

# Effects of Tocopheryl Acetate on *FLP1* Expression, Growth Rate, and Feeding Behavior of *Tetrahymena thermophila*

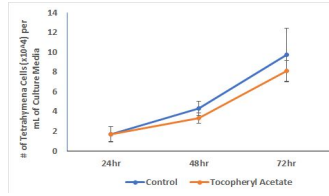
## Introduction

- *Tetrahymena thermophila* is a unicellular, eukaryotic ciliate that is popular as a model organism in molecular biology.
- It has been proposed that tocopheryl acetate, a chemical used as a thickening agent in THC-containing vaping products, is associated with vaping-related illness (EVALI).
- In the presence of high amounts of tocopherols, phosphatidylcholines undergo a phase change from a gel to a liquid crystalline phase, reducing the ability of the surfactant to maintain the surface tension necessary for respiration
- *FLP1* encodes Flippase, which is used to transport lipids across biogenic membranes.
- **Hypothesis:** We hypothesized that if *T. thermophila* were exposed to tocopheryl acetate, then cell growth, expression of the *FLP1* gene, and feeding behavior would all decrease.

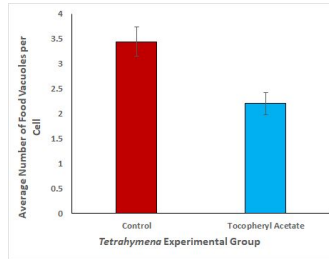
## Methods

- **Primer synthesis:** Oligoanalyzer software was used to design primers for *FLP1* replication
- **Culturing:** *T. thermophila* cultures were maintained in NEFF media. Cultures were then transferred into SPP media and experimental cultures were exposed to a single 72-hr dose of 1.0 mM tocopheryl acetate in ethanol.
- **RNA extraction:** total RNA was extracted through the use of Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** cDNA was produced using RevertAid RT enzyme.
- **Quantitative PCR** was performed with PowerUp SYBR Master Mix. Expression of the *BTU1* gene was monitored and used as a positive control.
- **Cell Counts** were performed using hemocytometers.
- **Feeding Assay** observed vacuoles in cells through use of India ink dye

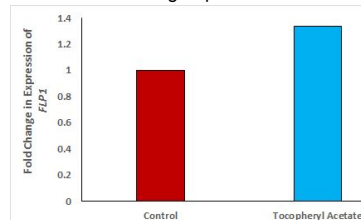
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**Results**



**Figure 1:** Number of cells per milliliter of culture media for control *T. thermophila* and *T. thermophila* exposed to tocopheryl acetate (24hr:  $p=0.982$ , 48hr:  $p=0.316$ , 72hr:  $p=0.594$ ).  $n=3$  for each group at each time interval



**Figure 2:** Number of food vacuoles per cell after 72 hour treatment ( $p=0.001$ ).  $n=4$  for each group.



**Figure 3:** Fold change in expression of *FLP1* in *Tetrahymena* after 72 hour treatment. Fold change is relative to control.  $n=2$  for each group.

## Conclusion

- Cell counts were inconclusive, but overall, *T. thermophila* cells exposed to tocopheryl acetate grew slower than control cells ( $p=0.982$ ,  $p=0.316$ ,  $p=0.594$ ).
- The feeding assay showed significantly less feeding activity in *T. thermophila* exposed to tocopheryl acetate than the control group ( $p=0.001$ ).
- One round of qPCR shows an increase in expression of *FLP1* in *T. thermophila* exposed to tocopheryl acetate, but sample size is too small to be conclusive.
- The hypothesis about feeding behavior was correct, while hypotheses concerning cell growth and expression of *FLP1* are inconclusive.
- Conclusions are limited by small sample size. Future research should seek to expand replicates of cell count assay, feeding assay, and qPCR.

## References

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