Observing chemotaxis in *T. thermophila* in the presence of glucose, lactose, and fructose

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Abstract

Tetrahymena thermophila is a free-living, unicellular eukaryote that belongs to the ciliated Protozoa, a major, ecologically successful monophyletic group (Oraisis, 2011). *T. thermophila* is lined by many cilia and uses them to move through water, as well as to sweep bacteria and other small debris into its mouth (Bozzone, 2000). Chemotaxis is a phenomenon when cell movement is directed by an extracellular chemical gradient. During this chemosensory behaviour, cells can change their locomotion as they detect changes in the concentration of the chemical. Cells can be attracted as positive chemotaxis or repelled as a negative chemotaxis by various chemical signals. Cell migration is essential for both multicellular and unicellular organisms. Chemotaxis is especially important for *T. thermophila* since it impacts the survival of *T. thermophila* in various environmental conditions. In order to observe the impact of each sugar on *T. thermophila,* the following experiment involved culturing *T. thermophila* in one of glucose, lactose, and fructose for 15 minute after starvation phase. The research conducted in this project can help identify an optimal concentration range of examined sugar at which a strong positive attraction can occur. The experimental findings were that there was no significance for all three sugar treatments. This finding contradicts the initial hypothesis and implies that the study needs a greater sample size in order to investigate an overall effect on different sugar treatments.

Introduction

Tetrahymena is a monophyletic genus containing 41 named species (Lynn & Doerder, 2012) of large, unicellular ciliates (Frankel, 1999), all characterized by their common 4-part oral structure (Lynn & Doerder, 2012). *Tetrahymena thermophila* is lined by many cilia and uses them to move through water, as well as to sweep bacteria and other small debris into its mouth (Bozzone, 2000). As is the case with many other organisms, *T. thermophila's* locomotion can be induced by the presence of some extracellular chemical gradient, such as that of a sugar. This phenomenon is called chemotaxis (Szemes, 2015).

In this study, samples of *T. thermophila* stock were incubated in starvation medium for 24 hours before being exposed to one of glucose, lactose, and fructose for 15 minutes, in order to determine the impact of each of these sugars on *T. thermophila* chemotaxis. These sugars were selected because of availability in the lab, and because they are commonly found in foods. The null hypothesis states that the presence of sugar, regardless of its type (of the three sugars tested), will have no effect on chemotaxis. It was hypothesized, through the alternative hypothesis, that sugar presence and type would have an effect on chemotaxis in *T. thermophila*. More specifically, it was predicted that glucose would be a strong chemoattractant, while fructose and lactose would have no positive effect on chemotaxis. This hypothesis is based, in part, on the results of a similar study, in which the effects of glucose, fructose, and lactose, among other sugars, on *Tetrahymena pyriformis* chemotaxis were observed. This study found that the rate of sugar-induced chemotaxis of *T. pyriformis* was determined to be a product of the physicochemical properties of the sugars, which determine how easily they can cross the organism's membrane as a means of ingestion (Szemes et. al., 2015). More specifically, there is evidence that *Tetrahymena* as a genus is particularly effective at internalizing glucose, relative to other sugars (Szemes et. al., 2015).

The findings of this study can be applied to numerous other organisms, as *T. thermophila's* cellular physiology is homologous with that of many mammalian models, including humans (Szemes et. al., 2015). In multicellular organisms, such as humans, these findings may have implications for fertilization, differentiation, and inflammatory responses (Szemes et. al., 2015).

Method

Starvation of *Tetrahymena thermophila*

To observe the chemotaxis in Tetrahymena thermophila, our culture needed to be starved. To starve the *T. thermophila*, we started by adding 35 ml of dense T.thermophila into two 50 ml centrifuge tubes and centrifuged at full speed for 10 minutes. Next, we used 10 ml pipettes to decant the natant layer, making sure to not stir up the pellet of T.thermophila. This process had to be done quickly as the T.thermophila would immediately start to dissipate away from the bottom. Once all of the natant layer has been removed, combine both pellets of T.thermophila and add 25 ml of starvation media into a 50 ml test tube. Around 5 ml of the starvation media was injected into the centrifuge tube that contained the pellets and poured into the 50 ml test tube in order to obtain the largest population possible. The mixture was then suspended with a micropipette and then 2 ml of the starved medium and T.thermophila was transferred into twelve 6 ml test tubes. The mixture was suspended with a micropipette before each transfer in order to add a uniform amount of T.thermophila into each test tube. Once all twelve 6 ml test tubes had 2 ml of T.thermophila, caps were placed on top of each test tube to keep any dust from falling into the tubes. The tubes were placed into a test tube rack and placed in a 21°C incubator for 24 hours.

Observing Chemotaxis

Once 24 hours had passed, 0.1 M solutions of glucose, fructose, and lactose were synthesized in 3 different 6ml test tubes and 5ml of the starvation medium was also added to a 6 ml test tube. To synthesize the glucose solution, 108.0 mg of glucose was added into 5 ml of

distilled water and mixed by swirling the test tube. The same procedure was done for fructose, using 180.16 mg, and for lactose, using 171.15 mg. These masses of sugar was obtained by using the formulas:

$$C_1V_1 = C_2V_2$$

and

$$M = \frac{n}{v}$$

These test tubes were labeled as their corresponding sugar solution and placed into a separate test tube rack. The test tube rack containing the starved T.thermophila was then removed from the incubator and labeled with a letter (C,G,F,L) corresponding with which sugar solution it would come in contact with and a trial number. As our group only had four micropipettes, 3 trials were performed using one glucose, fructose, lactose, and control solution. The test tube rack was then placed below a micropipette stand so a micropipette with a sugar solution could be placed into each section of the rack and the tip was suspended within the starved *T. thermophila* mixture. The setup can be observed in Figure 1.

Figure 1: Set-up of the experiment with four micropipettes hanging on the micropipette stand, with the tips suspended inside the starved T.thermophila mixture in each test tube. Each test tube and micropipette was labeled.

Once the setup was completed, the caps of the first trial test tubes were removed and four micropipettes, each containing 400 µl of either starvation media, glucose, fructose, or lactose, was placed into each test tube and suspended for 10 minutes. After ten minutes, the micropipettes were removed and the contents of each micropipette were ejected into a 2ml centrifuge tube. 40 µl of glutaraldehyde was added to each tube and the whole mixture was suspended using a micropipette, before 10 µl of each mixture was placed onto a haemocytometer. Then, the slide was placed under a microscope where we started to count the number of *T. thermophila* in each square. Figure 2 is an example of what the slide looked like under a microscope.

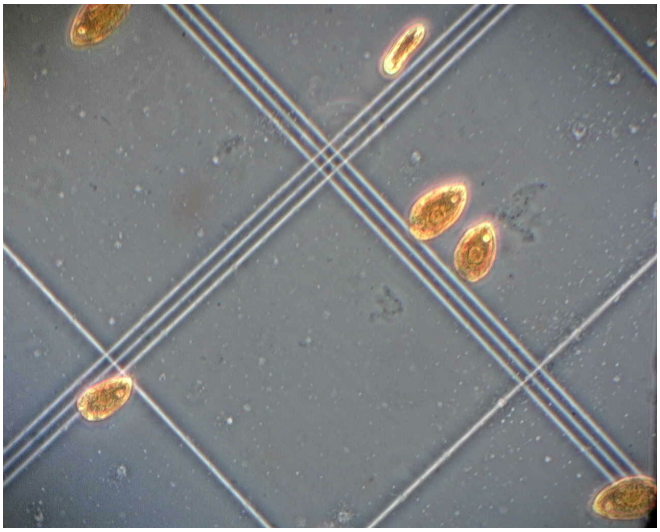

Figure 2: A test to observe what T.thermophila looks like the fixative under the compound microscope. Dense T.thermophila solution was used. Since there was not a lot of T.thermophila in the haemocytometers, all the T.thermophila inside the squares were counted. The data was then recorded into a table for statistical analysis.

Results

The number of *T. thermophila* attracted to the solutions of the four conditions was counted. The organisms were observed under hemocytometer, which allowed for conversion of the number of cells counted to the cell concentration in cells per mL (Figure 3). Due to the small number of the organism found under microscope, the number of cells in each small square of the hemocytometer have been disregarded. Instead, the cell concentration was calculated based on the number of cells found in the entire volume of the hemocytometer. The total volume of the hemocytometer was 1 nL, which is 10^{-4} mL. Therefore, the cell concentration was calculated by multiplying the number of *T. thermophila* counted by 10000. Figure 4. illustrates the summary of the cell concentrations under the four treatments.

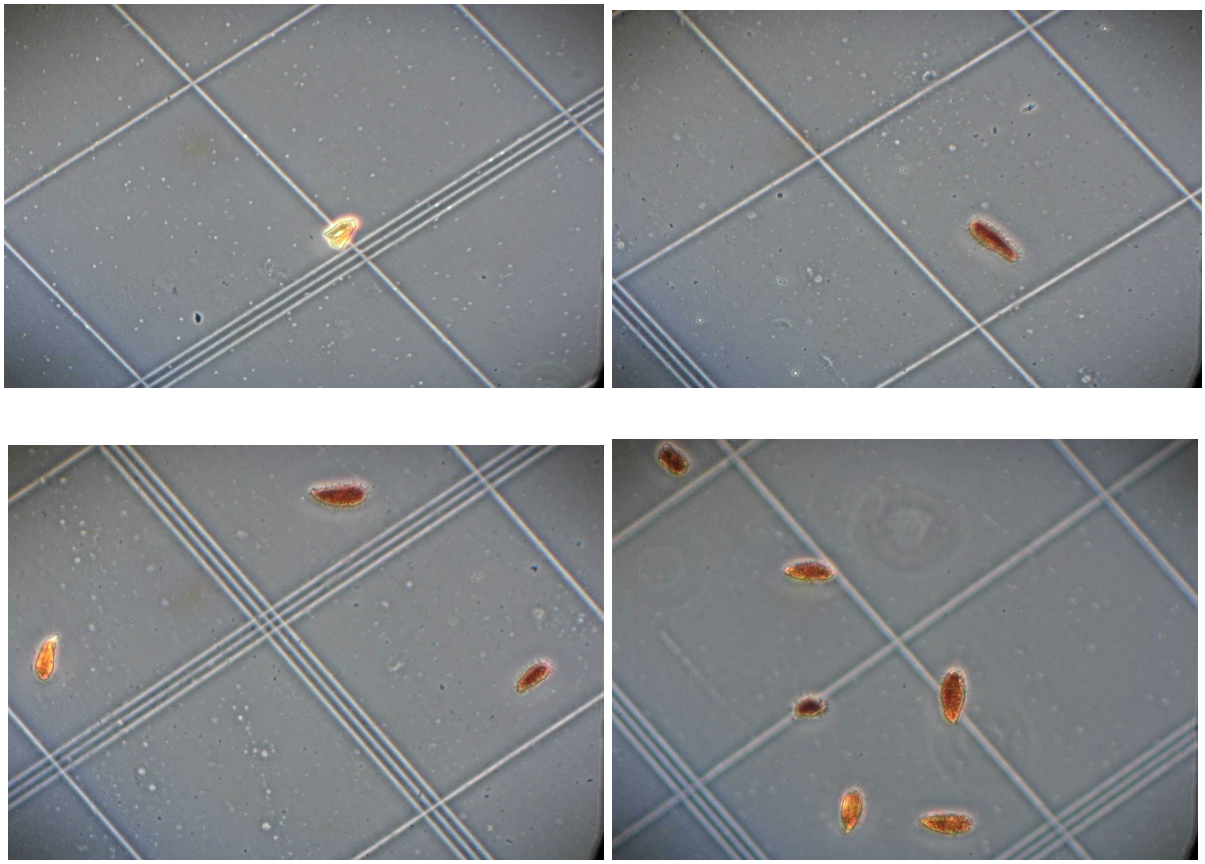

Figure 3. Photos of *T. thermophila* observed under microscopes. The photos include chemoattraction of the organisms to control (top left), glucose (top right), fructose (bottom left), and lactose (bottom right)

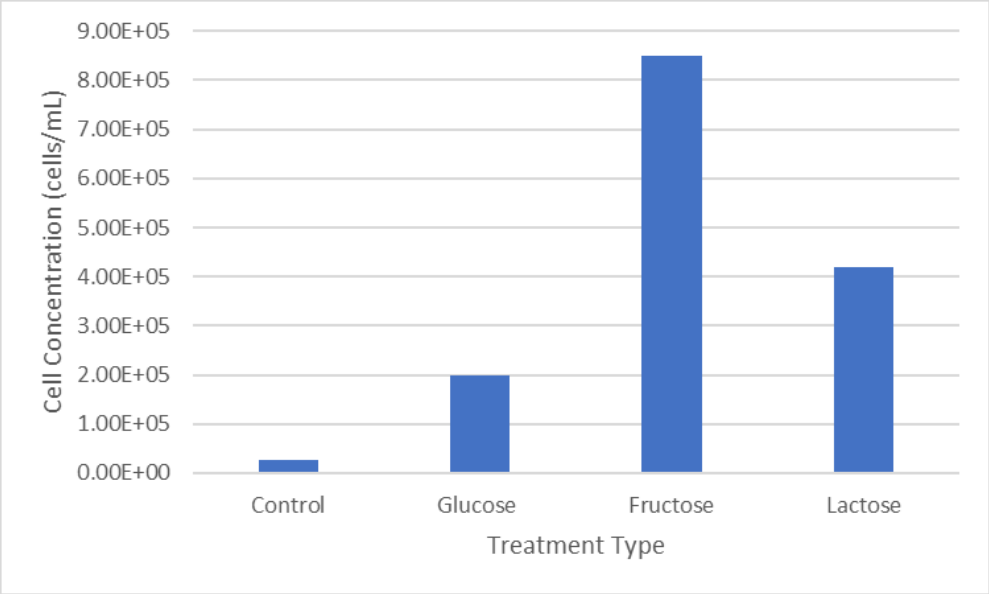

Figure 4. The figure of the number of chemoattracted *T. thermophila* counted under four different treatments (control, glucose, fructose, and lactose). The unit conversion was performed from the number of cells to cells per mL. Three replicates of each treatment have been performed.

The sample mean and the standard deviation of *T. thermophila* counted for the control was $2.7x10^4$ and $2.0\ x10^4$ cells/mL, respectively. For glucose and fructose, the cell concentrations were $2.0x10^5$ and $8.5x10^5$ cells/mL, and $1.9\ x10^5$ and $9.0\ x10^5$ cells/mL for standard deviations. Lastly, for lactose the sample mean was $4.2x10^5$ cells/mL with the standard deviation of $9.2\ x10^4$ cells/mL. Therefore, with regards to the magnitude of sample mean, the largest cell concentration was found in lactose and the smallest found in the control. Whereas, with regards to the standard deviation, fructose was largest, while the control had the smallest.

In order to determine the significance of the differences in the sample means, a one-way ANOVA statistical test was performed. The statistical test resulted in the F-statistic value of 1.9219 and the p-value of 0.2046, which is larger than the 0.05 threshold. Therefore, the experiment fails to reject the null hypothesis. In other words, there is no statistically significant

difference between the samples of different treatments. In addition, although the Tukey-Kramer test usually is not performed, unless a significant difference is found in ANOVA, but the Tukey-Kramer was performed as an assurance of the result. The Tukey-Kramer also could not find any significant differences between any of the treatment pairs. The largest Tukey p-value was 0.9 between the control and glucose, whereas the smallest p-value was 0.2053 between the control and fructose. Despite the variabilities in the p-value, even the smallest p-value found exceeds the threshold of 0.05; thus, the test again fails to reject the null hypothesis.

Discussion

The objective of this study was to determine how chemotaxis in *T. thermophila* is impacted in the presence of either glucose, lactose, or fructose. With a p-value of 0.2065, which is less than 0.05, our results are statistically insignificant. Additionally, a Tukey HSD test found all pairings to be statistically insignificant. Thus, we cannot reject the null hypothesis, which states that the presence of either glucose, lactose, or fructose positively impacts chemotaxis in *T. thermophila.* These results align with our prediction that lactose and fructose would have no positive effect on chemotaxis, but they conflict with our prediction that glucose would act as a chemoattractant.

These unexpected results may be caused by some flaw in our methodology or execution. One possibility is that we did not perform enough trials, as we only performed three trials per treatment group. This yielded only three data points per treatment group, without any apparent consistency between any group other than the control group. In particular, we were surprised by the fact that the third glucose-treated sample exhibited a complete absence of *T.*

thermophila cells on the haemocytometer. This brings to light the possibility of further inconsistencies or errors in our procedure. For example, the suspension angle of the micropipettes in each of the test tubes containing the starved *T. thermophila* was inconsistent. Certain angles may have hindered chemotaxis of the *T. thermophila* cells, despite the chemoattraction that they experienced.

Similarly, it is possible that in any of the samples, some of the treatment solution leaked out of the micropipette into the *T. thermophila* due to gravity. In similar studies, more sophisticated methods were used to prevent the leakage and unintentional mixing of solutions. For example, Szemes, F. et. al. (2015) used a two-chamber capillary chemotaxis assay, while Leick and Helle (1983) designed an apparatus that allowed for horizontal migration of *T. thermophila*, eliminating the unwanted impacts of gravity and pipette angle on chemotaxis.

An additional potential source of error was the starvation time of 24 hours, which may not have been sufficient to elicit a level of starvation that would render a significant migration toward a potential food source within the 15-minute window allotted for chemotaxis. That said, Szemes, F. et. al. (2015) did not undergo a starvation period and were observed after allowing 20 minutes for chemotaxis in the presence of the treatment sugars. Thus, this is less likely to be a source of error in our experiment.

If this experiment were to be performed in future, more conclusive results may be achieved by modifying the method to eliminate the aforementioned potential sources of error.

Conclusion

In conclusion, with a p-value of 0.2065 which is less than 0.05. This indicates that our results are statistically insignificant therefore, we fail to reject the null hypothesis. Our initial prediction was that glucose would be a strong attractant, while fructose and lactose would have no positive effect on chemotaxis. This hypothesis was also supported by past study, in which the rate of sugar-induced chemotaxis of *T. thermophila* was determined to be a product of the rate of ingestion of the given sugar by *T. thermophila*, which could be attributed to the sugar's level of topological polar surface area (Szemes, F. et. al., 2015). However, none of the examined sugars was a good selector for chemotaxis. The optimal range of sugar to trigger a positive chemotaxis within T. thermophila cannot clearly be determined based on the findings of this research.

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