

Exposure to HFPO-DA in *C. Elegans* reveals increased gene expression levels for genes gcs-1 and lmd-3

Alondra, S., Logan, O., Deanna, G., Dana, O.

Sweetwater High School, Tesoro High School, Gateway High School, Boz Life Science Research and Teaching Institute, University of California San Diego Extension

Summary

Null Hypothesis: There is a statistically significant difference in *lmd-3* and *Gcs-1* expression in *C.elegans*.

The gene expression for the *Lmd-3* and *Gcs-1* gene was assessed using qRT-PCR.

Gcs-1 expression was significantly different compared to *Lmd-3* expression in *C.elegans* under HFPO-DA exposure

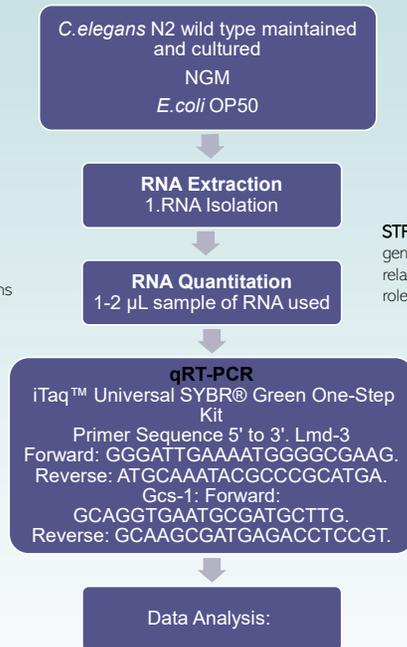
Abstract

The gene *lmd-3* role is to respond to oxidative stress, while *gcs-1* consults refusal to arsenic which activates oxidative stress. This study investigated if growing *C. elegans* in the presence of gen x will cause an induction or repression of gene expression. Under controlled laboratory conditions qRT-PCR analysis it shows that both genes *gcs-1* and *lmd-3* were upregulated. Therefore, the results did demonstrate significant gene expressions in *gcs-1* and *lmd-3* when *C.elegans* were under HFPO-DA conditions.

Introduction

- If the worms (*C.elegans*) are being exposed to *E.coli* incubated with HFPO-DA, genes *gcs-1* and *lmd-3* will be upregulated.
- C. elegans* are transparent nematodes that are directly applicable to human development and diseases because 83% of the *C. elegans*' proteome contains human homologous genes. *C. elegans* mapped out primitive neural circuit and ability to react to chemicals has made them an important model organism to study gene function. In the past the *C. elegans* have been used in scientific studies which has led to a breakthrough in nicotine dependence, aging, and Alzheimer's disease.
- Our genes of interest are *hsp-70*, *nhr-49*, *pxmp-4*, *pqm-1*, *gst-4*, *daf-16*, *sir-2.1*, *prx-11*, *lmd-3*, and *gcs-1*. When trying to group these, *lmd-3*, *gcs-1*, and *nhr-49* all have a response to oxidative stress. Whereas *daf-16*, *pqm-1*, and *hdp-70* all play an important role in longevity in *C.elegans*. *Sir-21* as well as *DAF-16* work together as antagonist to maintain balance between cell survival and development. *DAF-16*, and *Sir-21* both bind in heat stress. Lastly *prx-11* and *pxmp-49* are both proteins with peroxisomes that regulation of size.
- HFPO-DA, commonly referred to as GenX, is a compound difficult to detect. Some previous research done with this stressor was used to analyze acid on environmental level which is what this compound is of interest for (environmental monitoring).
- Commonly throughout our life, harmful chemicals are exposed to us, coming from incinerators, factories, or even as food. Specifically, chemicals referred to as per- and polyfluoroalkyl substances (PFAS) are tough to break down and have been found to make us sick or even cause cancer through protein aggregation. HFPO-DA is a proposed chemical which may be a safer alternative, and through testing it with *C. elegans* which contain many of the same genes as humans we can determine if this is a safer chemical or even more dangerous to expose to humans.

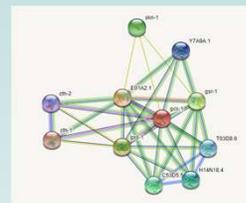
Methodology



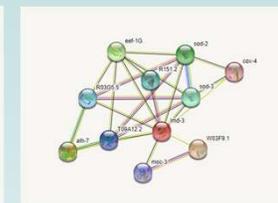
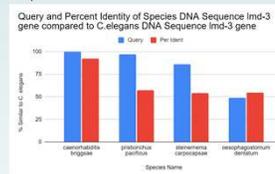
Materials and methods

- Control : Median worms are grown @MGM, temperature is 23 degrees Celsius
- Experimental condition: fed with *E.coli* that were incubated with HFPO-DA @280 nanograms per liter.
- Guanidinium Thiocyanate Phenol-Chloroform Extraction used for RNA isolation.
- Nanodrop Spectrophotometer used for RNA Quantitation.
- $\Delta\Delta Ct$ Data used to calculate average fold change for the treated and the control.
- SYBR Green 1 DNA Binding dye used to detect fold changes

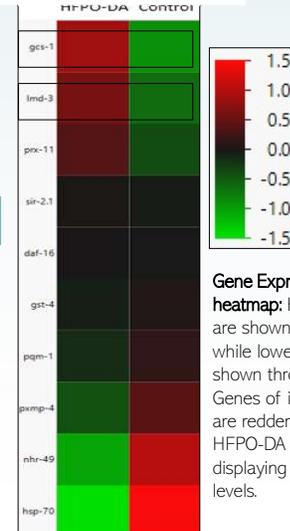
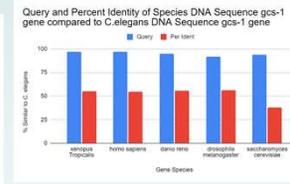
Results



STRING Figure: Displays connections between genes related to *gcs-1*, contains mainly genes related to resistance to arsenite and may play a role in response to oxidative stress.



STRING Figure2: Displays connections between genes related to *lmd-3*, mainly genes related to response to oxidative stress.



Gene Homology Graphs

(*lmd-3*=left, *gcs-1* right). *Gcs-1* homology displays a wide range of organisms, including homo sapiens, while homology for the *lmd-3* gene contains mainly nematodes, or roundworms, suggesting it is much newer gene compared to *gcs-1*.

Gene Expression shown through heatmap: Higher expression levels are shown through more red colors while lower expression levels are shown through more green colors. Genes of interest *gcs-1* and *lmd-3* are redder with the addition of HFPO-DA compared to the control, displaying higher gene expression levels.

Discussion / Conclusion

Using standard lab equipment has allowed us to have a baseline for the *gcs-1* and *lmd-3* gene expressions in *C. Elegans*. Our main finding was that *C. Elegans* exposure to HFPO-DA increased gene expression levels for both *gcs-1* and *lmd-3*. These results are consistent with *gcs-1*'s role of resisting arsenic-induced oxidative stress and *lmd-3*'s role of oxidation resistance. Our results suggest that exposure to HFPO-DA has negative effects on *C. Elegans* and therefore humans because it upregulates stress indicator gene expressions found in their homologous genomes.

Study limitations:

- Individual differences between the worms were not considered. It could be possible that not all worms reacted the same since we are just testing a group of worms together instead of testing each worm 1 by one and observing how they react to the *E.coli* incubated with HFPO-DA
- Limited access to equipment in an online environment during a pandemic, we couldn't get the full hands-on experience.

Future Directions:

In this experiment, we studied the effect exposure to HFPO-DA has on gene expression levels within *C. elegans* in order to determine if HFPO-DA known as GenX, would serve as a safer alternative to chemicals in our environment.

References

- Lai, C. H., Chou, C. Y., Ch'ang, L. Y., Liu, C. S., & Lin, W. (2000, May). *Identification of novel human genes evolutionarily conserved in Caenorhabditis elegans by comparative proteomics*. Genome research. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC310876/#:~:text=elegans%20protein%20sequences%2C%20our%20results,matching%20kown%20human%20gene%20transcripts>.
- UniProt, Consortium. (2002-2021). UniProtKB - G5EFY7 (G5EFY7 - CAEEL) National Institute of Health EMBL - EBI <https://www.uniprot.org/uniprot/G5EFY7>
- Caroline Kumsta, Maike Thamsen, and Ursula Jakob (2010 Mar 22; Revised 2010 Jul 19; Accepted 2010 Jul 20) Effects of Oxidative Stress on Behavior, Physiology, and the Redox Thiol Proteome of *Caenorhabditis elegans* <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3052275/>