same at the optimal temperature (35^{o}C).

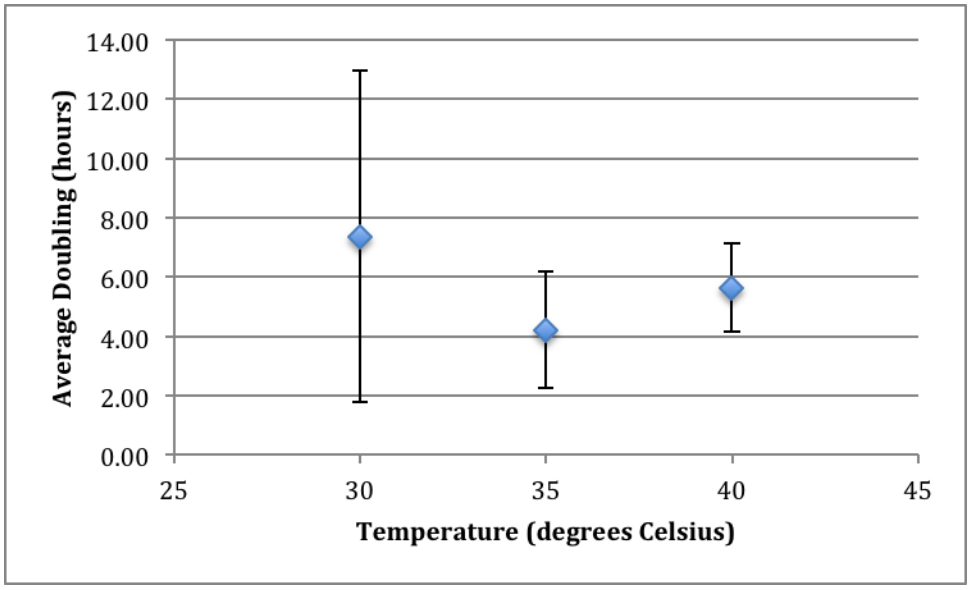

Figure 4. The mean doubling time of the wild type *T. thermophila* calculated during 6h to 10h for three temperature treatments (30°C, 35°C, and 40°C). Bars represent 95% confidence intervals, n=4.

Discussion

Based on our one-way ANOVA statistical test, we fail to reject our null hypothesis due to our calculated p-value of 0.49 being greater than p-alpha of 0.05. As well the confidence intervals within treatments overlapped; therefore, there is strong evidence to fail to reject the null hypothesis which states that the doubling time of wild type *T. thermophila* is longer or the same at the optimal temperature (35°C).

Although we failed to reject our null hypothesis, trends were still consistent with our alternate hypothesis, which states the doubling time of wild type *T. thermophila* is shortest at the optimal temperature (35°C). Frankel and Nelsen (2001) found that *T. thermophila* achieve maximum growth between 35-39°C, which is consistent with our observations in which doubling times were shortest when cells were incubated at an optimal temperature of 35°C, as can be seen in Figure 4. This is due to the inhibitory effect on reproduction that hot and cold environments exert on *T. thermophila* cultures, when induced to temperatures that deviate from their optimal temperature. Thormar (1962) showed that

longer lag periods in reproduction cycles are exhibited by *T. thermophila* cultures in temperatures that vary from optimal. This is a result of adjustments in their macromolecular structure over two to three generations in response to the hotter or colder temperature climate. This adjustment to temperature may be a reason for the longer 6 hour lag period observed in our *T. thermophila* cultures. With this lag period, we were only able to calculate exponential growth from 6 to 10 hours, which decreased the amount of data used for calculating doubling times of our cells. This may have accounted for our large confidence intervals, since small variations in data would have had a more pronounced effect on small sample sizes. Data should have been taken across a longer time period to account for the longer lag period. This is also supported by a similar experiment conducted by Frankel in which samples were counted 14-16 hours after incubation the next day (Frankel *et al* 2001). This may have minimized the variation in our data and may be the reason why we failed to reject our null hypothesis but still followed trends similar to our alternate hypothesis.

Within all treatment levels, minimal growth is observed up until the 6 hour mark. A potential biological explanation for this result, despite the fast doubling times of *T. thermophila,* are temperature-sensitive periods that *T. thermophila* exhibit when induced to sudden temperature shifts (Frankel *et al.* 1980). This temperature-sensitive period, also known as the excess-delay phenomenon, comes from sudden temperature shifts in *T. thermophila* cultures. This in turn brings about long excess-delays in cell proliferation despite *T. thermophila's* ability to rapidly grow (Frankel 1999). This phenomenon may be a result of constantly removing our replicates from their respective water baths every three hours when obtaining samples for counting. Replicates were also kept out of their water baths for up to 30 minutes while samples were fixed. It was also noted that the water baths were not stable throughout the experiment. Fluctuations of 5°C below the set temperature were noted in the 35°C and 40°C water baths which could have also contributed to possible errors in our results. Therefore, *T. thermophila* cultures were induced to many temperature changes over the course of our 12-hour experiment. This may have

contributed to the slow growth period observed initially within all our treatments. Ideally, more consistent results would have come from taking samples from our replicates without removing them from their respective water baths; however this procedure would make sterile technique extremely difficult.

Similar to the excess-delay phenomenon, rapid temperature changes have been accompanied with reversible structural transitions within *T. thermophila* cultures. Studies have shown that rapid temperature changes can cause abnormal movement of membrane components, which can affect transport processes associated with cell membranes (Volker and Wunderlich 1973). Since *T. thermophila* nutrient uptake is done via phagocytosis, any deviations in transport processes can in turn affect phagocytosis processes. Specifically, alterations in the transport of signal molecules can directly affect the production and release of growth factors, which directly determine when proliferation occurs (Christensen *et al.* 1995). Therefore throughout the experiment, any rapid temperature change could result in a direct alteration of transport processes, which would decrease proliferation in all *T. thermophila* cultures.

Laun *et al.* (2012) found that an increase in temperature resulted in a significant increase in the number of food vacuoles observed in *T. thermophila.* Production of food vacuoles is dependent on the activity of enzymes which function ideally at optimal temperature. This provides support for our observations in which we found highest cell density and lowest doubling time at an optimal temperature of 35°C. As our highest population density was observed at optimal temperature, it is possible that an increase in the number of food vacuoles could have resulted in faster transition through the cell cycle resulting in faster doubling time (Alfred *et al.* 2001). Alternatively, *T. thermophila* incubated at lower temperatures may not meet their nutritional requirements for growth due to fewer food vacuoles resulting in a longer replication time and smaller cell density. Similarly, we believe that temperatures

above optimal may disrupt the tertiary structure of proteins involved in the production of food vacuoles, resulting in a decline of cell growth due to insufficient nutritional requirements.

Sterile technique also plays a large role in how successful *T. thermophila* cultures are at reproducing. Despite the extremely short generation time, susceptibility to bacteria despite all precautions can easily contaminate *T. thermophila* cultures (Frankel 1999). Generally, contaminants grow faster than *T. thermophila* cells and can cause cell death. Therefore, using proper sterile technique is crucial in the beginning stages of cell growth. Bacterial contamination can be detected by qualitative analysis of the samples, where cloudiness in cell cultures is an indication of bacterial contamination (Cassidy-Hanley 2012). Although we did not observe any cloudiness in the samples, it is possible that bacterial contamination resulted in cell death resulting in inconsistent data within our initial measurements. Coupled with the fact that contamination of *T. thermophila* cells can cause cell death, and that *T. Thermophila* cultures are extremely sensitive below threshold density (Christensen *et al.* 1995), sterile technique plays a crucial role in the beginning stages of cell growth. Initial data taken at our three hour mark was not done with sterile technique; therefore contamination of our samples may have played a large role in the inconsistent data within our initial measurements.

Another source of variation was that not all samples were counted by the same individual. Ideally, possible variation in counting could have been avoided if one person counted all the replicates. Also, all samples were mixed by pipetting before the cells were counted. Variations in proper pipetting techniques may have resulted in extraction of samples that were not representative of the true cell densities of the replicates. These factors could have contributed to the large confidence intervals (Figure 4), especially observed at the 30°C treatment.

Hallunglarson *et al.* (1992) found that shaking cultures of *T. thermophila* can significantly lower growth rates almost by half. This could also lead to variation in our results as due to human error it is

possible that tubes were shaken when taking samples from the replicates or when transporting replicates to their respective water baths. This may have affected our cell densities in our replicates.

Conclusion

We failed to reject our null hypothesis which states that the doubling time of *T. thermophila* is longer or the same at the optimal temperature (35^{o}C). Therefore the doubling time of wild type *T. thermophila* is not significantly shortest at the optimal temperature. Trends however were observed that *T. thermophila* exhibits faster doubling time at its optimal temperature.

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