Section II: Methodology

Role of Student vs. Mentor

Throughout the research project, the student conducted all background research, designed the project and methodology, prepared all materials, performed the data collection, analyzed the results, and wrote papers on their own. The mentor provided feedback and assisted with technical questions when needed. Agar plates, E. coli cultures, and other communal supplies were made by the student, the mentor, and other student researchers. The time spent on this project was 5 months.

Equipment and Materials

Proper lab attire, gloves, and protective goggles were worn at all times. To maintain the worms, agar plates (Nematode Growth Medium, NGM) seeded with OP50 *Escherichia coli* liquid culture were used as per standard practice. A cooling chamber set to 16°C was used to grow the worms and prevent the premature expression of Amyloid beta. A stereo microscope [Model SMZ1500, Nikon] and an aluminum worm pick were used to observe and transfer the worms. To wash worms, 1mL pipettes and standard M9 buffer were used. To synchronize the *C. elegans*, a centrifuge, a 1 mL pipette, M9 buffer, and standard worm bleach were used. A 91% ethanol solution was used to clean surfaces such as the worm pick before and after use.

A 30°C incubator was used to induce Amyloid plaques in the worms. The worms were kept on agar plates seeded with OP50 *E. coli* for this process.

To stain the worms, a 1% stock solution of Congo red prepared according to the Highman method (Highman, 1946) was diluted to a concentration of 0.1% with M9 buffer. For the detection of Congo red, a stereo microscope with a red filter was placed on the objective and a 497 nm wavelength LED was used. For the detection of GFP, a stereo microscope with a yellow filter covering the objective and a 395 nm wavelength LED were used. A camera [44423, Celestron] connected to a computer

running the Celestron Digital Imager HD application was used to record and take images of the worms. Lastly, Fiji was used to analyze the images of the worms and output data on their pixel intensity (PI) in Arbitrary Units (AU).

Strain Growth and Maintenance

The CL2355 (smg-1(cc546) dvls50 I) and CL691 (dvls19 III; skn-1(zu67) IV/nT1 [unc-?(n754) let-?] (IV;V)) strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN, USA). All strains were maintained at 16°C. Worms at the L4 and young adult stages were transferred onto recently seeded agar plates to continue the strain.

Crossbreeding

Crossbreeding the CL2355 and CL691 strains allowed for the creation of a new strain with the desired genotype for this experiment. This was necessary as there was no existing strain able to express Amyloid beta when heated and GFP when the worm has oxidative stress.

L4 and young adult *C. elegans* of the CL2355 and CL691 strains were transferred onto the same seeded agar plate and kept for 3 days until the plate had hundreds of worms.

A plate of the crossbred worms was heated at 35°C for 30 minutes to induce Amyloid plaques and oxidative stress in the worms. After being heated, the worms were washed once with a 0.1% solution of Congo red in M9 buffer and pipetted onto a new seeded agar plate. Next, the worms were checked for GFP using the yellow filtered stereo microscope and a 395 nm wavelength LED angled at 45° relative to the horizontal towards the worms (Chagas et al., 2017). Then the worms were checked for Congo red using the red filtered stereo microscope and a 497 nm wavelength LED angled at around 45° relative to the horizontal towards the worms. Images of the fluorescence were captured using the Celestron Digital Imager and a computer.

This strain was maintained at 16°C. Worms at the L4 and young adult stages were transferred onto recently seeded agar plates to continue the strain.

Induction of Amyloid Plaques

One plate of CL691 worms of all stages and one plate of MO1 worms were heated at 30°C for 30 minutes to cause the expression of Amyloid beta. Another plate of MO1 worms remained at 16°C to prevent the expression of Amyloid beta for use as a control. The CL691 worms were used as a control for the levels of oxidative stress produced in the heating process. The age ranges of all worms were determined visually based on size.

Fluorescence Imaging

Fluorescence imaging is a commonly used method for detecting Amyloid plaques (Highman, 1946) and oxidative stress (Korovesis et al., 2023).

To stain the worms for the detection of Amyloid plaques, the worms were washed once with a 0.1% solution of Congo red in M9 buffer and pipetted onto a new seeded agar plate. Then, the worms were checked for GFP using the yellow filtered stereo microscope and a 395 nm wavelength LED angled at around 45° relative to the horizontal towards the worms. Then the worms were checked for Congo red using the red filtered stereo microscope and a 497 nm wavelength LED angled at around 45° relative to the horizontal towards the worms. Images of the fluorescence were captured using the Celestron Digital Imager and a computer.

The fluorescence images collected from the Celestron Digital Imager were opened in Fiji. Using the Freehand Sections option, the worms were traced. Then, the software was used to determine the mean image intensity of the worm with the measure option. The range of pixel intensities was from 0,

representing the darkest pixel value, to 255, representing the brightest pixel value. This was performed for all images of test worms and controls. The data was further analyzed in Excel.

Statistical Tests

After running the experiment, the results were graphed. The differences between pairs of conditions were compared using a one-tailed t-test to determine if the amounts of Amyloid plaque-induced oxidative stress were greater in the older worms. We compared the mean pixel intensities of heated MO1 worms with the mean pixel intensities of unheated MO1 worms, then compared the heated MO1 worms to the heated Cl691 worms to determine whether the methodology was successful.