Effect of EDTA on CalModulin 1 (CAM1) Gene Expression and Cell Viability in Tetrahymena thermophila

Introduction

- *Tetrahymena thermophila* (*T. thermophila*) is a unicellular, eukaryotic ciliate that is utilized as a popular model organism.
- Ethylenediaminetetraacetic acid (EDTA) is a metal ion chelator present in many body cleansing products. Calcium (Ca²⁺), a metal ion present throughout the body, would be sequestered by EDTA.
- Calmodulin, short for calcium-modulated protein, is an intracellular target activated by the secondary messenger Ca²⁺.
- The calmodulin-1 gene (*CAM1*) codes for the CAM1 protein needed for many pivotal biomolecular pathways.
- **Hypothesis:** If *T. thermophila* are exposed to EDTA, then *CAM1* gene expression will increase, and cell viability along with number of feeding vacuoles will decrease.

Methods

- **Primer synthesis** for *CAM1* was completed through the software Oligoanalyzer.
- **Culturing** *T. thermophila* was initiated in NEFF media. Upon experimentation, all cultures were transferred into SPP media and experimental cultures were exposed to a single 24-hour dose of 5.0 mM EDTA.
- **RNA extraction** was completed with Qiagen's RNeasy Mini Kit.
- **RevertAid Reverse transcription** was used to synthesize cDNA.
- **Quantitative PCR** was performed using PowerUp SYBR Master Mix. *BTU1* gene expression was used as positive control.
- **Cell Counts** were performed with hemocytometers.
- **Feeding Assays** were performed with India Ink.

Results

- The number of feeding vacuoles was decreased due to EDTA treatment in *T. thermophila*.
- SYBR Master Mix was used to perform qPCR on the experimental cultures.
- The results agree with our hypothesis regarding the number of feeding vacuoles and cell growth. In addition, the appearance of increased gene expression further supports our hypothesis.

**Figure 1:** Chemical structure of tetrasodium EDTA

**Figure 2:** Image of *T. thermophila* with dyed feeding vacuoles

**Figure 3:** Average number of feeding vacuoles (p=1.27x10⁻⁷; n=3 for each group)

**Figure 4:** Average number of cells per milliliter of media (Hour 6: p=0.781; Hour 12: p=0.0979; Hour 18: p=0.0365; Hour 24: p=0.0188, n=4 for each group)

**Figure 5:** Fold change in expression of *CAM1* (n=2 for each group)

Conclusion

- The number of feeding vacuoles was significantly increased in the control treated *T. thermophila*.
- Cell counts showed significant differences in the 18 and 24-hour mark where *T. thermophila* were more abundant in the control.
- The single round of qPCR could not be statistically analyzed, but it did show promising results with an upregulation in *CAM1* expression in the EDTA treated cells.
- The results agree with our hypothesis regarding the number of feeding vacuoles and cell growth. In addition, the appearance of increased gene expression further supports our hypothesis.

References

- Esposito, E., Knauth, T., Ohnstad, A., Effect of EGTA on SIT1 SIT1 Scramblase Gene Expression, Cell Viability, and Cell Growth in Tetrahymena thermophila. Carroll College Student Undergraduate Research Festival. Helena, MT.

Acknowledgements

We would like to thank our faculty advisor, Dr. Stefanie Otto-Hitt for her superlative guidance, enduring patience, and unwavering support throughout the course of this process. This project was funded in part by a supplies grant from the Ciliate Genomics Consortium.