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Effect of Potassium Bromate on OXR1 Gene Expression and Cell Growth in Tetrahymena thermophila

Brendan McMahon
bjmcmahon@carroll.edu

Scott Kahle
skahle@carroll.edu

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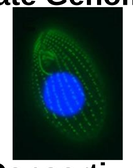
Ciliate Genomics Consortium



Effect of Potassium Bromate on *OXR1* Gene Expression and Cell Growth in *Tetrahymena thermophila*

Scott Kahle and Brendan McMahon
Department of Biology, Carroll College

Carroll College

Introduction

- The unicellular, eukaryotic ciliate *Tetrahymena thermophila* (*T. thermophila)* is a useful model organism in molecular biology.
- The protein encoded by *OXR1* is required to prevent oxidative damage resulting from energy production.
- Potassium Bromate ($KBrO_3$) is a strong oxidizing agent that is used as a leavening agent in baked goods.
- Previous studies have shown that $KBrO_3$ is toxic to living organisms.
- **Hypothesis:** If *Tetrahymena* are exposed to $KBrO_3$ in their environment, oxidative damage will be induced and, as a result, expression of *OXR1* will increase while cell growth rate decreases.

Methods

- **Primer synthesis:** IDT software was used to design primers for the *OXR1* gene.
- **Culturing:** *T. thermophila* cultures were maintained in NEFF media. During treatment, experimental cultures were transferred to culture dishes with SPP media containing 0.5mM $KBrO_3$. Control cultures were grown in pure SPP media.
- **RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** RevertAid was used to synthesize cDNA from extracted mRNA.
- **PCR** was performed using GoTaq master mix, and *BTU1* gene expression was used as a positive control.
- **Gel electrophoresis** was performed on RT-PCR reactions. Relative PCR signal intensities were analyzed using ImageJ.
- **Cell Growth rate** was determined by counting cell number with a hemocytometer twice a day over a 72 hour period.

Results

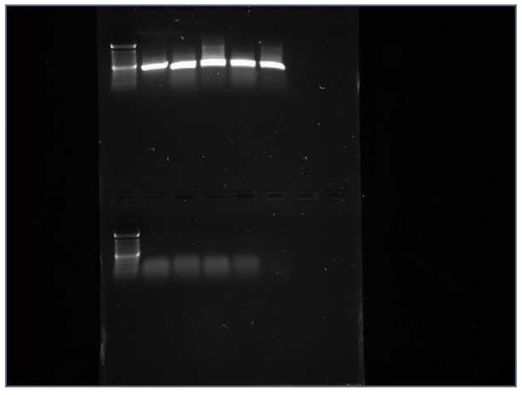


Figure 1: Round 1 *OXR1* PCRs. Both control (lanes 3-4) and experimental (lanes 5-6) groups show *OXR1 expression*

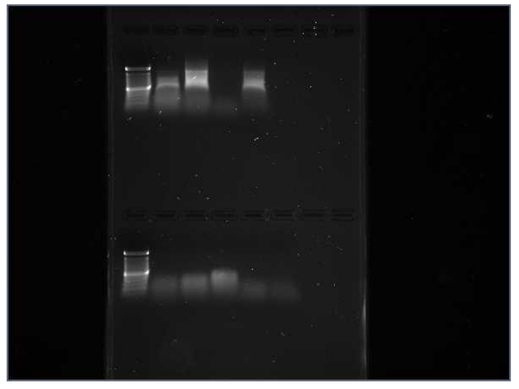


Figure 2: Round 1 *BTU1* PCRs. Three control (top) groups show *BTU1* expression, while negative controls do not.

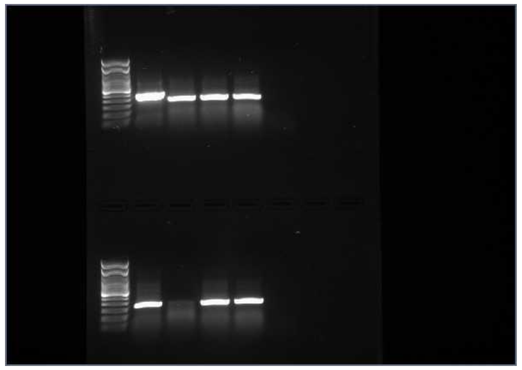


Figure 3: Round 2 *OXR1* PCRs. The control (lanes 3-4), one experimental (5) and three negative control groups show (b) *OXR1* expression.

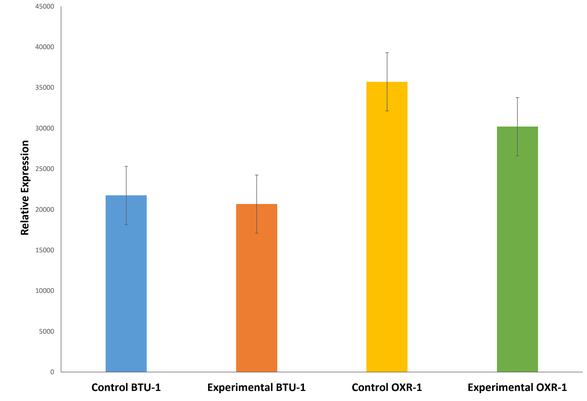


Figure 7: Relative expression of *OXR1* and *BTU1.* Error bars represent standard error. (p=0.25 for *OXR1* and p=0.93 for *BTU1*)

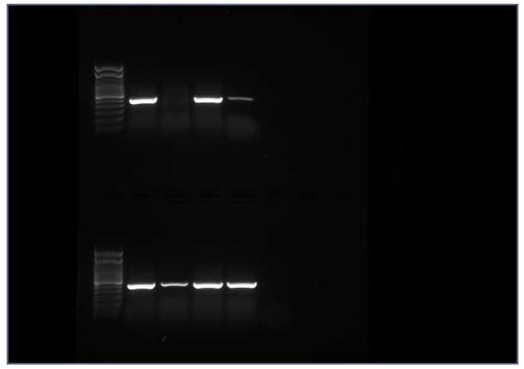


Figure 4: Round 2 *BTU1* PCRs. Three positive controls (top) and all negative controls (bottom) show BTU1 expression.

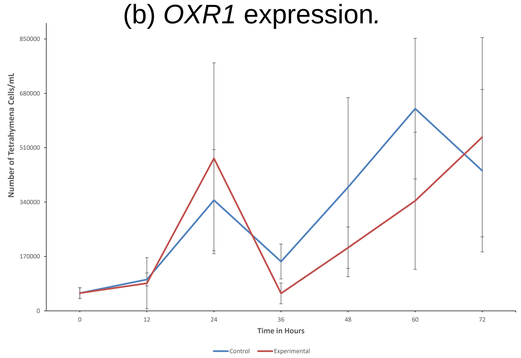


Figure 5: Growth curve of control and $KBrO_3$treated *Tetrahymena* cultures measured over a 72 hour period. From 0-72hrs, p-values: 1, 0.97 0.71, 0.18, 0.56, 0.38, 0.80

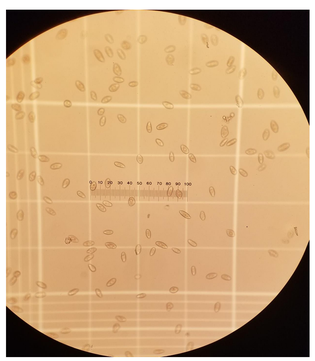


Figure 6: Image of *Tetrahymena* cells fixed in glutaraldehyde for counting with a hemocytometer.

Conclusions

- Both experimental trials indicated that exposure to $KBrO_3$did not induce any major change in expression of *OXR1*, as seen in Figures 1 and 3.
- There was no significant increase in the quantitative relative RT-PCR signal of *OXR1*, as shown in Figure 7 (p=0.25).
- $KBrO_3$ exposure had no significant effect on cell growth as seen in Figure 5. (p-values = T0:1, T12: 0.97, T24: 0.71, T36: 0.18, T48: 0.56, T60: 0.38, T72: 0.80).
- The results disagreed with our hypothesis in regard to both gene expression and cell growth rate.
- Future Directions: Optimize the concentration of $KBrO_3$, and increase the number of experimental trials.

Acknowledgements

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