Exposure to an Environmental Contributor of Parkinson's Disease: *S. venezuelae* impacts *daf-16* signaling and lifespan in *C. elegans*

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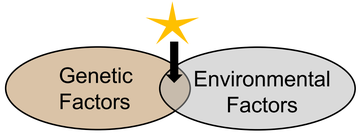
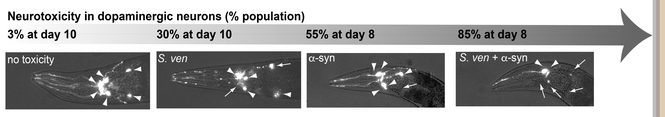
Abstract

Parkinson's Disease (PD) is characterized by the loss of dopaminergic (DA) neurons and the formation of protein inclusions that contain the α-synuclein (α-syn) protein. Overexpression of human α-syn in the eight DA neurons of *C. elegans* causes neurodegeneration in an age- and dose-dependent manner, like that observed in human pathology. Only 5-10% of PD cases have a direct genetic origin; however, exposure to herbicides, pesticides, and interaction with soil are all potential risk factors. A soil bacterium, *Streptomyces venezuelae* (*S. ven*), produces a secondary metabolite that causes age- and dose-dependent DA neurodegeneration in *C. elegans*; it also exacerbates α-syn-induced DA neurodegeneration. Initial studies from our lab determined that exposure to the *S. ven* metabolite caused oxidative stress and upregulation of reactive oxygen species (ROS). These studies identified that the metabolite worked through the transcription factor *daf-16* to activate *sod-3*. Furthermore, *daf-16* nuclear translocation assays revealed that upon metabolite exposure produced significantly more *daf-16* accumulation within nuclei. When mitochondrial homeostasis is perturbed, in part due to external stressors, mitochondrial toxicants, or the aging process, these disruptions impairs the normal metabolic activity of mitochondria leading to increased ROS and impaired protein folding. We found that *S. ven* toxicity negatively impacts mitochondrial function, including lowering ATP production, and inhibiting electron transport chain complex I activity. It is also associated with increased mitochondrial fragmentation and up- and down-regulation of *drp-1* and *fzo-1* gene expression, respectively. Recently, we have discovered that exposure to the *S. ven* metabolite causes a decrease in *C. elegans* lifespan. However, when used at lower concentrations, the metabolite can cause lifespan extension, suggesting a potential hormetic effect. We have subjected mitochondrial and transcription factor mutants to lifespan assay to investigate the impacts of *S. ven* exposure on the aging process.

S. ven metabolite causes DA neurodegeneration



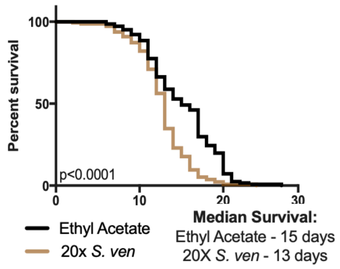
Environmental factors have been found to contribute to PD-like symptoms alone; while also exacerbating conditions when paired with genetic cofactors. *Streptomyces venezuelae (S. ven)* is a common soil dwelling bacterium, known to produce compounds related to disease-causing factors. Because of this connection, semi-purified compound from this bacterium was tested on both the *C. elegans* PD model and GFP only worms. Following exposure, DA neuronal health was assessed at various timepoints.

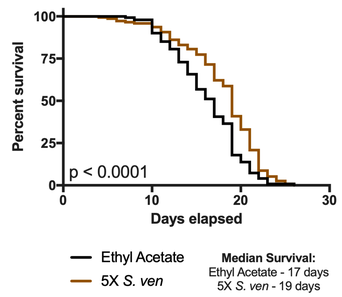


DA neuronal death in GFP only animals occurred at Days 9-12, whereas neurons containing α-syn degenerated as early as day 6 following *S. ven* exposure.

S. ven exposure causes a hormetic aging response in *C. elegans*

We were interested in whether exposure to the *S. ven* metabolite altered lifespan in *C. elegans*. Therefore, we exposed worms to two concentrations of *S. ven* metabolite, 20X and 5X, and monitored lifespan.

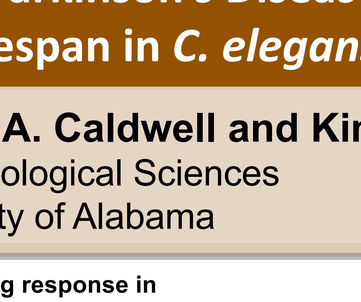




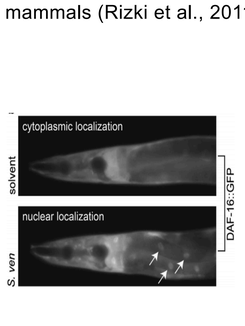
We found that exposure to *S. ven* metabolite caused a decrease in lifespan at higher concentrations, while extending lifespan at lower concentrations; suggesting a hormetic response.

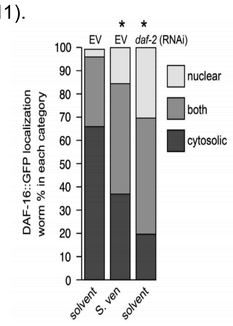
S. ven metabolite impacts *daf-16*

We further explored whether the transcription factor DAF-16 was impacted following metabolite exposure. DAF-16 is a FOXO transcription factor downstream of the DAF-2/insulin/IGF-1 signaling pathway. DAF-16 plays a critical role in this pathway to regulate longevity and stress response in both *C. elegans* and mammals (Rizki et al., 2011).



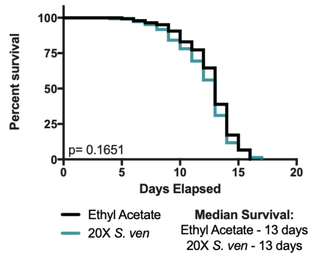
Rizki et al., 2011

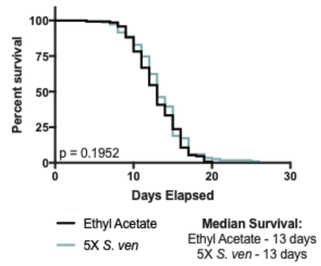




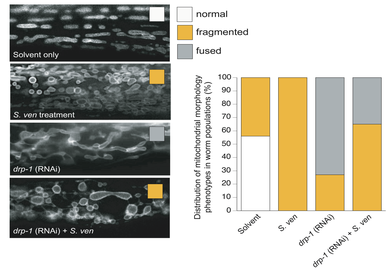
Using a DAF-16::GFP translational fusion, we investigated whether metabolite exposure induced DAF-16 nuclear localization. We found that worms displayed more DAF-16 in nuclei in the presence of the metabolite compared to solvent controls.

We further investigated whether the hormetic response was dependent on *daf-16*. We found no significant difference in median survival between the two treatments at either concentrations suggesting that the hormetic response is dependent on *daf-16* activity.





S. ven metabolite causes mitochondrial dysfunction

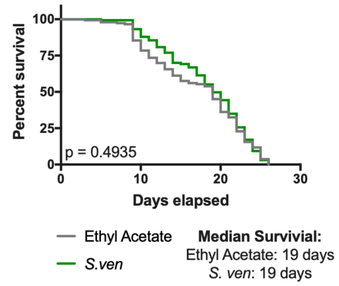


We further sought to investigate the impacts of metabolite exposure on mitochondria. We found that worms exposed to *S. ven* metabolite displayed increased mitochondrial fragmentation, an indicator of cell death.

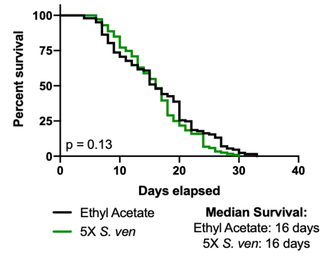
Mitochondrial mutants show differences in lifespan

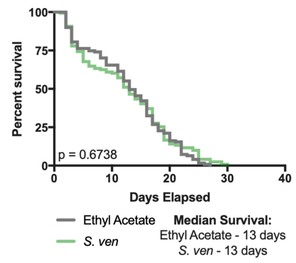
With knowledge of how the metabolite impacts mitochondrial fission, we were interested in testing *drp-1* in a lifespan assay. Additionally, *drp-1* has been shown to interact with the insulin signaling pathway (IIS).

In mammals, mitochondrial fission plays a crucial role in insulin secretion. Inactivation of *drp-1* in *C. elegans* was demonstrated to enhance and synergize with IIS mutants extending lifespan (Sun et al., 2017).



Byrne et al., 2019 showed loss of *drp-1* reduced lifespan. Here we found *drp-1* mutants exhibited prolonged lifespan in the presence of 20X metabolite compared to N2 animals, while exposure to 5X metabolite showed no significant difference compared to N2 animals. Thus, exposure to *S. ven* metabolite may elicit an interaction with IIS components to extend lifespan.





Likewise, we tested *fzo-1* mutants in the presence of 20X *S. ven* and found no significant difference compared to N2 animals, suggesting the mitochondrial fission plays a larger role in animals adapting to stressors and lifespan.

Future Directions

- Complete *daf-2* lifespan under both treatment conditions
- Complete neurodegeneration studies of IIS candidates
- Further evaluate pathways that converge onto *daf-16*

Acknowledgements

Department of Biological Sciences