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

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Introduction

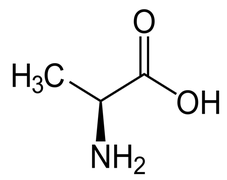
- The unicellular, eukaryotic ciliate *Tetrahymena thermophila* (*T. thermophila)* is a popular model organism in molecular biology.
- It has been proposed that ***β***-Methylamino-L-alanine (BMAA), a non-proteinogenic amino acid secreted by Cyanobacteria, is a hyper-excitatory neurotoxin.
- If hyper-excitation of cells were to occur, a strong influx of calcium ions would incduce 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

a.



b.

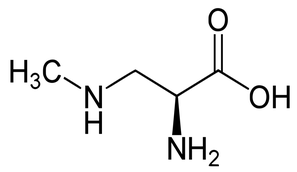


Figure 1: (a.) Chemical structure of the standard amino acid L-alanine. (b.) Chemical structure of the non-proteinogenic amino acid BMAA.

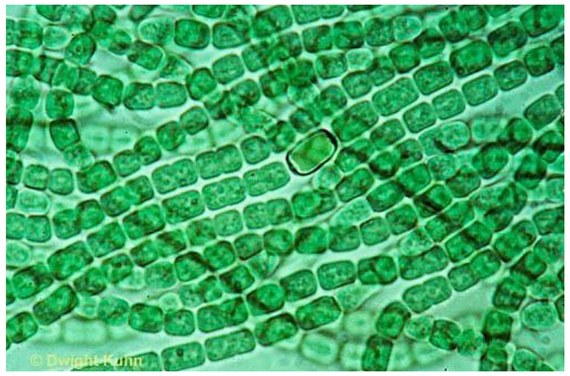


Figure 2: Cyanobacteria, which naturally produces and secretes BMAA.

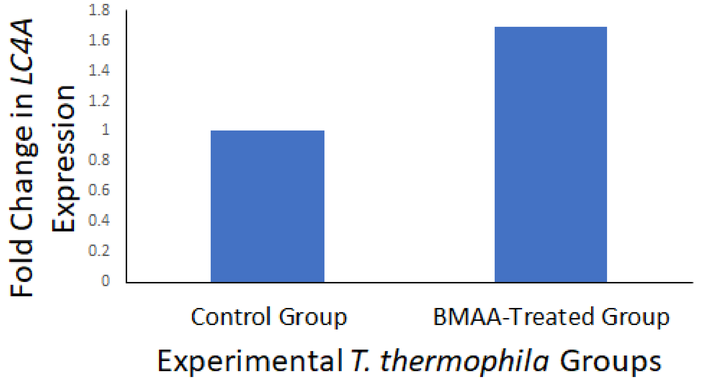


Figure 3: Fold change in expression of *LC4A.* (p=0.332; n = 4 for each group).

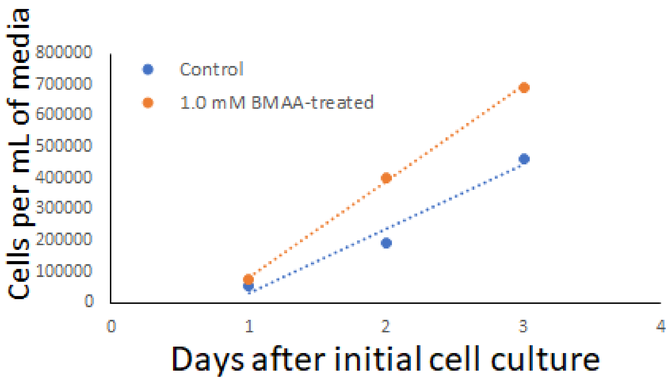


Figure 4: Number of cells per milliliter of media, averaged between round 1 and round 2. (Day 1: p=0.346; Day 2: p=0.189; Day 3: p=0.245, n = 4 for each group).

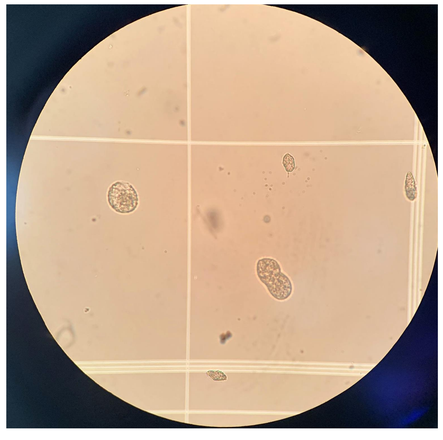


Figure 5: Image of *T. thermophila* on a hemocytometer under a microscope.

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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