Effects of Microbiome Diversity on Stress Response in *C. elegans* Strains with HIF Mutations

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Abstract

A study published in 2013 showed that several different species of test subjects grown in sterile environments from birth were less adaptive to stress than their wild counterparts. They also expressed higher levels of stress-related hormones, such as adrenocorticotropic hormone (ACTH) and corticosterone. Hypoxia-inducible factor (HIF) is a pathway that regulates the response to hypoxia and stress. HIF uses the gene *hif-1a*, which is a transcription blocker. The question addressed in this study is whether or not the microbiome has an effect on responses to stress that involve this pathway. This study used *C. elegans* strains with mutations in the HIF pathway as a model to look at the correlation between microbiome diversity and heat stress response. It used four different strains of *C. elegans* with varying tolerances to heat stress. From highest to lowest heat tolerance, these are *vhl-1*, *egl-9*, *N2*, and *hif-1*, where *N2* is wild type. These strains were grown on plates with a single strain, two strains, or four strains of bacteria to promote varying levels of microbiome diversity in the worms. The worms were then incubated, and their response to the 37˚C heat stress was recorded. At hour three, the death rate of the *vhl-1* and *egl-9* worms scaled inversely with the diversity of the microbiome. At hour five, the pattern changed slightly, as the worms grown on two strains of bacteria had approximately 15% less death than those grown on four strains. The remaining strains, *N2* and *hif-1*, had normal and reduced tolerance, respectively. They both followed roughly the same pattern as the other two strains, except that the worms grown on four strains of bacteria had approximately 20% less death than those grown on two strains. Overall, this suggests that a higher level of microbiome diversity decreased the probability of death in response to heat stress response. All four strains also showed abnormal behavior when experiencing heat stress, changing from the typical sinusoidal movements to repetitious coiling and seizing movements, as recorded in digital time lapses. Further studies will investigate how *C. elegans* with multiple of the mutations respond in circumstances similar to those used in this study. An enzyme-linked immunosorbent assay will also be utilized to observe levels of the stress hormone corticosterone.

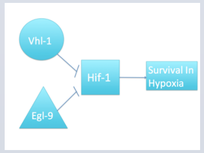
Caenorhabditis elegans

C. elegans is a nematode commonly used as a test organism in scientific studies. It serves well as a model organism because of its fully mapped neurological system, its completely sequenced genome, its consistent number of adult somatic cells, and the availability of hundreds of mutant strains (Edgley 2015). In the lab, *C. elegans* is grown on plates of nutrient agar typically seeded with one of two non-pathogenic strains of *E. coli* as a food source.

The Microbiome

The microbiome is the collection of living organisms living on or in an organism (Rea et al. 2016). Of particular interest to this study are those that populate the gut. The microbiome consists of bacteria, Archaea, fungi, and viruses. The majority of the microbes in the microbiome can be found in the gastrointestinal tract; they are also commonly found on the genitals and skin. The microbiome has been shown to have a substantial role in regulating the immune system (Round and Mazmanian 2009). As the *C. elegans* diet consists largely of bacteria in the wild, the microbiome may also play an important role in the worms' life and stress response. For example, serotonin also plays a role in the stress response of *C. elegans*. The goal of this research is to test whether diversifying the microbiome of laboratory strains of *C. elegans* will enhance their resistance to heat stress.

Hypoxia Inducible Factor (HIF)



This study utilized three of the hif-1 mutant *C. elegans* strains: *vhl-1* (OK161), *egl-9* (N571), and *hif-1* (IA4). *Vhl-1* is a gene that promotes the degradation of *hif-1*. The first two strains result in an increased tolerance to hypoxia. The *vhl-1* mutant has a deletion of the *vhl-1* gene, meaning that the *hif-1* is no longer being degraded. *Egl-9* is a gene that negatively regulates *hif-1*. The *egl-9* mutant has a substitution for *egl-9*.

Methods

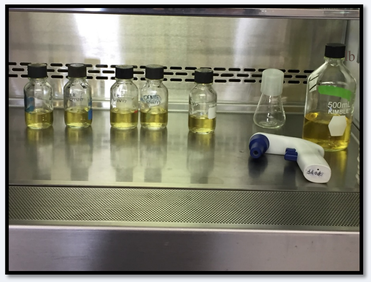


Figure 1

Figure 1 shows the sterile hood in which the media and bacteria used in the experiment were prepared. In the hood are containers of Luria Broth (LB) in which the bacteria were suspended.

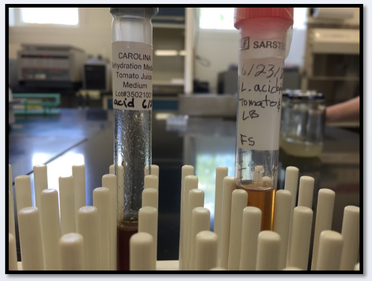


Figure 2

Figure 2 shows two vials which contain the *Lactobacillus acidophilus* used in the experiment.

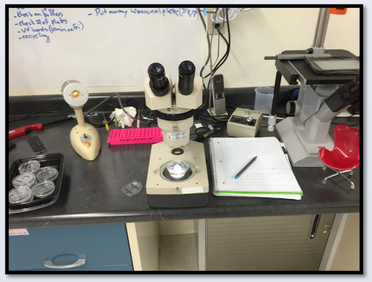


Figure 3

Figure 3 shows the microscope used to observe the *C. elegans* after they were exposed to heat stress.

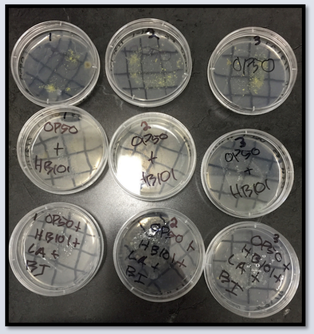
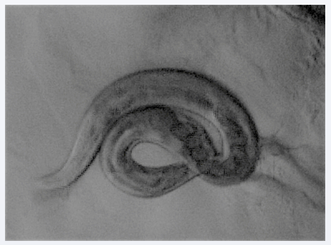
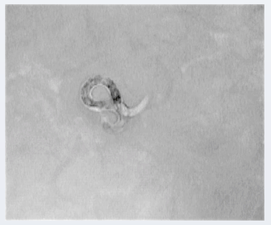


Figure 4

Figure 4 shows plates of *C. elegans* used in the experiment. The labels represent different bacterial mixes. The grids were used to increase accuracy when counting the worms.

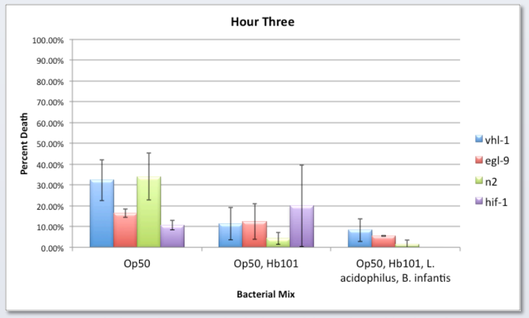
Results





The behavior of the *C. elegans* was observed and recorded immediately after two hours after being incubated at 37˚C. All worms displayed the typical sinusoidal movement prior to heat shock. At two hours, worms in all groups began to display abnormal behaviors such as thrashing, rolling, moving back and forth, and moving only one half of their bodies. The worms also laid eggs at an accelerated rate during the heat exposure. These eggs were observed for several days, and many of them did not hatch. Though not quantified, both the change in movement and the accelerated egg laying indicate a stressed state.

These photos show *C. elegans* strains *hif-1* and *egl-9* that had been exposed to heat stress for two hours.



The above graph shows the percent death observed for each combination of worm strain and bacterial mix after three hours of heat exposure. Each bar is the average of three plates that contained approximately 50 worms each. A trend can be seen on this graph where the groups with more diverse microbiomes have less death. ANOVA tests were performed among the bacterial groups within each strain. *Vhl-1* had a p-value of 0.05125, *egl-9* had a p-value of 0.07949, *N2* had 0.0057, and *hif-1* had 0.46759. These show that there was a significant difference within all groups except for *hif-1*.

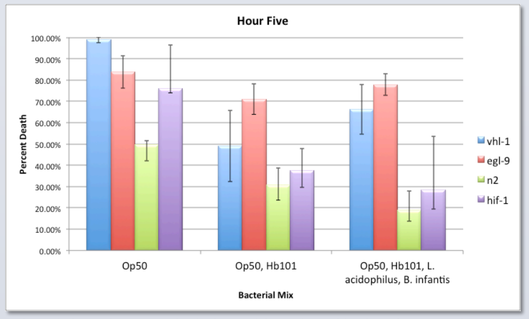


Figure 2 shows the percent death observed for each combination of worm strain and bacterial mix after five hours of heat exposure. Each bar is the average of three plates that contained approximately 50 worms each. The groups with more diverse microbiomes exhibit less death than those with one or two bacterial strains. ANOVA tests were performed between the bacterial groups of each strain. *Vhl-1* had a p-value of 0.01107, *egl-9* had a p-value of 0.18388, *N2* had 0.01477, and *hif-1* had 0.20409. These show that there was a significant difference within the *vhl-1* and *N2* strains.

Discussion

The non-sinusoidal movement and accelerated egg laying recorded at hours of heat stress are recognized signs of increased stress in *C. elegans*. The worms were likely stressed because of the increased heat and the correlated reduction of available oxygen.

At hour three, there was a general trend in which the groups with more diverse microbiomes had less death than those with less diverse microbiomes. The groups grown on only Op50 all had death rates of above 10%, with one as high as approximately 35%, whereas the groups grown on all four bacteria all had death rates below 10%, with one having 0% death. The only worm mutant that did not follow this trend was the *hif-1*, as the worms grown on two *E. coli* strains had a higher death rate than those grown on only Op50.

At hour five, there were higher death rates in all strains than at hour three. The same trend of correlation of lower death rates to higher microbiome diversity was also present. However, at this later time there was less significant difference within the *vhl-1* and *egl-9* groups. The groups grown on Op50 had death rates ranging from approximately 50% to 100%, with three of the four groups above 75%. The groups grown on all four bacteria had death rates ranging from approximately 20% to 75%. This suggests that the diversity of the microbiomes had a direct effect on the tolerance to heat stress. The lowered difference between death rates in the different strains at this time could be attributed to the extremity of the temperature and the time length.

At both hours three and five, *vhl-1* and *egl-9* had the highest death rate. *Egl-9* also did not show a significant difference in death rate between the control bacterial group and the experimental groups at hour five. This is contrary to what was expected, because *vhl-1* and *egl-9* have mutations that should make them more resistant to hypoxia, which is a component of heat stress. Heat tolerance is not wholly dependent on the HIF pathway, so there could be other factors at play. It is also possible that the hypoxia level created by the heat and CO_2 in the incubator was not significant enough for the mutants to be affected differently than wild type. The Caenorhabditis Genetics Center at the University of Minnesota states that the *hif-1* mutant has the most significant difference from wild type in conditions of 1% oxygen.

Further experimentation will be done to determine the roles different mutations play in the results by repeating the experiment with worms that have multiple of the mutations used. To determine whether it is the combination of bacteria or the individual probiotic strains that contribute most to the decreased stress response, the worms will be grown on each strain of bacteria individually. To understand the role of hormones in this experiment, an enzyme-linked immunosorbent assay (ELISA) will also be performed to observe the role of corticosterone in the stress response that is being exhibited.

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