4-28-2015

Wild-type and Mutant Escherichia coli Fitness After Consumption by Drosophila melanogaster Fruit Flies

Cynthia Lannette Stanley

Wild-type and Mutant *Escherichia coli* Fitness after Consumption by *Drosophila melanogaster* Fruit Flies

by

Cynthia Lannette Stanley

An Honors Thesis
submitted in partial fulfillment of the requirements
for the Honors Diploma
to

The Honors College

of

The University of Alabama in Huntsville

April 28, 2015

Abstract

The current study was designed to examine and analyze how sustainable it can be in the gut of *Drosophila melanogaster*. The goal of the current study was to identify the differences in fitness between two strains of *E. coli*, one with the *tnaA* operon, and one without. The strain of *E. coli* containing the Tryptophanase enzyme (*tnaA*) was predicted to be able to catalyze the cleavage of Tryptophan, whereas the strain of *E. coli* lacking this gene was predicted to lack this ability, decreasing its fitness. It was hypothesized that W3110 *E. coli* would have a greater survival rate in the gut of *D. melanogaster* than *SVS1144 E. coli*, based on the lack of indole production in *W3110*, allowing biofilm production. To test this hypothesis, first instar larvae of *D. melanogaster* were treated with antibiotics, placed onto plates of *E. coli* and were allowed time to ingest the bacteria. After ingestion, larvae were removed from the bacterial plates and placed on regular media. Larvae as well as matured flies were then tested for the presence of *E. coli* in the gut. Fitness was not tested due to the inability to amplify the *E. coli* strains via PCR.

Honors Thesis Advisor: Dr. Luciano M. Matzkin
Assistant Professor, Department of Biological Sciences

Advisor _____ Date _____

Department Chair _____ Date _____

Honors College Dean _____ Date _____

Table of Contents

Abstract

The current study was designed to examine and analyze how sustainable *Escherichia coli* can be in the gut of *Drosophila melanogaster*. The goal of the current study was to identify the differences in fitness between two strains of *E. coli*, one with the *tnaA* operon, and one without. The strain of *E. coli* containing the Tryptophanase enzyme (*tnaA*) was predicted to be able to catalyze the cleavage of Tryptophan, whereas the strain of *E. coli* lacking this gene was predicted to lack this ability, decreasing its fitness. It was hypothesized that W3110 *E. coli* would have a greater survival rate in the gut of *D. melanogaster* than *SVS1144 E. coli*, based on the lack of indole production in *W3110*, allowing biofilm production. To test this hypothesis, first instar larvae of *D. melanogaster* were treated with antibiotics, placed onto plates of *E. coli* and were allowed time to ingest the bacteria. After ingestion, larvae were removed from the bacterial plates and placed on regular media. Larvae as well as matured flies were then tested for the presence of *E. coli* in the gut. Fitness was not tested due to the inability to amplify the *E. coli* strains via PCR.

Introduction

Gut Microbiota

Animals commonly contain a very biologically diverse group of bacteria in their gut, which is a crucial location for nutrient acquisition and immune recognition (Lize et al., 2014, Newell & Douglas, 2014). Also, the bacteria that make up the gut microbiota vary both between and within species. An individual organism's gut bacteria makeup depends greatly on external factors, specifically on the diet of the host organism. There are several mammalian characteristics that have been found to be affected by the composition of an organism's gut bacteria, including social behaviors, mate preferences, bodily scents, and kin recognition (Lize et al., 2014, Newell & Douglas, 2014). Gut microbiota have also been thought to influence food digestion and energy extraction (Lee & Lee, 2014). In fact, the microbiota found in the gut is so influential to activities that occur within the host that they are now viewed as if it were internal organ that affects various important signaling pathways. Conversely, the lack of activity produced by gut microbiota has been found to lead to many diseases such as chronic inflammation and diabetes.

Drosophila melanogaster

Drosophila melanogaster, the fruit fly, is a well studied organism that is used in multiple fields of research, including genetics, evolution, embryonic development, learning and behavior, aging, microbiology, regenerative biology, and regenerative medicine (Jennings, 2011). Many advantages have led to the use of *D. melanogaster* as a research model. They are easy and inexpensive to culture and maintain in a lab setting, they have a fairly short life cycle, and they lay large numbers of fertilized embryos externally, allowing for genetic modifications. As such a versatile model organism, *Drosophila* has allowed for major advances in the understanding of biology and medicine to date.

Drosophila melanogaster undergo a four stage life cycle, during which they exist as egg, larva, pupa, and adult (Jennings, 2011). After fertilization, the embryo develops in an egg for approximately

one day and then hatches as a larva. The larval stage lasts five days, and during this time the larva eats continuously, until on the fifth day it pupates. Over the course of four days, the pupa undergoes metamorphosis into the adult fly stage, until it hatches on the fourth day. As adult flies, *D. melanogaster* can be anaesthetized with carbon dioxide (CO_2) in order to manipulate individual flies without their escape. While anaesthetized, flies are often moved around with a fine tipped paintbrush allowing examination under a stereomicroscope.

Gut microbiota in the *Drosophila* species are widely studied. While varying types of research has been done on this topic, the findings concur that gut microbiota are very influential and fundamental to the survival of these flies. *Drosophila melanogaster* feed on mostly decaying fruits, which contain a variety of living microorganisms (Buchon et al., 2013). Because a small percentage of the microorganisms ingested have the potential to be pathogenic, causing infection, *D. melanogaster* maintain a spectrum of immune responses to protect themselves as well as responses that maintain intestinal tissue homeostasis. This wide range of responses allows the flies to dampen immune responses in the presence of wanted gut microbiota, trigger such responses for unwanted bacteria, and promote tissue regeneration after intestinal damage. Immune responses activated for native and pathogenic microorganisms are similar in type, however, the level of the response and in turn the amount of damage done in the presence of the indigenous microorganisms is much lower.

Research has shown that gut microbiota is not essential to fly development or survival, although the absence of the gut bacteria does alter several host attributes (Buchon et al., 2013). For example, removal of gut bacteria in larvae has been shown to negatively influence development (Wong et al., 2013, Buchon et al., 2013). This disruption has been tied to insufficient insulin signaling in the absence of gut microbiota, and leads to a delay in larval development. Also, flies lacking a gut microbiota demonstrate a lower mitotic index, suggesting that the presence of microbiota directly influences intestinal turnover and helps maintain basal tissue homeostasis. Finally, the lack of gut microbiota has

been found to increase susceptibility to infection, suggesting the need for microbiota in immune defense.

Two known microbicidal effects are the generation of antimicrobial peptides (AMPs) and the production of reactive oxygen species (ROS), which have both been found to be significant modes of resistance against pathogen infection (Zhu et al., 2014). Triggered by the immune deficiency pathway, the generation of AMPs induces gut immunity defense mechanisms. Similarly, the production of ROS leads to bacterial DNA, RNA, and protein damage while also promoting oxidative degradation of lipids in the microbes' cell membranes. Nonetheless, an excess of ROS could potentially harm the host, so it is important that a balance between the production and removal of ROS be maintained by the host.

Although there are five types of gut bacteria, all of which fall under the *Acetobacter and Lactobacillus* species, which are commonly found among the *Drosophila* species, research suggests that there is not one core microbiota for the species as a whole (Wong et al., 2013). Instead, it appears that each species of *Drosophila*, feeding on a variety of different food sources, maintains a different array of gut bacteria.

In a study examining the effects of microbiota on kin recognition in *D. melanogaster* specifically, researchers found that individual flies feeding on the same food source shared similarities in regards to gut bacteria (Lize et al., 2014). It was posited that flies that were reared on different food types would use olfaction to decide on mating partners, ultimately choosing mates with a smell similar to their own. To test this, a follow up study was performed, and antibiotics were used to alter the gut bacteria of some flies. Findings supported this prediction and demonstrated that gut microbiota influenced mating choices. Flies were more likely to mate with flies that had similar gut bacteria to their own, however, the flies also demonstrated kin recognition by having shorter copulation duration with mates who were related. Conclusions based on this research suggest that either the gut bacteria play a direct role in

choosing mates, or that the gut composition alter the physiology of the flies in a more general manner, possibly by changing their overall scent.

Escherichia coli

Different Strains of *Escherichia coli* Used

SVS1144 and W3110. The first two strains of *E. coli* used differed based on their production of Tryptophanase; one wild-type strain that produces Tryptophanase, *SVS1144*, and one mutant strain that does not produce Tryptophanase, *W3110*. Tryptophanase is an enzyme that catalyzes the cleavage of Tryptophan, an amino acid that is necessary for normal growth and development, into indole, pyruvic acid, and ammonia. It is predicted that the strain of *E. coli* that produces Tryptophanase would have greater fitness in environments with higher tryptophan levels due to the ability to catabolize this amino acid. To differentiate between the two strains, different operons within the bacteria's DNA were amplified via PCR. For the mutant strain, PCR targeted the Lacz operon and for the wild-type strain, PCR targeted the aTna-lacz operon.

PKQv4 plasmid. Alternate strains of *E. coli* (*MG1655ΔpNK and +A75lins)* were also used, in an attempt to differentiate between wild-type and mutant strain fitness. However, when unable to differentiate between them due to primer failure, a *PKQv4* plasmid was inserted into a strain of *E. coli* as a last resort. The *PKQv4* plasmid was used due to its Ampicillin resistance (Aggarwal & Lee, 2011). Unfortunately, I was not able to PCR amplify the plasmid. Due to this, the fitness of *E. coli* in the gut of *D. melanogaster* was not measured.

Tryptophanase (*tna*) Operon

The Tryptophanase operon is essential to bacteria because it is an enzyme that allows them to take advantage of tryptophan as a source of carbon, nitrogen, and energy (Konan & Yanofsky, 1997). This enzyme catabolizes L-tryptophan into indole, pyruvate, and ammonia. This reaction is also reversible, so bacteria can synthesize tryptophan when indole, pyruvate, and ammonia are all present.

The *tna* operon of *E. coli* contains two structural genes, *TnaA* and *TnaB*. In *E. coli*, the *TnaA* gene encodes for Tryptophanase while *TnaB* encodes for a low-affinity tryptophan permease. Catabolite repression and tryptophan-induced transcription anti-termination regulate the transcription of the *tna* operon (Konan & Yanofsky, 1997). Catabolite repression is responsible for regulating transcription, while excess tryptophan induces anti-termination (Gong & Yanofsky, 2001). *TnaC* is a gene that codes for the leader region consisting of 319-nucleotides. This leader region encodes a 24-residue peptide, *TnaC*. Without the translation of this gene, tryptophan induction does not occur. This has been attributed to the presence of Rho-dependent transcription sites located in the leader region. Altering any of these Rho-dependent sites alters the induction of TnaA & TnaB at some level; however while working models have been proposed, the exact mechanisms of tryptophan induction have yet to be determined. Due to the complexity of the mechanisms responsible for tryptophan induction, the current study was designed to assess the fitness of these mechanisms in the *E. coli* inside the gut of *D. melanogaster*.

Experiments Performed to Date

***D. melanogaster* Growth**

Drosophila melanogaster obtained in Huntsville, Alabama were used for the current research. The id number of the strain used is 043014.01. The *D. melanogaster* were placed onto antibiotic banana media which contained 125 μg/mL of Ampicillin and 12.5 μg/mL of Tetracycline. Before mixing the antibiotics into the media, the Ampicillin and Tetracycline were dissolved in 50% EtOH. Flies were placed onto antibiotic media for three generations, and then placed on regular media for an additional three generations before being placed onto *E. coli* plates.

***E. coli* culturing**

Culturing the *E. coli* from a single colony was a three day process. All materials used were sterilized prior to use. Day 1 triggered growth in LB broth, day 2 consisted of creating a serial dilution

and placing dilution onto LB plates, and on day 3, the plates of *E. coli* were ready for use. On day 1, in the proximity of a flame, an extractor tool was used to grab a single colony of each strain and each colony was placed into a tube containing 5 mL of LB broth. For two strains, two separate tubes were prepared. The tubes were then placed into an incubator overnight at a temperature of 37 Degrees °C. On day 2, 3 small tubes were used to create a serial dilution. 900 µL of LB broth was placed into each small tube. The tubes prepared on day 1 were taken out of the incubator and checked for growth. If the liquid in the tubes was clear, resembling regular LB, this signified that growth did not occur. If the liquid was cloudy, then growth was successful. When growth was present, in the proximity of the flame, 100 µL of the growth-containing LB was pipetted from the large tube into a smaller tube and gently mixed by pipetting up and down 3-4 times. Then 100 µL of the liquid from the 1st small tube was pipetted into the 2nd small tube and gently mixed. Finally, 100 µL of the liquid from the 2nd tube was pipetted into the 3rd small tube and gently mixed to create a concentration of 10^{-3}. This was performed for both strains. 300 µL was then pipetted from each serial dilution and placed onto the side of individual petri dishes which contained regular LB media. A glass swizzle tool was used to push the liquid up and down on the layer of LB, while slowly spinning the petri dish to evenly spread the bacteria-containing liquid. Once the desired amount of plates was prepared, they were placed in an incubator at 37 Degrees Celsius upside down overnight. The plates were ready for use the following morning.

Feeding the *D. melanogaster*

For *D. melanogaster*, larvae enter their first instar stage about 48 hours after they are laid as eggs. In order to obtain first instar larvae, individual vials of flies were transferred one day to begin the count. Then, 48 hours later they were transferred again, using the newly emptied vial as a source of larvae. A small tool with a fine needle at the end was sterilized with 70% EtOH and used to remove larvae one at a time from the media and they were placed onto the *E. coli* plates. The larvae were left on the *E. coli* plates for 6-96 hours, depending on protocol. After the allotted time, the larvae were

removed either with the same sterilized tool or with slightly larger sterilized tweezers and placed into a 0.6 μL tube half-filled with Distilled water and vortexed for 10-15 seconds to rinse off the *E.coli*. Then, the larvae were re-placed onto regular media. After 48 hours, 1-2 larvae (per strain of *E. coli* used) were removed from the media and frozen for future PCR testing. This removal and freezing sample procedure was repeated until the larvae reached adulthood, then 1-2 adult flies (per strain of *E. coli*) was removed from the media and frozen.

Protocol Variations

Length of Time on E. coli Plates. When first beginning the current research, the larvae were placed onto the *E. coli* petri plates for 48-96 hours each time. The survival rate of the larvae was not as high as desired, though. In *Table 1* and *Table 2*, the survival rates from placement onto *E. coli* plates to re-placement onto regular media are recorded. In order to increase the survival rates of the larvae, the length of time left on the plates was shortened by increments of 12 hours, until a final length of time of 6 hours was reached. In Table 3 and Table 4, the survival rates from placement onto *E. coli* plates to re-placement onto regular media are recorded for 24 hours and the final length of time, 6 hours.

Introduction of a control. When the current research first began, flies were placed on either a mutant *E. coli* strain or a wild-type *E. coli* strain. As the studies progressed, instead of comparing a mutant to a wild-type strain, fly survival rates were compared between being placed onto an *E. coli* covered LB plate and a plain LB plate as a control, while analyzing *E. coli* survival as well. Initial studies were targeted to identify the differences between mutant and wild-type *E. coli* survival; however with the occurrence of continuous obstacles, mere survival in the gut of *E. coli* became first priority.

Polymerase Chain Reaction

PCR Protocol. For the PCR reaction, a master mix was created, which contained 10x buffer, dNTPs, $MgCl_2$, Forward and Reverse primers, Taq, dH_2O. All ingredients were mixed in order. Once all was added, the master mix was vortexed for 15-30 seconds. Then, 24μl of the master mix was added to

strip tubes and 1 μl of DNA was added when appropriate (the Negative control did not get DNA). For each PCR ran, there was a positive and a negative control. The Positive control received 1μl of the *E.coli* DNA and the Negative control received no DNA. Then the strip tubes were placed in the PCR cycler. The protocol was as follows: Step 1: 95 °C, 2 min, Step 2: 95 °C, 30 sec, Step 3: 50 °C, 30 sec, Step 4: 72 °C, 45 sec, Step 5: GOTO Step 2 (34 times), Step 6: 72 °C, 4 min, Step 7: 4 °C, Infinite (Cycle complete). In order to obtain the *E. coli* DNA for the positive control, a pipette tip was used to grab 1-3 colonies and they were placed in 25ul of distilled water, then vortexed for 30-40 seconds. Once amplified, the samples were run in a 1% agarose gel using a mid-ladder for 30-60 minutes at 130 volts.

Tables and Figures

Hours on *E. coli*	**Mutant (*TnaA2)***
96	7 out of 15
48	5 out of 8
48	7 out of 15

Hours on *E. coli*	**Wild-type (*TnaA)***
96	5 out of 15
48	4 out of 10
48	8 out of 15

Table 1 and Table 2. Survival Rate of *D. melanogaster* after being placed on *E. coli* plates for 48-96 hours. As you can see from the tables above, survival rates were as low as 33% when left on the plates for 2-4 days. In order for the fitness of the *E. coli* to be measured, the *D. melanogaster* needed to survive for several days after being placed and removed from the *E. coli* plates. Due to these poor

survival rates, the length of time that the larvae were left on the *E. coli* plates was drastically reduced. See *Tables 3 and 4*.

Hours on *E. coli*	**Wild-type**	**Strain**
24	29 out of 40	(MG1655)
6	9 out of 20	(PKQv4 plasmid)

Hours on *E. coli*	**Mutant**	**Strain**
24	32 out of 44	(+A75lins)
6	13 out of 20	(LB with Amp.)

Table 3 and Table 4. Survival Rate of *D. melanogaster* after being placed on *E. coli* plates for 6-24 hours. Here are the survival rates for two different sets of strains used. The second set (on which the *D. melanogaster* were placed for 6 hours) consisted of an *E. coli* strain and a control plate. As you can see from *Table 3 and 4*, survival rates were still not as good as desired; however, they did reach as high as 72%. Only one round was conducted with each set of strains due to the inability to obtain positive PCR results for the strains.

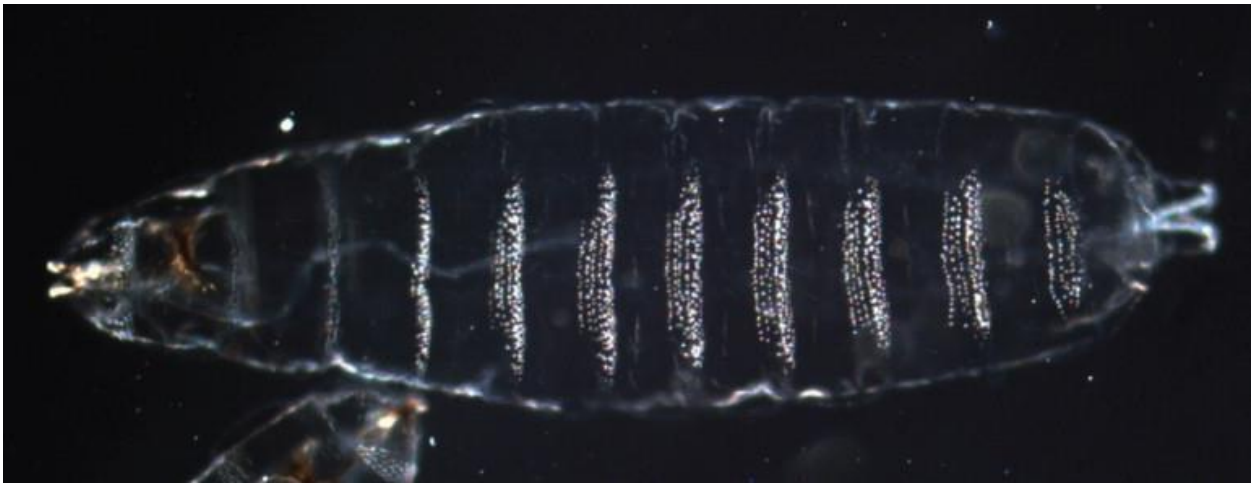

Figure 1. This is a dark field microscopy image showing the cuticle (exoskeleton) of a young (1st instar) larva. The head is to the left of the image (Jennings, 2011). Here you can also see the two points from which tweezers will be used to pull the larvae apart in future research in order to separate the gut from the rest of the larvae parts (the two white "v" shaped points at either end of the larvae).

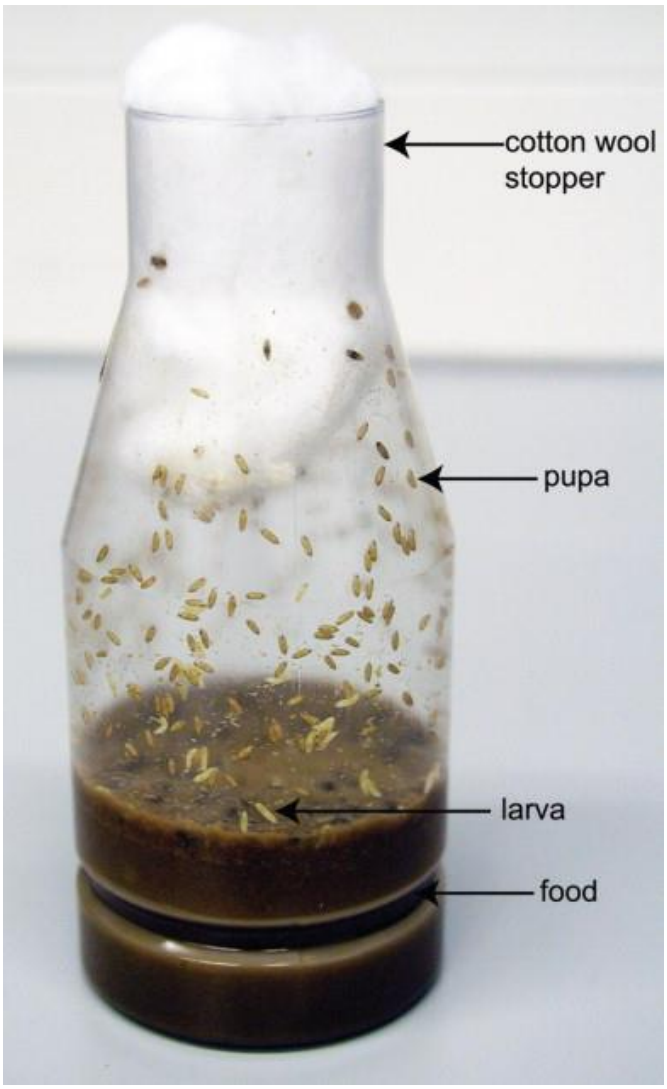

Figure 2. Here is a bottle containing Drosophila larvae and pupae (Jennings, 2011). The current studies used vials that were designed and plugged just like this one. In order to obtain 1st instar larvae, a small amount of food was scraped from the bottom of the vial, and placed under a stereomicroscope (see *Figure 3*). In order to obtain adult flies, the cotton plug remained on the vial, and a small needle was slid in-between the plug and the glass, allowing CO_2 to enter the vial, temporarily sedating the flies.

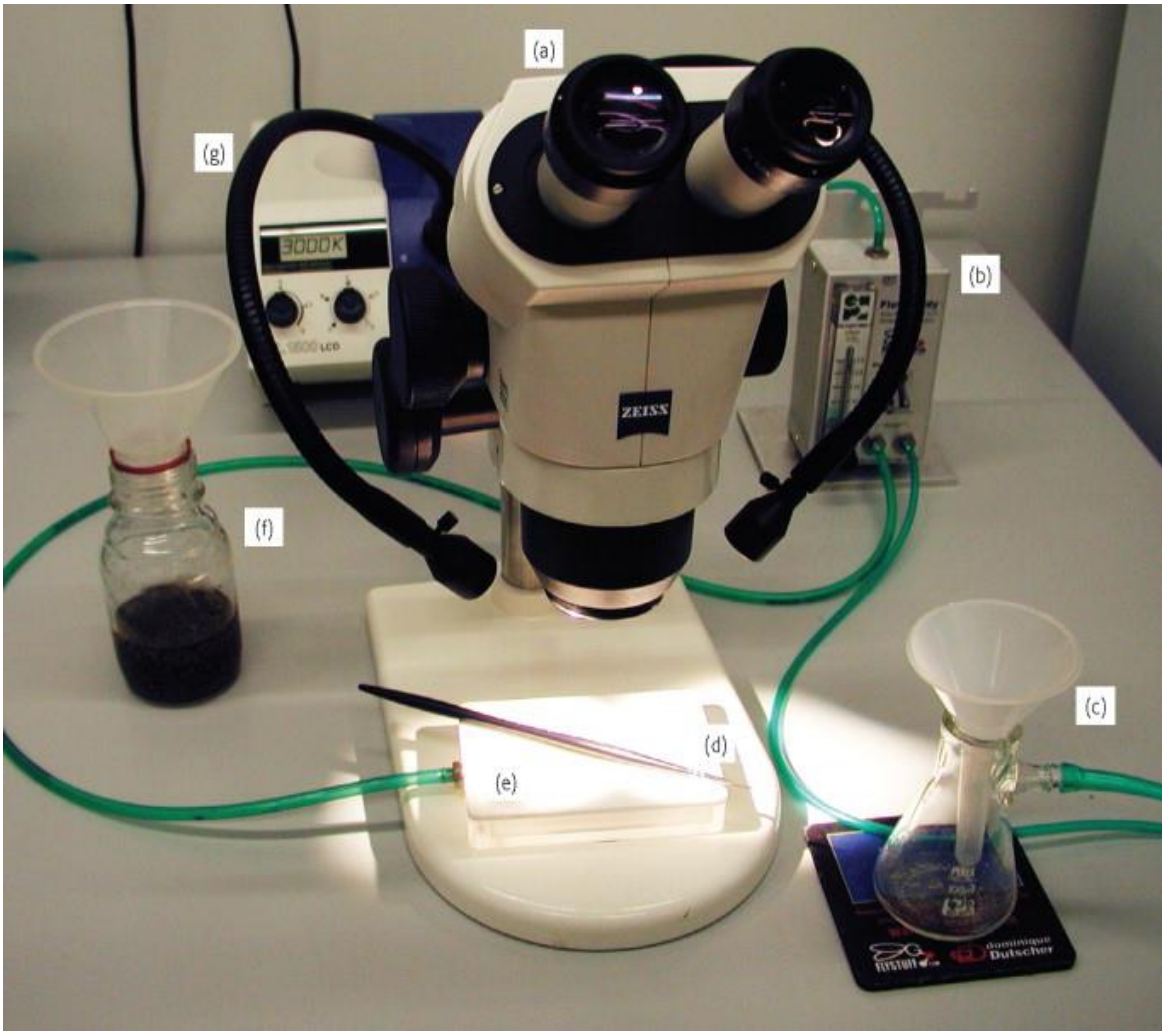

Figure 3. A typical bench set up for work with *Drosophila*: (a) stereomicroscope, (b) CO_2 regulator, (c) chamber for anesthesia, (d) paint brush, (e) porous pad connected to CO_2, (f) a fly morgue: a bottle containing methanol, and (g) a cold light source (Jennings, 2011). Note that (b) and (c) from the above picture were not used in the current experiments; instead, a CO_2 tank and CO_2 gun with a needle attached were used (as discussed under *Figure 2*).

Figure 4. Here is an image of a petri dish covered in *E. coli* (image obtained from iGEM 2010 website).

The *D. melanogaster* used in the current experiments were raised on Ampicillin antibiotic food, allowing them to survive on Ampicillin-resistant *E. coli*, as seen in the image.

Obstacles

Different *Escherichia coli* Strains and Primers

E. coli strains. The current research began using the *W3110* and *SVS1144 E. coli* strains; however, due to the inability to differentiate between these two, two new strains were used. The second set of *E. coli* strains tested were *MG1655ΔpNK* and *+A75lins*; and finally, an Ampicillin resistant *E. coli* strain with an inserted PKQv4 plasmid was used. Unfortunately, none of these strains could be identified individually via PCR, so their fitness inside the gut of *D. melanogaster* was not able to be tested.

Primers. Multiple primers were used in the current research. The first set of primers was unable to successfully distinguish between the *W3110* and *SVS1144* because the two strains were too similar. For this reason, the primers were amplifying genes from both strains, making it impossible to compare individual fitness. So, a second set of primers was ordered to specifically target the *ArgF* gene in the mutant strain. The *ArgF* gene is deleted from the wild-type genome. Unfortunately, the primers created to amplify this gene were unsuccessful. The final set of primers used was created to target a plasmid that was placed into an Ampicillin resistant *E. coli* strain. The primers were made to target the inserted plasmid, PKQv4. However, using the PCR protocol discussed previously, the primers failed to amplify the plasmid DNA.

Discussion

Importance of the Research

The physiological responses exhibited by *D. melanogaster* are quite similar to those of humans, specifically in regards to immune responses. Due to the similarity in genes and immune responses, understanding the mechanics behind that of *D. melanogaster* will better the understanding of human mechanics. The potential findings that can be obtained via studying *D. melanogaster* are limitless,

therefore the continued research of the fruit fly and its internal mechanics are crucial to furthering the field of biology and the surrounding fields of science.

Understanding the mechanisms responsible for tryptophan induction will further the understanding of bacterial defense, while inside the gut of its host. By better understanding these defenses, the discovery of future human defenses against bacterial infection may be accomplished. Currently, there is much known about *E. coli*, however, there is a plethora of mechanisms still yet to be understood.

Why *Drosophila melanogaster* and *Escherichia coli*?

Over the past 100 years, many developments in the field of biology have been contributed to the research performed with *D. melanogaster* (Jennings, 2011). While *D. melanogaster* and humans look very different in their appearance, through comparisons of fully sequenced *melanogaster* and human genomes, it has been found that approximately 75 % of known human disease genes can be matched with genes located in the genome of Drosophila. Due to these resemblances, more genetic tools have been developed for *D. melanogaster* research than from any other multicellular organism.

Drosophila melanogaster is a model organism for research for several reasons. Fruit flies are easy to obtain, maintain, and study (Jennings, 2011). They have many features similar to that of humans, such as their intricate immune responses used for gut homeostasis, their genetic composition, and their gut microbiota composition is unique to their species, like with humans. Also, many of the genes found to be crucial for fly development have also been found to be important for all animal development, including human development. There are very few restrictions on the use of *D. melanogaster* in the laboratory, making the study of various diseases possible with minimal ethical and safety concerns. The ability to reduce expression of individual genes in *Drosophila* has allowed for over 88 % of *melanogaster* genes to undergo knockdown, leading to over 22,000 different transgenic fly lines

(Jennings, 2011). These genetically altered strains have led to a powerful strategy for studying the roles of individual genes in various biological processes.

Escherichia coli is a widely studied bacteria. Similar to *D. melanogaster*, *E. coli* are fairly easy to culture and maintain in the lab. *E. coli* has not been studied in the gut of *D. melanogaster* to date, so it is unknown whether the bacteria could survive against the immune defenses of *D. melanogaster*. Nonetheless, the Gram-negative nature of *E. coli* may provide protection for the *E. coli* inside the fly gut that Gram-positive bacteria would lack (Buchon et al., 2013). In Gram-negative bacteria, the cell wall protects the Peptidoglycan layer inside the periplasmic space, preventing the layer from being detected. Since one of the initial recognition mechanisms used by *D. melanogaster* identifies Peptidoglycan, *E. coli* may go unnoticed long enough to colonize inside the gut, allowing the bacteria to survive.

Plans for Future Research

After confirmation of the *E. coli's* presence in the gut, the *D. melanogaster* larvae/flies will be placed on different types of media, each containing different levels of tryptophan. This will allow the fitness of the *E. coli* inside the gut to be measured and further understood, among the presence of tryptophan. As mentioned earlier, the Tryptophanase operon is essential for *E. coli* because it allows the bacteria to use tryptophan. By examining the fitness of wild-type and mutant *E. coli* in the presence of the *D. melanogaster* gut, the mechanisms of the Tryptophanase operon can be better understood. Also, additional mechanisms related to the cleavage of tryptophan may be identified.

Initial research, to be continued, is interested in whether *E. coli* can survive in the gut of *D. melanogaster*. For this reason, whole larvae and whole flies were and will be used in order to obtain DNA to be used in PCR reactions. Future research will dissect the larvae and flies in order to obtain the digestion tract alone, and run PCR on these specific tissues. Also, future research plans include creating an *E. coli* mutant by eliminating the *TnaA* gene from the bacteria's genome. When eliminating the *TnaA*

gene, the gene will be replaced with a type of fluorescent protein, or something similar that can easily be traced.

References

Aggarwal, K. & Lee, K. (2011). Overexpression of cloned RhsA sequences perturbs the cellular translational machinery in *Escherichia coli*. *American Society for Microbiology, 193*, pp. 4869-4880. doi: 10.1128/JB.05061-11

Broderick, N. A., Buchon, N., & Lemaitre, B. (2014). Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. *Mbio, 5*, e01117-e01114. doi:10.1128/mBio.01117-14

Buchon, N., Broderick, N. A., & Lemaitre, B. (2013). Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nature Reviews Microbiology, 11*, 615-626. doi:10.1038/nrmicro3074

Coelho, A., Fraichard, S., Le Goff, G., Faure, P., Artur, Y., Ferveur, J., & Heydel, J. (2015). Cytochrome P450-Dependent Metabolism of Caffeine in *Drosophila melanogaster*. *Plos ONE, 10*, 1-15. doi:10.1371/journal.pone.0117328

Gong, F., & Yanofsky, C. (n.d). Reproducing tna operon regulation in vitro in an S-30 system - Tryptophan induction inhibits cleavage of TnaC peptidyl-RNA?. *Journal of Biological Chemistry, 276*, 1974-1983

Jennings, B. H. (2011). Drosophila—a versatile model in biology & medicine. *Materials Today, 14*, 190-195.

Konan, K. V., & Yanofsky, C. (1997). Regulation of the *Escherichia coli* tna operon: nascent leader peptide control at the tnaC stop codon. *Journal of Bacteriology, 179*, 1774-1779

Lee, K. A. & Lee, W. J. (2014). Drosophila as a model for intestinal dysbiosis and chronic inflammatory diseases. *Developmental and Comparative Immunology, 42*, 102-110. doi: 10.1016/j.dci.2013.05.005

Lizé, A., McKay, R., & Lewis, Z. (2014). Kin recognition in Drosophila: the importance of ecology and gut

microbiota. *ISME Journal: Multidisciplinary Journal of Microbial Ecology, 8*, 469-477. doi:10.1038/ismej.2013.157

Newell, P. D. & Douglas, A. E. (2014). Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in *Drosophila melanogaster*. *Applied and Environmental Microbiology, 80*, 788-796.

Takayuki, K., Aki, H., & Shoichiro, K. (2013). Host-microbe interactions in the gut of *Drosophila melanogaster. Frontiers in Physiology, 41*. doi:10.3389/fphys.2013.00375

Teixeira, L. (2012). Whole-genome expression profile analysis of *Drosophila melanogaster* immune responses. *Briefings in Functional Genomics, 11*, 375-386

Wong, A. C., Chaston, J. M., & Douglas, A. E. (2013). The inconstant gut microbiota of Drosophila species revealed by 16S rRNA gene analysis. *ISME Journal: Multidisciplinary Journal of Microbial Ecology, 7*, 1922-1932. doi:10.1038/ismej.2013.86

Woodward, S. (2015). *E. coli*: a brief overview. *British Journal of Nursing, 24*, 158-160. doi:10.12968/bjon.2015.24.3.158