

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

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Experiment Type: Reverse transcriptase (RT) PCR

Feature: Oxidative Resistance

Gene: OXR1: Protein of unknown function required for oxidative damage resistance

1 Introduction

The purpose of our experiment was to answer the question: Does oxidative damage, induced by potassium bromate (KBrO_3), affect growth rate and expression of the OXR1 gene in *Tetrahymena thermophila*? Potassium Bromate was chosen as the environmental stressor because it is a strong oxidizing agent that is used as a leavening agent in baked goods. It was hypothesized that expression of OXR1 would increase in *Tetrahymena* cells that were exposed to potassium bromate and that their growth rate would decrease. The exact mechanism and function of the OXR1 gene is still unknown; however, the literature suggests that it is required for oxidative damage resistance. To test the hypothesis, *Tetrahymena* were randomly assigned to either a control group or a test group. A solution of potassium bromate that was pre-determined to be non-lethal to the *Tetrahymena* was added to the test groups culture media and both the control and test groups were cultured under ideal conditions for 72 hours. During the 72hr time-period, *Tetrahymena* were counted twice a day to determine their growth rate. Following the 72-hour treatment period, RNA was extracted from the control and test groups. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed on the extracted RNA, followed by gel electrophoresis and a semi-quantitative analysis of OXR1 expression. Due to the strong oxidizing capacity of potassium bromate, it was predicted that there would be significant oxidative damage resulting in increased expression of OXR1 in the *Tetrahymena* cultures that were treated with potassium bromate.

2 Methods

Primer synthesis: Primers for OXR1 were designed using Integrated DNA Technologies PrimerQuest. The sequences of the forward and reverse primers targeting OXR1 are as follows: AGCACTCCAATGTACTGACC (Forward) and ACCACAGCCAACCTCCTAATC (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATGGCAGCA (Reverse).

Culturing Tetrahymena: *T. thermophila* cultures were maintained in NEFF media as previously described (Cassidy-Handley, 2012). During treatment, experimental cultures were transferred to culture dishes with SPP media containing 0.5mM KBrO₃ while control cultures were grown in pure SPP media (Cassidy-Handley, 2012).

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures following 72hrs of exposure to 0.5mM KBrO₃ using Qiagen 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) 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.

Growth Rate: The growth rate of control and experimental Tetrahymena cultures was determined by counting cells with a hemocytometer (Bright-line (TM), Sigma) twice a day over a 72 hour period. In preparation for counting, cells were first treated with a 5% solution of glutaraldehyde (Sigma). More specifically, 190uL of cell culture was mixed with 10uL of 5% glutaraldehyde.

3 Results

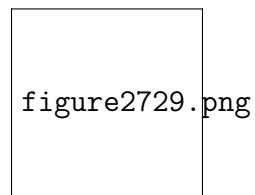
The results of these experiments indicate that exposure of Tetrahymena to KBrO₃ for 72hrs does not induce any major change in expression of the OXR1 gene, as seen in Figure 1 (p=0.25). Furthermore, KBrO₃ exposure had no significant effect on cell growth, as seen in Figure 2 (p-values = 0hr:1, 12hr: 0.97, 24hr: 0.71, 36hr: 0.18, 48hr: 0.56, 60hr: 0.38, 72hr: 0.80). Collectively, these results disagreed with our hypothesis in regards to both gene

expression and cell growth rate.

Future research on this project should optimize the concentration of KBrO₃ used to treat the Tetrahymena cultures and increase the number of experimental trials. A more quantitative measure of gene expression should also be used to more accurately determine whether KBrO₃ affects OXR1 expression.

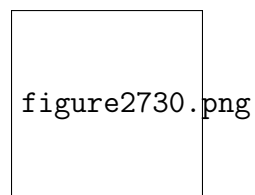
4 Figures

4.1 Relative Expression of OXR1 in Control and Potassium Bromate Treated Tetrahymena Cultures



The relative expression of OXR1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four KBrO₃ treated (experimental) Tetrahymena cultures. The error bars represent the standard error of the means for each condition. A two-tailed t-test assuming unequal variance was performed to determine the significance of changes in gene expression between the control and experimental cultures, with $p=0.25$ for OXR1 and $p=0.93$ for BTU1.

4.2 Growth Rate of Control and Potassium Bromate Treated Tetrahymena Cultures



The growth rate across four control and four KBrO₃-treated Tetrahymena cultures was measured over a 72- hour period. A two-tailed t-test assuming unequal variance was performed to determine the significance of changes in cell counts between the control and experimental cultures, with $p= 1$ (0hr), 0.97 (12hr), 0.71 (24hr), 0.18 (36hr), 0.56 (48hr), 0.38 (60hr), 0.80 (72hr).

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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