Effects of Curcumin on Inflammatory Pathways of *Caenorhabditis elegans* During Heat Shock Response

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Introduction

The bioactive compound of the turmeric plant, curcumin, has long been used in traditional Ayurvedic medicine as an anti-inflammatory and antioxidant. The biological activities of curcumin have even revealed it as a therapeutic agent against cancer (Katsori 2011). With historical roots in South Asia, Ayurvedic practices have yet to be fully understood and accepted from a Western-scientific perspective. This study aims to empirically test the efficacy of curcumin on inflammation and pinpoint its impact on eukaryotic inflammatory pathways.

The microscopic roundworm *Caenorhabditis elegans* is a suitable organism for studying the immune system. For the purposes of this study, *C. elegans* allows for rapid testing, simple manipulation, and visualization of the organism due to its short lifetime, low maintenance needs, and small, transparent body. *C. elegans* is a model organism, and thus, its genome has been fully sequenced, elucidating the many genes and pathways of innate immunity that are homologous to humans (Diogo and Bratanich 2014).

One essential pathway of immunity, conserved among all eukaryotes, is the mitogen-activated protein kinase (MAPK) pathway. When activated by cellular damage, this signal transduction cascade mediates regulation of gene expression, survival, metabolism, and inflammation (Troemel et al. 2006). In observation of curcumin's effect on inflammation, the *C. elegans* were put under stress through heat shock, and various transcripts of the MAPK pathway were evaluated: DAF-16, HSF-1, and HSP-70. The transcription factor DAF-16 serves as an inducer to many heat shock proteins, and is also implicated in increased longevity. The transcription factor HSF-1 mounts the stress response by binding to HSPs such as HSP-70, which aids in thermo-tolerance and promotes cell survival (Tullet 2015). The objective of this project is to assess the effect of curcumin on inflammation pathways by analyzing the transcription factors and heat shock proteins induced by heat stress.

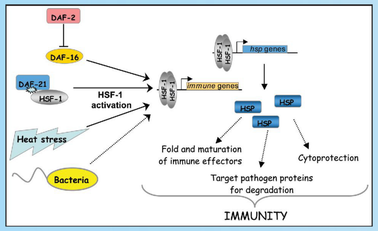

Figure 1. HSF-1 pathway in innate immunity.

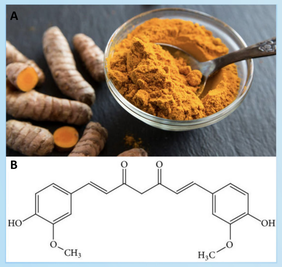

Figure 2. (A) Turmeric plant from which curcumin (B) is derived.

Results

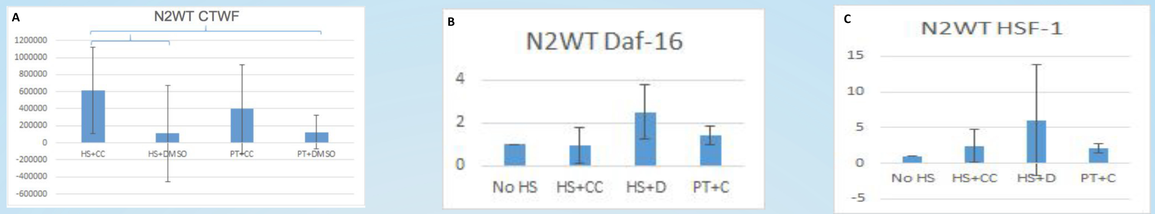

Figure 3. Corrected total worm fluorescence (CTWF) and transcript analysis of N2-WT worms. (A) CTWF measurements were manually taken with ImageJ. Blue bars indicate a significant difference between the two treatment groups (p-value < 0.05). The HS+CC treatment group was found to have significantly more fluorescence when compared to the DMSO groups regardless of pre-treat conditions. **(B)** q-PCR was used to quantify the level of expression of transcripts Daf-16 and **(C)** HSF-1. Expression relative to the no HS control is conveyed through fold differences. No significant differences were found.

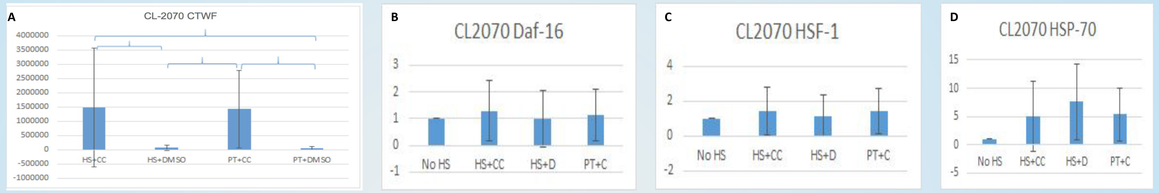

Figure 4. Corrected total worm fluorescence (CTWF) and transcript analysis of CL-2070 worms. (A) CTWF measurements were manually taken with ImageJ. Blue bars indicate a significant difference between the two treatment groups (p-value < 0.05). Notably, fluorescence was significantly higher in the groups treated with curcumin as compared to the groups treated with DMSO. **(B)** q-PCR was used to quantify the level of expression of transcripts Daf-16 and **(C)** HSF-1 and **(D)** HSP-70. Expression relative to the no HS control is conveyed through fold differences. No significant differences were found.

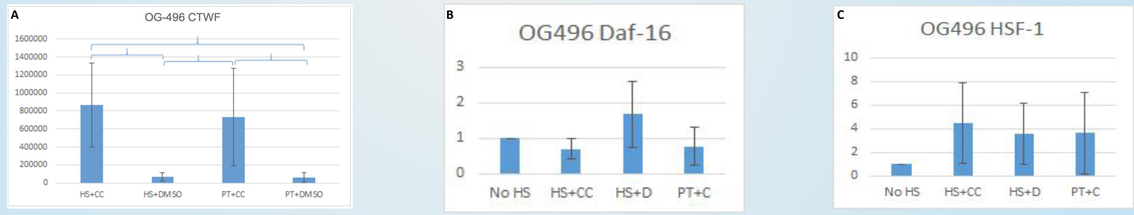

Figure 5. Corrected total worm fluorescence (CTWF) and transcript analysis of OG-496 worms. (A) CTWF measurements were manually taken with ImageJ. Blue bars indicate a significant difference between the two treatment groups (p-value < 0.05). Trends in CTWF were similar to that of strain CL-2070; the OG-496 worms fluoresced significantly more in the groups treated with curcumin as compared to the groups treated with DMSO. **(B)** q-PCR was used to quantify the level of expression of transcripts **(B)** Daf-16 and **(C)** HSF-1. Between treatment groups, there were no significant differences in expression of **(B)** Daf-16 or **(C)** HSF-1.

Conclusions

- qPCR analysis reveals curcumin does not significantly affect transcription levels of Daf-16, HSF-1, or HSP-70
  - N2-WT worms treated with HS+DMSO resulted in the highest level of expression of Daf-16 and HSF-1 transcripts
  - CL-2070 worms treated with curcumin had the highest level of expression of Daf-16 and HSF-1 transcripts, while worms treated with HS+DMSO had the highest level of expression of the HSP-70 transcript
  - N2WT and CL2070 worms treated with curcumin had an increase in expression of Daf-16 and HSF-1 transcripts compared to the control
  - OG-496 worms treated with HS+DMSO resulted in the highest level of expression of Daf-16, while worms treated with curcumin had the highest level of expression of the HSF-1 transcript
  - It appears that OG-496 worms treated with curcumin showed a decrease in Daf-16 expression compared to the control
- Fluorescence microscopy data indicated curcumin significantly increased expression of heat shock proteins during heat stress
  - A longer duration of curcumin treatment appears to have no significant impact

Future Directions

- Further assess HSP-70 expression between treatment groups after heat shock (due to lack of time and reagents, HSP-70 transcription levels could not be quantified for N2-WT or OG-496 strains)
- Additional replicates to confirm that duration of curcumin treatment is inconsequential

Methods

1. The following was performed in triplicate for each of 3 different worm strains: N2-WT (wild-type), CL-2070 (HSP-16.2 GFP), OG-496 (HSF-1 GFP)
2. C. elegans were grown on NGM agar plates with E. coli OP50
3. Worms were synchronized and assays were carried out using adult stage worms
4. Worms were washed in M9 + DMSO or M9 + Curcumin and placed in the following treatment groups:

Control	Heat Shock	Pretreat
No HS = control in DMSO ○ 25 °C for 1 hr	**HS + DMSO = heat shock in DMSO** ○ 35 °C for 1 hour **HS + CC = heat shock in curcumin** ○ 35 °C for 1 hour	**PT+DMSO+HS = pretreat in DMSO** ○ 25 °C for 1 hour ○ 35 °C for 1 hour **PT+CC+HS = pretreat in curcumin** ○ 25 °C for 1 hour ○ 35 °C for 1 hour

5. Worms from each group were collected on slides and GFP expression was visualized through fluorescence microscopy
6. Worm RNA was isolated in TriZol, cDNA was synthesized, and q-PCR analysis of transcripts performed

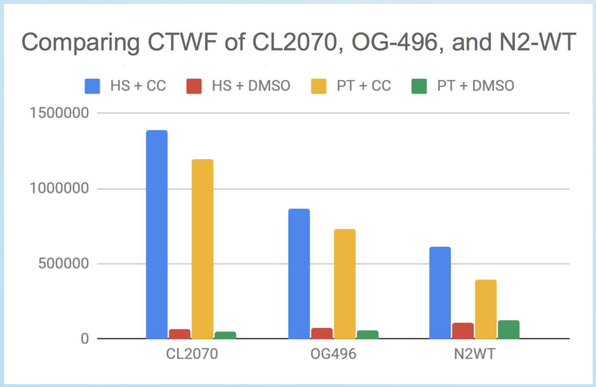

Figure 6. Average CTWF for each treatment group of strains CL-2070, OG-496, and N2-WT. A summary of Figures 3A, 4A, & 5A. Trends in fluorescence amongst the treatment groups are parallelled between all 3 strains, with CL-2070 (HSP-16.2 GFP) emitting the greatest intensity of fluorescence, followed by OG-496 (HSF-1 GFP), and finally the wild-type strain N2-WT

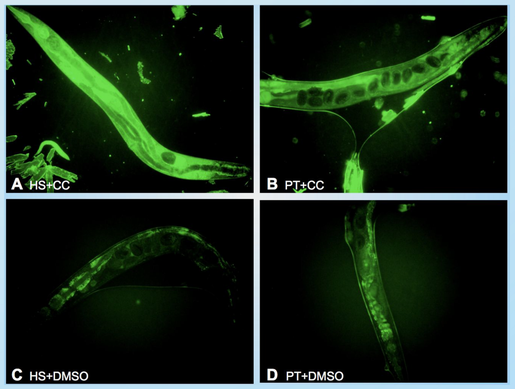

Figure 7. N2WT confocal images from each treatment group. After heat shock, worms were immediately washed in M9, anesthetized with sodium azide, and mounted on slides for viewing under the confocal microscope. CTWF measurements were sourced from these images using ImageJ. Pictures were taken with the same exposure settings for each treatment group: **(A)** HS+CC, **(B)** PT+CC, **(C)** HS+DMSO, and **(D)** PT+DMSO.

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Citations

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