Apr 25th, 2:45 PM - 3:45 PM

Effects of Ethanol on the Gene Expression of Uracil DNA N-Glycosylase 1 (UNG1) and Growth Rate in Tetrahymena thermophila

Samantha Mundel
Carroll College, smundel@carroll.edu

Seth Berkowitz
Carroll College, sberkowitz@carroll.edu

Effects of Ethanol on the Gene Expression of *Uracil DNA N-Glycosylase 1* (*UNG1*) and Growth Rate in *Tetrahymena thermophila*

Samantha Mundel and Seth Berkowitz
Department of Biology, Carroll College

Introduction

- The single celled eukaryotic ciliate *Tetrahymena thermophila* (*T. thermophila)* is a widely used model organism in many fields of biology.
- Uracil DNA N-Glycosylase 1 (UNG1) is a base excision repair enzyme that facilitates the removal of deaminated bases.
- Ethanol (EtOH), interferes with human health in a variety of ways by inducing heart damage, liver damage, and DNA damage.
- **Hypothesis:** Expression of *UNG1*, the gene that codes for the enzyme Uracil DNA N-Glycosylase 1, would increase and that growth rate would decrease in *T. thermophila* cells exposed to EtOH.

Methods

- **Primer synthesis:** Primers for *UNG1* were designed using IDT Oligoanalyzer software.
- **Culturing:** *T. thermophila* were cultured in NEFF media. Control cultures were transferred into SPP media with no ethanol and experimental cultures were transferred into SPP media with 1.5% EtOH for 72 hours.
- **Cell Counts:** Cells were treated with 5% glutaraldehyde and counts were performed using hemocytometers every 12 hours.
- **RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** cDNA was synthesized using RevertAid.
- **Quantitative Polymerase Chain Reaction (qPCR):** Was performed using PowerUp SyBr Master Mix to determine expression of *UNG1*.

Results

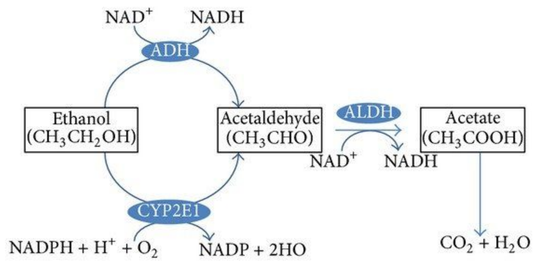

Figure 1: EtOH metabolism pathway in eukaryotes.

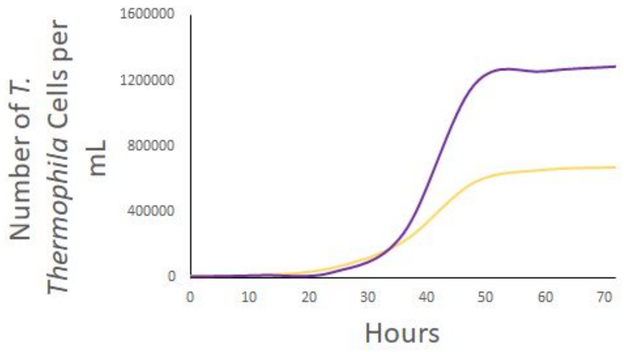

Figure 2: Average growth of control (purple) and EtOH-exposed (gold) *T. thermophila* over a 72 hour period, p-values: 0.45 (0 hrs), 0.42 (24 hrs), 0.42 (48 hrs), 0.28 (72 hrs).

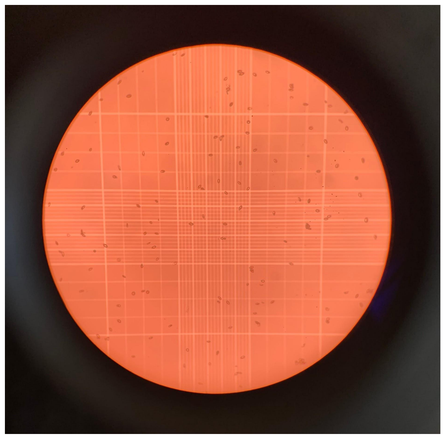

Figure 3: Image of hemocytometer and *T. thermophila* under a microscope used for cell counting.

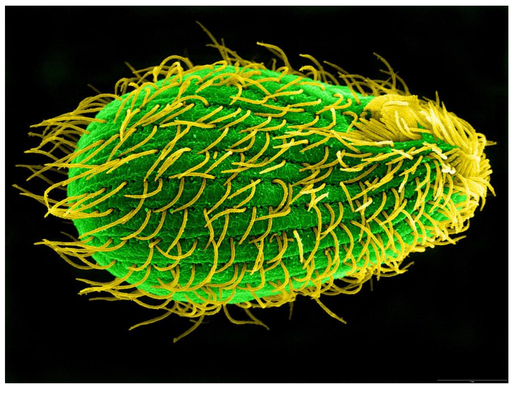

Figure 4: Color enhanced scanning electron micrograph of *T. thermophila.*

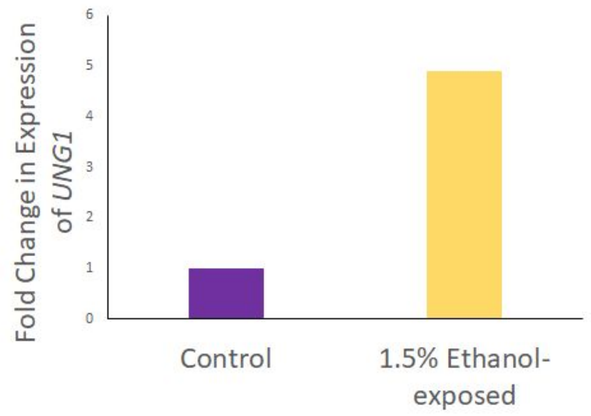

Figure 5: Relative expression of *UNG1* in control and EtOH-exposed *T. thermophila* (p=0.28).

Conclusion

- The growth rate curves in **Figure 2** suggest that *T. thermophila* undergo a decreased growth rate when exposed to ethanol.
- The increased expression of *UNG1* in EtOH-exposed *T. thermophila* compared to control *T. thermophila* in **Figure 5** is not significant (p=0.28).
- Based on our findings we cannot reject the null hypothesis that EtOH-exposure effects the growth rate and *UNG1* expression of *T. thermophila.*

References

Bell, A. J. (2017, February 6). *Tetrahymena Thermophila Sem* [Photograph found in Tetrahymena Photographs, FineArtAmerica].

Guo L, Yang, Y, Wu F. 2008. Oxidative DNA Damage Induced by Ethanol in Mouse Peripheral Leucocytes. Basic & Clinical Pharmacology & Toxicology, 103: 222-227.

Kawaratani H, Tsujimoto T, Douhara A, Takaya T. The Effect of Inflammatory Cytokines in Alcoholic Liver Disease. Mediators of Inflammation, 2013(3):1-10.

Nilsson J. 2005. Ethanol Affects Endocytosis and Proliferation of Tetrahymena pyriformis GL and Promotes Encystment. Acta Protozool. 44: 293-299.

Acknowledgements

We would like to thank our faculty advisor, Dr. Stefanie Otto-Hitt for her guidance throughout this process.