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Effect of Turmeric on Oxidatively Stressed Tetrahymena thermophila Cells

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Introduction

- The unicellular, eukaryotic ciliate *Tetrahymena thermophila* (*T. thermophila)* is a popular model organism in molecular biology.
- *OXR1* is an oxidative stress response gene encoding an enzyme that assists mitochondria in their response to Reactive Oxygen Species (ROS).
- Turmeric contains Curcumin which preliminary studies have shown has the ability to help mitigate the issues associated with Oxidative Stress.
- Oligomycin is a toxin that blocks the Electron Transport Chain, inducing oxidative stress within the mitochondria.
- **Hypothesis:** If *T. thermophila* are exposed to Turmeric, then the *OXR1* gene will be expressed at the same levels and the cells will die off at the same rate as the untreated cells.

Methods

- **Primer synthesis:** Primers for *OXR1* were designed using IDT Oligoanalyzer software.
- **Culturing:** *T. thermophila* cultures were maintained in NEFF media. Upon experimentation, all cultures were transferred into SSP media and were all exposed to Oligomycin. The treatment cultures were supplemented with 0.5% by volume Turmeric essential oils.
- **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 positive control.
- **Cell Counts** were performed with hemacytometers.
- **Growth Curve Assays** were performed with Cell Counting over time.

Results

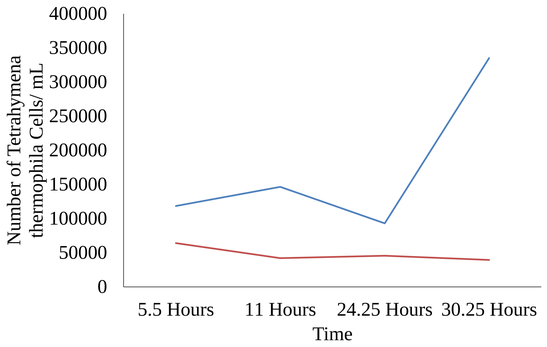

Figure 1: Growth Curve Assay of *Tetrahymena thermophila* over 30 hour period. (n=3, p-value = 5.5 hrs:0.2004, 11 hrs:0.1737, 24.25 hrs:0.2186, 30.25 hrs:0.0863)

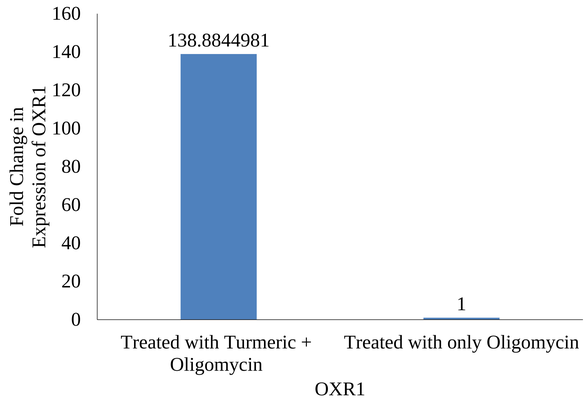

Figure 2: Fold Change of *OXR1* expression in the control and treatment *Tetrahymena* cell cultures (p=0.385239 and n=4)

	Average RQ (OXR1)	Fold Change
Treated with Turmeric + Oligomycin	0.014636063	138.8844981
Treated with only Oligomycin	0.000105383	1

Table 1: Results of the qPCR results of the *OXR1* gene's expression in *T. thermophila*

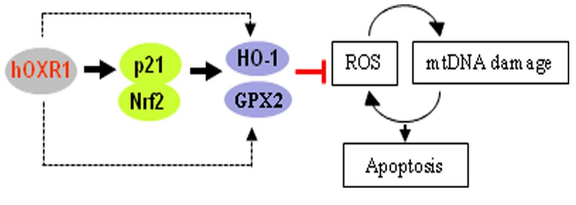

Figure 3: A schematic that describes the effect that the *OXR1* gene product has upon ROS.

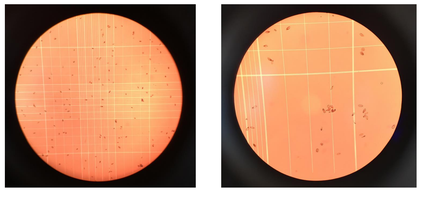

Figure 4: Images of *T. thermophila* when being observed in a hemocytometer.

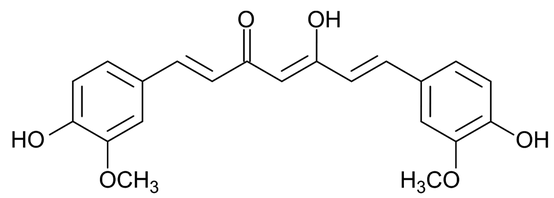

Figure 5: Chemical Structure of Curcumin, a molecule commonly found in turmeric roots and the subject of our study.

Conclusion

- The *OXR1* gene in *Tetrahymena thermophila* was not significantly influenced by the presence of curcumin (turmeric essential oil). A qPCR assay did not show a statistically significant impact of curcumin on *T. thermophila* in an oxidative environment.
- A growth curve assay of *T. thermophila* revealed that the turmeric at a 5% concentration by volume killed all the cells. The oligomycin did not kill the cells at a concentration of 2.5*10^-3 ug/mL. These results lead to the conclusion that too high of a concentration of turmeric essential oil was used.
- Interestingly, mRNA expression revealed through qPCR analysis showed a 139-fold increase in OXR1 gene expression in the experimental group. Though intriguing, this result was not statistically significant.

References

- Chemical structure of Curcumin was grabbed from https://www.selleckchem.com/products/Curcumin.html
- Yang, Mingyi, et al. "Human OXR1 Maintains Mitochondrial DNA Integrity and Counteracts Hydrogen Peroxide-Induced Oxidative Stress by Regulating Antioxidant Pathways Involving p21." Free Radical Biology and Medicine, vol. 77, 2014, pp. 41–48., doi:10.1016/j.freeradbiomed.2014.09.003 (Figure 4 was provided from this paper).

Acknowledgements

We would like to thank our faculty advisor, Dr. Stefanie Otto-Hitt for her superb guidance throughout this process. This project was funded in part by a supplies grant from the Ciliate Genomics Consortium.