The effect of temperature on growth rate for wild-type and mutant *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila is a motile eukaryote that has been broadly studied due to the fact that it exhibits dimorphism (two nuclei), and is also studied for cilia biogenesis. In this study, the main objective was to compare the population sizes of the wild-type and low vacuole mutant strains of *T. thermophila* under a variety of different temperatures. Our goal was to observe whether the mutation or temperature was responsible for varying population sizes. We failed to reject all three of our null hypotheses as all of our p-values were > 0.05 (0.77, 0.30, and 0.40 respectively); failing to support our predictions that temperature would have an effect on the growth rate of *T. thermophila*. Our data did support our prediction that the presence of the mutation did not have an effect on population size.

Introduction

Tetrahymena thermophila is a small, motile, phagocytic, unicellular eukaryote found in mainly temperate freshwater environments (Collins & Gorovsky 2005). This particular organism is considered an important model organism, due to the fact that it displays a unique characteristic known as nuclear dimorphism, meaning that *T. thermophila* has two nuclei: a macronucleus and a micronucleus (Asai & Forney 1999). In addition to nuclear dimorphism, the organism is also studied for cilia biogenesis, telomerase structure and function, which are of importance in many eukaryotes (Collins & Gorovsky 2005).With a doubling time of approximately two hours, *T. thermophila* is widely considered a model organism (Asai & Forney 1999).

In this experiment we utilized a low vacuole mutant strain of *T. thermophila* in addition to the wild type for comparison. The mutant strain has fewer vacuoles present in comparison to its wild type. Vacuoles are necessary for cell survival in that they act as storage space for food and nutrients, and also serve in a variety of different pathways within the organism (Suhr-Jessen

& Orias 1979). As the mutant strain of our organism contains fewer vacuoles, we predicted we would observe a decrease in population growth in comparison to the wild type.

The main objective of our study was to compare growth rates between the wild-type and low vacuole mutant strains of *T. thermophila,* and also to examine whether or not temperature had a significant effect on the growth rate of these strains. We predicted that both the presence of the mutation and temperature will have an effect on population growth of *T. thermophila*. The reasoning behind our predictions are that *T. thermophila* is known to have to an optimal temperature of growth of 35ºC (Frankel & Nelsen 2001), and also because a lower number of vacuoles corresponds to low metabolism rates, and thus mutant populations are likely to grow slower than the wild-type (Asai & Forney 1999).

The implications of our research do not expand into merely population size. The results obtained from this laboratory can be utilized in other areas of research that also pertain to *T. thermophila.* As well, this may provide insight into new information about low vacuole mutants, and also tie into what we already know about the importance of vacuoles.

H_{O1}: Temperature has no effect on the growth rate of *Tetrahymena thermophila.*

H_{A1}: Temperature has an effect on the growth rate of *Tetrahymena thermophila.*

H_{O2}: The presence of the mutation has no effect on the growth rate of *Tetrahymena thermophila*.

H_{A2}: The presence of the mutation has an effect on the growth rate of *Tetrahymena thermophila*.

H_{O3}: The effect of temperature on the growth rate of the wild type *Tetrahymena thermophila* is the same as the effect of temperature on the growth rate of the mutant *Tetrahymena thermophila.*

H_{A3}: The effect of temperature on the growth rate of the wild type *Tetrahymena thermophila* is not the same as the effect of temperature on the growth rate of the mutant *Tetrahymena thermophila*.

Methods

Preparation

We used wild-type *T. thermophila* strain B2086, and the mutant cells were *T. thermophila* low vacuole strain, *TtVPS13A*. We determined the concentration of *T. thermophila* cells in the undiluted stock solution using a haemocytometer and found that the wild-type stock solution had 6.497×10^4 cells/mL and the mutant stock solution had 2.585×10^5 cells/mL. Both the wild type and mutant strains were diluted with SSP medium consisting of 2% proteose peptone, 0.1 % yeast extract, 0.2% glucose, and 33 μM $FeCl_3$. Then, we determined the ratios of stock solution and SSP media needed in order to create starting cell cultures of 20,000 cells/mL for both the wild-type and mutant strain. Sterile technique was used throughout the experiment to avoid sample contamination.

Counting Cells

In order to count the cells in the wild-type and mutant stock solutions, we swirled the flasks containing the *T. thermophila* first until they were homogenous and transferred 100μL of solution from a test tube and 10μL of the gluteraldehyde fixative into a microcentrifuge tube. We placed 20μL of the mixed fixative and solution onto the haemocytometer counting chamber and counted the number of cells in the blue square through the Axiostar compound microscope at 10X objective lens magnification.

Process

To prepare the wild-type *Tetrahymena* cell culture, we placed 30.8mL of wild type *Tetrahymena* stock solution and 69.2mL of SSP medium in a 250mL Erlenmeyer flask (Figure 1). To prepare the mutant *Tetrahymena* cell culture, we placed 7.7mL of mutant *Tetrahymena* stock solution and 92.2mL of SSP medium in a 250mL Erlenmeyer flask (Figure 1). These

volumes were determined through dilution calculations to produce approximately 20,000 cells/mL for the starting cell culture.

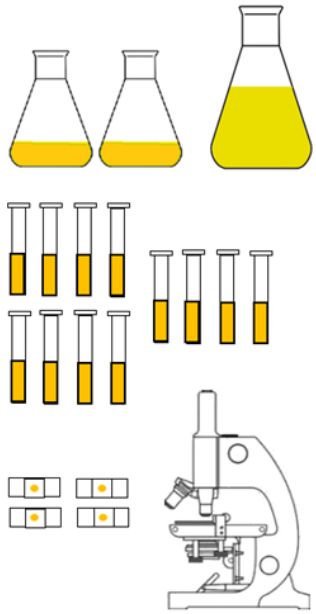

Figure 1. Diagram of experimental setup.

We pipetted 5mL of the diluted wild-type solution into 12 test tubes and 5mL of the diluted mutant solution into 12 test tubes (Figure 2).

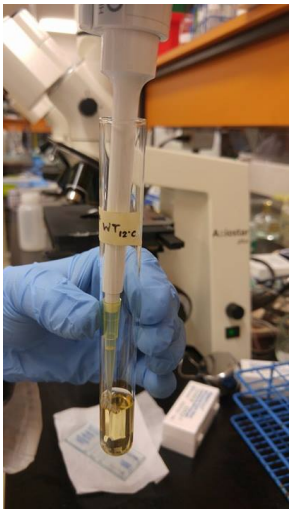

Figure 2. Transferring the diluted *T. thermophila* stock solution into test tubes.

We took an initial count of the *T. thermophila* in each test tube by pipetting 100µL of solution from a test tube and 10µL of the gluteraldehyde fixative into a microcentrifuge tube. We placed 20µL of the mixed fixative and solution onto the haemocytometer counting chamber, covered it with a coverslip and counted the number of cells in the blue square through the Axiostar compound microscope at 10X magnification.

We repeated this for each of the 24 test tubes and labelled each test tube wild type or mutant, replicate number, and the temperature it would be incubated at. Then, we arranged the labelled test tubes on a test tube rack and put four replicates of wild-type and mutant *Tetrahymena* into three different incubators with temperatures of 12°C, 20°C, and 30°C (Figure 3). After two hours, we used the same method to count the cells with the haemocytometers and we repeated this process three times for a total of six hours.

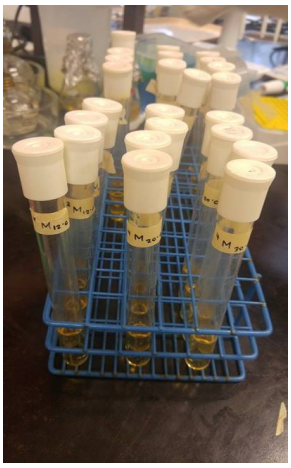

Figure 3. Preparing labelled test tubes for moving into the incubator.

Other Factors

We took note of the temperature in the lab room, and ensured that cell counts were performed in the same area of the lab in order to keep light and other factors constant. We also observed that samples had cloudiness and murkiness that may have indicated bacterial contamination or high population densities (Cassidy-Hanley, 2012). We used a two-way

ANOVA, 95% C.I., and t-tests to analyze the difference in the wild type and mutant populations of *T. thermophila.*

Results

Figure 4 shows a general trend of an increase in the mean growth rate of the mutant with increasing temperature over the six-hour period of measurement. The mean growth rate was calculated to be 1105, 1253 and 1656 cells/mL/hr corresponding to the treatment levels of 12ºC, 20ºC and 30ºC respectively. The opposite trend was observed for the wild-type strain where the mean growth rate was the greatest at the treatment level of 12ºC and decreased at the following temperatures of 20ºC and 30ºC. The mean growth rate for the wild type corresponding to treatment levels at 12ºC 20ºC and 30ºC was determined to be 1499, 525 and 515 cells/mL/hr, respectively.

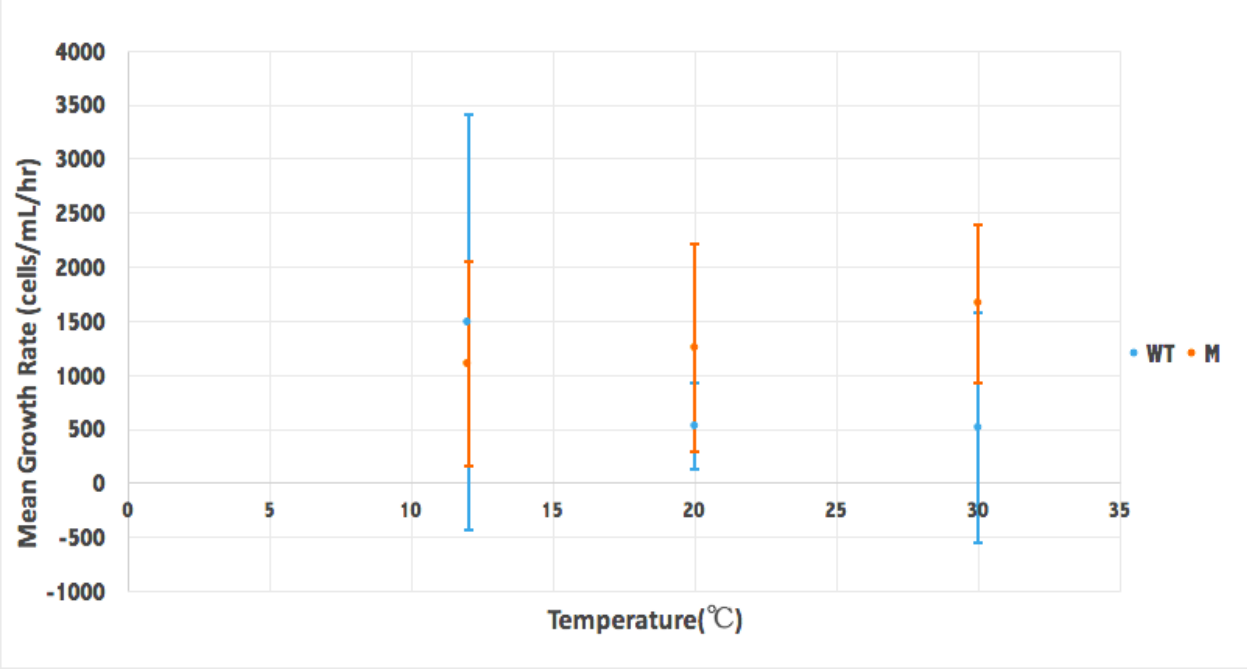

Figure 4. Mean growth rate measured over a six- hour period of *T. thermophila* wild-type (WT) and mutant *TtVPS13A* strain (M) at temperatures of 12℃, 20℃ and 30℃. The error bars indicate 95% C.I. n=4 for each strain at each treatment level. $p>0.05$ for H_1, H_2 and H_3.

Analysis of the mean growth rate at each treatment level for the wild-type and the mutant strain indicates a lack of statistically significant differences as is apparent by the overlapping 95% confidence intervals in Figure 4. H_{O1}, H_{O2} and H_{O3} all had corresponding p-values greater than 0.05 and were calculated to be 0.77, 0.30 and 0.40, respectively. The p-values computed using the two-way ANOVA for each H_{O1}, H_{O2} and H_{O3} also conclude that the differences in the mean growth rate trends of the wild-type and mutant strain of *T. thermophila* are not statistically significant.

Growth rate was determined by graphing the cell concentration for each replicate at zero, two, four, and six hours, and using excel to determine the slope. We then found the mean of the four replicates of each cell type at their respective temperatures to calculate their mean growth rate.

Discussion

We fail to reject all three aforementioned null hypotheses due to $p > 0.05$ for all. The data obtained for the wild-type and the *TtVPS13A* mutant was analyzed using a two-way ANOVA which produced p-values of 0.77, 0.30 and 0.40 corresponding to H_{O1}, H_{O2} and H_{O3}, respectively. Since all three p- values are greater than 0.05, we can conclude that differences in the mean growth rates deduced from our results are not statistically significant and thus we fail to reject H_{O1}, H_{O2} and H_{O3}.

The statistical analysis of our results computed $p\ (0.77) > 0.05$ for H_{O1} and therefore we fail to reject that temperature has an effect on the growth rate of wild-type *T. thermophila*. Our prediction therefore was not supported by the results since we expected the growth rate to significantly increase at the higher temperature treatment levels relative to the growth rate at the lowest temperature of 12ºC The expected results were based on a previous study by Frankel and

Nelsen stating that *T. thermophila* exhibits optimal growth and exponential cell division at 35ºC (2001). They also concluded that *T. thermophila* has the ability to multiply close to 40ºC without abnormal development (Frankel & Nelsen 2001). Thus in the chosen range of 12ºC to 30ºC for this experiment, we expected to see a statistically significant increase in the growth rate of *T. thermophila* upon exposure to temperatures approaching the optimal. Although we had expected the data to support that temperature does have an effect on the growth rate of wild-type *T. thermophila*, there is a plausible explanation for the results observed. Similar to many other eukaryotes, *T. thermophila* has mechanisms that sustain the survival of the organism upon changes in environmental conditions and regulation of gene expression is one of them. Normal protein synthesis is under stress when the cell is in an environment differing from the optimal and results in defective protein folding and thus function. An exposure to temperatures deviating away from the optimal to non-lethal temperatures induces production of heat shock or cold shock proteins which are metabolically stable in the new environment (Fink & Zeuthin 1980). These specific, environmentally regulated proteins are present at the cellular membrane surface approximately one hour after incubation and are transcribed at a faster rate as transcription of normal temperature protein decreases (McMullin & Hallberg 1987). Although the exact function of these proteins is not yet clearly understood, the inability to produce the proteins leads to cell death upon exposure to temperatures that would otherwise be non-lethal to *T. thermophila* (McMullin & Hallberg 1987). An example of such a protein is hsp70, a chaperone protein studied by Fukuda *et al*. (2015) which assists normal protein folding and thus function of the otherwise reduced protein synthesis. The presence of the normal, functional proteins at new temperatures can thus support cell division however at relatively reduced levels. This can potentially explain the survival of the wild-type *Tetrahymena* at the three treatment levels of

temperatures deviating away from the optimal. The generally decreasing growth rate of the wild type, although not statistically significant, can also be justified by the presence of a reduced number of normal proteins in the cell and consequently depreciating cell division. Using the previously stated information, we assume that significantly different results would be produced at temperatures at extreme ends of the spectrum such as 10ºC and 40ºC where normal protein synthesis would be unsalvageable.

Furthermore, the generally decreasing mean growth rate trend visible in Figure 4 for the wild type could have also occurred due to unintentional mishandling of the test tubes. A study by Hellung-Larsen & Lyhne (1992) detected patterns of low growth due to inhibited cell division caused by the handling of the cell culture tubes. The doubling time increased by twofold upon shaking cell cultures at 28ºC (Hellung-Larsen & Lyhne 1992). The shaking has no effect on the cell size and only inhibits cell division for cell concentrations under 10^4 cells/mL, consistent with the concentrations used in our experiment. It is proposed that the decreased growth rate is a result of hyper-oxygenation of the medium which disrupts cells division. It is however not clear as to what stage of cell division is more vulnerable to this phenomenon. Hellung-Larsen and Lyhne (1992) also concluded that the effect of the shaking is immediate and the growth rate is the same at the start and at the end of the incubation period. Therefore, it is possible that the cell counts taken after every two hours were subject to the initial impact of shaking. In our experiment, shaking of the cell culture test tubes was inevitable such as moving them to incubators and removal from the incubators for cell counts along with transportation of test tubes to and from incubation spots. These phenomena potentially played a role in the unexpected results as handling plays a powerful role in impacting cell division and therefore overall population growth rate.

Furthermore, we also fail to reject H_{O2} that temperature has no effect on growth rate of the low vacuole mutant strain of *T. thermophila*. The p-value calculated was 0.30 which is greater than 0.05 and thus the means for cells numbers of the *TtVPS13A* strain at each temperature level were not statistically different. We had expected significant results in order to support H_{A2} and initially predicted that the formation of a relatively low number of vacuoles in the mutant would result in decreased nutrient uptake which would then decrease the rate of cell division (Asai & Forney 1999). In a similar study, Orias and Rasmussen (1976) tested the growth of a mutant with heat-sensitive development of the oral apparatus involved in the phagocytic feeding mechanism accompanied by the decreased formation of food vacuoles. The mutant in this study is similar to *TtVPS13A* since both are characterized by a low number of vacuoles. They observed that at 28ºC the mutant strain continued to form vacuoles and exhibited similar growth as the wild-type. Orias and Rasmussen's (1976) study also observed that at temperatures at the extreme end of the spectrum such as at 37ºC the mutant displayed growth even with the absence of vacuoles. The key finding of their study was that this ciliated protozoan has a dual capacity for nutrient uptake. Phagocytosis is necessary for particles greater than 0.5 μm in diameter. However, small food particles can be absorbed via the surface of *T. thermophila* and this allows the cell to sustain its growth (Orias & Rasmussen 1976). Using this study as support, our data can be explained since at the highest temperature of 30ºC the mean growth rate was the highest although not significantly, compared to the other two treatment levels of lower temperatures. Since the mutant strain in this case is only lacking a functional *VPS13A* gene, we assume that it has all the other mechanisms that sustain survival at temperatures that vary from the optimal as discussed above. Consistent with Orias and Rasmussen's (976) study, mutant *TtVPS13A* continued to grow at all temperatures regardless of a low number of vacuoles. The

exact function of the *VPS13A* is yet to be clarified. Therefore, we can say that although the *VPS13A* protein is essential for mediating phagocytosis due to its association with the phagosome membrane, the lack of this protein is not lethal for the cells.

The differences in growth rates between the wild-type and the mutant strain were also concluded to be not statistically significant upon assessment of p (0.40) > 0.05. Thus, we fail to reject H_{O3}. This is apparent in Figure 4 where the growth rates at each temperature treatment of the wild-type and the mutant strain are close and consistent with overlapping confidence intervals. Our findings were consistent with a study by Samaranayke (2011) in which the mutant was also observed to have no significant impact on the growth rate. Samaranayke (2011) observed how the mutant type *T. thermophila* and the wild-type *T. thermophila* did not seem to have significantly different growth rates, similar to the trends observed in our findings. The main difference between the mutant and wild type comes in the form of their vacuoles, as the mutant type *T. thermophila* was observed by Samaranayke (2011) to have an inability to form properly food vacuoles properly due to deletion of a specific protein that was involved in phagocytosis. Further studies were done by Samaranayke (2011) to assess whether the inability to properly form food vacuoles through phagocytosis may impair their cellular function. Samaranayke (2011) found that the doubling times of the mutant strains were not significantly different from those of the wild-type cells in situations where phagocytosis was not required, however in situations where phagocytosis was required, the mutant with the knockout gene grew significantly slower, almost double the time of the wild type cells. The biological evidence for this was provided by Suhr-Jessen & Orias (1976) where they created a mutant *T. thermophila* cell with a defective oral apparatus, as discussed previously. They observed that *Tetrahymena* have two efficient and sufficient forms of nutrient uptake systems. One is called the oral uptake

system, which involves food vacuole formation via uptake from the oral apparatus, and the other is a surface uptake system where the cell is capable of taking in most small inorganic and organic molecules from the medium. As our experiment provided our *Tetrahymena* with an environment where phagocytosis is not required, we would expect the fact that our mutant *Tetrahymena* to not differ much from our wild type in terms of growth rate.

A source of error that may have contributed to our experiment could potentially be from the use of the haemocytometer, which was used for cell counts. As all members of our group were counting cells during different times of the day due to time constraints as well as availability, the way each individual member counted may not have been consistent. Optimally, one person would count all the replicates to reduce possible errors. Another potential source of error and variation may have occurred during the transferring of material from the test tube to the counting tubes to the haemocytometer slides. As the person doing each step was different, the pipetting and mixing of the *Tetrahymena* cell cultures could have been affected. For example, some of the members may have pipetted at the top of the cell culture and others may have pipetted at the bottom. As the density of the cell culture may have been different at the different areas, this could influence our counts when we used the haemocytometer.

Conclusion

After analysis of the wild type and mutant population sizes of *T. thermophila*, we fail to reject our three null hypotheses that temperature and the presence of the low vacuole mutation affect population size, and that wild-type and mutant *T. thermophila* would exhibit the same growth pattern at different temperatures.

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