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

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Introduction

- *Tetrahymena thermophila* (*T. thermophila)* is a unicellular eukaryote and a freshwater organism commonly used as a model organism in molecular biology.
- The use of sodium chloride (NaCl) as road salt has been shown to increase the salinity of freshwater lakes and streams, altering the diversity of freshwater ecosystems.
- The *CRP1* gene encodes Calcium regulator protein 1, an exchanger protein that regulates cellular calcium concentrations based on the cellular sodium concentrations
- **Hypothesis:** If the sodium concentration is increased in SPP media, *T. thermophila* growth should decrease while expression of the *CRP1* gene should increase.

Methods

- **Primer synthesis:** Primers for *CRP1* were designed using Ciliate Genome and Integrated DNA Technologies websites.
- **Culturing:** *T. thermophila* was cultured in NEFF media and then transferred into nutrient-rich SPP media for testing. SPP media for experimental cultures was supplemented with 2.7 mg/mL of NaCl.
- **RNA extraction:** RNA extractions were performed using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** RevertAid was used to synthesize cDNA. *Gapdh* was used as a positive control to confirm the kit's effectiveness.
- **PCR** was performed using GoTaq polymerase. *BTU1* gene expression was used as a positive control.
- **Gel electrophoresis** was used to visualize PCR reactions. Band intensities were analyzed using ImageJ software.
- **Cell Counts** were performed using hemocytometers.

Results

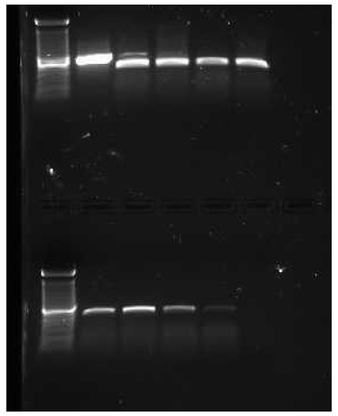

Figure 1: Round 1 RT-PCRs. Control and experimental *CRP1* with RT (top) Control and experimental *CRP1* without RT (bottom).

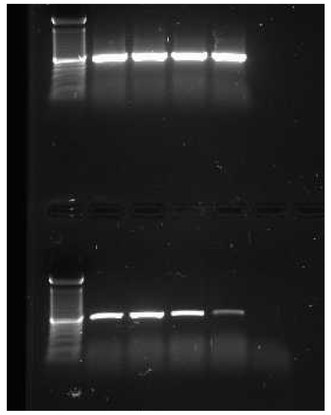

Figure 2: Round 1 RT-PCRs. Control and experimental *BTU1* with RT (top) Control and experimental *BTU1* without RT (bottom).

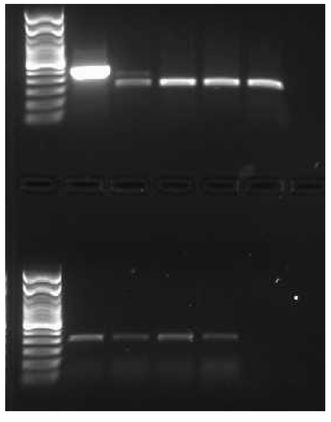

Figure 3: Round 2 RT-PCRs. Control and experimental *CRP1* with RT (top) Control and experimental *CRP1* without RT (bottom).

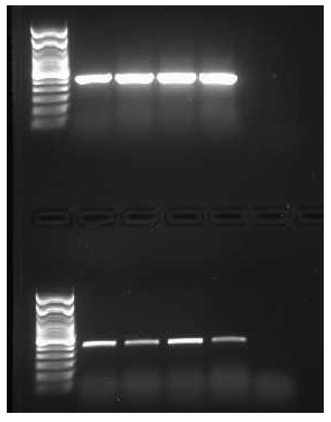

Figure 4: Round 2 RT-PCRs. Control and experimental *BTU1* with RT (top) Control and experimental *BTU1* without RT (bottom).

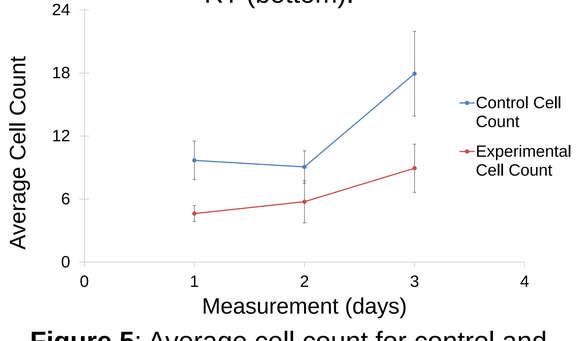

Figure 5: Average cell count for control and experimental groups over a three day interval (M1 p=0.06, M2 p=0.24, M3 p=0.11)

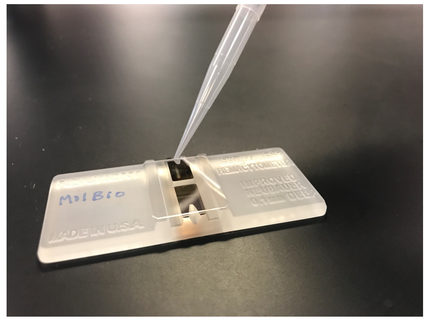

Figure 6: Hemocytometer used for cell counting.

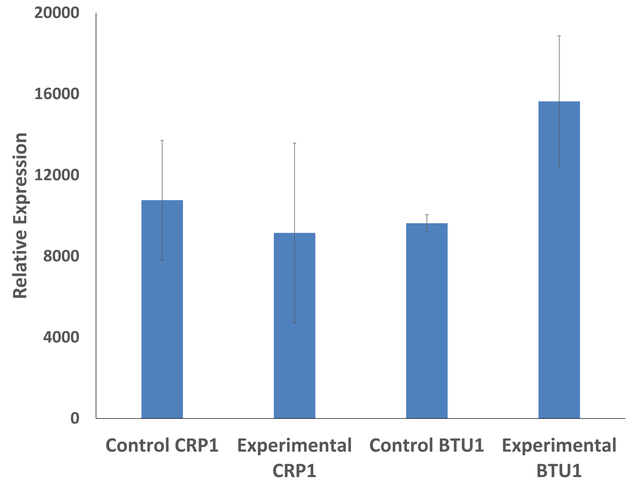

Figure 7: Relative expression of *CRP1* and *BTU1.* Error bars represent standard error. (p=0.79 for *CRP1* and p=0.32 for *BTU1*)

Conclusions

- Both PCR rounds demonstrated that increased salt concentration resulted in no significant change in expression of *CRP1*, as seen in Figures 1 and 3.
- There was a slight decrease in the C*RP1* relative gene expression, as shown in Figure 7, but it was not statistically significant.
- The experimental treatment decreased cell growth as seen in Figure 5, but it was not statistically significant.
- Collectively, our findings do not support our hypothesis that salt exposure would increase *CRP1* expression and decrease growth rate.
- Future Directions: Increasing the number of experimental samples and varying salt concentrations would strengthen the design of this experiment.

Acknowledgements

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