The Effect of Glucose Concentration on *Tetrahymena Thermophila* Growth Rate

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Abstract

Tetrahymena Thermophila is a unicellular eukaryote with a doubling time of approximately 2 hours, making it ideal to study population growth. In the presence of nutrients, namely glucose, its growth rate can be further increased. The objective of our study was to determine how differing glucose concentrations would have an impact on the growth rate of *T. Thermophila*, using treatment concentrations of glucose at 0.2%, 1%, 3%, and 5%. We hypothesized that with increasing glucose concentration, growth rate would increase, with peak growth at 3% glucose. In this study, we placed *T. Thermophila* in media with varying glucose concentrations, and did trials at 3, 21, 24, and 27 hours, with cells kept incubated at 35℃ to promote optimal growth. Cell density was then determined using a haemocytometer. We found that the average population growth rate increased slightly from 0.2% glucose to 1%, before it significantly increased at 3% where it displayed the maximum growth rate, and then sharply declined at 5% glucose. Using a one-way ANOVA test, it was revealed that our results were not statistically significant ($p=0.0768$) therefore we were unable to reject our null hypothesis that there was no significant difference between increasing glucose concentrations on mean growth rate.

Introduction

Tetrahymena thermophila, a member of the Tetrahymenidae family, is known to be a unicellular eukaryote that swims and resides in temperate freshwater environments (Collins & Gorovosky, 2005). This particular organism was chosen for our research experiment as it is a ciliate model organism. *T. thermophila* has a unique and easily manipulated single-cell life cycle and also is able to grow in culture with little to no difficulty (Smith et al., 2013). Furthermore, it also has a rapid growth rate, under optimal conditions the cells can double in less than 2 hours (Cassidy-Hanley, 2012). To achieve the most favourable growing conditions, *T. thermophila* cells must be grown at the optimal temperature of 35°C (Asai & Forney, 1999).

T. thermophila meet their nutritional needs and uptake food through the process of phagocytosis where specialized cells called phagocytes ingest and break down other microorganisms and then create a food vacuole for storage (Jacobs et al., 2006). The stimulation of phagocytosis in *T. thermophila* is dependent on the concentration of the medium it grows in (Quiñones-Maldonado & Renaud, 1987), meaning that the growth rate of the organism is dependent on the components of the medium. Previous studies done by Szablewski et al. (1991), and Lee et al. (2015) show that the presence of glucose in the

medium increases the growth rate of *T. thermophila*, however, only a limited amount of glucose can be present in the growing media without jeopardizing the rate of cell division itself.

The objective of our study was to determine how differing glucose concentrations would have an impact on the growth rate of *T. thermophila*, building off of a study done previously by Lee et al. (2015). The study found that the 4% glucose concentration resulted in the largest number of cells and cell density, while there was a detrimental impact on the cell density of *T. thermophila* at the 6% glucose concentration. Their treatment concentrations were 0.2%, 2%, 4% and 6%, therefore we decided to test the interval values, using treatment concentrations of glucose at 0.2%, 1%, 3%, and 5%. In order to determine whether the 4% glucose concentration was in fact the optimal glucose concentration that resulted in the largest cell density and growth rate, we tested the surrounding values. Our null hypothesis was that there is no significant difference in the growth rate with the differing glucose concentrations. Thus, our alternative hypothesis predicted that the growth rate with the differing glucose concentrations would be significantly different.

Methods

Experimentation started with a cell culture stock of 50 mL suspended in standard *T. thermophila* media (0.2% glucose concentration). Before setting up the trials, we took the stock culture and conducted an initial cell count. We took 100 µL of the stock culture and mixed 10 µL of the fixative in a small eppendorf tube. We then took 20 µL of the solution and added it onto a hemocytometer and placed a cover clip on top to hold the solution in place. Using a Axiostar compound microscope at 10x magnification, we then counted the number of cells within each of the 16, 1 mm x 1 mm squares. If the number of cells was too high, we averaged the number of cells per square counted already and extrapolated the number to the rest of the 16 squares. We were then able to calculate the number of cells per millimeter and this was our initial cell count. We then set up the different glucose concentration media (0.2, 1, 3, and 5% glucose concentration) by adding calculated

values of the cell culture stock solution, 50% glucose concentration media, and 0.2% standard media. We set up 3 replicates of all concentrations, leading to a total of 12 trials, illustrated in Figure 1. After calculations, we added 388 µL of the standard *T. thermophila* cell stock in all the trial tubes, before adding the according amount of glucose and standard media to reach 1 mL. For example, our 1% trial used 20 µL of the 50% glucose concentration media, 388 µL of the standard cell stock, and 592 µL of the standard media.

Figure 1. Replicates for 0.2% (labeled as CA, CB, or CC), 1%, 3%, and 5% treatments

After the initial set up was complete, we set the trial tubes into a 35 °C incubator to allow for cell growth. We came back in 3 hour intervals to sample the tubes. After proper mixing of the solution, we pipetted 100 µL out of the trial tubes into smaller Eppendorf tubes and mixed it with 10 µL of the fixative and placed the samples in the fridge to inhibit any more growth. We did this a total of 4 times, resulting in 48 total samples where there were 12 samples for each sampling time. When sampling was complete, we acquired the samples and pipetted 20 µL onto the hemocytometer to conduct the cell counts for each sample, similar to the initial cell count.

After the cells were counted using the haemocytometer, they were converted to cell concentrations (cells/mL) by multiplying the cell counts by 2 dilution factors. The first dilution factor was multiplying by 1.1, which represented the 10 µL of fixative that was added to 100 µL of the cells before counting. The second dilution factor was a varying dilution factor that depended on the square

size in the haemocytometer that counted approximately 150 cells. Once the cell concentrations were calculated, the average cell growth rates for each replicate in each treatment was then calculated by plotting the cell concentration against time. Then, the slopes of each replicate were taken and inputted into GraphPad Prism 9 to plot the average growth rate against glucose concentration (Figure 2). Error bars indicate 95% confidence intervals, which represent the interval of values that has a 95% probability of containing the true mean of the group. Lastly, a one-way ANOVA test was also conducted through GraphPad Prism 9 to determine if there was a statistically significant result between glucose concentration and average cell growth rate. Due to the findings being statistically insignificant, there was no post hoc analysis performed.

Results

The average growth rate of *T. thermophila* placed in 4 different glucose concentrations is shown in Figure 2. For each of the 4 glucose concentrations, there were 3 total replicates (n=3). The findings suggest that *T. thermophila* exhibited different average growth rates by being placed in environments with different glucose concentrations over 2 days, where the control (0.2%) glucose concentration had an average growth rate of 977 cells/mL/h, the 1% treatment group's growth rate was 1213 cells/mL/h, the 3% treatment group's growth rate was 4749 cells/mL/h, and the 5% treatment group had a growth rate of -401 cells/mL/h. This trend suggests that average growth rate increased slightly starting from the control 0.2% concentration to 1%, before it drastically increased when it reached 3% concentration where it displayed the maximum optimal growth rate, before finally sharply declining at 5% glucose concentration. Furthermore, *T. thermophila in* 5% glucose concentration was the only group that displayed a negative growth rate as the other 3 glucose concentrations all had positive average growth rates. Error bars plotted represent 95% confidence intervals (Figure 2). A one-way ANOVA test was then conducted to determine if there was a statistically significant difference between the average

growth rates of the 4 groups. A p-value of 0.0768 was calculated, indicating that there were no statistically significant differences between the average growth rates of the 4 glucose groups.

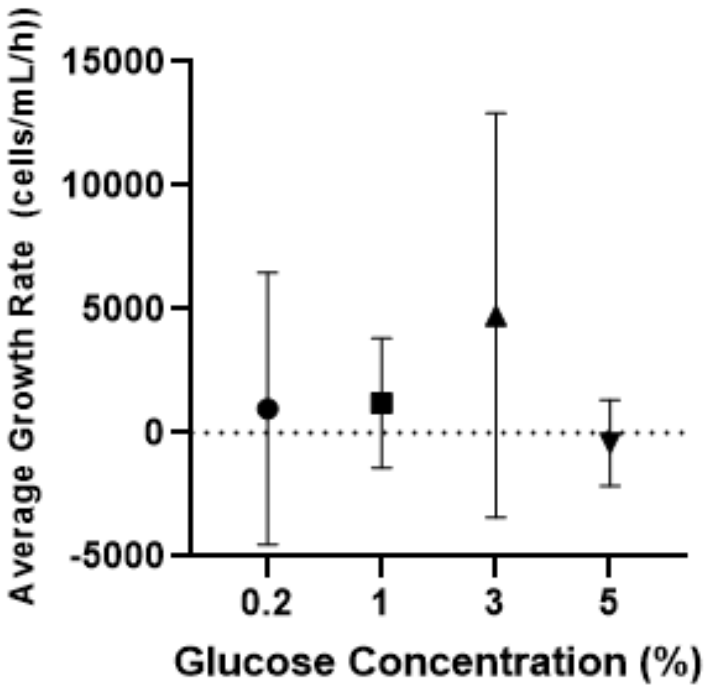

Figure 2. The average growth rate of *Tetrahymena thermophila* in 4 different glucose concentrations (n=3). Error bars represent 95% confidence intervals. The p-value calculated through a one-way ANOVA test is 0.0768.

Discussion

The purpose of the study was to examine the role of different glucose concentrations on the growth rate of *T. thermophila.* We predicted that a 3% glucose concentration would be the optimal glucose treatment for maximal *Tetrahymena* growth. Based on the results of the one-way ANOVA ($p=0.0768$, $p> 0.05$), we reject the alternative hypothesis that there is a difference in the average growth rate of *T. thermophila* in the 3% glucose control in comparison to the 0.2%, 1%, and 5% treatments. Our statistical tests, as observed in Figure 2, demonstrate statistical insignificance in the 3% glucose

treatment relative to the other treatment groups. Furthermore, there is a relatively higher average growth rate (cells/mL/h) in the 3% glucose concentration compared to the other treatment groups, however as indicated by of our results, we cannot state with statistical significance that the 3% glucose concentration group is the optimal growth treatment of *T. thermophila,* thus failing to reject the null hypothesis.

While our results are not statistically significant, the raw data does demonstrate a relatively higher average growth rate in 3% glucose concentration compared to other treatment groups. To explain this occurrence in parallel with the findings of our statistical tests, we looked into previous literature that investigated the role of differential glucose concentrations on the growth rates of *Tetrahymena*. Kiy and Tiedke (1992) found that with increased glucose concentration treatment the doubling time for *T. thermophila* increased. Thus, explaining why at 3% glucose concentration the concentration of cells at the end of the test period is significantly higher than the 0.2%, 1% and 5% treatments. Yet, the 3% glucose treatment had a low and stagnant number of cells relative to the other treatment groups until the last sampling time. This is consistent with the literature, where higher glucose concentration treatments have an initial lower growth rate due to a longer doubling time (Kiy and Tiedke, 1992; Cassidy-Hanley, 2012).

In Figure 2, the 5% glucose treatment demonstrates an average negative growth rate. To explain this trend, we considered how *Tetrahymena* growth rate and cell division are determined by food vacuole production and nutrient availability (Rasmussen, 1973; Seaman, 1961). Nutrients are taken up by phagocytosis, theoretically with an increase in nutrients, additional food vacuoles can be produced to aid in cell division. Yet, at 5% glucose concentration, we see the opposite effect. It is possible that the over-saturation of glucose acted as a limiting growth factor in metabolic processes (Blum, 1970). With an increase in glucose, previous research found a link to a lower oxygen pressure correlated with the saturation of carbohydrates within the contained media (Blum, 1970). Furthermore, based on studies on closely related species *Tetrahymena pyriformis*, a decrease in oxygen availability is tied to a

switch from aerobic to anaerobic metabolism and the inhibition of key enzymes in the gluconeogenic pathway (Blum, 1970). These factors lend an explanation to why the 5% glucose treatment demonstrates an average negative growth rate, as there isn't sufficient ATP production to foster substantial cell growth. Therefore, while glucose is a beneficial medium to aid cell division, high concentrations within a culture have disadvantageous impacts on cell growth.

Additionally, there are sources of variation and uncertainty that could have influenced the results. The initial culture of cells experienced a consistent lag phase in their growth cycle over the first week where we attempted to obtain data. Due to this, we had two entire previous failed experimental samples prior to the final experimental samples. Furthermore, our initial cell culture during our failed attempts was held in capped glass test tubes that got observably cloudy and contaminated. Overall, the multiple attempts and handling of the experimental materials could have led to variability within our study and impacted the acquired results. Based on the raw data trends at the end of the sampling period, our statistical test results being very close to a p-value of 0.05 and Kiy and Tiedke's findings, this indicates that a potentially longer sampling period could have produced different results. Therefore, further experimentation could expand on longer sampling periods and potentially study the adverse effects of low oxygen pressure with high glucose saturation on *Tetrahymena* growth rates.

Conclusion:

In our study, we found that the average population growth rate of *T. thermophila* increased slightly from 0.2% glucose concentration to 1%, before it significantly increased at 3% where it achieved its optimal growth rate, and then sharply declined at 5% glucose. Our one-way ANOVA test revealed that our results were not statistically significant ($p=0.0768$), and therefore we cannot reject our null hypothesis that there are no significant differences in growth rate between different glucose concentrations. However, with further testing and more replicates the findings that 3% glucose has a higher average growth rate may have a higher chance to be statistically significant.

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Literature Cited

Asai, J.D., & Forney, J.D. (1999). Cell biology of Tetrahymena thermophila. *Methods in Cell Biology, 62*, 31.

Blum, J.J. (1970). On the regulation of glycogen metabolism in *Tetrahymena*. *Archives of biochemistry and biophysics, 137*, 65-74. https://doi.org/10.1016/0003-9861(70)90411-X.

Cassidy-Hanley, D. M. (2012). Tetrahymena in the laboratory: Strain Resources, methods for culture, maintenance, and storage. *Methods in Cell Biology, 109*, 237-276. https://doi.org/10.1016/B978-0-12-385967-9.00008-6.

Collins, K, & Gorovsky, M.A. (2005). Tetrahymena thermophila. Current Biology, 15(9), R317-8. https://doi.org/10.1111/j.1550-7408.1991.tb04802.x.

Jacobs, M.E., DeSouza, L.V., Samaranayake, H., Pearlman, R.E., Siu, K.W., Klobutcher, L.A. (2006). The Tetrahymena thermophila phagosome proteome. *Eukaryot Cell., 5*(12), 1990-2000. https://doi.org/10.1128/EC.00195-06.

Kiy, T., & Tiedtke, A. (1992). Mass cultivation of Tetrahymena thermophila yielding high cell densities and short generation times. *Appl Microbiol Biotechnol* 37, 576–579. https://doi.org/10.1007/BF00240728

Lee, G., Magarian, A., Ng, V., & Pirzada, S. (2015). Effect of increasing glucose concentrations on the growth rate of Tetrahymena thermophila. *The Expedition, 4*.

Quiñones-Maldonado, V., & Renaud, F.L.(1987). Effect of biogenic amines on phagocytosis in Tetrahymena thermophila. *J Protozool., 34*(4), 435-8. https://doi.org/10.1111/j.1550-7408.1987.tb03208.x

Rasmussen, L.B. (1973). On the role of food vacuole formation in the uptake of dissolved nutrients by Tetrahymena. *Experimental cell research, 82*(1), 192-196. https://doi.org/10.1016/0014-4827(73)90261-9.

Seaman, G. R. (1961). Acid phosphatase activity associated with phagotrophy in the ciliate, *Tetrahymena*. *Journal of Protozoology, 8*(2), 204-212. https://doi.org/10.1083/jcb.9.1.243.

Smith, J.J., Wiley, E.A., & Cassidy-Hanley, D.M. (2012).Tetrahymena in the classroom. *Methods Cell Biol., 109*, 411-430. https://doi.org/10.1016/B978-0-12-385967-9.00016-5.

Szablewski, L., Andreasen, P., Tiedkte, A., Florin-Christensen, J., Florin-Christensen, M., & Rasmussen, L. (1991). Tetrahymena thermophila: Growth in synthetic nutrient medium in the

presence and absence of glucose. *The Journal of Protozoology, 38*(1), 62-65. https://doi.org/10.1111/j.1550-7408.1991.tb04802.x.