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Effects of β-Methylamino-L-Alanine (BMAA) on LC4A Expression and Growth of Tetrahymena thermophila

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Introduction
- The unicellular, eukaryotic ciliate Tetrahymena thermophila (T. thermophila) is a popular model organism in molecular biology.
- It has been proposed that β-Methylamino-L-alanine (BMAA), a non-proteinogenic amino acid secreted by Cyanobacteria, is a hyper-excitatory neurotoxin.
- If hyper-excitation of cells were to occur, a strong influx of calcium ions would induce unregulated cell signaling, which would need to be modulated by a Calmodulin protein in order to rescue homeostatic cell processes and prevent cell death.
- LC4A is a calmodulin homolog which sequesters calcium ions.
- Hypothesis: It is hypothesized that presence of BMAA in the media will decrease Tetrahymena thermophila growth and increase LC4A expression in order to help regulate calcium ion concentrations within the cell.

Methods
- Primer synthesis: Primers for LC4A were designed using Oligoanalyzer software.
- Culturing: T. thermophila cultures were maintained in NEFF media. Upon experimentation, all cultures were transferred into SPP media and experimental cultures were exposed to a single three-day dose of 1.0 mM BMAA.
- RNA extraction: RNA was extracted using Qiagen’s RNeasy Mini Kit.
- Reverse transcription: cDNA was synthesized using RevertAid.
- Quantitative PCR was performed using PowerUp SYBR Master Mix. BTU1 gene expression was used as positive control.
- Cell Counts were performed with hemocytometers.

Results

Figure 1: (a.) Chemical structure of the standard amino acid L-alanine. (b.) Chemical structure of the non-proteinogenic amino acid BMAA.

Figure 2: Cyanobacteria, which naturally produces and secretes BMAA.

Figure 3: Fold change in expression of LC4A. (p=0.332; n = 4 for each group).

Figure 4: Number of cells per milliliter of media, averaged between round 1 and round 2. (Day 1: p=0.346; Day 2: p=0.189; Day 3: p=0.245, n = 4 for each group).

Figure 5: Image of T. thermophila on a hemocytometer under a microscope.

Conclusion
- Cell counts were inconclusive, but overall, T. thermophila treated with BMAA were more abundant than the control cells.
- Both rounds of quantitative PCR demonstrated that BMAA exposure resulted in no major change in the expression of LC4A.
- There was an increase in fold change in expression of LC4A, as shown in Figure 3, but it was not statistically significant (p=0.332).
- The results disagree with our hypothesis regarding both gene expression and cell growth.

References

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