Determining the effect of glucose concentration on the chemotactic response of *Tetrahymena thermophila*

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Abstract

Chemotaxis is vital for the survival of *Tetrahymena thermophila* in various environmental conditions. Thus, the chemotaxis of *T. thermophila* was investigated in efforts to find an optimal concentration range of glucose at which a strong positive response occurs. *T. thermophila* were starved in 10mM Tris-HCl, pH = 7.5 for 24 hours then subjected to our glucose and control treatments using a 2-chamber assay apparatus. This was set up by suspending a 2 mL pipette containing 1mL of the glucose solution into a 6 mL test tube containing 2 mL of the starved *T. thermophila*. After fixing the cells with 1% glutaraldehyde, the cells in each replicate were counted using haemocytometer slides. A one-way ANOVA test followed by a Tukey HSD test showcased a statistically significant difference in glucose concentrations of 1×10^{-1} M and 1×10^{-6} M, however there was no statistically significant difference among the other treatments. Our study demonstrated that *T. thermophila* exhibits a stronger chemotactic response to higher concentrations of glucose. However, an optimal glucose concentration range for strong chemotaxis response was not determined.

I. Introduction

Tetrahymena thermophila (*T. thermophila*) are ciliated, non-parasitic protozoans abundant in freshwater environments (Collins, 2005). As protozoans, *T. thermophila* perform essential functions in aquatic ecosystems such as: processing of dead organic matter and debris, recycling and redistribution of mineral nutrients (Pratt and Cairns, 1985), and preventing virus propagation through phagocytosis (Pinheiro et al., 2007). Therefore, *T. thermophila* play a crucial role in the aquatic food chain (Pratt and Cairns, 1985) for enabling the survival of higher marine organisms (Stoecker and Pierson, 2019) which include keystone species, such as the Pacific Salmon in British Columbia (Bass et al., 2017).

Chemotaxis is defined as the directed movement of an organism induced by chemical gradients (Szemes et al., 2015). In *T. thermophila*, chemotaxis allows for feeding, avoidance of toxic substances, and prompt response to environmental stimuli, therefore it is essential for survival (Szemes et al., 2015). Studying the chemotactic response of *T. thermophila* can provide insight on its behaviour in varying conditions, as well as environmental conditions beneficial to salmon ecology and the rest of the aquatic food web.

Several studies have found *T. thermophila* to be suitable organisms to study chemotaxis for their rapid swimming speeds, and that their starvation in a Tris aminomethane hydrochloride buffer (Tris-HCl) media elicits a prompt chemotactic response (Chen and Leick, 2004; Hellung-Larsen et al., 1986; Leick and Helle, 1983; Szemes et al., 2015). Therefore, the starvation of *Tetrahymena* provides ideal conditions when studying chemotaxis in a laboratory setting. Previous studies have found glucose to be a necessary component for the growth of *T. thermophila* (Cassidy-Hanley, 2012; Hellung-Larsen et al., 1986). The chemotactic response of *Tetrahymena pyriformis* to various chemical stimuli such as amino acids, peptides, aroma compounds, and other sugars has also been studied previously (Hellung-Larsen et al., 1986; Láng et al., 2011; Szemes et al., 2015).

Hellung-Larsen et al. (1986) determined that glucose concentrations between 10^{-4} to 10^{-2} M elicited a negative chemotactic response in *T. pyriformis*. Meanwhile, Szemes et al. (2015) found that concentrations between 10^{-7}-10^{-8} M elicited a strong positive chemotactic response in *T. pyriformis.* Both studies utilized a two-chamber capillary assay to investigate chemotaxis in *Tetrahymena*. The concentration ranges and chemotaxis assay outlined by Hellung-Larsen et al. (1986) and Szemes et al. (2015) provide a basis for the methods of this study.

The purpose of this study is to investigate the chemotactic response of *T. thermophila* to varying concentrations of glucose, and to observe if an optimal concentration exists to elicit the strongest chemotactic response. The strength of the response will be measured by the amount of *T. thermophila* cells that travel towards each glucose solution, relative to the control. Based on the literature findings, we hypothesize that *T. thermophila* will exhibit the strongest chemotactic response to the highest concentration of glucose, the 1 x 10^{-1} M treatment.

II. Methods

Starvation of *Tetrahymena thermophila*

In order to get a prompt response from our *T. thermophila,* we starved our culture in a starvation media of 10 mM Tris-HCl, pH=7.5. We began by adding dense *T.thermophila* stock into two 50mL centrifuge tubes up to the 35 mL mark for each, using sterile technique by flaming the flasks. Next, we centrifuged both tubes containing our culture at full speed for 10 minutes. As soon as the centrifuge tubes were out of the centrifuge, the natant layer was quickly decanted using 10 mL pipettes, while being careful not to touch the pellet at the bottom. This step was important to complete promptly to get as much of the *T. thermophila* before they began swimming up to the natant layer. We combined the decanted *T. thermophila* into one centrifuge tube to avoid having unequal amounts of cells divided in the test tubes. Then, 25 mL of the starvation media (10mM Tris-HCl, pH=7.5) was pipetted into the centrifuge tube containing our *T. thermophila* using the 10 mL pipette. In order to mix the starvation media with the culture, we resuspended them using the pipette. With the same pipette, 2 mL of the *T. thermophila* in starvation media was added to sterile 6mL test tubes using sterile technique. This was repeated for a total of 12 test tubes

containing 2 mL of *T. thermophila* in starvation media. The *T. thermophila* in starvation media were then incubated at 25°C for 24 hours.

Preparation of Glucose

Starvation media was used as our control with no glucose added. Our varying concentrations of glucose were: 1×10^{-1} M, 1×10^{-3} M, and 1×10^{-6} M glucose in starvation media. We used varying concentrations of glucose in starvation media in order to keep the media the same as in the test tubes with the *T. thermophila*. We were provided 1×10^{-1} M glucose in starvation media by Mindy, our lab technician. We calculated the necessary volumes for each dilution using the formula:

$$C_1V_1 = C_2V_2$$

Two 10-fold serial dilutions were performed on the 1×10^{-1} M glucose to make 4 mL of 1×10^{-3} M glucose solution to have enough for the treatments and the second serial dilution. From the 1×10^{-3} M glucose solution we made, we performed three 10-fold serial dilutions to get 4 mL of our 1×10^{-6} M glucose solution.

Set up for 2-chamber assay apparatus

There were a total of 12 test tubes for 3 replicates for each of the 4 treatments. For each replicate, we pipetted 1 mL of each glucose concentration solution into a 2 mL pipette. Next, we suspended the 2 mL pipettes into the 6mL test tubes containing 2 mL of the starved *T. thermophila* for 15 minutes to give enough time for the *T. thermophila* to swim into the pipettes. When suspending the pipettes, we tilted them in the test tubes to prevent leaking of the glucose solutions (Figure 1). After 15 minutes, the contents of the pipette were emptied into a sterile 1.5 mL Eppendorf tube that was labeled. We then proceeded to micropipette 200 µL of 1% glutaraldehyde

to the Eppendorf tubes to fix the cells. To confirm whether the *T. thermophila* had swum into the pipettes, we prepared haemocytometer slides to count the cells. After mixing the sample, we micropipetted 20 uL of each sample and placed it under the cover slip on a haemocytometer slide. The number of cells were counted with a compound microscope on phase 1, using the 10x objective lens (Figure 2). We divided the number of cells by the number of squares multiplied by the dilution factor of the haemocytometer square and the correction for the fixative to obtain the number of cells in each sample:

$$\#\text{ of cells in sample (cells/mL)} = (\#\text{ of cells})/(\#\text{ of squares}) \times (8 \times 10^4) \times (1.2)$$

We made 3 counts for each sample and calculated the average number of cells from each sample. A one-tailed ANOVA test and Tukey HSD test were conducted for statistical analysis of our data through the socscistatistics program.

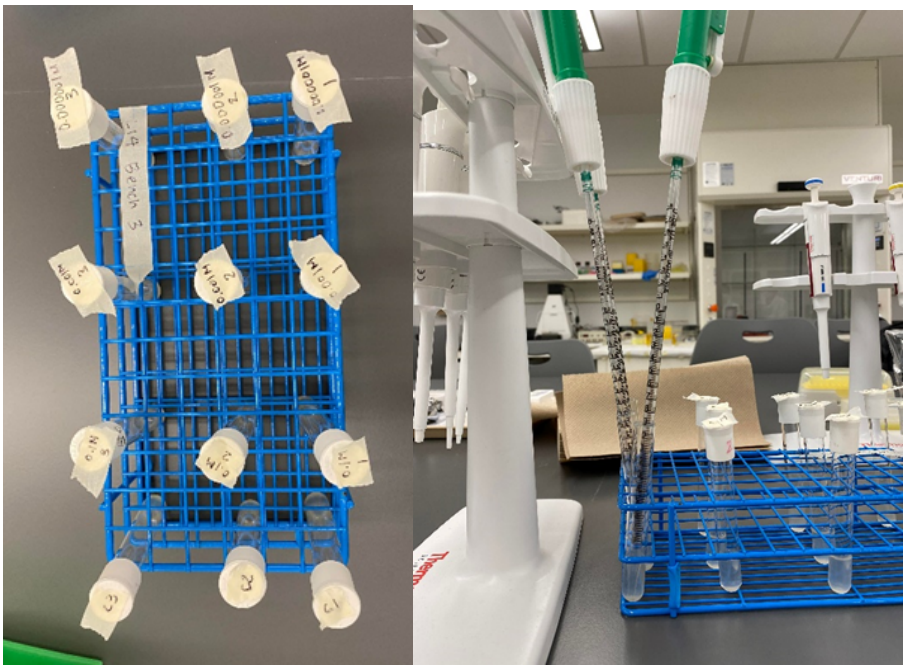

Figure 1. Experimental set up for two-chamber assay apparatus. The picture on the left shows our *T. thermophila* test tubes for a total of 12 replicates. On the right are our glucose solutions in the 2 mL

pipettes suspended in the test tubes containing the starved *T. thermophila* (note the pipettes have purposely been tilted to allow space for *T. thermophila* to travel).

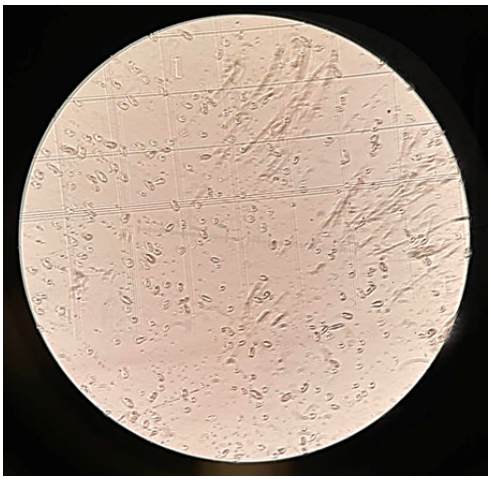

Figure 2. *T. thermophila* on a haemocytometer seen under a compound microscope with the 10X objective lens. *T. thermophila* are the oblong shapes seen in the squares. The triple-lined borders mark the bigger squares, while the single-lined borders mark the smaller 0.25mm x 0.25mm squares we used for our counting.

III. Results

Between all four treatments, the largest average cell concentration was found in *T. thermophila* exposed to the glucose treatment of 1 x 10^{-1} M with a standard deviation (SD) of 47737.09 cells/mL and 95% confidence interval (CI) of the mean of 172726.98 cells/mL ± 31187.66. This is followed by the control at 0 M glucose with a SD of 133000.65 cells/mL and 95% CI of the mean of 124162.92 cells/mL ± 86892.16. Treatments levels of 1 x 10^{-3} M and 1 x 10^{-6} M glucose showcased SD values at 73761.15 cells/mL and 95% CI of the mean of 102661.28 cells/mL ± 48189.73, and 20352.17 cells/mL and 95% CI of the mean of 26875.00 cells/mL ± 13296.51, respectively. The F-value for our treatments was found to be 5.13628.

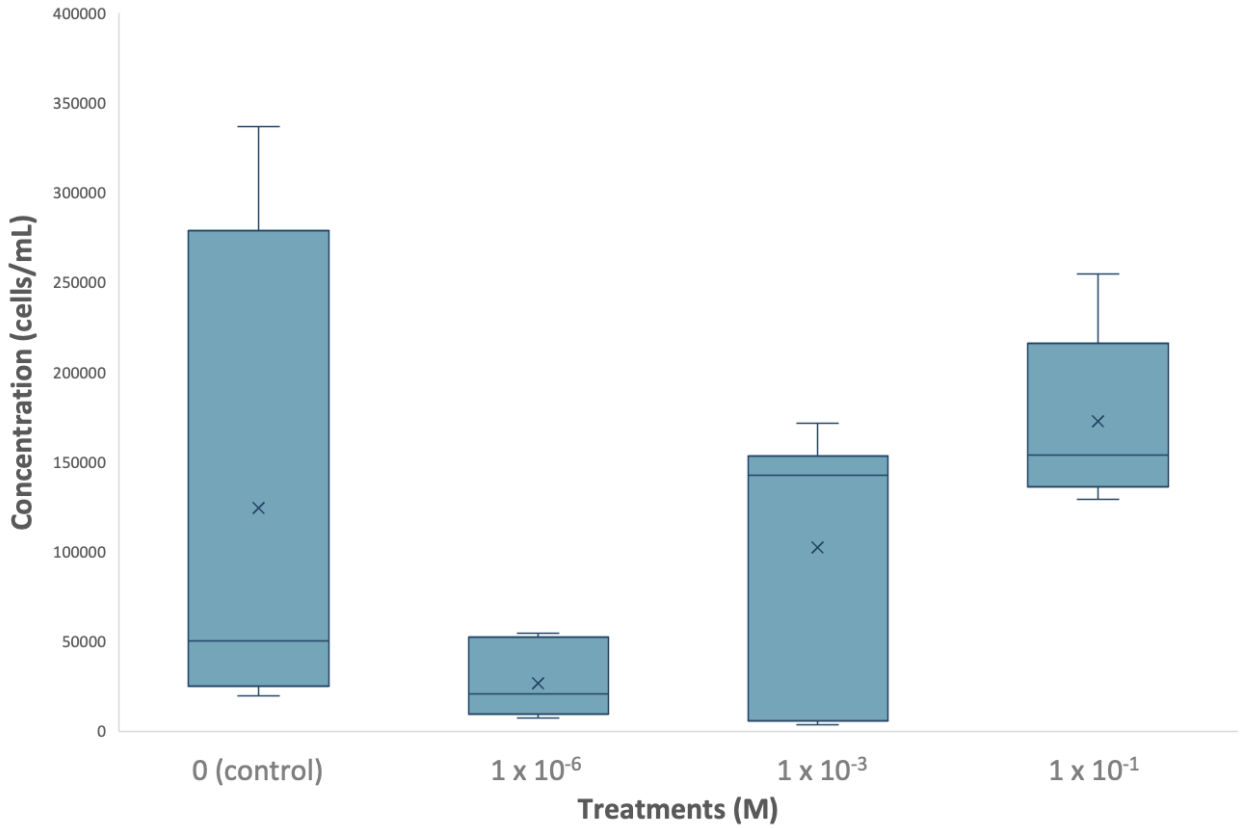

Figure 3. **Comparison of average cell concentrations of *T. thermophila* at treatment levels of 0 M, 1 x 10^{-6} M, 1 x 10^{-3} M, and 1 x 10^{-1} M (N=9).** Box plot represents 25% and 75% percentiles. The flat horizontal line marks the median (50250.00 for 0 M, 20625.00 for 1 x 10^{-6} M, 142601.94 for 1 x 10^{-3} M, 154000.00 for 1 x 10^{-1} M) while "x" marks the mean (124162.92 for 0 M, 26875.00 for 1 x 10^{-6} M, 102661.28 for 1 x 10^{-3} M, 172726.98 for 1 x 10^{-1} M). Upper and lower whiskers showcase the maximum and minimum values calculated. P-value = 0.005171 ($P < 0.05$).

As shown in Figure 3., the dispersion of average cell concentration varies for each treatment. First, the control showcases a large positively skewed distribution (Q1 = 19875.00 cells/mL, Q2 = 50250.00 cells/mL, Q3 = 337116.28 cells/mL, and standard error of means (SEM) = 44333.55 cells/mL). At a glucose concentration of 1 x 10^{-6} M, the range is fairly smaller but also positively skewed (Q1 = 7500.00 cells/mL, Q2 = 20625.00 cells/mL, Q3 = 54750.00 cells/mL, and SEM = 6784.05 cells/mL). Whereas at treatment level of 1 x 10^{-3} M there is a large negatively skewed distribution (Q1 = 3570.00 cells/mL, Q2 = 142601.95 cells/mL, Q3 = 171670.58 cells/mL, and SEM = 24587.05 cells/mL). Finally, at a glucose concentration of 1 x 10^{-1} M, the distribution

shows a smaller positively skewed range (Q1 = 129428.57 cells/mL, Q2 = 154000.00 cells/mL, Q3 = 254896.55 cells/mL, and SEM = 15912.36 cells/mL).

A one-tailed ANOVA test was conducted to determine if a statistically significant difference exists among our results. At an $\alpha = 0.05$, the overall p-value was found to be less than $\alpha = 0.05$ at 0.005171. Additionally, a Tukey HSD test was performed to determine where our difference lies within each individual treatment. It was discovered that only our treatments at 1×10^{-6} M and 1×10^{-1} M are statistically significant from one another with a p-value of 0.00285, whereas the cell concentration in other treatments overlap and are not statistically significant with individual p-values greater than the $\alpha = 0.05$.

IV. Discussion

The objective of our study was to determine whether *T. thermophila* exhibit different chemotaxis responses towards varying glucose concentrations. With a p-value of 0.0051741, our results are statistically significant as the $p < 0.05$. Therefore, we reject the null hypothesis and can confirm that *T. thermophila* exhibits a stronger chemotaxis response towards higher concentrations of glucose (Figure 1.). An analysis using the Tukey HSD test results show that a statistically significant difference only lies between the glucose treatments 1×10^{-6} M and 1×10^{-1} M. Our results demonstrate a general trend where a higher glucose concentration leads to increased *T. thermophila* cell concentration. The large standard deviations, large range in cell concentration, and an F-value of 5.13628 can be attributed to multiple sources of error.

A significant source of error in our experiment was that a small fraction of the glucose solution with the *T. thermophila* spilled out while emptying into the Eppendorf tube in one of the control replicates. This likely resulted in a loss of *T. thermophila* cells leading to inaccurate counts.

Additionally, the angle at which the pipettes were suspended in each test tube replicate was not consistent; some angles may lead to leakage, while some may prevent *Tetrahymena* from swimming up the pipette. Evidently, solution leakage may have occurred from a loose pipette bulb into the test tube given that the glucose solutions in the 2 mL pipettes were slightly below the initial 1 mL mark at the end of the treatment time. The pipette of Replicate 1 of the 1 x 10^{-3} M treatment, in particular, contained only 0.6 mL of glucose solution after 15 minutes. This would mean glucose was present in both chambers, leading to the loss of the glucose gradient to effectively trigger chemotaxis. Ultimately, all instances would have led to a less accurate final cell concentration. Our experimental set-up had no precedents and lacked a sophisticated apparatus compared to previous research on *T. thermophila* chemotaxis. For example, Leick and Helle (1983) used capillary tubes and poked holes in the two chambers such that the tetrahymena migrated horizontally, effectively eliminating leakage.

The study on chemotaxis in *T. thermophila* done by Leick and Helle (1983) noted that the cell concentration in the outer compartment should not be over 1-2 x 10^4 cells/ml to avoid high values in the control treatment. Additionally, the cell concentration should be below 2 x 10^5 cells/ml to induce maximal chemotactic effects during starvation (Koppelhus et al., 1994). For our study, we did not fix our cell concentrations in the test tubes (the outer compartment), which could explain the wide variances in our control replicates. We did not count our cell concentrations beforehand, thus, each test tube would have varying concentrations of *T. thermophila* to begin with. Accuracy could have been improved if we set a fixed starting concentration of cells in the outer compartment. Additionally, a major source of variance in our control replicates could be explained by the observation that the control replicate with the high cell count was murky in

appearance. This likely means that the cells were not fixed properly with our fixative (1% glutaraldehyde) so that a proper count would not have been taken.

Szemes et al. (2015), found that there was a significant positive chemotactic response to glucose in their chemotaxis study on *T. pyriformis*. However, their maximal chemoattractant effect of glucose observed at a concentration of 10^{-7} – 10^{-8} M, contradicts our results which showed a response at our highest concentration of 1 x 10^{-1} M. A second study found that *T. pyriformis* showed a negative response to glucose concentrations of 10^{-2} – 10^{-4} M (Hellung-Larsen et al., 1986). Upon further research, the varying results across glucose concentrations for each study and ours, could be explained by the results of Szemes et al's study (2015). They found that the strong positive responsiveness toward glucose was independent of concentration. However, with an insulin treatment, the *T. pyriformis* showed an increased and concentration dependent chemotactic response to glucose. Insulin induces the breakdown of intracellular glucose such that a positive chemotactic response is correlated with the instant metabolization of glucose as an energy source. Thus, the metabolic state of the cells could largely affect the chemotaxic response of the Tetrahymena to glucose (Szemes et al., 2015).

V. Conclusion

A statistically significant positive chemotactic response was demonstrated between the glucose treatments of 1 x 10^{-1} M and 1 x 10^{-6} M. Results were insignificant in the 1 x 10^{-3} M treatment, and large variances existed within the control group. Overall, the methods utilized in this study provide a solid basis for future experiments aiming to investigate chemotaxis. The optimal range of glucose to trigger a strong chemotactic response within *T. thermophila* cannot clearly be determined based on the findings of this study.

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References

Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2017). A survey of microparasites present in adult migrating Chinook salmon (Oncorhynchus tshawytscha) in south-western British Columbia determined by high-throughput quantitative polymerase chain reaction. *Journal of Fish Diseases*, *40*(4), 453–477. https://doi.org/10.1111/jfd.12607

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

Chen, F., & Leick, V. (2004). The protozoan tetrahymena as a bioindicator to screen bioactive substances. *Journal of Microbiological Methods*, *59*(2), 233–241. https://doi.org/10.1016/j.mimet.2004.07.003

Collins, K., & Gorovsky, M. A. (2005). Tetrahymena thermophila. *Current Biology*, *15*(9), R317–R318. https://doi.org/10.1016/j.cub.2005.04.039

Hellung-Larsen, P., Leick, V., & Tommerup, N. (1986). Chemoattraction in Tetrahymena: On the Role of Chemokinesis. *Biological Bulletin*, *170*(3), 357–367. https://doi.org/10.2307/1541847

Koppelhus, U., Hellung-Larsen, P., & Leick, V. (1994). Physiological Parameters Affecting the Chemosensory Response of Tetrahymena. *The Biological Bulletin*, *187*(1), 1–7. https://doi.org/10.2307/1542159

Láng, J., Rákász, V., Magyar, A., Pállinger, É., & Kohidai, L. (2011). Chemotactic effect of odorants and tastants on the ciliate Tetrahymena pyriformis. *Journal of Receptor and Signal Transduction Research*, *31*(6), 423–433. https://doi.org/10.3109/10799893.2011.625428

Leick, V., & Helle, J. (1983). A quantitative assay for ciliate chemotaxis. *Analytical Biochemistry*, 135(2), 466–469. https://doi.org/10.1016/0003-2697(83)90713-3

Pinheiro, M. D. O., Power, M. E., Butler, B. J., Dayeh, V. R., Slawson, R., Lee, L. E. J., Lynn, D. H., & Bols, N. C. (2007). Use of Tetrahymena thermophila To Study the Role of Protozoa in Inactivation of Viruses in Water. *Applied and Environmental Microbiology*, 73(2), 643–649. https://doi.org/10.1128/aem.02363-06

Pratt, J. R., & Cairns, J. (1985). Functional Groups in the Protozoa: Roles in Differing Ecosystems. *The Journal of Protozoology*, *32*(3), 415–423. https://doi.org/10.1111/j.1550-7408.1985.tb04037.x

Stoecker, D., & Pierson, J. (2019). Predation on protozoa: its importance to zooplankton revisited. *Journal of Plankton Research*, *41*(4), 367–373. https://doi.org/10.1093/plankt/fbz027

Szemes, F., Lajkó, E., Láng, O., & Kőhidai, L. (2015). Chemotactic effect of mono- and disaccharides on the unicellular Tetrahymena pyriformis. *Carbohydrate Research*, 407, 158–165. https://doi.org/10.1016/j.carres.2015.02.009