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Effects of Salt Concentration on Tetrahymena thermophila Growth and CRP1 Gene Expression

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: Ion exchange
Gene: CRP1: Sodium/calcium exchanger protein

1 Introduction
The use of salt to remove ice from roads has resulted in increased salinity in many freshwater lakes in North America. For this project, we wanted to explore how environmental salt concentrations influence the rate of growth of Tetrahymena thermophila and expression of the CRP1 gene. We hypothesized that if the sodium concentration is increased in the media, Tetrahymena growth would decrease and expression of the CRP1 gene would increase. The CRP1 gene encodes a protein that helps regulate calcium concentrations within a cell based on the concentration of sodium ions. The media of the experimental group was treated with sodium concentrations reflective of the increasing salt concentration of freshwater lakes. Cultures were randomly assigned to either the control group, containing no added sodium, or to the experimental group. The course of treatment lasted for 3 days and growth of the Tetrahymena was measured every 24 hours during the treatment period. Immediately following the 72-hour treatment, RNA extraction procedures were followed and gene-specific Reverse Transcription Polymerase Chain Reactions (RT-PCRs) were performed on both the control and experimental groups to measure expression of CRP1. Increased sodium concentration in the media was predicted to decrease Tetrahymena thermophila growth and increase CRP1 expression in order to help regulate ion concentrations within the cell.

2 Methods
Primer synthesis: Primers for CRP1 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies PrimerQuest. The sequences of the forward and reverse primers targeting CRP1 are as follows: TTGCTTACCTTCTTCTTGCTATTTC
(Forward) and CCAGCAAAGCATCCAGTTAAAG (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATGGCAGCA (Reverse).

Culturing Tetrahymena: T. thermophila were cultured in NEFF media and then transferred into nutrient-rich SPP media at the time of the experiment (Cassidy-Handley, 2012). The SPP media for experimental cultures was supplemented with 2.7 mg/mL of NaCl.

RNA extraction: RNA was extracted from control and experimental T. thermophila cultures following 72hrs of exposure to 2.7mg/mL of NaCl using Qiagens 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% agaorse 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) once 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 the RT-PCR experiments demonstrate that increased salt concentrations do not affect expression of CRP1, as seen in Figure 1 (p=0.79). Furthermore, there was no change in growth rate between the control and salt-treated Tetrahymena cultures over the course of 72hrs, as seen if Figure 2 (24hr: p=0.06, 48hr: p=0.24, 72hr: p=0.11). Collectively, our findings do not support our hypothesis that salt exposure would increase CRP1 expression and decrease growth rate.

Future studies should focus on increasing the number of experimental samples and optimizing salt concentrations. A more quantitative measure of gene expression should also be used to more accurately determine whether increased salt concentrations affect CRP1
4 Figures

4.1 Relative Expression of CRP1 in Control and NaCl-treated Tetrahymena thermophila Cultures

The relative expression of CRP1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four NaCl-treated Tetrahymena cultures. The error bars represent the standard error of the means for each condition. A student’s t-test was performed to determine the significance of changes in gene expression between the control and experimental cultures, with p=0.79 for CRP1 and p=0.32 for BTU1.

4.2 Growth Rate of NaCl-treated Tetrahymena Cultures

The growth rate across four control and four NaCl-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=0.06 (24hr), 0.24 (48hr), 0.11 (72hr).
5 References


6 Acknowledgements

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