Analyzing Egg Laying Behaviors in *C. elegans* Based on Bacterial Food Sources

by

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Presented in partial fulfillment of the requirements

for

Departmental Honors

in the

Department of Biology

Hood College

April 2016

Abstract

Caenorhabditis elegans is a non-parasitic nematode that feeds on soil bacteria. It has been shown that these organisms prefer to consume strains of *E. coli* bacteria, which are Gram negative, over strains of *Bacillus* bacteria, which are Gram positive. It was hypothesized that the food preference of the nematodes relates to the survival of the fittest concept, so that worms who consume Gram negative bacteria have greater reproductive success than worms who consume Gram positive bacteria. To test this hypothesis, the nematodes were fed two types of Gram negative bacteria and one type of Gram positive bacteria, and reproductive success was measured as the number of eggs the worms could lay after a specific time period. The results showed that the number of eggs laid by the worms fed Gram positive bacteria was significantly less than the number of eggs laid by worms fed Gram negative bacteria. In addition, a gene expression study using real-time PCR to amplify two egg-laying genes and two dauer-formation genes was conducted. Three of the four genes investigated are part of the TGFβ superfamily of genes that are known to be involved in many stages of reproduction and embryogenesis. The results showed that worms fed Gram negative bacteria had a higher expression of the genes tested than the worms fed Gram positive bacteria, concluding that reproductive abilities of nematodes can be affected by a food source. The mechanism by which bacterial food source affects reproduction needs further investigation, but these data indicate there may be a link between the immune system, diet, and reproduction.

Introduction

Caenorhabditis elegans, commonly referred to as nematodes, are free-living organisms found in temperate soil environments (Altun and Hall 2006). These worms are considered model organisms as researchers use them in genetic, developmental, and neurological studies. *Caenorhabditis elegans* are ideal for these kinds of studies because they are microscopic and easily manipulated in the lab. Their small bodies, measuring approximately 1 mm in length when fully grown, are composed of nearly one thousand cells (Corsi et al. 2015). The nematodes have six pairs of chromosomes, five pairs of autosomes and one pair of sex chromosomes. *Caenorhabditis elegans* have two sexes, hermaphrodite (XX) and male (XO) (Hodgkin 2005). The males are rare in nature as they only account for 0.1% of the population. Typically the male genotype is produced by nondisjunction of chromosomes (Altun and Hall 2006). However, the male has a relatively higher frequency in the lab when selective mating occurs.

The worms appear transparent when viewed under the microscope which allows for easy identification of structures. Their overall physical appearance is smooth and non-segmented. The head of the organism is slightly rounded, whereas the tail is very slim and pointed. However, in the male, the tail is shaped more like a fan, as shown in Figure 1 (Emmons 2005).

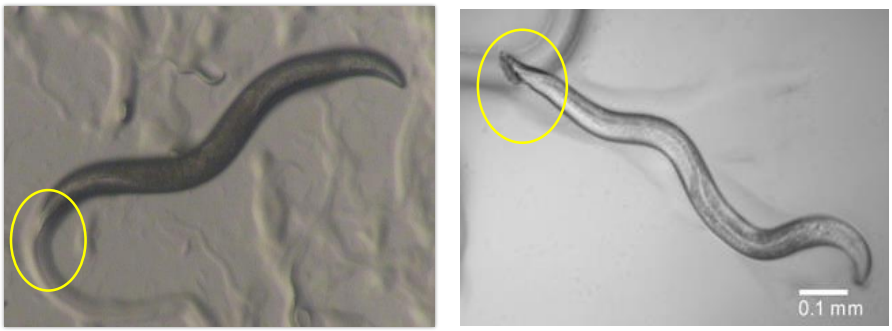

Figure 1: The hermaphrodite (left) with a slim tail and the male (right) with a fanned tail (Emmons 2005).

The body structure of the nematode primarily consists of an outer tube and an inner tube. The outer tube contains the cuticle, muscles, and neurons. The cuticle acts as a barrier separating the internal structures of the worm from its environment. The muscular system helps the worm make undulatory motions that assist in locomotion. In the nervous system, most of the neurons are located in the head and around the pharynx. The inner tube mainly consists of the digestive and reproductive organs (Altun and Hall 2006).

The digestive and reproductive systems are fairly simple systems given the limited cell count of the organism. The digestive system contains the mouth, pharynx, pharyngeal valve, intestine, and anus. *Caenorhabditis elegans* feed on soil bacteria found naturally in the environment, and on *Escherichia coli OP50* in the lab. Food enters through the mouth and is passed through the pharynx by a process called pharyngeal pumping. Food continues to travel from the pharynx to the intestine through the pharyngeal valve. The intestine is a long tube that continues the length of the body until it reaches the anus, located just before the tip of the tail. Defecation occurs when the digested food leaves the body through the anus (Altun and Hall 2006).

The reproductive system in this hermaphrodite consists of two gonad arms and two spermatheca organs which are located on either side of the central uterus, and a vulva (Altun and Hall 2006). The two gonad arms accompany a relatively large amount of space in the organism as they make a ‘u’ shape and a single loop around the intestine. Germ cells begin to develop in the distal gonad arms and continue to mature into oocytes in the proximal gonad arms. The mature oocytes then pass through the spermatheca, a structure that stores sperm, and are fertilized. The fertilized egg, or zygote, is then stored in the central uterus in the middle of the body. The uterus collects zygotes from both

spermatheca organs and is able to hold ten to fifteen eggs at a time. An egg is ready to be laid when it consists of thirty cells. The vulva, attached to the uterus, is the structure that allows the eggs to be expelled from the uterus and deposited into the environment. An adult worm can lay three hundred or more eggs in their life time (Altun and Hall 2006). Figure 2 shows how the digestive and reproductive systems appear under a microscope.

Figure 2: The digestive system of an L4 hermaphrodite (left) showing the pharynx and pharyngeal valve. The line down the middle of the body is the intestine. The right picture shows the reproductive system of an adult hermaphrodite with the darkened patch representing the uterus.

Caenorhabditis elegans, on average, take three days to develop and mature after uterine development. Once the eggs are expelled from the uterus, they continue to develop for about nine hours until they hatch into the first larval (L1) stage. From this stage they continue to progress through the second larval (L2), third larval (L3), and fourth larval (L4) stages until they mature into adult worms, who are capable of reproducing. The main difference between the four larval stages is the cuticle structure and internal organ development of the worm (Altun and Hall 2006). An adult worm can live for three or more weeks if environmental conditions are optimal, in which case the adult worms continue to reproduce. However, if conditions are not sustainable for life, an

L1 or L2 stage worm can enter into an alternative stage called dauer larval stage (Altun and Hall 2006). The dauer worms are longer and skinnier than the L2 worms, making the differentiation between the dauer worms and other larval stage worms possible. The dauer stage allows the worms to continue to survive even given the unfavorable conditions. Two main functions that are different between dauer worms and worms past the L2 stage are their reproductive abilities and metabolism. The dauer worm is unable to reproduce as their reproductive organs are not fully formed and do not continue to form during the dauer stage. Furthermore, worms in the L2 stage and above use aerobic respiration; however, dauer worms continue to use the glyoxylate cycle that is present in the L1 stage worms (Hu 2007). This type of metabolism allows the organism to avoid metabolic stress and live longer.

A worm will enter the dauer stage if the following environmental changes occur: starvation, crowding, or increased temperature (Altun and Hall 2006). When there is an insufficient amount of food present in the environment the worm will enter the dauer stage. Furthermore, if there are too many worms present in the environment or the temperature is too high, the worm will enter the dauer stage. Worms can survive in the dauer stage for several months. If optimal conditions return, the dauer worm can enter into the late L3 stage and continue into adulthood. Since the nematode does not continue to grow and develop during the dauer stage, the adult lifespan is unaffected after the nematode exits dauer (Altun and Hall 2006).

Several experiments have been conducted using *C. elegans* as model organisms because of their small size and ability to survive harsh conditions. In particular, a study by Shtonda and Avery (2006) explored the dietary choices of *C. elegans*. They found that

when given the option between strains of *Escherichia coli* and *Bacillus,* the worms tended to choose the *E. coli* bacteria as their food source, concluding that it was the higher quality food source when compared to *Bacillus* (Shtonda and Avery 2006). In terms of the survival of the fittest concept, it can be hypothesized that the *E. coli* is preferred as the favorable food source because it allows for greater reproductive success. Therefore, this hypothesis would suggest that the nematodes choose *E. coli*, which is Gram negative, over *Bacillus*, which is Gram positive, because consuming *E. coli* allows them to produce more progeny. Furthermore, in the broad spectrum of bacteria, it can be hypothesized that consuming Gram negative bacteria allows the worms to lay more eggs than when they consume Gram positive bacteria.

Testing the reproductive success of *C. elegans* can be accomplished by counting the number of eggs they lay when fed different types of bacteria. *Escherichia coli OP50* acts as the control bacterial food source as this is the type of bacteria the nematodes are typically fed in laboratory experiments. The experimental samples include *E. coli HB101* and *E. coli K12*, which are both Gram negative bacteria, and *Bacillus cereus* and *Bacillus subtilis*, which are Gram positive bacteria. Based on the food preference of the organism, it is hypothesized that dauer worms consuming the *E. coli HB101* or *K12* would lay more eggs than the dauer worms who consume the two strain of *Bacillus.*

There are several addition factors to consider when testing for egg laying behaviors which include; temperature, the amount of food present, the larval stage, and contamination. Two conditions that promote dauer formation are temperature and food availability (Altun and Hall 2006). If the worms are exposed to high temperatures, they will enter dauer, resulting in no egg-laying. Furthermore, if there is an insufficient

amount of food on the plate, the worms may enter dauer sooner than expected. In addition, the larval stage of the worms could influence the results of an egg-laying assay. For example, an L4 worm is closer to reproductive age than an L1 worm. Therefore, it is important to choose worms who are in the same stage for the experiment so they reach the reproduction state at the same time. Finally, it is important to eliminate contaminating bacteria from entering the environment of the worms because the worms would consume the foreign bacteria. If the worms eat foreign bacteria and continue to lay eggs, the results could be skewed as the identity of the bacteria would be unknown and the food source could have an unknown effect on the reproductive abilities of the worms.

After observing egg laying behaviors in the worms, an additional experiment can be used to monitor gene expression. The purpose of analyzing gene expression would be to examine genes involved in egg laying and how they are differentially expressed in the worms based on the food source consumed. Furthermore, analyzing gene expression could potentially uncover a mechanism by which bacterial food sources affect reproduction. If the number of eggs laid by worms consuming different types of bacteria is determined to be significantly different, then it would be expected that genes involved in egg laying are being expressed differently. For example, it is hypothesized that *C. elegans* will lay more eggs in Gram negative bacteria. Therefore, it can hypothesized that worms fed Gram negative bacteria will have a higher expression in genes that are involved in egg laying than worms fed Gram positive bacteria. Furthermore, if an anomaly is discovered in which one gene shows a different trend among the treatments, it could be hypothesized that that gene is involved in the mechanism of how the genes are expressed.

A method to examine gene expression involves real-time PCR which can amplify specific gene sequences of cDNA (derived from mRNA) that are involved in egg laying to determine their level of expression. Among the several genes that control egg laying in *C. elegans*, four were examined in this study; *egl-4*, *egl-21*, *daf-4*, and *daf-7*. The *egl-4* (egg-laying) gene encodes for a kinase that interprets sensory cues that control egg laying, dauer formation, chemosensory behavior, and foraging (Horvitz 2015). The other egg-laying gene, *egl-21*, encodes a carboxypeptidase and controls normal egg laying (Horvitz 2015). The third gene examined, *daf-4* (dauer-formation), encodes for a transmembrane kinase and assists in dauer formation and egg laying (Riddle 2015). Finally, *daf-7* is in a family of growth factors that monitor environmental conditions to regulate pathways that affect dauer formation, egg laying, and feeding behavior (Riddle 2015). Real-time PCR requires the use of a house keeping gene that should show the same level of expression in all treatment groups and serves as a baseline for gene expression. The house keeping gene used in this experiment is *ama-1* which encodes for RNA polymerase II which assists with mRNA transcription (Riddle 2015).

To determine if the food preference of *C. elegans* is related to the survival of the fittest concept, worms are exposed to several types of Gram negative and Gram positive bacteria and allowed to reproduce. It is hypothesized that eating Gram negative bacteria will result in a larger progeny from the worms. Furthermore, when looking at egg-laying gene expression, it is hypothesized that worms who lay more eggs will have a higher gene expression over worms who lay few eggs.

Methods

Egg Laying Behavioral Assay

Several timeline experiments were conducted to determine the amount of time it took a dauer worm to reach the reproductive stage. This was necessary as different laboratory conditions can lead to slight variations in the growth and development of the worms. These experiments consisted of plating dauer worms onto *E. coli OP50* seeded plates and monitoring when the worms first began laying eggs. Following these tests, the different kinds of bacterial sources were made. Two types of Gram negative bacteria and one type of Gram positive bacteria were used and compared to a Gram negative control. The two Gram negative bacteria used were *E. coli HB101* and *E. coli K12*, and the Gram positive bacteria used was *B. cereus*. Originally, *B. subtilis* was an additional Gram positive variable but the bacteria would not grow properly on the nematode growth medium (NGM) plates. The bacteria was grown into a liquid culture by isolating and selecting a single colony and inoculating it in trypticase soy broth. The NGM plates were seeded with three to four drops of the appropriate type of bacteria. Three plates were used for each type of bacteria and five N2 (wild-type) dauer worms were picked onto each seeded plate from a starved stock plate. The worms were then kept at room temperature. After sixty-four, sixty-six, and sixty-eight hours, the number of eggs present on each plate were counted. The egg laying behavioral assay was repeated for a total of three trials. The total number of eggs counted at the sixty-four hour time mark was used for data analysis. A one-way ANOVA test was used to determine significance.

qPCR Gene Expression

The *C. elegans* were harvested and cleaned using 1X phosphate buffered saline after the final egg laying trial. RNA extraction was conducted as per the PureLink RNA Mini Kit protocol (Life Technologies 2012). The concentration of the extracted RNA was determined to be too low for cDNA synthesis; therefore, the RNA was concentrated using sodium acetate and ethanol. The newly concentrated RNA was used to make cDNA as per the instructions in the SuperScript® VILO cDNA Synthesis Kit, following the times and temperatures as indicated by the manufacturer (Life Technologies 2015). The forward and reverse primers of *egl-4*, *egl-21*, *daf-4*, *daf-7*, and *ama-1* were suspended and a working stock was made to a final concentration of 10 mM. Real-time PCR was conducted using SYBR green as the fluorescing agent which binds to double stranded DNA and serves as an indirect reporter of gene amplification. Each treatment with each individual primer set was run in triplicates. The average fold change of gene expression for each treatment and gene was then calculated.

Results

Egg Laying Behavioral Assay

Escherichia coli OP50 fed worms were used to determine the length of time required for a dauer worm to reach the reproductive stage. Monitoring the worms during this experiment revealed that a dauer worm was able to reproduce approximately six-four hours after being introduced to a food source. This time frame was used as a reference to determine when the worms should be capable of laying eggs during the behavioral assay.

Dauer stage *C. elegans* were fed either Gram negative or Gram positive bacteria to determine the number of eggs they could lay. These results were compared to worms

fed a Gram negative control. The number of eggs were counted after sixty-four, sixty-six, and sixty-eight hours of the initial plating of five worms per plate. Figure 3a-d show one plate per bacterial source after approximately 115 hours of the first trail. The *E. coli OP50* control plate had multiple worms in different stages of life, showing multiple generations have occurred. The Gram negative *E. coli HB101* plate also shows multiple generations of worms, but appears to have more worms present when compared to the control plate, suggesting more eggs were laid. Furthermore, the control plate and the *E. coli HB101* plates had no more food present on them indicating the newly hatched and soon to be hatched worms would soon enter dauer. The other Gram negative bacterial plate, *E. coli K12*, also shows multiple generations of worms on the plate, but closer to the same amount present on the control. Moreover, the *E. coli K12* bacteria is still present on the plate which indicates the worms would continue through the larval stages. Finally, the *B. cereus* plate, which has bacteria still present, only shows the original worms plated and only approximately seventy eggs at this time point.

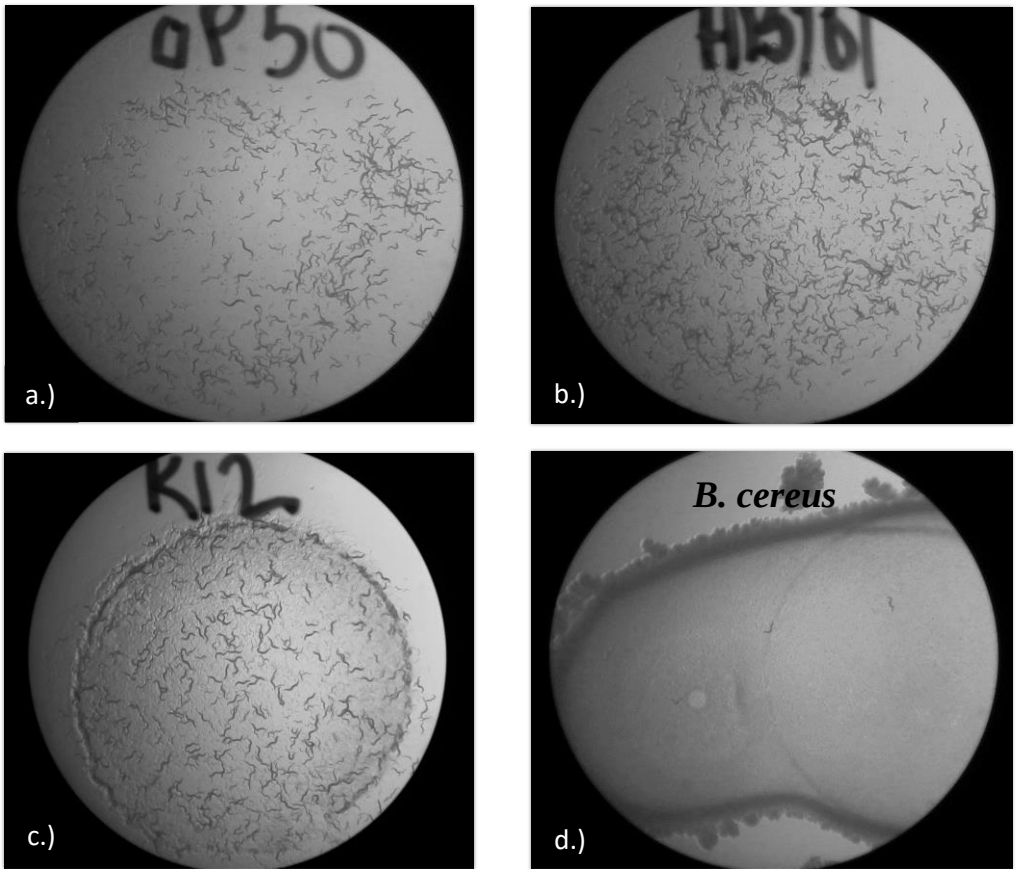

Figure 3: The appearance of each plate after 115 hours for a.) the *E. coli OP50* seeded plate b.) the *E. coli HB101* seeded plate c.) the *E. coli K12* seeded plate and d.) the *B. cereus* seeded plate.

Counting the worms at sixty-four, sixy-six, and sixty-eight hours after the initial plating was completed to determine if the number of eggs laid by worms consuming each type of bacteria increased at a different rate. However, the rate of eggs being laid every two hours was not different between the treatment groups, specifically in the Gram negative fed worms. The number of eggs laid by the Gram positive fed nematodes was zero for all three time points so their rate did not increase. Therefore, only data collected at the sixty-four hour time mark was used for analysis.

To determine the average number of eggs laid per worm when fed each type of bacteria, the number of eggs present on all three plates per trial were added and divided

by the total number of worms present on all three plates. Then those three averages were used to determine the average number of eggs a worm can lay in each type of bacteria, as shown in Figure 4.

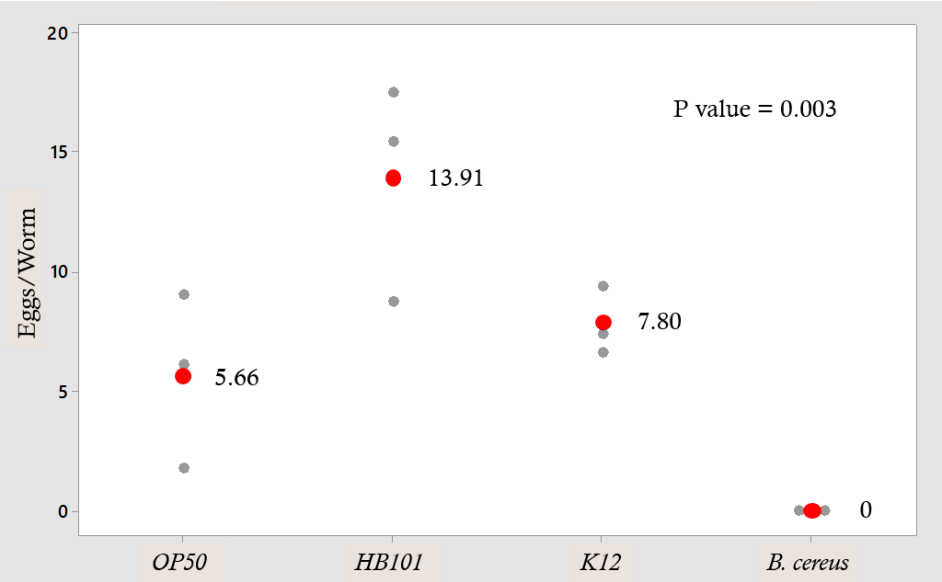

Figure 4: The average number of eggs a worm lays when fed *E.coli OP50*, *HB101*, *K12*, and *B. cereus.* The gray points represent the average for each trial and the red points represent the overall average per treatment. Using an ANOVA test, the p value was 0.003 on a 95% confidence interval indicating the number of eggs laid by worms consuming Gram negative bacteria is significantly higher than by the worms consuming Gram positive bacteria.

Caenorhabditis elegans who consumed *E. coli OP50* laid an average of six eggs after sixty four hours of being exposed to the bacteria. When the nematodes were fed either of the two Gram negative bacteria, *E. coli HB101* or *E. coli K12*, the total number of eggs laid per nematodes was approximately 14 and 8, respectively. The worms fed the *B. cereus* bacteria showed zero eggs per worm after the allotted sixty-four hours. These values indicate that there is a significant difference between the number of eggs a worm lays after consuming *B. cereus* and the number of eggs a worm lays after consuming the Gram negative bacteria ($p = 0.003$). However, when only analyzing the two Gram

negative bacteria treatments against the control bacteria, the number of eggs laid by the worms does not show a significant difference (p = 0.063), as shown in Figure 5.

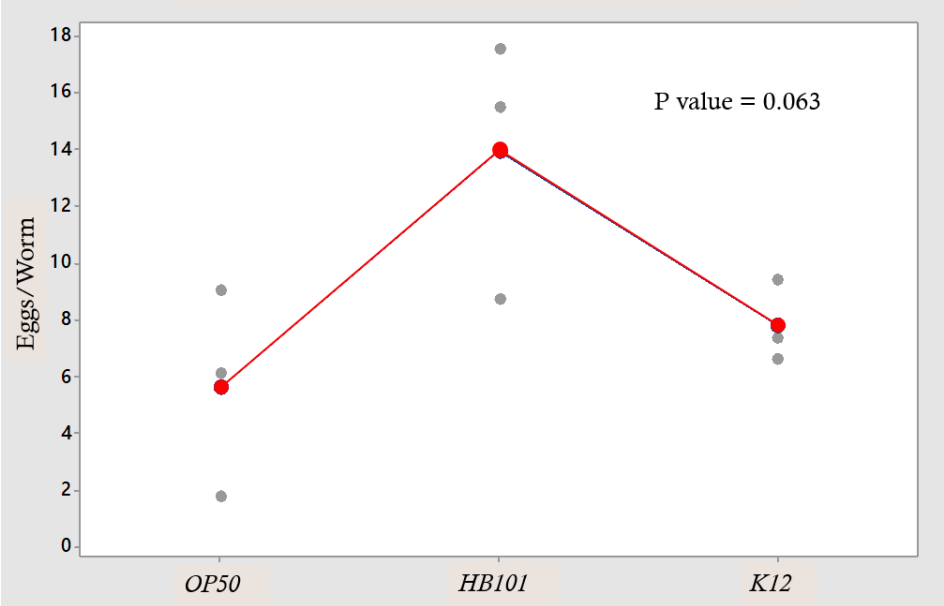

Figure 5: The average number of eggs a worm lays when fed *E.coli OP50*, *HB101*, and *K12*. The gray points represent the average for each trial and the red points represent the overall average per treatment. Using an ANOVA test, the p value was 0.063 on a 95% confidence interval indicating the number of eggs laid by worms consuming Gram negative bacteria is not significant.

Real-time PCR Gene Expression

The four genes of interest, *egl-4*, *egl-21*, *daf-4*, and *daf-7* were amplified and analyzed using real-time PCR. The relative gene expression of all three independent variables were compared to the gene expression shown in the *E. coli OP50* control. Figure 6 shows the fold change in gene expression in the *egl-4* gene. Since worms fed *E. coli OP50* acted as the control, their expression of each gene was 1. The worms fed the Gram negative bacteria, *E. coli HB101* and *E. coli K12*, showed approximately a 3 and 4.5-fold change, respectively, when compared to the control. The *B. cereus* fed worms showed approximately a 1.5-fold change when compared to the control.

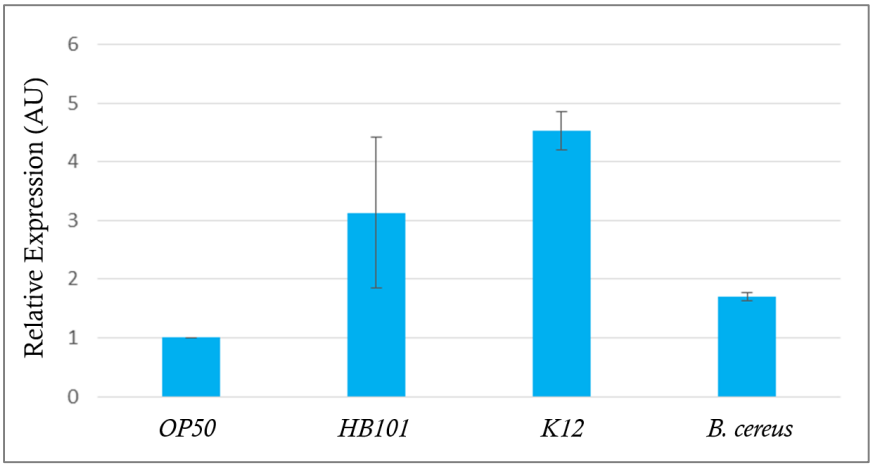

Figure 6: The relative *egl-4* gene expression of worms fed the treatment bacteria (*E.coli HB101*, *K12*, and *B. cereus*) compared to worms fed the *E. coli OP50* control. The error bars represent one standard error for triplicates within one real-time PCR experiment.

The relative gene expression of the *egl-21* gene is shown in Figure 7. Similar to the results from the *egl-4* gene, nematodes fed *E. coli K12* and *E. coli HB101* had the highest expression of *egl-21* with approximately an 11 and 6-fold change, respectively. *B. cereus* fed nematodes continued to show a lower level of expression when compared to the other treatment groups.

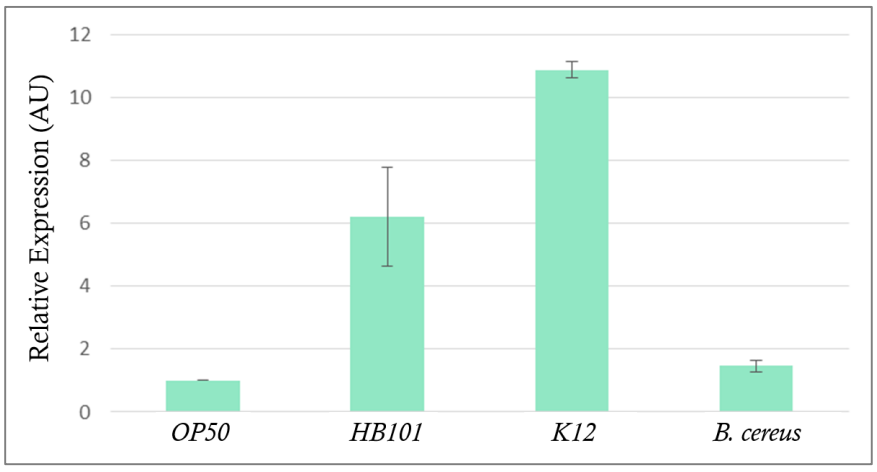

Figure 7: The relative *egl-7* gene expression of worms fed the treatment bacteria (*E.coli HB101*, *K12*, and *B. cereus*) compared to worms fed the *E. coli OP50* control. The error bars represent one standard error for triplicates within one real-time PCR experiment.

The relative gene expression for the *daf-4* gene, shown in Figure 8, is slightly different from the egg-laying genes analyzed previously. Like the *egl-4* and *egl-21* genes, the worms fed *E. coli K12* continued to show over expression, nearly an 11-fold change, when compared to the control. However, worms fed the Gram positive bacteria showed the second highest gene expression with approximately a 5-fold change.

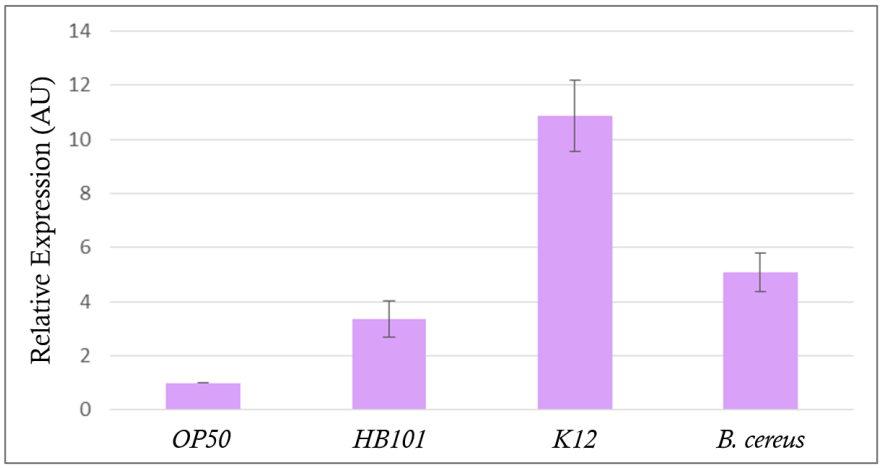

Figure 8: The relative *daf-4* gene expression of worms fed the treatment bacteria (*E.coli HB101*, *K12*, and *B. cereus*) compared to worms fed the *E. coli OP50* control. The error bars represent one standard error for triplicates within one real-time PCR experiment.

Finally, the gene expression for *daf-7* was consistent with the *egl-4* and *egl-7* gene expression. Nematodes fed *E. coli K12* and *E. coli HB101* showed the highest gene expression with 85 and 2-fold change, respectively, as shown in Figure 9. Overall, worms fed *E. coli K12* showed the highest gene expression in all four genes, and in most cases worms fed *E. coli HB101* showed the second highest level of gene expression.

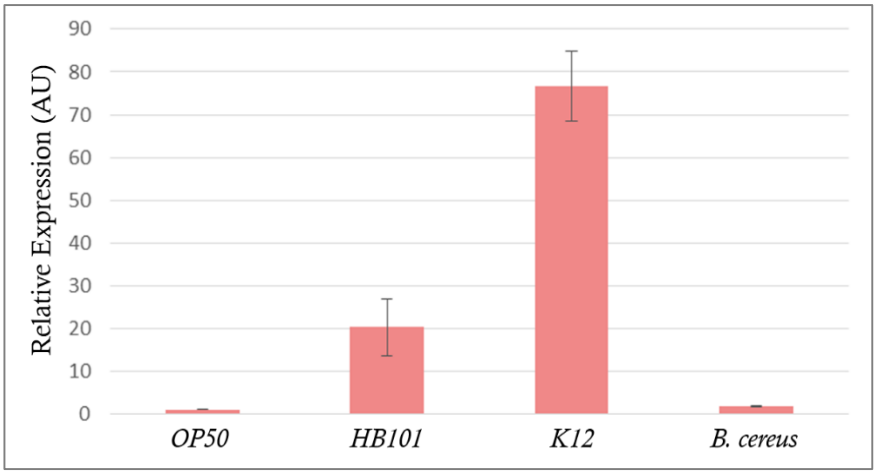

Figure 9: The relative *daf-7* gene expression of worms fed the treatment bacteria (*E.coli HB101, K12,* and *B. cereus*) compared to worms fed the *E. coli OP50* control. The error bars represent one standard error for triplicates within one real-time PCR experiment.

Discussion:

Caenorhabditis elegans were hypothesized to have better reproductive success when fed Gram negative bacteria as opposed to Gram positive bacteria. An egg laying behavioral assay was conducted to determine how many eggs the worms can lay when consuming different types of bacteria. The results showed that while the Gram negative bacterial fed worms laid eggs after sixty-four hours, the *B. cereus* fed worms did not. This difference in egg laying was significant as the p value was 0.003 on a 95% confidence interval. Furthermore, between the Gram negative bacterial variables and the control, it appeared that the worms fed *E. coli HB101* had a higher reproductive success as they laid approximately fourteen eggs per worm and the *E. coli K12* and *E. coli OP50* fed worms laid approximately eight and six eggs per worm, respectively. However, an ANOVA test conducted between only the Gram negative bacteria fed worms resulted in a p value of 0.063 which is not significant at the 95% confidence interval. The p value is too close to the accepted p value of 0.05 indicating that more data has to be collected to

better determine the real significance between the *E. coli HB101* fed worms and the other Gram negative fed worms.

As hypothesized, the nematodes were able to lay more eggs while consuming the Gram negative bacteria than the nematodes consuming the Gram positive bacteria for this experiment. However, only one type of Gram positive bacteria and two types of Gram negative bacteria were used. Therefore, it is difficult to conclude that the *C. elegans* would have the same reproductive abilities in all Gram negative or Gram positive bacteria; however, it can be speculated that this could be the case based on the results from this experiment.

Following the egg-laying assay, a real-time PCR experiment was conducted. The real-time PCR was used to determine if genes involved in egg laying were being affected by the bacterial sources and to possibly understand how they were being affected. At this point, based on the results from the egg laying assay, it was hypothesized that the worms feeding on Gram negative bacteria would show an over expression in egg-laying genes compared to worms that were fed the Gram positive bacteria based on the significant difference in the number of eggs laid. Furthermore, because the worms fed *E. coli HB101* appeared to show slightly higher egg laying numbers than the worms in other treatments, it was hypothesized that they would have slightly higher gene expression compared to the other worms. The gene expression in the *E. coli K12* and the *E. coli OP50* fed worms were expected to be similar as they had similar egg laying results.

Based on the relative fold change from the real-time PCR results, there was a consistent pattern where worms that were fed *E. coli K12* had higher levels of expression in the *egl-4*, *egl-21*, *daf-4*, and *daf-7* genes. Furthermore, worms fed *E. coli HB101*

tended to have the second highest gene expression in all of the genes except in the *daf-4* gene where worms fed *B. cereus* actually had a slightly higher gene expression. This is contradictory to the original hypothesis that worms fed *E. coli HB101* would have the highest gene expression. However, it can be concluded that the timing of the RNA extraction may have skewed the results of the data. At the time of RNA extraction the worms on the *E. coli HB101* plate were starting to starve, as there was no food present. This would indicate that the worms would soon be entering dauer and no longer actively laying eggs. The *E. coli K12* plate still had bacteria present indicating that the worms were still actively reproducing and continuing the normal life cycle. Since the results showed that nematodes fed *E. coli K12* had higher gene expression, it could be possible that this is because they were still actively laying eggs unlike the *E. coli HB101* fed worms. It is likely that the four genes analyzed in this experiment were being suppressed more in the *E. coli HB101* fed worms than the other Gram negative fed worms because of their diminished egg-laying activity. In order to obtain a more accurate level of gene expression in the Gram negative bacteria fed worms, the RNA extraction would have to be completed when the nematodes on both plates were still actively laying eggs.

However, just as the *E. coli K12* plate still had bacteria present, so did the *B. cereus* plate. The worms from the *B. cereus* plate had started laying eggs and some had started hatching at the time of the RNA extraction, but they still did not show the same level of gene expression as the worms from the *E. coli K12* plate. This indicates that even when the *B. cereus* fed worms were actively laying eggs, their egg-laying gene expression was suppressed compared to the worms fed *E. coli K12*. Furthermore, it was concluded that at the time of the RNA extraction, the *E. coli HB101* fed worms were not

actively laying as many eggs as they had been, but these worms still showed a higher gene expression in most genes when compared to the *B. cereus* fed worms who were laying eggs. These results indicate that the egg-laying genes in the *B. cereus* fed worms were being negatively affected based on the food source. The original hypothesis stated that Gram negative feed worms would have higher reproductive success than the Gram positive fed worms, and the results indicate that this is the case for the bacterial food sources studied in this experiment as genes related to egg laying were expressed differently. However, even though it can be concluded that egg laying is affected based on the available food source, it is difficult to determine how the genes are being affected from the results of this experiment because all the treatments showed similar trends among the genes. It could be possible that a gene not tested in this experiment is affecting the four genes tested. Further research is needed to determine what genes are involved in the mechanism by which diet affects reproduction.

Future experiments need to be performed to optimize the real-time PCR experiment as there were a few unexpected issues with the house keeping *ama-1* gene. The house keeping gene showed slight variations in gene expression across all four treatments which would have slightly skewed the data. However, the raw inverse Ct values from the real-time PCR experiment showed that nematodes fed the *E. coli K12* and *E. coli HB101* still had the highest level of expression when compared to nematodes fed *B. cereus*. This suggests that egg-laying is still being genetically affected, and that the only results being affected by the house keeping gene variability is relative fold change. It is still concluded that egg laying is being negatively affected in *B. cereus* fed worms;

however, another house-keeping gene such as *act-1* could be used to optimize the experiment.

Three of the genes analyzed in this experiment, not including *egl-21*, are all part of a transforming growth factor β super family of genes (Horvitz 2015; Riddle 2015). *Caenorhabditis elegans*, humans, and other species express the TGFβ super family of genes which control several aspects of embryogenesis and reproduction. Specifically in the human female reproductive system, the TGFβ super family of genes help with follicle development, especially in the maturation of the oocytes, ovulation, and corpus luteum development. Furthermore, it is expected that polymorphisms in the TGFβ receptors could be causing premature ovarian failure in women (Gordon and Blobe 2008). This experiment looked strictly at reproductive abilities in nematodes, but possible links to immunology could be made. Specifically in the *B. cereus* fed worms, their gene expressions were relatively low, and their egg laying abilities were diminished. Perhaps the *B. cereus* food source is causing suppressions in the immune system of the organism and causing symptoms that are similar to ovarian failure, which could be causing changes in the TGFβ super family of genes. Future experiments could determine if another gene, such as *dbl-1*, which is part of the TGFβ super family of genes that helps to resist infection of pathogens in nematodes, is being suppressed in the worms fed Gram positive bacteria (Ewbank 2006). Furthermore, the future studies could determine if similar polymorphisms that occur in the human female reproductive system are also occurring in the hermaphrodite nematode. The interaction of the food sources, immune system, and the TGFβ super family of genes in *C. elegans* needs further investigation.

Acknowledgements

Thank you to Professors Georgette Jones, Oney Smith, and James Devilbiss, and the Hood College Department of Biology.

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