

Effect of Alcohol on PFK1 Gene Expression and Feeding Activity in *Tetrahymena thermophila*

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Experiment Type: Reverse transcriptase (RT) PCR

Feature: Glycolysis

Gene: PFK1: Alpha subunit of heterooctameric phosphofructokinase

1 Introduction

The purpose of this experiment was to test whether alcohol induces starvation conditions in *Tetrahymena thermophila*. For this experiment, it was hypothesized that alcohol induces starvation due to a lack of energy available in the organism for feeding. This hypothesis was tested by monitoring food vacuole formation and expression of the PFK1 gene in *Tetrahymena* that were exposed to alcohol. The PFK-1 gene was chosen because its encoded protein plays an essential role in cellular metabolism. For the experiment, control and experimental cultures of *Tetrahymena thermophila* were maintained in a nutrient rich media, with the media of the experimental group being supplemented with 1.75% ethanol. The production of food vacuoles was monitored using India ink over a course of 24 hours following the addition of alcohol. After 24 hours, RNA was extracted from the *Tetrahymena* and semi-quantitative Reverse Transcription -Polymerase Chain Reactions (RT-PCRs) were performed to determine the expression of the PFK-1 gene. We predicted that alcohol would cause *Tetrahymena thermophila* to starve by reducing their ability to produce food vacuoles. Furthermore, it was predicted that the inability of *Tetrahymena* to produce food vacuoles in the presence of alcohol would lead to a reduction in the expression of PFK-1.

2 Methods

Primer synthesis: Primers for PFK1 were designed using the Integrated DNA Technologies program, PrimerQuest. The sequences of the forward and reverse primers targeting PFK-1 are as follows: CGGTGAGGAAGGTGTGTTAG (Forward) and ATCCAGTAAAGCCTGCCATAG (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA

(Forward) and TAACACCAGACATGGCAGCA (Reverse).

Culturing Tetrahymena: A NEFF nutrient media was used to maintain *T. thermophila* cultures as previously described (Cassidy-Handley, 2012). During the experiment, all cultures were transferred into SPP media (Cassidy-Handley, 2012) and experimental cultures were supplemented with 1.75% ethanol.

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures after 24hrs of ethanol exposure using Qiagens RNeasy Mini Kit as per the manufacturer's instructions. All cultures were centrifuged for three minutes at 3,000rpm and washed with 5mL of 10mM Tris (pH 7.4) before processing.

RT-PCR: cDNA was synthesized using the RevertAid RT kit (ThermoScientific) and following the manufacturer's protocol. PCR was performed using GoTaq Green PCR Master Mix (Promega) using the manufacturer's protocol. PCR amplification of BTU1 cDNA was used as a positive control for the Tetrahymena RT-PCRs while Gapdh was used as a positive control for the entire RT-PCR experiment as the reagents for this sample were provided in the RevertAid kit. The No Template Control (NTC) for the reactions contained all reagents for the RT-PCR reaction, except nuclease-free water was added in place of RNA template.

Gel electrophoresis: The RT-PCR reactions were electrophoresed on a 1% agarose gel (BioRad) in 1xTBE (VWR). The ImageJ gel analysis program was used to determine the relative intensities of the PCR products for semi-quantitative analysis.

Vacuole Counts: Feeding behaviors of the control and experimental cultures were measured by supplementing the SPP media with a 1% solution of India ink and using a hemocytometer to count the number of food vacuoles that formed after 10 minutes of feeding.

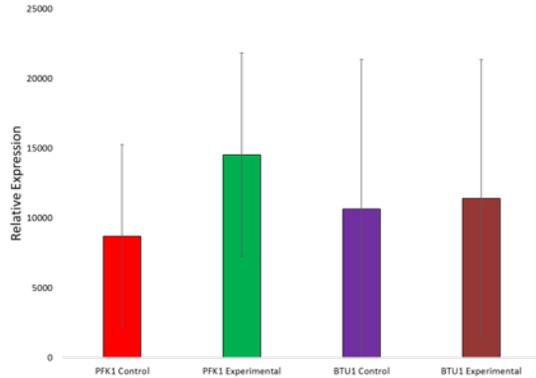
3 Results

The semi-quantitative RT-PCR results indicate that exposure of *Tetrahymena thermophila* cultures to alcohol for 24hrs did not result in a significant change in expression of PFK1, as seen in Figure 1. However, exposure to alcohol did cause a decrease in the uptake of India ink as indicated by a decrease in the formation of vacuoles indicated in Figure 2. Collectively, these results support our hypothesis that alcohol exposure decreases food vacuole formation; however, our results do not support the hypothesis that alcohol exposure causes a decrease in expression of the PFK1 gene.

Future research on this project should focus on testing the effects of different concentrations of alcohol on feeding behavior and PFK1 expression. Furthermore, a more quantitative measure of PFK1 expression should be performed along with increasing the sample size for the experiment.

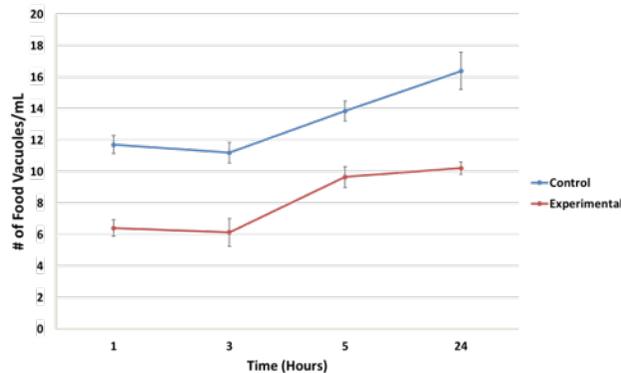
4 Figures

4.1 Relative Expression of PFK1 in Control and Ethanol-treated *Tetrahymena thermophila*



The relative expression of PFK1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four ethanol-treated *Tetrahymena* cultures. The error bars represent the standard error of the means for each condition. A student's t-test was performed to determine the significance of changes in gene expression between the control and experimental cultures, with $p=0.336$ for PFK1 and $p=0.927$ for BTU1.

4.2 Feeding Behavior of Ethanol-treated *Tetrahymena* Cultures



The feeding behavior of control and ethanol-treated *Tetrahymena* cultures was measured at 1, 3, 5 and 24hrs following exposure to ethanol. The graph represents the mean number of India ink-containing feeding vacuoles across four control and four ethanol-treated cultures at the time points indicated. Error bars represent the standard error of the means for each data set and a two-tailed t test assuming unequal variance was performed to determine significance, with $p=1.12E-8$ for hour 1, $p=4.47E-5$ for hour 3, $p=1.87E-5$ for hour 5, and $p=2.78E-5$ for hour 24.

5 References

Cassidy-Hanley DM. Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage. *Methods Cell Biol.* 2012;109: 237-76. doi: 10.1016/B978-0-12-385967-9.00008-6.

6 Acknowledgements

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