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Effect of Lead on VMA1 ATPase Gene Expression and Feeding Behavior in *Tetrahymena thermophila*

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Effect of Lead on *VMA1* ATPase Gene Expression and Feeding Behavior in *Tetrahymena thermophila*

Introduction

- Tetrahymena thermophila* (*T. thermophila*) are eukaryotic, freshwater ciliates that are a prime model organism.
- The *VMA1* gene encodes the protein VMA1 (Vacuolar Membrane ATPase) which assists in regulating active transport systems on the vacuolar membrane.
- Lead is an environmental toxin that has been shown to have deleterious effects on human health including neurological defects, developmental delays, and body pain.
- Hypothesis:** If *T. thermophila* are exposed to lead, then the *VMA1* ATPase gene would show altered expression as well as altered feeding behavior.

Methods

- Primer synthesis:** Primers for *VMA1* ATPase were designed using Oligoanalyzer software.
- Culturing:** *T. thermophila* cultures were maintained in NEFF media. Upon experimentation, cultures were transferred into one control culture containing only SPP, and two experimental cultures, one with SPP and 12 mM sodium nitrate, and the other with SPP and 3 mM of lead nitrate.
- RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- Reverse transcription:** cDNA was synthesized using RevertAid.
- qPCR** was performed using PowerUp Sybr Master Mix. *BTU1* gene expression was used as a positive control.
- Feeding Assay** was performed using 5% glutaraldehyde to immobilize *T. thermophila* and India Ink to quantify the number of vacuoles per cell.

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Results

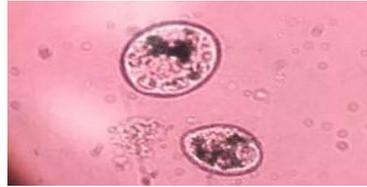


Figure 1: Feeding Assay of Lead Nitrate Group after six hours of exposure.



Figure 2: Feeding Assay of Sodium Nitrate Group after six hours of exposure.

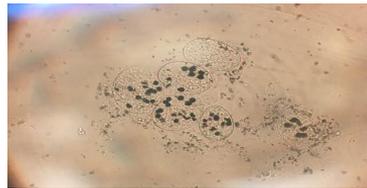


Figure 3: Feeding Assay of Lead Nitrate Group after eighteen hours of exposure.



Figure 4: Feeding Assay of Sodium Nitrate Group after eighteen hours of exposure.

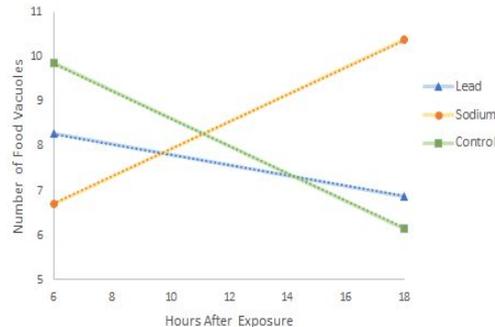


Figure 5: Graphic representation of number of vacuoles formed in presence of SPP, Sodium Nitrate, and Lead Nitrate n=2.

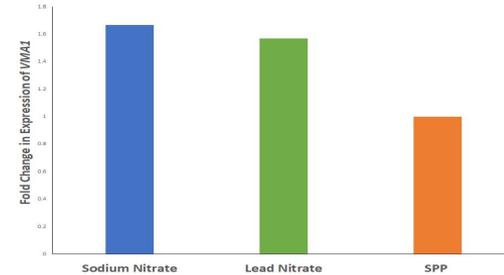


Figure 6: Relative expression of *VMA1* in sodium, lead, and control. P-value: sodium-SPP 0.325; Lead-SPP 0.16; Sodium-Lead 0.837.

Conclusions

- Both qPCR rounds demonstrated that lead nitrate exposure resulted in no major change in the expression of *VMA1* (Figure 6).
- There was an observed increase in the expression of *VMA1* in lead and sodium-treated *T. thermophila*; however, they were not found to be statistically significant (Figure 6).
- The feeding assay showed that in sodium more vacuoles are produced, while in lead or SPP the vacuoles produced decreased (See Figure 5).
- The results failed to reject the null hypothesis, supporting that lead did not have an effect on the gene expression of *VMA1* or feeding behavior in *T. thermophila*.

References

- Nilsson, J. R. (1979). Intracellular Distribution of Lead in *Tetrahymena* During Continuous Exposure to the Metal. *Biologists Limited*, (39), 383-396.

Acknowledgements

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