

Effect of Nitrate Pollution on *Tetrahymena thermophila*

Reegan DeBruycker, Hannah Sylvester, and Stefanie Otto-Hitt, PhD
Carroll College

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: TTHERM.00327340
Gene: CDC16: TPR domain containing protein; subunit of anaphase promoting complex

1 Introduction

The question under investigation in this project was: Does an elevated concentration of nitrates have a negative effect on cell growth and expression of the CDC16 gene in the organism *Tetrahymena thermophila*? Exposure of *Tetrahymena* cultures to nitrates mimics the exposure of these organisms to nitrate pollution in the environment, which can occur due to septic tank leakage, nitrogen-rich fertilizer runoff, and agricultural processes. The EPA standard for nitrate concentrations in drinking water is a maximum of 10ppm.

In our experiment, *Tetrahymena* cultures were treated with media that contained 30ppm of nitrates for one week. The 30ppm nitrate concentration has been proven acceptable for freshwater fish; therefore, we predicted that it would have more of an impact on our *Tetrahymena* cells which are far less complex. The CDC16 gene encodes a Cell Division Cycle protein that contains an anaphase-promoting complex (APC). This APC is involved in Cyclin degradation and promotes the movement of the cell out of mitosis. During the one-week exposure to 30ppm nitrates, the growth rate of *Tetrahymena* cultures was determined using a hemocytometer. Furthermore, expression of the CDC16 gene was analyzed using semi-quantitative RT-PCR following the weeklong exposure. We predicted that a high concentration of nitrates would decrease the growth rate and expression of CDC16 in our *Tetrahymena* cultures.

2 Methods

Primer synthesis: Primers for CDC16 were designed using the *Tetrahymena* Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify CDC16 are as follows: GCATGGATTGGTATGGCTCACTC (Forward)

and ACAATCTGGCTCATACCTGTAGAAAAATGAAAG (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 control Tetrahymena cultures were treated with a nitrate solution of 10ppm while the experimental cultures were treated with a nitrate solution of 30ppm for one week.

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures following the weeklong incubation 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) over the course of the week-long experiment. 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

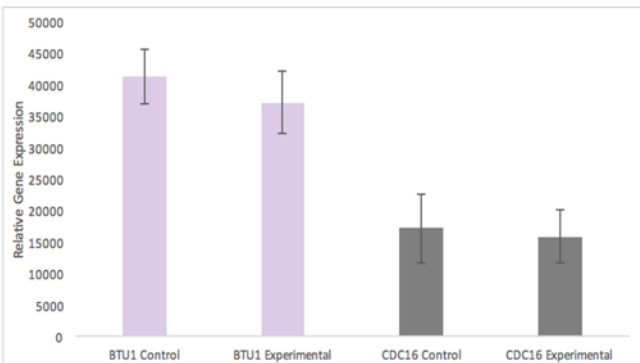
The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells to a 30ppm nitrate solution has no significant effect on the expression of CDC16 compared to control cells that were treated to 10ppm, as indicated in Figure 1 ($p = 0.65$). The growth curve analysis does indicate that there was a slight decrease in the growth rate of Tetrahymena that were subjected to the 30ppm nitrate solution. Collectively, these results support our hypothesis regarding nitrate exposure and its effects on growth rate; however, they do not support our hypothesis regarding expression of CDC16.

Future studies should focus on varying the concentrations of nitrates the Tetrahymena

cultures are exposed to, as well as increasing the number of replicates. Furthermore, a more quantitative measure of gene expression should be used to better determine whether nitrate pollution affects CDC16 expression.

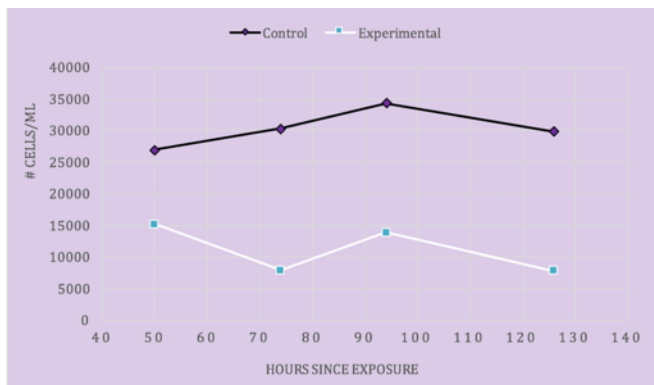
4 Figures

4.1 Relative Expression of CDC16 in Nitrate-exposed *Tetrahymena thermophila* Cultures



The relative expression of CDC16 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control (10ppm nitrate) and four experimental (30ppm nitrate) *Tetrahymena* cultures after one week of culturing. 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 $p=0.65$ for CDC16 and $p=0.55$ for BTU1.

4.2 Growth Rate of Nitrate-exposed *Tetrahymena* Cultures



The growth rate across four control (10ppm nitrate) and four experimental (30ppm nitrate) *Tetrahymena* cultures was measured over the course of the weeklong experiment.

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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