The effect of time and food concentration on vacuole formation in *Tetrahymena thermophila*

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Abstract

The objective of this experiment was to study how time in food and food concentration affects food vacuole (FV) formation in *Tetrahymena thermophila.* This experiment gives us insight into the process of phagocytosis, a crucial biological process for *T. thermophila* to uptake nutrients. We diluted SSP medium containing Congo red (CR)-stained *Saccharomyces cerevisiae* (yeast) into concentrations of 8×10^7, 4×10^7, and 2×10^7 cells/mL added them to *T. thermophila* cultures, and allowed feeding for 60 minutes. samples were taken at 1, 15, 30 and 60 minutes. *T. thermophila* were then examined under the microscope to count the number of CR-stained FVs that were formed. After conducting a two-way ANOVA to analyze our data, we determined that the concentration of food available to *T. thermophila* in the medium had no significant effect on the number of FVs formed, but the length of feeding time was statistically significant. It was also found that these two independent variables were not interacting. *T. thermophila* is biologically limited in how quickly phagocytosis can be performed by the maximum capacity of its oral groove, which can explain the significant effect of time on FV formation.

Introduction

T. thermophila is a ciliated protozoan; it is an ideal model organism and can be used as a valuable resource to study unicellular processes (Bozzone 2000). To uptake nutrients, *T. thermophila* utilizes phagocytosis: a critical biological process where a phagocyte surrounds a food particle and engulfs it to form a food vacuole (FV) within the cell, as seen in Figure 1 (Gronlien *et al.* 2002). Following this formation, lysosomes then fuse to the FV to break down its contents so the cell can absorb the nutrients (Bozzone 2000). Cilia are crucial to this protozoan's holozoic nutrition; their coordinated motion creates a current to move food to the location of phagocytic intake, called the oral groove (Gronlien *et al*. 2002). Time and concentration of food particles are just a few of the important factors that can have an effect on phagocytosis (Fok *et al*. 1988).

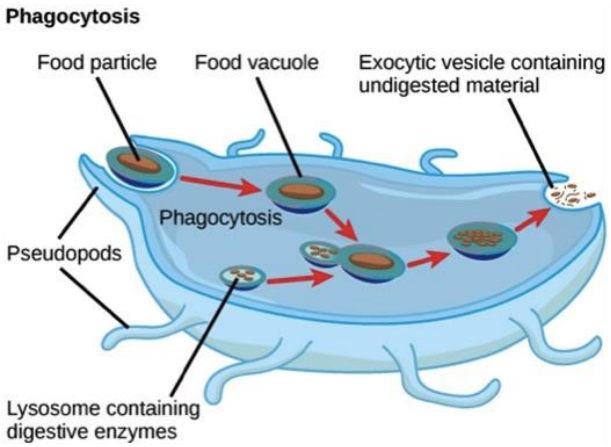


Figure 1. An overview of the process of phagocytosis in *Tetrahymena thermophila*, showing the cilia (labeled pseudopods), which moves the food to the oral groove where phagocytosis occurs and forms food vacuoles within the cell (Microbiology 2015).

The objective of our experiment was to test how the progression of time and different concentrations of Congo red (CR)-stained yeast in the medium would affect the number of FVs formed in wild-type *T. thermophila*. This is to help us understand the efficiency of phagocytosis in *T. thermophila*, which, despite being a good model organism, has had little study in this specific area. As such, to make predictions for our experiment we reviewed studies performed on *Paramecium*, a ciliary protozoan very similar in structure and function to *T. thermophila*. Fok et al. (1988) investigated *Paramecium* and using latex beads for the food medium, determined that the number of FVs formed increased as the number of beads in the medium increased. Based on these results, we predicted that an increased concentration of yeast in the medium will lead to higher numbers of FVs formed in the cell. *T. thermophila* is one of the most efficient filter feeders because it is covered in cilia. Therefore, if the concentration of yeast in the medium increases, then more food will be moved towards the oral groove, resulting in higher FV formation (Fok *et al.* 1988). Based on a study by Bozzone (2000) we also predicted that as the time that *T. thermophila* spends in the yeast medium increases, the number of FVs formed in the cell will also increase.

The first null hypothesis (H_{o1}) for our experiment is that time in food will not affect the number of FVs formed in *T. thermophila*. The alternate hypothesis (H_{a1}) is that time in food will affect the number of FVs formed in *T. thermophila*. Our second null hypothesis (H_{o2}) is that the concentration of yeast in the medium will not affect the number of FVs formed in *T. thermophila*. Alternatively, our second alternative hypothesis (H_{a2}) is that the concentration of yeast in the medium will affect the number of FVs formed in *T. thermophila*. Finally, our third null hypothesis (H_{o3}) is that time in food will have the same effect on FV formation at all concentrations of yeast in the medium. Our third alternate hypothesis (H_{a3}) stating is that time in food will have the same effect on FV formation at all concentrations of yeast in the medium.

Methods

To prepare the yeast, *Saccharomyces cerevisiae*, we incubated them in a 50°C water bath for ten minutes to kill them. We added the stain, Congo red (CR) at a 1:1 ratio of yeast to CR. We incubated the yeast overnight to allow the CR to stain the yeast.

After 24 hours, the yeast were centrifuged and the pellets were resuspended in SSP. We centrifuged and decanted a second time, and these pellets were then resuspended in SSP. Next we added 0.05 mL of glutaraldehyde fixative to 36 microcentrifuge (mcf) tubes, which were set aside for later. We then prepared and conducted a yeast concentration count using a haemocytometer. The yeast concentration obtained was used to calculate our highest yeast concentration treatment, and the medium and low concentration treatments were made from this initial amount. The final concentrations for all three treatments of stained yeast were: 8×10^7, 4×10^7, and 2×10^7 cells/mL.

Starting with treatment 1, we added 0.5 mL of the 8×10^7 yeast cells/mL solution to 0.5 mL of *T. thermophila* in SSP and mixed via finger vortexing. After 1 minute elapsed (t=1), we

pipetted 0.1 mL of this mixture into one of the mcf tubes with the glutaraldehyde fixative pre-added, and mixed via micropipette resuspension. This was repeated three times to obtain our three replicates. For treatment 2, the same steps were followed but with 0.5 mL of the 4×10^7 yeast cells/mL solution. For treatment 3, the same steps were followed but with 0.5 mL of the 2×10^7 yeast cells/mL solution. We then continued sampling each replicate at t=15, t=30, and t=60 minutes. Finally, we stored our 36 mcf tubes with samples at 4°C overnight. FV counts were done the next day by micropipetting 0.1 mL from each of the 36 mcf tubes onto a microscope slide, and viewing under an Axiostar compound microscope at 400x magnification. The slide was observed in a right to left and top to bottom manner to ensure no *T. thermophila* was counted twice, and then 10 random individual *T. thermophila* were examined and the number of red FVs were recorded. Only intact *T. thermophila* that were not in the process of dividing were counted. Figure 2 shows the view of *T. thermophila* under the microscope. We calculated the mean number of FVs for each slide and statistically analyzed our data using a two-way ANOVA test.

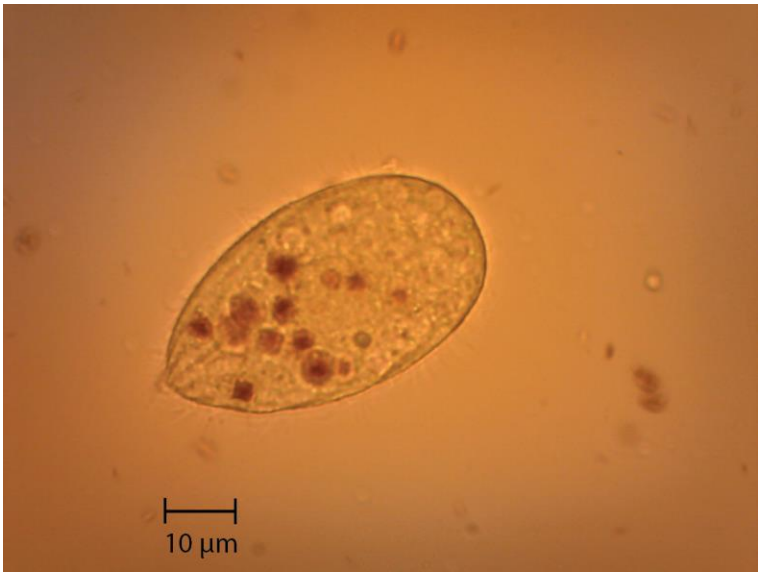


Figure 2. Observed *T. thermophila* exhibiting presence of food vacuoles as highlighted by the Congo red-stained yeast. The photo was taken using a DinoXcope Digital Microscope camera in conjunction with an Axiostar compound microscope at 400x magnification.

Results

Our data show that there is generally an increase in the mean number of FVs as time increases (Figure 3). This relationship can be observed in the mean numbers of FV's for each of the three treatments. For the 4×10^7 yeast cells/mL treatment at times 1, 15, 30, and 60 minutes, the mean numbers of vacuoles were 3.9±0.9, 2.9±0.9, 4.8±3.0, and 6.4±6.7, respectively (Figure 3). For the 2×10^7 yeast cells/mL treatment, the mean numbers of vacuoles at times 1, 15, 30, and 60 minutes were 1.7±0.1, 2.0±0.2, 3.6±1.8, and 7.9±2.4, respectively (Figure 3). For the 1×10^7 yeast cells/mL treatment, the mean numbers of vacuoles at times 1, 15, 30, and 60 minutes were 1.6±0.1, 1.5±0.7, 2.2±0.2, and 5.6±2.5, respectively (Figure 3). There is a significant difference in the mean numbers of vacuoles at the different times in food (p-value = 6.2×10^{-4})'

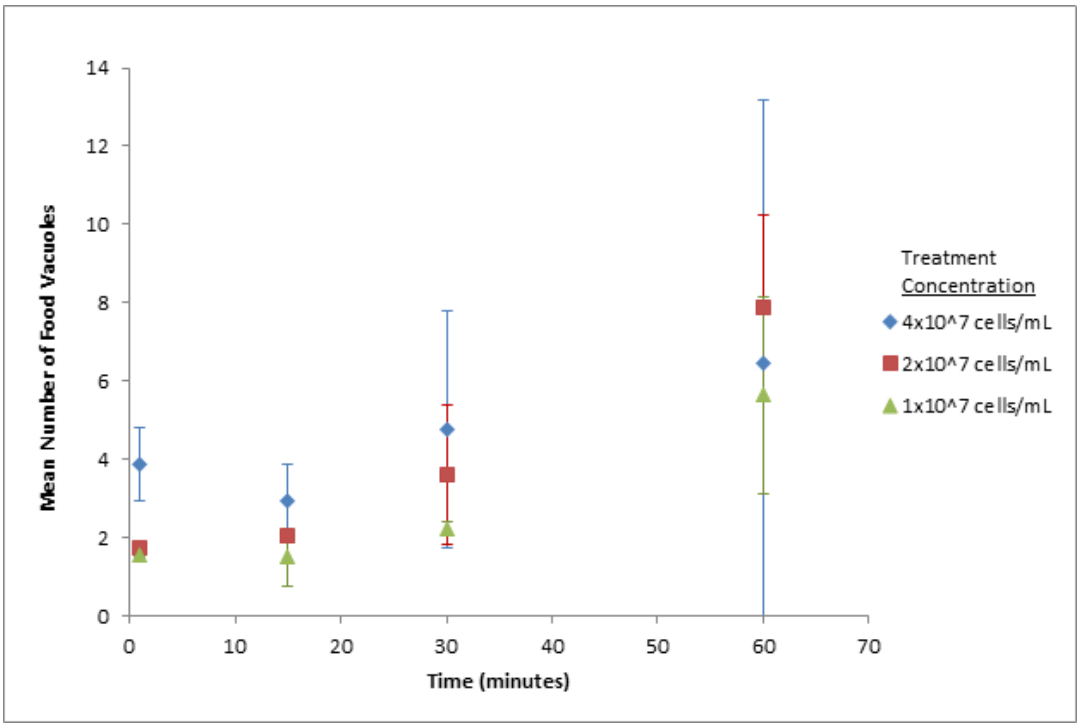
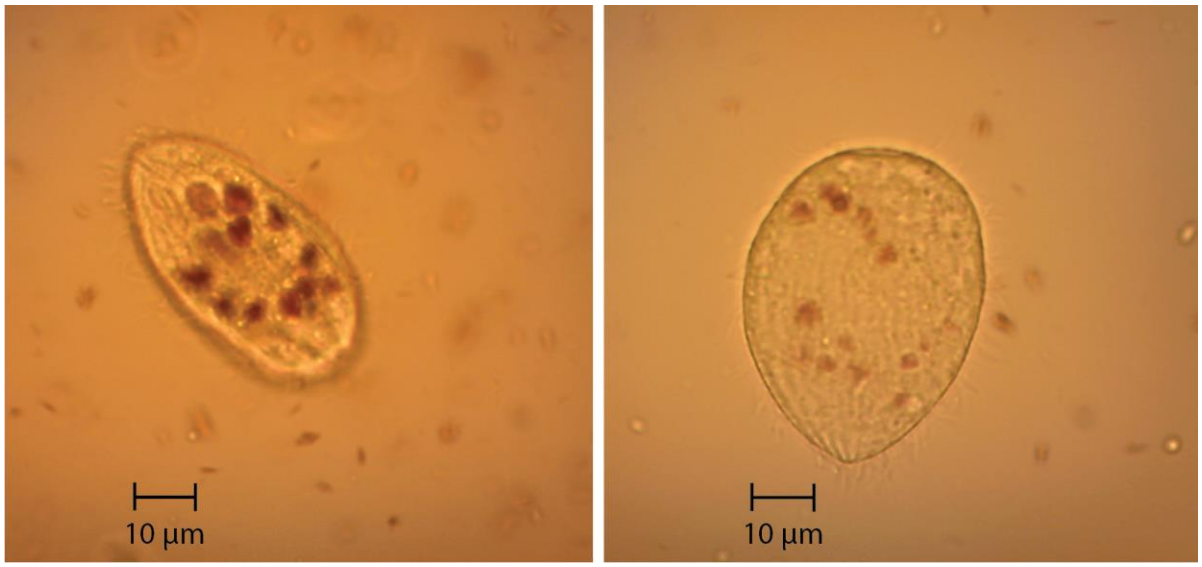


Figure 3. Mean number of FV's formed in wild-type *Tetrahymena thermophila* at times 1, 15, 30, and 60 minutes and yeast concentrations of 1×10^7, 2×10^7, and 4×10^7 cells/mL. Error bars represent 95% confidence intervals, n=3 for each treatment concentration. The p values calculated using a two-way ANOVA for H_1, H_2 and H_3 are $p = 6.2\times10^{-4}$, $p = 1.5\times10^{-1}$, and $p = 8.7\times10^{-1}$ respectively.

Figure 3 also shows that the amount of variation between the replicates of a treatment generally tends to increase as time increases. All the error bars for the 4×10^7 cells/mL treatment overlap, while only the t=15 and t=30 error bars overlap for the 2×10^7 cells/mL treatment, and only the t=15 error bar overlaps with the t=1 and t=30 error bars for the 1×10^7 cells/mL treatment. By examining the 95% confidence intervals in Figure 3, we can determine that the data from the 2×10^7 and 1×10^7 cells/mL treatments are most likely contributing to statistical differences among the times.

Furthermore, we found that the mean number of FVs generally tended to increase as concentration of yeast increased, though this trend was not completely consistent at t=60 (Figure 3). This trend was not statistically significant, with a *p*-value of 1.5×10^{-1}. There was also no statistically significant interaction between the time in food and concentration of food on FV formation ($p = 8.7 \times 10^{-1}$).

While collecting data, we noticed that there was variation in the intensity of red colour among FVs, even within the same replicates (Figure 4). Although the higher concentration treatments tended to exhibit darker colours, faintly red FVs were observed in all replicates.



(a) 4×10^7 cells/mL treatment at 60 minutes (b) 1×10^7 cells/mL treatment at 60 minutes

Figure 4. *T. thermophila* exhibiting Congo red stained food vacuoles (FVs). (a) Darkly stained FVs are visible in the high concentration treatment, while only (b) faintly stained FVs are visible in the low concentration treatment. This photo was taken using a DinoXcope Digital Microscope camera in conjunction with an Axiostar compound microscope at 400x magnification.

There was also variation in the size and shape of FVs, with some being more distorted than the typical spherical appearance. The *T. thermophila* themselves exhibited a range of appearances, from slightly-collapsed to slightly-inflated, though with fairly uniform lengths of about 50 µm (Figure 4).

Discussion

Based on the first *p* value obtained from our two-way ANOVA test ($p = 6.2 \times 10^{-4}$), we are able to reject H_{o1} and provide support for H_{a1}, which is that time in food has an effect on the number of FVs formed in *T. thermophila*. This trend can be seen in the results of our data, as displayed in Figure 3. This relationship is expected, as it supports our prediction based on research conducted by Fenchel (1980), who suggested that ciliated protozoa reach a saturation point in FV formation because they are limited by how fast the protozoa can perform phagocytosis. This is also supported by Weisman *et al.* (1967) who examined the uptake of latex

beads in a similar protozoan and determined that the uptake by phagocytosis shows saturation kinetics, with a mean maximal ingestion rate of 1.5 beads/cell per minute. This allows us to conclude that *T. thermophila* is limited by the rate at which phagocytosis can occur, as only a certain number of vacuoles can be formed at one time in *T. thermophila's* oral groove. Therefore the number of FVs formed will only be as great as the speed at which phagocytosis can be completed at *T. thermophila's* single food uptake site. Due to this, time is a biologically limiting and statistically significant factor for FV formation. Again, this is consistent with Weisman *et al.*'s (1967) findings which determined that the uptake of nutrients is proportional to the length of the incubation period.

From our analysis on our second hypothesis we obtained a p value of 1.5×10^{-1}, and thus we fail to reject H_{o2} and therefore fail to support H_{a2}. This means our experiment offers evidence that the concentration of yeast in the medium does not have an effect on the number of FVs formed, at treatment concentrations of 4×10^{7}, 2×10^{7}, and 1×10^{7} yeast cells/mL. This is contradictory to our prediction, which was that at a higher concentration of yeast in the medium, a higher number of FVs would be formed. Weisman *et al.* (1967) found that the number of FVs formed is directly proportional to the concentration of food available, and from this we expected to see a statistically significant difference. Our results also contradict the findings of Fok *et al.* (1988), who determined that the number of FVs formed is proportional to the food concentration in the medium, as was investigated on a ciliated protozoan similar to *T. thermophila.* The reason our statistical findings might disagree with other literary sources could be because we analyzed 10 cells per time, per treatment, whereas Fok *et al.* (1988) analyzed up to 4000 cells per replicate and our statistical result could simply be a consequence of not looking at enough replicates. Therefore, the trend represented by Figure 3 accurately depicts our predication and is relevant

based on literature, although not statistically significant. We also determined that even though the concentration of yeast available to *T. thermophila* in the medium does not have a significant effect on the number of FVs formed in our experiment, the concentration of yeast could have an effect on the density of the contents within the FVs, as can be seen in Figure 4. This shows the possibility that as concentration increases, so does the intensity of the colour present in the FV's formed.

Finally, the last *p* value obtained from our two-way ANOVA was $p = 8.7\times10^{-1}$. This causes us to fail to reject H_{o3} and also fail to support H_{a3}. This means there is no interaction between our independent variables, time in food and yeast concentration in the media. As a result, we cannot provide support for our prediction that if *T. thermophila* is fed in a high concentration of yeast medium over a longer period of time, a higher number of FVs would form. This statistical result is important because, although it does not support our prediction, in combination with our other hypotheses it tells us that only time, not the yeast concentrations we tested, has an effect on the number of FVs formed. The trend represented in our data in Figure 3 shows that FV formation increases with time as well as with yeast concentration in the medium, and suggests that although the time and concentration may not be interacting themselves, a combination of the two still affect the number of FVs formed.

Finally, a possible source of experimental error we encountered during our experiment was that we mistakenly did not fully mix the *Tetrahymena*-yeast solution and the fixative for our replicates at t=1 minute, so biological processes may have continued longer than we had anticipated. At t=15, we began mixing the two solutions via resuspension with a pipette to ensure the effectiveness of the fixative. The effect of this experimental error can be seen in Figure 3, where t=1 shows a higher mean number of FV's formed than at t=15.

Conclusion

We reject H_{o1}, providing support that there is an increase of FV formation as time progresses. Based on our results, we fail to reject H_{o2}, providing support that the yeast concentration in the medium was not a significant factor in the number of FVs formed in *T. thermophila*. Alternatively, we failed to reject H_{o3} providing support that the yeast concentration and time did not interact with each other. This provides support that time significantly affects phagocytosis and therefore the number of FVs formed because the cell can engulf a higher number of food particles over time.

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Literature Cited

Bozzone, D.M. 2000. Investigating phagocytosis in *Tetrahymena*: an experiment system suitable for introductory & advanced instruction. The American Biology Teacher, **62** (2): 136-139.

Fenchel, T. 1980. Suspension feeding in ciliated protozoa: functional response and particle size selection. Microbial Ecology, **6**: 1-11.

Fok, A.K., Sison, B.C., Ueno, M.S. and Allen, R.D. 1988. Phagosome formation in *Paramecium*: effects of solid particles. Journal of Cell Science, **90**: 517-524.

Gronlien, H.K., Berg, T., and Lovlie, A.M. 2002. In the polymorphic ciliate *Tetrahymena vorax*, the non-selective phagocytosis seen in microstomes changes to a highly selective process in macrostomes. The Journal of Experimental Biology, **205:** 2089-2097.

Microbiology for agronomists. 2015 [online] Available from http://web2.mendelu.cz/af_291_projekty2/vseo/print.php?page=4535&typ=html [accessed 15 November 2015]

Weisman, R., Korn, E. 1967. Phagocytosis of latex beads by *Acanthamoeba*. I. Biochemical Properties. Biochemistry, **6**: 485-497.