Characterizing a *C. elegans* Model for Oxidative Stress Response

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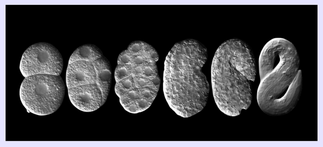
Abstract

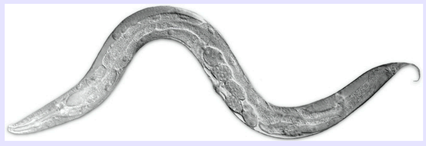
Neurodegenerative diseases such as Parkinson's and Alzheimer's disease are characterized by progressive neuronal loss, often driven by oxidative stress. The accumulation of reactive oxygen species (ROS) contributes to cellular damage, making oxidative stress a key factor in disease pathology. Caenorhabditis elegans, a genetically tractable model with conserved stress response pathways and neuronal structures, provides an effective system for studying oxidative stress and neurodegeneration. This study aims to establish an optimized oxidative stress assay in C. elegans to evaluate protective effects against ROS-induced damage. Wild-type (N2) C. elegans were synchronized via a bleaching protocol to generate a uniform population of young adults. Lifespan and survival assays were performed using tert-butyl hydroperoxide (tBHP) to induce oxidative stress, testing concentrations of 10, 1, 0.1, and 0.01 mM. Higher concentrations (10 and 1 mM) resulted in rapid mortality of C. elegans within 3 and 9 hours, respectively, whereas lower concentrations (0.1 and 0.01 mM) allowed survival beyond 12 hours. Based on these findings, an optimal tBHP concentration will be used to further refine this oxidative stress model. This study provides foundational data for investigating the efficacy of potential antioxidant molecules in reducing ROS-related damage. By using the C. elegans model, future research will focus on identifying molecular mechanisms of oxidative stress response and evaluating therapeutic candidates for neurodegenerative diseases.

Introduction

- Model Organism: *C. elegans* is a transparent nematode widely used in genetic and neurodegenerative disease research.
- Life Cycle:
  - Lifespan: ~2–3 weeks when grown under standard lab conditions.
  - Development: Embryonic stage → 4 larval stages (L1–L4) → Adult nematodes (around day 3).
  - Temperature Effects: *C. elegans* lives longer at lower temperatures (12°C), and has a shorter lifespan at higher temperatures (25 °C).
- *C. elegans* for Neurodegenerative Research:
  - ~38% of *C. elegans* genes have human orthologs.
  - Transparent body allows real-time observation of cellular processes.
  - Short lifespan and rapid development enable efficient study of aging and disease progression.



C. elegans embryonic development



C. elegans nematode

Methods

C. elegans age-synchronization procedure:

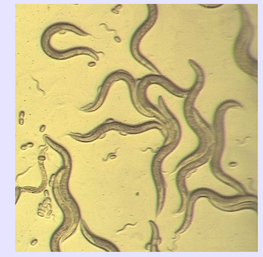
- Gravid adults were washed off plates with DI water, spun down (2000 rpm, 2 min), and treated with bleach/KOH solution for ~4 min, with gentle mixing by inversion.
- Most of the solution was removed, worms were washed with DI water, and embryos were plated onto fresh NGM plates, seeded with *E. coli*.

Preparing tBHP solutions:

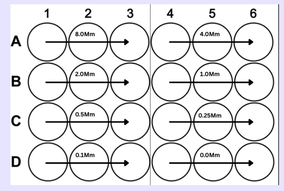
- tBHP was prepared from a 7.7 M stock and diluted with M9 buffer to obtain different working concentrations.
- Final concentrations assayed: 10, 8, 4, 2, 1, 0.5, 0.25, 0.1, and 0.01 mM of tBHP.

Survival Assay:

- 50 µL of age-synchronized worms in DI water were plated into 24-well plates, then treated with 500 µL of assigned tBHP concentration.
- Worm survival was assessed hourly by movement or lack thereof to plot survival curves over 12 hours.



Age- synchronized adult *C. elegans*



Experimental plan: 24-well plate with different concentrations of tBHP

Results

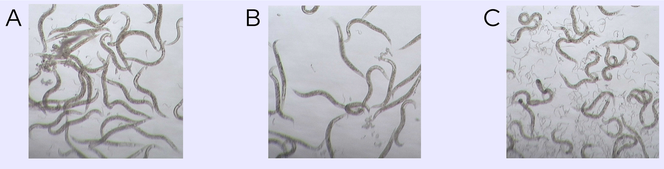


Figure 1. N2 *C. elegans* in M9 buffer with no exposure to tert-butyl hydroperoxide (tBHP). (A) 0 hours, (B) 2 to 6 hours, (C) 6 to 12 hours

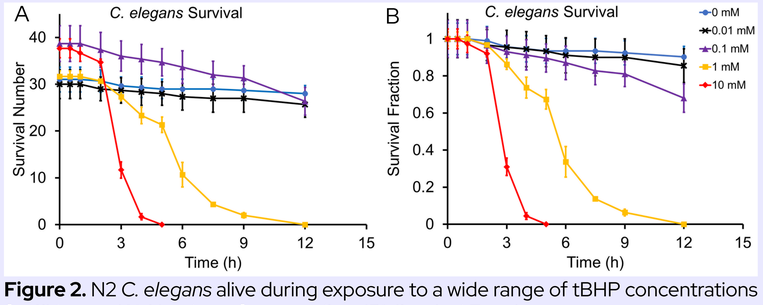


Figure 2. N2 *C. elegans* alive during exposure to a wide range of tBHP concentrations for 12 hours. (A) Number of worms alive. (B) Survival Fraction

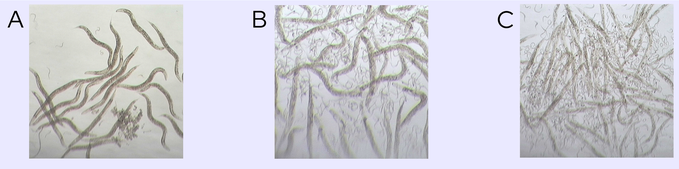


Figure 3. N2 *C. elegans* exposed to tert-butyl hydroperoxide (tBHP) in M9 buffer. (A) 0 hours, (B) 2 to 6 hours, (C) 6 to 12 hours

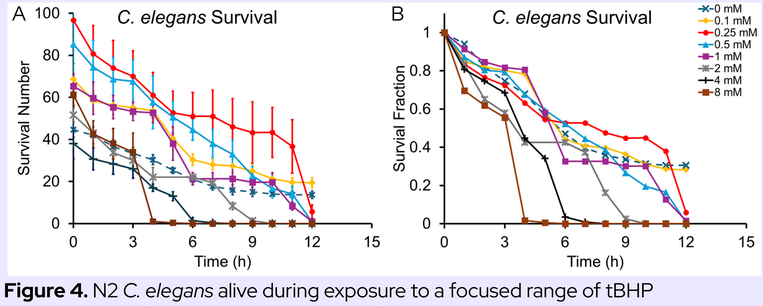


Figure 4. N2 *C. elegans* alive during exposure to a focused range of tBHP concentrations for 12 hours. (A) Number of worms alive. (B) Survival Fraction

Discussion

- 0.25 mM and 0.5 mM tBHP are optimal concentrations for inducing ROS in *C. elegans:* just enough to cause oxidative stress damage, without exerting too much damage.
- We plan to use these optimized concentrations of tBHP to test the protective effects of an antioxidant drug (L2) using survival and lifespan experiments to determine if it prevents oxidative damage in *C. elegans*.
- Our research aims to identify treatments that help reduce oxidative stress-related damage in diseases like Alzheimer's, Parkinson's, and cardiovascular disorders.

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Oxygen-related damage to cells is a key contributor to neurodegenerative diseases like Alzheimer’s and Parkinson’s. Our study used *C. elegans* as a model organism to develop an oxidative stress assay using a stress-inducing molecule, tert-butyl hydroperoxide (tBHP). By exposing young adult worms to various concentrations of tBHP, we determined that 0.25–0.5 mM reliably induced oxidative stress without killing the worms too quickly. This model can now be used to test antioxidants for their protective effects. These findings lay the groundwork for identifying molecules that reduce oxidative damage and help delay the progression of diseases associated with reactive oxygen species (ROS) and nerve damage.