ABSTRACT

Staphylococcus aureus Infection Modulates the Egg-laying Behavior of the Nematode *Caenorhabditis elegans*

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Caenorhabditis elegans provides a convenient tool to study host-pathogen interactions. In my research, I exposed worms with *Staphylococcus aureus*, the commensal bacteria that can utilize virulence factors to cause diseases in human. *S. aureus* infection caused egg-laying defect in *C. elegans*, which was characterized as the incapability to produce fertilized eggs and the difficulty in expelling eggs. Biofilm formed by *S. aureus* was confirmed to play a role in inducing the defect. Besides, part of the research was dedicated to isolate small molecules secreted by *S. aureus* and grow *C. elegans* in its mixture. Severe egg-laying defect was observed in worms under this situation, indicating the potential roles small molecules of *S. aureus* might be playing in *C. elegans* egg-laying. The defect could be alleviated by either placing worms back to a normal condition not long after the infection took place, or by providing worms with the neurotransmitter serotonin.

Staphylococcus aureus Infection Modulates the Egg-laying Behavior of the Nematode *Caenorhabditis elegans*

by

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A Thesis

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER ONE

Introduction

Caenorhabditis elegans

Caenorhabditis elegans is free-living nematode, about 1 mm in length, which lives in soil and feeds primarily on bacteria (Brenner, 1974). Most of the *C. elegans* individuals are hermaphrodites, with a minority of worms being true males (Hodgkin *et al.*, 1978). As showing in figure 1 (Grant and Wilkinson, 2003), the major anatomical structures of a hermaphrodite *C. elegans* include a pharynx, intestine, gonad, vulva, and anus.

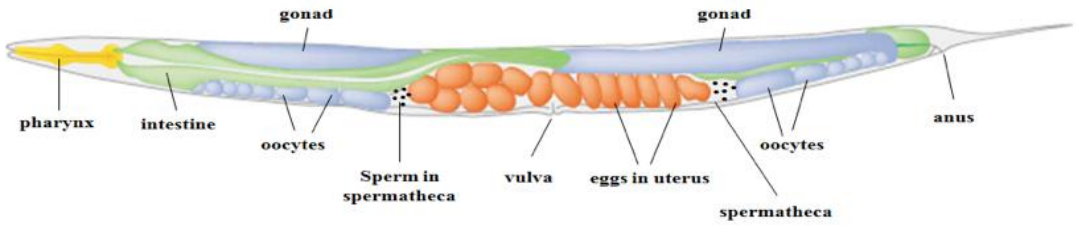

Figure 1. *C. elegans* hermaphrodite anatomical structure (Grant and Wilkinson, 2003)

The hermaphrodite *C. elegans* animals reproduce by self-fertilizing, which allows for homozygous worms to generate genetically identical progeny (Han *et al.*, 2010). Under environmental conditions that are in favor for reproduction, hatched larvae develop through four stages or molts, designated as L1 to L4 (Klass and Hirsh, 1976). When conditions are harsh, for example, if food is scarce or temperature is extreme, *C.*

elegans can enter an alternative third larval stage that is called the dauer state (Nicole and Adam, 2008). At this stage, the old cuticle is replaced by a special, relatively impermeable cuticle unique to dauer larvae (Cassada and Russel, 1975). As figure 2 (Christ and Dietmar, 2010) shows, the life cycle of *C. elegans* comprises of the embryonic stage, 4 distinct larval stages (L1-L4), and the adulthood. A molt marks the end of each larval stage when a new, stage-specific cuticle is synthesized as the old one is shed.

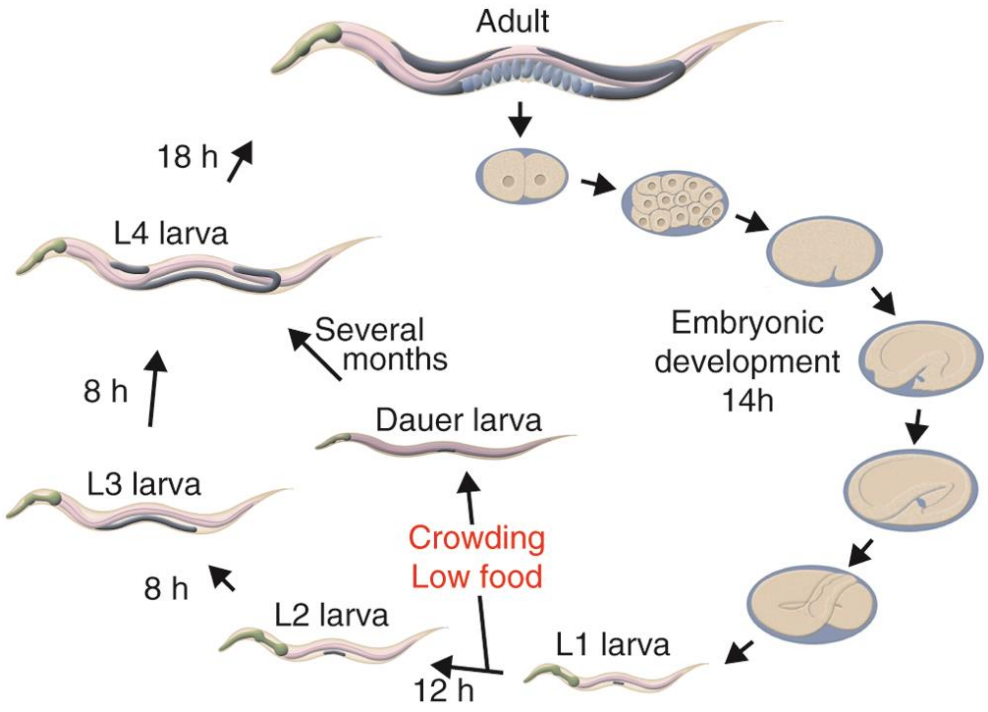

Figure 2. *C. elegans* life cycle (Chris and Dietmar, 2010)

In laboratories, *C. elegans* uses *Escherichia coli* strain OP50 as food source (Brenner, 1974). *E. coli* OP50 lacks uracil, which is used to prevent overgrowth of the bacterial lawn (Brenner, 1974), and to enable a better observation of worms.

C. elegans has been frequently used in scientific researches because of its many intriguing features. It is easy to culture and observe. It has short life cycle and reproduces fast. Most importantly, depending on the particular bioinformatics method used, *C. elegans* homologues have been identified for 60-80% of human genes (Harris *et al*., 2004). This is also the reason why the nematode is considered to be a powerful experimental tool to study a large variety of biological processes and events that are closely related to human diseases, such as cell signaling, cell cycle, apoptosis, aging, sex determination and so forth (Riddle, 1997).

Staphylococcus aureus

C. elegans establishes a convenient model to study host-pathogen interactions (Kim, 2008). The pathogen of interest in my study is *Staphylococcus aureus*. It is a facultative anaerobic gram-positive coccal bacterium also known as the "golden staph" (Liu *et al*., 2005). When viewed under a microscope, *S. aureus* appears as grape-like clusters and has large, round, yellowish colonies. It is part of the human microbiota (Javier, 2010), as it is commonly found in the human respiratory tract and on the skin. Although *S. aureus* is not always harmful, it is extremely versatile and a very important pathogen for humans and many other animals (Archer, 1998). It can utilize virulence factors to cause human diseases as diverse as superficial skin infections, respiratory disease, soft tissue abscesses, and life-threatening infections such as toxic shock syndrome (Deurenberg, 2008). Notably, the treatment aimed for overcoming *S. aureus*

infections has been complicated by the emergence of strains that are resistant to multiple antibiotics (Santos *et al*., 2000).

The disease-associated *S. aureus* strains often induce infections by producing a wide array of pathogenic factors, which can be subdivided into 3 major groups: Cell-associated products, secreted exoproteins and regulatory loci (Sifri *et al.*, 2003). Typical examples for cell-associated products include the adhesins of the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) and capsular polysaccharides, which aid the bacteria in attaching to host cells and evading host immune mechanisms (Sifri *et al*., 2003). Secreted exoproteins include cytolysins (e.g., alpha-hemolysin) and extracellular proteases (e.g., V8 protease), which play potential roles in combating host defenses, as well as facilitating nutrient acquisition and tissue invasion (Sifri *et al*., 2003). Regulatory loci represent a number of genes that regulate the expression of these pathogenicity factors in response to a variety of environmental conditions, such as osmolarity, oxygen concentration and PH (Goerke *et al.*, 2001). The most extensively studied regulators are the global virulence regulatory loci *agr* and *sarA* (Luong *et al*., 2003).

In addition, many nosocomial infections and infections on indwelling medical devices caused by *S. aureus* involve biofilm formation (Otto, 2008). As previously stated, *S. aureus* are commensal microbes that colonize human skin and mucous surfaces, making it possible for the bacteria to contaminate any medical devices that penetrate skin or mucous surfaces (Otto, 2008), and induce pathogenic complications.

C. elegans *Egg-laying Behavior*

Egg-laying behavior of *C. elegans* upon interaction with *S. aureus* was specifically studied in my research.

Egg-laying is among the most important *C. elegans* life events. It is regulated by a number of environmental conditions that, if favorable, occurs in a specific temporal pattern (Waggoner *et al*., 1998) (Zhou *et al*., 1998). *C. elegans* hermaphroditic individuals self fertilize, producing sperm first that are stored in the spematheca, and then producing oocyte (Schafer, 2005). Within the first day of L4 /adult molt, animals accumulate fertilized eggs in the uterus. At any given time, a young adult animal is able to hold up to 10-15 eggs in the uterus. When specialized sex-specific muscles contract, egg-laying occurs: animals open the vulva and allow eggs to be expelled (Schafer, 2005).

16 muscles are usually considered to be participating in the process of egg-laying. There are 2 sets of vulva muscles: vm1 and vm2, with each set containing 4 muscle cells (Schafer, 2005). The vm2 muscle groups are located in between the ventral margin of the body wall and the uterus-vulva junction. They are the most essential partners in egg-laying, and the only related muscles that receive significant synaptic input from the neurons that control the behavior (White *et al.*, 1986). Knocking out of the vm2 muscles leads to a total destruction of egg-laying (Trent, 1983). It is believed that the contraction of vm2 muscles is critical for opening the vulva. The 4vm1 muscles are located on the dorsal margin of the body wall. They work in a similar way with vm2 muscles, however, without innervation. The ablation of vm1 muscles does not significantly influence

egg-laying behavior. In addition, 8 uterine muscles could also play a role in the process. They form bands that surround the anterior and posterior sides of the uterus. They are thought to promote laying eggs because the contraction of those muscles can constrict the uterus, furthermore pushing eggs out of the vulva. Although, destroying all of the 8 muscles does not cause any significant defect in egg-laying.

Various environmental factors affect egg-laying event. Firstly, a favorable living condition for *C. elegans* is advantageous for egg-laying. For example, worms have higher egg-laying rate in the presence of abundant food than in the absence of food (Trent, 1982). Also, if worms are kept in a temperature that is lower than 15 °C, they will have lower egg-laying rate than if kept in their optimal growing temperature such as 20 °C. Secondly, a high salty environment inhibits egg-laying behavior of the worms. If worms are kept in a hypertonic M9 solution, they will have a significantly reduced egg-laying rate (Horvitz *et al*., 1982). Thirdly, environmental forces that affect the worms or the culture media could also suppress egg-laying, such as vibration and shaking.

C. elegans *Egg-laying and Nervous System*

Behavior depends on the ability of the nervous system to establish temporal patterns of muscle contractions and to modulate these patterns in response to sensory information (Waggoner *et al.*, 1998). The hermaphrodite specific neurons (HSNs) play a critical role in the normal execution and regulation of *C. elegans* egg-laying event. HSN cell bodies are located posterior and lateral to the vulva, extending long ventrally to the

ventral nerve cord and anteriorly to the nerve ring. They make important neuron-muscle junctions with the vm2 muscles (Schafer, 2005). Removal of the HSNs greatly reduces egg-laying rate, and in many egg-laying defective mutants, the function and development of the HSNs are abnormal (Desai and Horvitz, 1989).

The HSNs use at least three neurotransmitters: serotonin (Horvitz *et al*., 1982) (Desai *et al*., 1988), acetylcholine (Duerr *et al*., 2001), and one or more neuropeptides (Schinkmann and Li, 1992) (Kim and Li, 2004). Studies have shown that exogenous serotonin rescues egg-laying defect (Trent *et al*., 1983), indicating the importance of serotonin level in maintaining normal egg-laying rate. As shown in figure 3 (David *et al.*, 1995), the HSNs release the serotonin receptor 5-HT, which takes actions on egg-laying muscles directly, leading to the contraction of the muscles. Figure 3 also depicts the role of an antidepressant imipramine in *C. elegans* egg-laying event. Imipramine is used in the treatment of depression, such as depression associated with agitation or anxiety. It is similar in efficacy to the antidepressant drug moclobemide. It has also been used to treat nocturnal enuresis because of its ability to shorten the time of delta wave stage sleep, where wetting occurs. In *C. elegans* egg-laying process, tt works by blocking the reuptake of 5-HT released by HSNs, increasing the level of 5-HT in the synaptic cleft (Briley, 1985). Although, the detailed mechanism of imipramine is not clear, and it did not show a specific important role in controlling *C. elegans* egg-laying in our experiment.

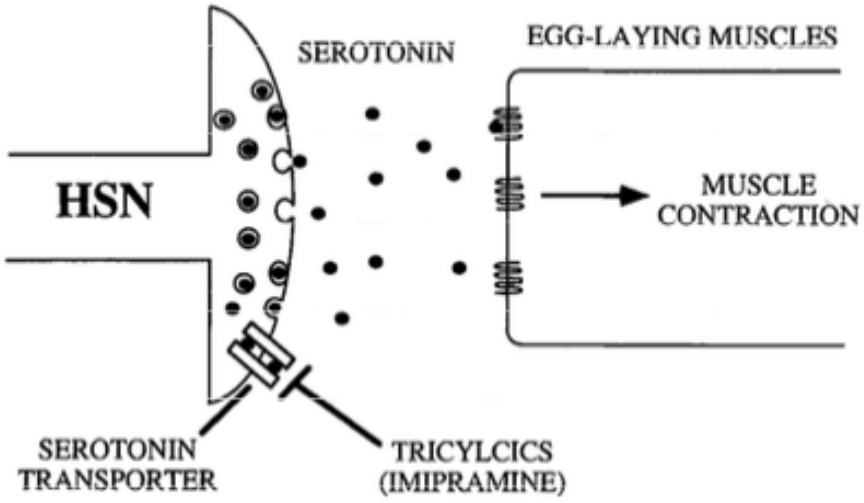

Figure 3. A schematic model of *C. elegans* egg-laying (David *et al*., 1995)

Research Rationale

As a potent pathogen, *S. aureus* is capable of causing a variety of human diseases in both hospitals and community settings. Importantly, the rapid emergence of antibiotic resistant *S. aureus* strains has highlighted the urgent need to develop newer and safer strategies to combat the bacterial infections (Kong *et al*., 2014). Unlike mammalian pathogenesis models that are generally difficult to establish, nematode *C. elegans* serves as a simple and effective model host in studying host-pathogen interactions (Alegado *et al*., 2003).

C. elegans egg-laying behavior was the focus of my research. It is one of the most important *C. elegans* life events. Besides, many neurotransmitters and neuronal signal transduction pathways have been shown to have specific effects on egg-laying behavior,

making it useful to analyze in order to understand many neurobiological problems in *C. elegans* (Geng *et al*., 2005).

Specific Aim

We were able to observe an egg-laying defect of *C. elegans* when growing in *S. aureus* lawn. Several strains of *C. elegans* were used, including wild type N2, *che-3 (e1124)*, *pat-3 (sp)* and *pat-3 (+)* (Kihira *et al*., 2012). They all showed signs of defect when feeding on *S. aureus*. We also tested different *S. aureus* strains and observed that those with prolific biofilm formation caused more severe egg-laying defect in worms than those that do not form biofilm, suggesting a potential role biofilm plays in inducing the defect. We transferred worms shortly after infected by *S. aureus* back to a normal condition with E. coli as food, and observed an increase in egg-laying rate, indicating that the defect was reversible. In addition, we isolated a mixture of small sized molecules (less than 10 kDa) secreted by *S. aureus* and fed to worms in 96 well plates. Worms showed similar egg-laying defect, which could be rescued by the adding of exogenous serotonin.

CHAPTER TWO

Materials and Methods

C. elegans

C. elegans Bristol strain N2 (wild type) nematodes were used in most of the experiments conducted in the lab. They were maintained on NGM (Nematode Growth Media) plates seeded with *Escherichia coli* strain OP50 as food source at 25 °C. CB1124 *che-3* (*e1124*) nematode strain was used in parts of the research. *che-3* gene encodes a dynein heavy chain (DHC) 1b isoform that takes effect on the establishment and maintenance of the structural completeness of sensory cilia, and also plays a role in intraflagellar transport (Jonathan, 1982). The protein product CHE-3 is expressed in ciliated sensory neurons, lack of which leads to a phenotype of disordered eating habits. All nematode animals were observed under SZ25 Olympus compound microscopes, handled with aseptic technique (a technique to prevent unwanted microorganisms in one's culture/media), and manipulated with standard established lab procedures (Lewis and J.T., 1995).

Bacterial Strains

Escherichia coli strain OP50 was used as *C. elegans* main food source. It was grown and cultured in standard Lysogeny Broth (LB), and inoculated onto the NGM plates 8-10 hours prior to being fed to worms. Various *S. aureus* strains were used in this

research. *S. aureus* RN4220 (Nair *et al.*, 2011), the most often used strain, is a key reference laboratory strain. It is also the most virulent strain of all that used; *S. aureus* dltA (Peschel *et al.*, 1999) strain has the enzyme DltA knocked out, which is needed for wall teichoic acid biosynthesis, causing the defect in forming biofilm. Notably, the parent strain of dltA is SA113, which forms biofilm rather well; *S. aureus* S30 (Tu Quoc *et al*., 2007) is a clinical strain, which forms prolific biofilm and is not resistant to any antibiotic except for penicillin. The *S. aureus* bacteria were grown and cultured in Tryptic Soy Broth (TSB) media.

Chemicals

A couple of antibiotics were used to inhibit the growth of *S. aureus*, in order to compare the effect of the antibiotics on the virulence of the bacteria. Vancomycin and oritavacin were tested. To make their stock solutions, vancomycin was dissolved in water and oritavacin was dissolved in dimethyl sulfoxide (DMSO). Afterwards the appropriate amount of the stock solutions was added to each plate containing 1ml of TSB with 1% w/v glucose. The concentration gradient for both of the antibiotics was 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 micrograms/milliliter.

MBX compound, which inhibits the formation of *S. aureus* biofilm, was used when testing the virulence of biofilm on *C. elegans*. Two types of MBX were used. They are MBX-1420 and MBX-1247 (Timothy *et al.*, 2009). They were dissolved in DMSO.

The concentrations tested, that were tolerable by *C. elegans* worms, were 0, 8, 32, 64, 128, 192 and 240 micrograms/milliliter.

Serotonin and imipramine were used to rescue the egg-laying defect in *C. elegans* caused by *S. aureus*. They were added in respected liquid media (LB/TSB/M9 buffer) at the concentration of 5 mg/ml.

Making Bacterial Seeded Plates

For control groups where *C. elegans* was fed with *E. coli* OP50, bacteria culture was incubated for 12-16 hours at 37 °C before inoculated on the standard NGM plates. 100 ml bacteria culture was used per NGM plate that is 60mm in diameter. They were then left on bench top in room temperature for 8 hours before used. For groups where *C. elegans* was fed with *S. aureus*, 10 µl of the bacteria frozen stock was taken from -80 degree freezer and was used to inoculate 1 ml of TSB media (1% inoculum). Then bacteria culture was incubated overnight at 37 °C with 180 rpm orbital shaking. After bacteria were fully grown, the culture was diluted at a 1:1 ratio with plain TSB media (Dilution is necessary because otherwise the bacteria colony would overgrow). 100 µl of the diluted culture was then pipetted to D=60mm NGM plates, spreading evenly with inoculating loops. All the NGM plated seeded with *S. aureus* were incubated at 37 °C overnight. During certain parts of our research, 24 or 96 well plates were used instead of NGM plates. When using wells, 20 µl of the diluted *S. aureus* culture was pipetted into

each well, which contained TSB agar supplemented with 1% w/v glucose. Afterwards the plates were incubated overnight at 37 °C.

Making S. aureus Spent Media

S. aureus frozen stock was incubated in LB or TSB overnight in a 1% inoculum (250 µl of stock culture was used to prepare 25 ml culture solution). It was then grown for 24 hours at 37 °C with 180 rpm orbital shaking. Fully grown bacteria culture was then disinfected by placing it in the boiling water bath for 30 min. Heat-killed bacteria solution was centrifuged (Beckman Coulter Allegra X-15R, with SX4750 rotor) at 4750 rpm for 15 min at 4 °C. Then the supernatant was collected and the pellet was discarded. Subsequently, the supernatant was filtered through a 10 kDa MWCO filter (EMD Millipore) and centrifuged at 4750 rpm (The amount of time it took varied depending on the experiment conditions). The filtered spent media was collected and stored at 4 °C, ready to be used.

Making S. aureus *"Dirty Water" Solution*

S. aureus stock solution was incubated the same way as the spent media. Afterwards the culture was centrifuged immediately, and the supernatant was discarded. An equal amount of autoclaved DI water was added in the *S. aureus* pellet, and the pellet was resuspended. The bacteria mix solution was then incubated for 4 hours at 37 °C with 180 rpm orbital shaking, following by the disinfection with boiling water bath for 30 min. The solution was then centrifuged down at 4750 rpm for 15 min at 4 °C. Afterwards the

supernatant ("dirty water") was collected and the pellet was discarded. "Dirty water" was then filtered through a 10 kDa MWCO filter (EMD Millipore) and centrifuged at 4750 rpm (the amount of time it took varied). "Dirty water" was collected and stored at 4 °C before putting in use.

Statistical Analysis

Program R (Version 3.2.0, 2005, The R foundation for statistical computing platform: x86_64-apple-darwin13.4.0) was used for all the statistical analysis in this thesis. Several statistical tests were performed depending on certain data type and conditions. Shapiro-Wilk normality test was performed at first to check if the data were normally distributed. If normally distributed, student's t test was performed to compare the difference in any given two sets of data. If not normally distributed (p-value>0.05), non-parametric Wilcox test was performed to compare the data. Specifically, one-tailed Wilcox test was performed for most of the data set in this thesis. Difference in two groups of data was considered significant with the associated p-value smaller than 0.05. When more than two sets of data needed to be compared at once, Kruskal-Wallis sum test was performed to decide whether the population distributions were identical without assuming them to follow the normal distribution. Chi-squared values were calculated and the data sets were considered independent: coming from unrelated populations if the associated p-values were smaller than 0.05.

CHAPTER THREE

Results

Survival Assay of C. elagans *that Fed on* E. coli *OP50 and* S. aureus *RN4220*

In order to test toxicity of *S. aureus* to *C. elegans*, I conducted short-term and long-term killing assays. In control groups, I transferred L4 stage *C. elegans* animals on NGM plates seeded with *E. coli* OP50 (one worm per plate); In experimental groups, I transferred L4 stage *C. elegans* animals on NGM plates seeded with *S. aureus* RN4220 (one worm per plate). Furthermore, *S. aureus* strains were divided into 3 subgroups and treated with one of the two types of antibiotics (oritavacin and vancomycin) at 3 different concentrations: 0, 8, and 64 micrograms/milliliter. For short-term killing assay, 40 worms were used in each condition. Data was taken at 0, 1, 2, 3, 4, and 5 hours after incubation. For long-term killing assay, 20 worms were used in each condition at their L4 stage or young adult stage. Data was taken at 0, 24, 48, and 72 hours after incubation. Number of worms alive was counted for each well. Result of short time killing assay with *S. aureus* RN4220 treated by oritavacin is recorded in table 1; result of short time killing assay with *S. aureus* RN4220 treated by vancomycin is recorded in table 2; result of long time killing assay with *S. aureus* RN4220 treated by oritavacin is recorded in table 3.

Table 1. Short-term killing assay with *S. aureus* RN4220 treated by oritavacin

Bacteria used		Number of worms alive after transferring					
		0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
E. coli OP50		40	40	40	40	40	40
S. aureus with oritavacin (μg/ml)	0	40	40	40	40	40	40
	8	40	40	40	40	40	40
	64	40	40	37	38	40	40

Table 2. Short-term killing assay with *S. aureus* RN4220 treated by vancomycin

Bacteria used		Number of worms alive after transferring					
		0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
E. coli OP50		40	40	40	40	40	40
S. aureus with vancomycin (μg/ml)	0	40	37	39	39	38	39
	8	40	39	40	40	40	40
	64	40	40	40	38	39	40

From the result, we did not observe *S. aureus* killing *C. elegans* within 5 hours, indicating that the bacteria strain we used, or the specific conditions in our experiments, were not toxic to *C. elegans*.

Table 3. Long-term killing assay with *S. aureus* RN4220 treated by oritavacin

		Number of worms alive after transferring			
Bacteria used		0 hr	24 hrs	48 hrs	72 hrs
E. coli OP50		20	20	20	20
S. aureus with oritavacin (μg/ml)	0	20	20	20	20
	8	20	20	20	20
	64	20	20	20	20

From the result, we did not observe *S. aureus* killing *C. elegans* within 5 hours, or within 3 days. However, comparing to the animals that fed on *E. coli* OP50 and the animals fed on *S. aureus* that were inhibited by antibiotics oritavacin or vancomycin, worms fed on virulent *S. aureus* grew slower and produced less progeny (laid less eggs). Therefore we analyzed egg-laying defect of nematode *C. elegans* when ingesting *S. aureus*.

C. elegans *Egg-laying Defect Induced by* S. aureus *Virulent Strain RN4220*

This experiment was conducted to quantify the egg-laying defect of *C. elegans* due to *S. aureus* infection. In each condition, 20 wild type N2 *C. elegans* worms were used, with one worm per plate. L4 stage or young adult animals were transferred on NGM plates seeded with *E. coli* OP50 and *S. aureus* RN4220, respectively. The number of progeny (eggs and larvae) on every plate was recorded 24 hours after incubation in table 4.

Table 4. Number of progeny produced by *C. elegans* under different conditions

Age of worms transferred	Bacteria fed	Number of progeny (mean ± sd)
L4	*E. coli* OP50	54.55±7.54 (n=20)
	S. aureus	3.65±4.09 (n=20)
Young Adult	*E. coli* OP50	79.05±17.41 (n=20)
	S. aureus	4.60±4.91 (n=20)

Note: In the conditions where L4 worms were used, the amount of progeny produced on *E. coli* OP50 was significantly higher than that on *S. aureus* (p <0.05). In the conditions where young adult worms were used, the amount of progeny produced on *E. coli* OP50 was also significantly higher than that on *S. aureus* (p <0.05).

From the results, we can see that *C. elegans* animals fed on *E. coli* produced much more progeny than those that fed on *S. aureus*, indicating a potential egg-laying defect caused by *S. aureus* infection. Also, the defect was observed for both L4 and young adult worms that ingested *S. aureus*, suggesting that the infection could disturb the fertilization of eggs, as well as the expelling of eggs after they were formed.

Biofilm Formation from S. aureus *Contributed to the Egg-laying Defect in Infected* C. elegans *Animals*

Since the strain of *S. aureus* used previously, RN4220, is a laboratory strain that forms a heavy amount of biofilm (Nair *et al.*, 2011), the first factor I was suspicious of for causing the egg-laying defect in *C. elegans* was biofilm. To test the effect of biofilm

formation in worms' egg-laying, I selected 3 different strains of *S. aureus* (refer to "bacterial strains" in "material and method" section). They are *S. aureus* RN4220, *S. aureus* S30 (a clinical strain that forms much biofilm) (Tu Quoc *et al*., 2007), and *S. aureus* dltA (a strain that's incapable of forming biofilm) (Peschel *et al.*, 1999). L4 *C. elegans* worms were transferred on NGM plates seeded with *E. coli* OP50 and 3 strains of *S. aureus,* 20 worms were used in each condition. After 24 hours of incubation, the number of progeny was counted and recorded in table 5. After 72 hours of incubation, the appearance of progeny was observed.

Table 5. Biofilm-formation effect on *C. elegans* egg-laying

Bacteria used	Number of progeny (mean ± sd)	Description of worms (after 72 hours)
E. coli OP50	46.90±8.47 (n=20)	Progeny normal
S. aureus RN4220	2.60±2.24 (n=20)	Progeny smaller and less
S. aureus S30	3.75±2.93 (n=20)	Progeny smaller and less
S. aureus dltA	37.45±7.63 (n=20)	Progeny normal

Note: Worms fed on *E. coli* OP50 laid significantly higher amount of eggs than all 3 groups of worms that fed on *S. aureus* strains ($p < 0.05$). Worms fed on *S. aureus* dltA laid more eggs than worms fed on *S. aureus* RN4220 and *S. aureus* S30 ($p < 0.05$).

There was an obvious difference in the egg-laying capability between *C. elegans* worms that fed on *E. coli* or non-biofilm forming *S. aureus* strain, and the worms that fed on prolific biofilm forming *S. aureus* strains, indicating a potential role biofilm might be playing in causing the defect. Worms that ingested virulent and biofilm-forming *S. aureus* strains laid less progeny, and the progeny showed delayed grown. Dead eggs were also seen on some plates seeded with RN4220.

To further analyze the biofilm effect on *C. elegans* egg-laying, I performed an experiment where I tested 3 distinct conditions. In the first condition, I used N2 worms to feed on the virulent *S. aureus* RN4220 bacteria. In the second condition, I used MBX-1247 compound (Timothy *et al.*, 2009) to inhibit the formation of biofilm from *S. aureus* RN4220, therefore worms would be eating the bacteria without biofilm affecting them. In the third condition, I grew N2 worms on *E. coli* OP50 lawn that had MBX-1247 added 3 days before the experiment started to select for the optimal MBX concentration that *C. elegans* worms could tolerate. The concentration of MBX-1247 was determined to be 240 micrograms/milliliter. After 3 days when worms gained tolerance for the chemical, L4 worms were selected and transferred onto plates seeded with *S. aureus* RN4220. 20 worms were used in each condition. The number of progeny on every plate was counted and recorded 24, 48, and 72 hours after incubation, respectively. Data is recorded in table 6.

Table 6. *C. elegans* egg-laying under different conditions

Condition	Number of progeny		
	After 24 hours (mean ± sd)	After 48 hours (mean ± sd)	After 72 hours (mean ± sd)
S. aureus RN4220	1.45±3.22 (n=20)	5.05±5.70 (n=20)	9.60±19.75 (n=20)
Biofilm inhibited	8.55±9.92 (n=20)	20.85±19.38 (n=20)	29.95±26.08 (n=20)
MBX tolerant	0.70±1.38 (n=20)	2.80±3.50 (n=20)	5.90±5.38 (n=20)

Note: At all three time points, worms fed with MBX inhibited *S. aureus* laid more progeny than worms fed with *S. aureus* (p <0.05), and than worms that were tolerant of MBX (p <0.05). Worms fed with *S. aureus* and worms that were tolerant of MBX did not show a significant difference in egg-laying (p >0.05).

We can see that worms laid significantly higher amount of eggs in *S. aureus* when the biofilm formation was inhibited. It is consistent with what I obtained from previous experiment when testing different strains of *S. aureus*, confirming that biofilm might be contributing to the egg-laying defect in *C. elegans*.

Egg-laying of C. elegans *Worms Infected by* S. aureus *Increased after Transferred to* E. coli *Seeded Plates within a Certain Short Period of Time*

In the previous experiments, after L4 worms were transferred to the respected plates, they were left to grow for 24 hours before the checking of egg-laying. 24 hours is considered to be a long period of time in *C. elegans* life cycle. Therefore, I performed the

experiment to first grow N2 worms in *S. aureus*, and then transfer a group of them to *E. coli* seeded plates after 8 hours, and another group of them after 24 hours. In the mean time, 2 control groups of worms were used, with 1 group growing in *E. coli* and the other in *S. aureus*. 10 worms were used in each condition. The number of progeny on every plate was recorded in table 7 after 24 hours of incubation.

Table 7. Transferring *S. aureus* infected *C. elegans* to *E. coli* lawn improved egg-laying

Bacteria fed		Number of progeny (mean ± sd)
Control	*E. coli* OP50	57.40±5.85 (n=10)
	S. aureus	1.20±1.89 (n=10)
		Transfer *C. elegans* from *S. aureus* to *E. coli* OP50
Transfer time		Number of progeny (mean ± sd)
		After transfer / Before transfer
After 8 hours		33.20±4.14 (n=10) / 0.00±0.00 (n=10)
After 24 hours		5.10±4.48 (n=10) / 0.90±1.64 (n=10)

Note: For control groups, worms fed on *E. coli* OP50 laid significantly more eggs than worms fed on *S. aureus* (p <0.05). Transferring worms back to *E. coli* after growing in *S. aureus* rescued egg-laying defect to some degree. When transferred after 8 hours, worms laid more eggs than before transfer (p <0.05), and than the control worms that fed on *S. aureus* (p <0.05). When transferred after 24 hours, worms also laid more eggs than before transfer (p =0.0076), and than control worms that fed on *S. aureus* (p =0.014). Notably, worms transferred after 8 hours had better egg-laying rate than those transferred after 24 hours (p <0.05). The control worms fed on *E. coli* OP50 still laid more eggs than either of the 2 groups after they were transferred (p <0.05).

In conclusion, comparing to worms fed on *E. coli*, those that fed on *S. aureus* RN4220 had significantly lower amount of progeny. However, we observed an improvement of egg-laying in *S. aureus* infected worms after they were transferred to *E. coli* OP50 seeded plates. The improvement was not a complete rescue, however, as can be seen from Table 7 that the number of progeny produced in the tested groups after transfer was still less than the worms that had been always fed on *E. coli* OP50, suggesting that worms might need longer time to recover than the 24 hours we used in the experiment, or the defect was too severe for worms to completely overcome. In addition, worms that were transferred after 24 hours of infection did not recover as well as the ones transferred after 8 hours, suggesting that the egg-laying defect could be recovered better if worms were transferred to a healthy environment earlier after infection took place.

S. aureus *Induced Egg-laying Defect in Cilia-defective* C. elegans *Strain*

To confirm that *C. elegans* animals were actually ingesting *S. aureus*, I observed the egg-laying of *C. elegans* strain CB1124 *che-3* (*e1124*) (Jonathan, 1982) when growing on *S. aureus*. It is a cilia-defective strain, which lacks the recognition and preference for food. L4 worms of wild type N2 and the mutant CB1124 were transferred to *E. coli* OP50 or *S. aureus* seeded plates, and were allowed to grow for 24 hours before the number of progeny on every plate was counted and recorded. 20 worms were used in each condition.

Table 8. Egg-laying of cilia-defective *C. elegans* strain in *S. aureus*

Worms strains	Bacteria fed	Number of progeny (mean ± sd)
N2	*E. coli*	46.15±6.22 (n=20)
	S. aureus	1.80±2.14 (n=20)
CB1124	*E. coli*	33.75±9.58 (n=20)
	S. aureus	0.00±0.00 (n=20)

Note: For N2 strain, worms laid more eggs when fed on *E. coli* OP50 than when fed on *S. aureus* (p <0.05); For CB1124 strain, worms also laid more eggs when fed on *E. coli* OP50 than when fed on *S. aureus* (p <0.05).

We can see from Table 8 that the cilia-defective strain CB1124 *che-3* (*e1124*) showed egg-laying defect when fed on *S. aureus* RN4220, indicating that *C. elegans* were actively ingesting *S. aureus* when growing in them.

Serotonin Improved C. elegans *Egg-laying after they were Infected by* S. aureus

As discussed in the introduction section, the HSNs are critical to the normal execution and regulation of *C. elegans* egg-laying. Ablation of the HSNs would result in a strong reduction in egg-laying rate, which has been proved in many egg-laying defective mutants that have abnormal HSN development or function (Desai and Horvitz, 1989). Since exogenous serotonin rescues the egg-laying defects (Trent *et al.*, 1983), it is therefore assumed that HSNs promote egg-laying at least in part by releasing serotonin as a neuromodulator. Imipramine, a tricyclic antidepressant, is also proved to ease the

egg-laying defect in certain mutants (Briley, 1985). Therefore, I performed experiments to test the effectiveness of these 2 drugs on rescuing *C. elegans* egg-laying defect after infected by *S. aureus* RN4220. L4 worms were transferred on *E. coli* or *S. aureus* seeded plates and left in the incubator to grow for either 8 hours or 24 hours. Then individual worms were transferred in 96 well plates where water, serotonin, or imipramine was added. The number of eggs laid was recorded in table 9 after 30 min and 90 min. 20 worms were used in each condition.

From Table 9, we can see that after worms were transferred into 96 well plates, those grew in *E. coli* OP50 laid more eggs comparing to worms that had fed on *S. aureus*. Worms infected by *S. aureus* almost couldn't lay eggs at all in water, confirming the egg-laying defect caused by *S. aureus*. However, if the infected worms were transferred in serotonin shortly (8 hours) after the infection, their egg-laying rate was increased, suggesting that the defect could be related to the disorder of neuronal pathway and that adding exogenous neurotransmitter serotonin could help overcome the defect. On the contrary, if worms were left in *S. aureus* for as long as 24 hours, the defect seemed not to be rescued by serotonin, suggesting that the defect might be too severe to be rescued after a long period of infection. In addition, imipramine did not work as well as serotonin, because it did not change the egg-laying rate of worms in any of the conditions significantly.

Table 9. Effect on *C. elegans* egg-laying by serotonin and imipramine

Worms grew on NGM for 8 hours before transferred to 96 well plates

Bacteria fed	Liquid in 96 wells	Number of eggs after 30 min	Number of eggs after 90 min
E. coli	Water	10.10±4.15 (n=20)	10.10±4.15 (n=20)
	Serotonin	9.32±2.64 (n=20)	9.32±2.64 (n=20)
	Imipramine	9.42±1.93 (n=20)	9.42±1.93 (n=20)
S. aureus	Water	0.00±0.00 (n=20)	0.00±0.00 (n=20)
	Serotonin	5.53±3.60 (n=20)	5.53±3.60 (n=20)
	Imipramine	0.74±0.91 (n=20)	0.74±0.91 (n=20)

Worms grew on NGM for 24 hours before transferred to 96 well plates

Bacteria fed	Liquid in 96 wells	Number of eggs after 30 min	Number of eggs after 90 min
E. coli	Water	5.47±2.60 (n=20)	5.47±2.60 (n=20)
	Serotonin	2.20±2.71 (n=20)	5.42±2.09 (n=20)
	Imipramine	4.20±3.49 (n=20)	4.89±1.80 (n=20)
S. aureus	Water	0.00±0.00 (n=20)	0.00±0.00 (n=20)
	Serotonin	0.60±1.20 (n=20)	0.84±1.76 (n=20)
	Imipramine	0.37±0.93 (n=20)	0.37±0.93 (n=20)

Note: For worms fed on *E. coli* OP50, there was no significant difference in egg-laying when they were placed in water, serotonin, and imipramine ($p > 0.05$); for worms that had grown in *S. aureus* seeded plates for 8 hours, they laid more eggs in serotonin than those in water ($p < 0.05$), or in imipramine ($p = 0.004$). But there was no big difference between worms in water and worms in imipramine ($p = 0.146$); for worms that had grown in S. aureus for 24 hours, egg-laying of worms in water, serotonin and imipramine had no significant difference ($p > 0.05$).

Small Molecules Secreted by S. aureus *RN4220 Induced Egg-laying Defect in* C. elegans*, and the Defect could be rescued by Serotonin*

Besides studying the relation between biofilm formation of *S. aureus* and *C. elegans* egg-laying defect, we were suspicious of other molecules or toxins secreted by *S. aureus* to be responsible for the defect. We were scaling down the molecules' range to test and decided to start from examining the smaller molecules secreted by *S. aureus* because they are the most easily to isolate. We prepared *S. aureus* spent media and dirty water (Refer to "material and method" section for how they were prepared), which were the mixture of molecules smaller than 10kDa. For the control groups, L4 worms were grown in *E. coli* or *S. aureus* for 24 hours, and then were transferred in wells with either water or serotonin added. For the test group, L4 worms were grown on *E. coli* OP50 for 24 hours, and then were transferred in wells added with *S. aureus* spent media, or with spent media plus serotonin. 10 worms were used in each condition. The number of eggs was counted in each well at 1, 2, 3, 4, and 7 hours post transferring and recorded in table 10.

To further test the effect from *S. aureus* small molecules on *C. elegans* egg-laying, I repeated the experiment with *S. aureus* dirty water. L4 worms were grown on *E. coli* OP50 for 24 hours, and then were transferred in 96 wells with water, water plus serotonin, dirty water or dirty water plus serotonin, respectively. 20 worms were used in each

condition. The number of eggs laid in each well was recorded 2 hours after worms were placed in the wells.

Table 10. Using *S. aureus* spent media to test *C. elegans* egg-laying

Bacteria used	Liquid media in wells	Number of eggs produced after certain hours (mean ± sd) (n=10)				
		1 hr.	2 hrs.	3 hrs.	4 hrs.	7 hrs.
E. coli	Water	2.70±2.19	2.70±2.19	2.70±2.19	2.70±2.19	3.10±1.92
	Serotonin	3.50±3.17	7.60±3.77	7.70±3.85	7.70±3.85	7.70±3.85
S. aureus	Water	0.30±0.64	0.30±0.64	0.30±0.64	0.30±0.64	0.30±0.64
	Serotonin	0.30±0.90	0.30±0.90	0.30±0.90	0.30±0.90	0.30±0.90
E. coli	SpentM	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.46
	SpentM +serotonin	0.10±0.30	8.30±3.03	11.0±3.74	11.8±3.06	16.7±3.83

Note: At 7 hours, worms fed with *E. coli* laid more eggs in serotonin than in water (p <0.05). Worms fed on *S. aureus* did not lay more eggs in serotonin than in water (p =0.708). They also had much less eggs comparing to the worms fed with *E. coli* (p <0.05). As for worms that were transferred into wells with *S. aureus* spent media, they laid significantly less eggs than *E. coli* fed worms in water (p <0.05). But their egg-laying rate was highly improved when serotonin was added in the *S. aureus* spent media (p <0.05).

Dirty water was also tested and data was recorded in table 11, after counting the number of eggs produced in the wells under each condition. Worms were left in wells for 2 hours to allow for the egg-laying to occur.

Table 11. Using *S. aureus* dirty water to test *C. elegans* egg-laying

Liquid media in the wells	Number of eggs produced in 2 hours (mean ± sd)
Water	6.45±3.50 (n=20)
Water + serotonin	10.65±2.61 (n=20)
S. aureus dirty water	2.05±2.18 (n=20)
S. aureus dirty water + serotonin	14.50±5.63 (n=20)

Note: Worms in water laid significantly more eggs than worms in *S. aureus* dirty water (p =0.046). Serotonin improved the egg-laying rate of worms in *S. aureus* dirty water (p <0.05). Worms in wells with dirty water plus serotonin laid similar amount of eggs with worms in water plus serotonin (p =0.498).

Similar result was obtained from *S. aureus* dirty water test and *S. aureus* spent media test. Comparing to the worms in water, those placed in *S. aureus* dirty water or *S. aureus* spent media laid significantly less eggs, suggesting that small molecules secreted by *S. aureus* could contribute to the egg-laying defect. Importantly, the defect was rescued by adding serotonin in the wells, suggesting that the defect might be neuronal, and similar with depression, could probably be improved by the releasing of more neurotransmitter.

CHAPTER FOUR

Discussion

S. aureus *did not Kill* C. elegans*, instead it Induced Egg-laying Defect in the Infected Nematode Animals*

In our experiments, we did not observe the dying of *C. elegans* when growing on *S. aureus* lawn (Table 1; Table 2; Table 3). However, it was consistently shown that *C. elegans* worms had egg-laying defect when feeding on *S. aureus* (Table 4). Different *C. elegans* and *S. aureus* strains had been used, and in the plates seeded with virulent *S. aureus* strains, worms showed difficulty in producing progeny even when the eggs were observed in their vulva. Comparing to wild type animals that ingested *E. coli* OP50, infected animals laid significantly less, if any, eggs. The few eggs they laid occasionally also took relatively longer time to hatch. Dead eggs were also seen often on the *S. aureus* plates, indicating a possibility of defective fertilization, or the expelling of unfertilized eggs.

In addition, one of our experiments showed that *C. elegans* egg-laying defect caused by ingesting *S. aureus* could be rescued to some extent if animals were transferred to a nonpathogenic food source, such as *E. coli* OP50 lawn (Table 7), indicating that the defect might occur early enough during the infection. And given enough time, the bacteria can be cleared out of *C. elegans* animals' intestine. Importantly, animals can only be rescued if they were transferred back to a normally

healthy environment within a short period of time after feeding on *S. aureus* (Table 7). If worms had lived on *S. aureus* lawn for as long as 24 hours, for example, the egg-laying defect still persisted after they were transferred to *E. coli* seeded plates. It was also tested for whether *C. elegans* worms were actually eating *S. aureus*, since animals tended to move away from *S. aureus* colonies on the plates possibly due to smells and the sticky surface. Animals could have problems in developing and egg-laying simply due to the lack of food and starvation. We used *C. elegans* strain CB1124 *che-3 (e1124)*, which is a cilia defectives train that cannot recognize smells and tend to ingest everything, even the agar. This strain of worms ingested *S. aureus* bacteria regardless of its smell. When growing in the *S. aureus* lawn, the animals also showed the same egg-laying defect (Table 8), confirming that the problem did come from worms' eating *S. aureus*.

In the meantime, our research devoted in finding out what virulence factor of *S. aureus* was responsible for inducing the egg-laying defect. The first thing we were suspicious about was biofilm formation from *S. aureus*, since there was a difference in *C. elegans* worms' behavior when grown on different *S. aureus* strains: Non-biofilm forming strains of *S. aureus* did not cause big problems to the nematode (Table 5). We designed the experiment where we tested the effect of biofilm simply by feeding a group of worms with *S. aureus* whose biofilm was removed by MBX compound. The result clearly showed that worms laid more eggs in *S. aureus* when the biofilm was inhibited than in prolific biofilm forming *S. aureus* (Table 6),

indicating the possible relation between biofilm formation and egg-laying defect of *C. elegans*. Noticeably, worms that were previously grown on plates with MBX-1247 added and expected to gain the immunity from biofilm did not show an improvement in egg-laying on *S. aureus*. It invalidated the designed model, which I thought could help worms overcome the effect of biofilm. It could be because the effect from the chemicals worn off fast enough to not be able to protect the worms when they were growing on *S. aureus* coated plates. Or it could be due to the fact that worms did not ingest enough chemicals, and there was no good way for us to confirm whether or not the chemicals were actually in the worms' body. In addition, since the worms laid the least eggs in this condition, there is a possibility that the chemicals were also somewhat harmful to the worms, which was counter effective when they were growing in an unfavorable environment. However, comparing to wild type animals that fed on *E. coli,* worms that ingested *S. aureus* without biofilm still laid less eggs, meaning that the removal of biofilm was not a completely rescue to worms regarding the egg-laying event. Therefore, there could be other factors that play an important role in inducing the egg-laying defect. There are many proteins and toxins *S. aureus* is capable of producing to cause infections in animals. More factors need to be considered and tested in order to elucidate the infection mechanism.

S. aureus *Small Molecules Contribute to the Egg-laying Defect in* C. elegans*, which can be Rescued by the Neurotransmitter Serotonin*

We started the process of finding more factors of *S. aureus* that could be responsible in inducing the *C. elegans* egg-laying defect by isolating the group of smaller molecules (smaller than 10 kDa), to be specific, small molecules. We designed different experimental settings for these tests, where we used 96 well plates in addition to NGM plates to conduct the experiments. *C. elegans* animals that fed on *E. coli* OP50 were transferred into wells where isolated *S. aureus* small molecules mixture was added. Comparing to wild type animals that were simply placed in wells with water, those animals showed signs of egg-laying defect (Table 10), indicating the possible influence from *S. aureus* small molecules. Noticeably, the defect took place quickly, usually within just a couple of hours after healthy worms were placed into the small molecule media.

We were also interested in discovering if there is a way to rescue the defect. Because serotonin plays an essential role in egg-laying event, and the antidepressant imipramine also has effect on the process (Briley, 1985), we tested whether the adding of serotonin or imipramine in wells with *S. aureus* small molecules media could rescue the egg-laying defect of *C. elegans* animals. From the result of repeated experiments, we saw that serotonin indeed increased the egg-laying amount in all groups of worms, and significantly improved the egg-laying rate in the defected worms that had grown in *S. aureus* small molecule mixture (Table 9; Table 10). We

can suggest that the virulent *S. aureus* interfered with the neuronal pathway of *C. elegans* and furthermore caused obstacle in egg-laying. Further research can be done to enable us to better understand the mechanism involved in the egg-laying. It is of great importance to study serotonin and neuronal pathway in *C. elegans* because the biochemical pathway of serotonin synthesis is conserved between *C. elegans* and humans (Sze *et al*., 2000) (Loer and Kenyon, 1993). Many neurotransmitters found in mammals are also found in *C. elegans* (Sulston, Dew, and Brenner, 1975). If the egg-laying defect we observed in *C. elegans* worms induced by *S. aureus* is in fact due to the disorder in the serotonergic targets, a more in-depth research in the *C. elegans* serotonin pathways could allow us to understand many of humans' defects that are related to neuronal disorders.

What Can be Done in the Future for a More In-depth Study?

To explore and understand better of the egg-laying defect and *C. elegans* interaction with the pathogen *S. aureus*, more in-depth experiments need to be done. We know that biofilm and small molecules secreted by *S. aureus* virulent strain affects *C. elegans* animals' health and egg-laying event, but it is not known to us for now what exactly the proteins are, and if other molecules, such as lipids, polysaccharides and glycopeptides, contribute to the defects as well. In addition, there are many crucial mammalian virulence factors in *S. aureus*, such as the global virulence regulators *agr* and *sarA*, which was proved to attenuate in *C. elegans*

pathogenesis process (Luong *et al*., 2003). Those regulators, as well as their downstream targets such as V8 protease and alpha-hemolysis (Tamber and Cheung, 2009), can all be tested in our research to establish a complete *C. elegans* and *S. aureus* pathogenesis model. On the other hand, from the host perspective, it was found in previous study that *C. elegans* animals that are unable to signal through the p38 MAPK cascade is more susceptible to *S. aureus* infection than wild type nematode (Irazoqui *et al*., 2008). A study into important pathways in *C. elegans* could also help with the advance of this research.

REFERENCES

Alper S, *et al*. 2008. Identification of innate immunity genes and pathways using a comparative genomics approach. *Proc. Natl. Acad. Sci*. 105:7016–7021.

Alegado RA, Campbell MC, Shen WC, Slutz SS, and Tan MW. 2003. Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. *Cell Microbiol*. 5:435-444.

Archer GL.1998. *Staphylococcus aureus*: a well-armed pathogen. *Clin Infect Dis.* 26:1179-81.

Brenner S. 1974. The genetics of *Caenorhabditis elegans*. Genetics. 77:71-94.

Chris M, Dietmar S. 2010. Histone tales: echoes from the past, prospects for the future. *Genome Biology*. 11:105.

Cassada RC, Russel RL. 1975. The dauerlarva, a post-embryonic developmental variant of nematode *Caenorhabditis elegans. Devl Biol*. 46:326-342.

David W, Gian G, and James T. 1995. Genetic and Pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. *The Journal of Neuroscience*. 15:6975-6985.

Deurenberg RH, Stobberingh EE. 2008. The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution*. 8:747-763.

Desai, C., Horvitz, H.R. 1989. *Caenorhabditis elegans* mutants defective in the functioning of the motor neurons responsible for egg laying. *Genetics.* 121:703–721.

Durr JS, Gaskin J, and Rand JB. 2001. Identified neurons in *C. elegans* coexpress vesicular transporters for acetylcholine and monoamines. *Am. J. Physiol. Cell Physiol*. 280:1616-1622.

Elizabeth M and Robin M. 2012. *Caenorhabditis elegans*, a Model Organism for Investigating Immunity. *Appl. Environ. Microbiol*. 7:2075-2081.

Grant BD, Wilkinson H. 2003. Functional genomic maps in *Caenorhabditis elegans*. *Curr Opin Cell Biol*. 15:206-212.

Gettner, S.N, C. Kenyon, and L.F. Reichardt. 1995. Characterization of *pat-3* heterodimers, a family of essential integrin receptors in *C. elegans*. *Journal of Cell Biology.* 129:1127-1141.

Goerke C, Fluckiger U, Steinhuber A, Zimmerli W and Wolz C. 2001. Impact of the regulatory loci *agr, sarA* and *sae* of *Staphylococcus aureus* on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis. *Mol Microbiol*. 40:1439-47.

Horvitz, H.R, Chalfie M, Trent C, Sulston J.E, and Evans P.D. 1982. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science. 216*:1012–1014.

Harris, T. W. *et al*. 2004. A multi-species resource for nematode biology and genomics. *Nucleic Acids Res.* 32:411–417.

Han SM, Cottee PA, and Miller MA. 2010. Sperm and oocyte communication mechanisms controlling C.*elegans* fertility. *Dev Dyn*. 239:1265-1281.

Hodgkin J, Horvitz HR, and Brenner S. 1978. Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics*. 91:67-9.

Hunt R, et al. 2007. High-throughput in vivo analysis of gene expression in *Caenorbabditis elegans*. *PloS Biol*. 5:e237.

Irazoqui JE, Ng A, Xavier RJ, Ausubel FM. 2008. Role for beta-catenin and HOX transcription factors in *Caenorhabditis elegans* and mammalian host epithelial-pathogen interactions. *Proc. Natl. Acad. Sci. U. S. A.* 105: 17469–17474.

Javier I, Emily R. 2010. Distinct pathogenesis and host response during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. 10:1371.

Justyna B, Olga S, and Przemyslaw B. 2011. Characterization of virulence factors of Staphylococcus aureus: Novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. *Journal of pathogens*. 10:4061.

Jonathan H. 1982. Male Phenotypes and Mating Efficiency in *Caenorhabditis elegans. Genetics*. 103:43-64.

Kuwabara, P. E, O'Neil N. 2001. The use of functional genomics in *C. elegans* for studying human development and disease. *J. Inherit. Metab Dis.* 24:127–138.

Kim D. 2008. Studying host-pathogen interactions and innate immunity in *Caenorhabditis elegans*. *Dis Model Mech*. 1:205-8.

Kim K and Li C. 2004. Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *J. Comp. Neurol*. 475:540-550.

Kristoffer TB, Dorte F, Adriana R, Christine B, Natalia R, Caroline M, William LK. 2013. Genetic Variation in the *Staphylococcus aureus* Strain Lineage Revealed by Whole-Genome Sequencing. 10:1371.

Klass M, Hirsh D. 1976. Non-aging development variants of *Caenorhabditis elegans*. *Nature*. 260:523-525.

Kihira S, Yu EJ, Cunningham J, Cram EJ, Lee M. 2012. A novel mutation in β Integrin Reveals an Integrin-Mediated Interaction between the Extracellular Matrix and *cki-1/p27^{KIP1}*. *Plos one*. 10.1371.

Kong C, Yehye WA, Rahman NA, Tan MW, and Nathan S. 2014. Discovery of potential anti-infectives against *Staphylococcus aureus* using a *Caenorhabditis elegans* infection model. *BMC Complementary and Alternative Medicine*. 14:1186-1472.

Lai CH, Chou CY, Chang LY, Liu CS, Lin W. Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. *Genome Res.* 10:703–713.

Lewis J. A, and J. T. Fleming. 1995. *Caenorhabditis elegans*: modern biological analysis of an organism. Ph.D thesis. Academic Press, San Diego, CA. pp. 3-29.

Levitan, D, Greenwald I. 1995. Facilitation of lin-12-mediated signaling by *sel-12*, a *Caenorhabditis elegans* S182 Alzheimer's disease gene. *Nature.* 377:351–354.

Lindsay JA. 2010. Genomic variation and evolution of *Staphylococcus aureus*. *Microbiol*. 300:98–103.

Luong TT, Newell SW, and Lee CY. 2003. *mgr*, a novel global regulator in *Staphylococcus aureus. Bacteriol*. 185:3703-3710.

Liu GY, *et al.* 2005. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *JEM.* 202:209-215.

Loer CM, Kenyon CJ. 1993. Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. *J. Neurosci.* 13:5407–5417.

Maertens D. 1975. Observations on the life cycle of *Prionchulus punctatus* (Cobb, 1917) (Nematoda) and some culture conditions. *Biol. Jaarb. Dodonaea 43*:197–218.

Nicole F, Adam A. 2008. *C. elegans* dauer formation and the molecular basis of plasticity. *Genes and Dev.* 22: 2149-2165.

Nair D, *et al*. 2011. Whole-genome sequencing of *Staphylococcus aureus* strain RN4220, a key laboratory strain used in virulence research, identified mutations that affect not only virulence factors but also the fitness of the strain. *J Bacteriol*. 193:2332-2335.

Otto M. 2008. Staphylococcal Biofilms. *Microbiol Immunol*. 322:207-228.

Peschel A, *et al.* 1999. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. *The journal of biological chemistry*. 274:8405-8410.

Ranganathan R, Sawin ER, Trent C, Horvitz HR. 2001. Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J. Neurosci.* 21:5871–5884.

Sulston J, Dew M, Brenner S. 1975. Dopaminergic neurons in the nematode *Caenorhabditis elegans. J Comp Neurol*.163:215-226.

Sifri CD, Begun J, Ausubel FM, and Calderwood SB. 2003. *Caenorhabditis elegans* as a Model Host for *Staphylococcus aureus* Pathogenesis. *Infect Immun*. 71:2208-2217.

Sze JY, Victo M, Loer C, Shi Y, Ruvkun G. 2000. Food and metabolic signaling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature.* 403:560–564.

Sulston JE and Horvitz HR. 1977. Post-embryonic cell lineages of the nematode, *Caenorhbditis eleggans*. *Dev Biol*. 1:110-56.

Santos S, Mato R, Lencastre H, and Tomasz A. 2000. Patterns of multidrug resistance among methicillin-resistant hospital isolates of coagulase-positive and coagulase-negative staphylococci collected in the international multicenter study RESIST in 1997 and 1998. *Microb. Drug Resist*. 6:199-211.

Sonnhammer EL, Durbin R. 1997. Analysis of protein domain families in *Caenorhabditis elegans*. *Genomics*. 46:200–216.

Sherrington R. *et al*. 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. 375;754–760.

Schafer RW. 2005. Egg-laying. *WormBook, ed*. The *C. elegans* research community, WormBook, doi/10.1895/wormbook.1.38.1.

Schinkmann K and Li C. 1992. Localization of FMRFamide-like peptides in *Caenorhabditis elegans. J. Comp. Neurol*. 316:251-260.

Tamber S, Cheung AL. 2009. *SarZ* promotes the expression of virulence factors and represses biofilm formation by modulating *SarA* and *agr* in *Staphylococcus aureus*. *Infect Immun*. 77:419-28.

Trent C, Tsuing N, Horvitz HR. 1983. Egg-laying defective mutants of the nematode *Caenorhabditis elegans. Genetics.* 104:619-47.

Trent C. 1982. *Genetic and Behavioral Studies of the Egg-Laying System of Caenorhabditis elegans*. Ph.D thesis. Massachusetts Institute of Technology, Cambridge, USA.

The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science*. 282:2012–2018.

Titus K, Michael H. 2006. Finding function in novel targets: C.*elegans* as a model organism. n*ature Reviews Drug Discovery* 5:387-399.

Timothy O, Steven K, John W, Atiyya K, Norton P, Donald M, and Terry B. 2009. Aryl Rhodanines Specifically Inhibit *Staphylococcal* and *Enterococcal* Biofilm Formation. *Antimicrobial Agents and Chemotherapy*. 4357-4367.

Tu Quoc PH, *et al*. 2007. Isolation and characterization of biofilm formation-defective mutants of *Staphylococcus aureus*. *Infect Immun*. 75:1079-1088.

White J, Southgate E, Thomson N and Brenner S.1986. The structure of the *Caenorhabditis elegans* nervous system. *Biol. Sci*. 314:1-340.

Waggoner L.E, Zhou G.T, Schafer R.W, and Schafer W.R. 1998. Control of alternative behavioral states by serotonin in *Caenorhabditis elegans*. *Neuron.* 21:203–214.

Wittenburg, N. *et al*. 2000. Presenilin is required for proper morphology and function of neurons in *C. elegans*. *Nature*. 406:306–309.

Zhou GT, Schafer WR, Schafer RW. 1998. A three-state biological point process model and its parameter estimation. *IEEE Trans on Signal Processing*. 46:2689-2707.